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Microbial diversity indices in burned soils estimated by Biolog and PLFA techniques

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Abstract

The BIOLOG and PLFA techniques were used to evaluate the short- and medium- term effects produced by high and low severity fires on microbial diversity. Two scrublands ecosystems from Galicia (NW Spain) with different post-fire treatments (seeding and mulching) were used, one affected by an experimental fire of low-severity and the other affected by a high-severity wildfire and the diversity indices (R, microbial richness; H, Shannon-Weaver diversity index; E, Shannon's eveness) were determined in soil samples collected from the top layer (0-2 cm) immediately and 90, 180 and 365 days after the fire. The H values calculated with the PLFA technique were strongly correlated with those obtained with the Biolog technique; thus, information concerning taxonomic diversity given by H-PLFA is consistent with that provided by H-Biolog, metabolic diversity. The values for the R, H and E indices tended to be higher in the burned soils than in the respective unburned control, indicating that the diversity was slightly higher and the microbial groups were distributed more equitably following the fire. These effects were more marked in high severity wildfire than in low severity fire and they persisted even one year after the fire. Independently of the method used (PLFA, BIOLOG) there was no evidence of any significant difference in the diversity indices among the two different post-fire treatments and the corresponding burned treatment on both sampling areas.

1 INTRODUCTION

Galicia is an area specially affected by wildfires, which are one of the main causes of soil degradation. Fire can produces important changes in physical, chemical and biological properties of soils such as number, mass, activity and diversity of soil microorganisms. However, 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 biochemical transformations in soils can be determined as the capacity of carbon substrates degradation by 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.

2 OBJECTIVE

The aim of this study was to evaluate the short- and medium- term effects produced by high and low severity fires on microbial diversity estimated by Biolog and PLFA techniques.

Table 1. Biolog and PLFA diversity indices in A Estrada and Laza soils (mean values ± SE of three field replicates). R, Microbial richness; H, Shannon-Weaver diversity index of fatty acid (PLFA) and substrate (Biolog); E, Shannon's eveness. U, unburned soil; B, burned soil; B+S, burned soil with seeding; B+M, burned soil with straw addition.

Soil	Diversity		Treatments							
	Index	Time (days)	U		B		B+S		B+M	
A Estrada	R-Biolog	1	21.45 ± 0.947	a	25.25 ± 0.750	a	24.25 ± 1.031	a	24.25 ± 1.031	a
		90	23.25 ± 0.479	a	26.75 ± 0.854	b	26.25 ± 1.031	b	26.50 ± 0.646	b
		180	23.00 ± 0.707	a	24.50 ± 0.866	a	25.75 ± 1.031	a	24.75 ± 0.629	a
		365	22.25 ± 1.652	a	26.00 ± 1.780	a	27.75 ± 1.780	a	26.25 ± 1.652	a
	H-Biolog	1	2.906 ± 0.036	a	3.033 ± 0.012	a	3.034 ± 0.045	a	3.018 ± 0.025	a
		90	2.970 ± 0.027	a	3.100 ± 0.045	b	3.110 ± 0.036	b	3.154 ± 0.029	b
		180	2.989 ± 0.032	a	3.058 ± 0.018	a	3.108 ± 0.040	a	3.084 ± 0.034	a
		365	2.964 ± 0.060	a	3.088 ± 0.078	a	3.136 ± 0.078	a	3.100 ± 0.077	a
	E-Biolog	1	0.945 ± 0.003	a	0.940 ± 0.006	a	0.952 ± 0.004	a	0.948 ± 0.005	a
		90	0.944 ± 0.004	a	0.943 ± 0.005	a	0.952 ± 0.003	ab	0.963 ± 0.004	b
		180	0.954 ± 0.002	a	0.957 ± 0.007	a	0.957 ± 0.004	a	0.962 ± 0.004	a
		365	0.958 ± 0.004	a	0.950 ± 0.007	a	0.944 ± 0.005	a	0.950 ± 0.007	a
	H-PLFA	1	2.898 ± 0.012	a	2.932 ± 0.005	a	2.924 ± 0.007	a	2.912 ± 0.009	a
		90	2.880 ± 0.023	a	2.940 ± 0.010	a	2.883 ± 0.032	a	2.894 ± 0.034	a
		180	2.903 ± 0.014	a	2.937 ± 0.008	a	2.924 ± 0.002	a	2.913 ± 0.002	a
		365	2.929 ± 0.007	a	2.948 ± 0.008	a	2.960 ± 0.005	a	2.948 ± 0.012	a
	E-PLFA	1	0.838 ± 0.002	a	0.846 ± 0.001	a	0.844 ± 0.002	a	0.840 ± 0.002	a
		90	0.835 ± 0.006	a	0.848 ± 0.006	a	0.834 ± 0.008	a	0.837 ± 0.008	a
		180	0.838 ± 0.004	a	0.848 ± 0.002	a	0.844 ± 0.000	a	0.841 ± 0.004	a
		365	0.845 ± 0.002	a	0.851 ± 0.002	a	0.854 ± 0.001	a	0.851 ± 0.003	a
Laza	H-PLFA	1	2.881 ± 0.001	a	2.913 ± 0.014	a	2.916 ± 0.004	a	2.916 ± 0.005	a
		90	2.880 ± 0.011	a	2.925 ± 0.009	b	2.912 ± 0.002	ab	2.938 ± 0.013	a
		180	2.925 ± 0.006	a	2.957 ± 0.006	b	2.962 ± 0.006	b	2.956 ± 0.000	b
		365	2.888 ± 0.009	a	2.931 ± 0.010	b	2.955 ± 0.007	b	2.940 ± 0.004	b
	E-PLFA	1	0.831 ± 0.000	a	0.840 ± 0.004	a	0.840 ± 0.004	a	0.841 ± 0.005	a
		90	0.830 ± 0.003	a	0.844 ± 0.003	b	0.840 ± 0.000	ab	0.848 ± 0.004	b
		180	0.844 ± 0.002	a	0.853 ± 0.002	b	0.855 ± 0.002	b	0.853 ± 0.000	b
		365	0.833 ± 0.003	a	0.846 ± 0.003	b	0.853 ± 0.002	b	0.848 ± 0.001	b

3 METHODOLOGY

Two scrublands ecosystems from Galicia (NW Spain) with different post-fire treatments were used, one located in A Estrada (Pontevedra) and affected by an experimental fire of low-severity in October 2009 and the other located in Laza (Ourense) and affected by a high-severity wildfire in September 2010. The soil samples were collected from the top layer (0-2 cm) immediately and 90, 180 and 365 days after the fire in the following treatments established by triplicate: unburned (U) control soil; burned soil (B); burned soil with a mixture of seeds (B+S); burned soil with straw mulch (B+M).

The Biolog ecoplates® technique were used to characterize the physiological profiles of the soil microbial community. The Biolog microbial richness (R-Biolog) was expressed as the number of oxidized C substrates. The Shannon-Weaver index (Shannon, 1948), which determines the substrate diversity (H-Biolog), was calculated as: $H=-\Sigma pi\ (\ln pi)$, where pi is the ratio of the activity of each substrate to the sum of the activities on all substrates. The same index was used to calculate the fatty acid diversity (H-PLFA) but, in this case, pi is the relative abundance of each fatty acid in the total sum. The microbial fatty acid abundance was determined by PLFA analysis using the procedure described by Frostegård et al (1993). The equitability on carbon source utilization and the fatty acids distribution was calculated with the Shannon's evenness index (E) [$E=H/\ln(R)$], where R is the Biolog microbial richness or the number of detected fatty acid, respectively (Shannon, 1948).

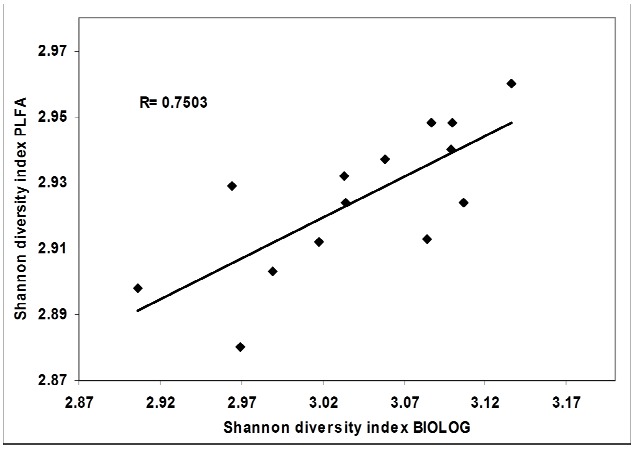

Figure 1. Correlation between fatty acid diversity (H-PLFA) and functional diversity (H-Biolog) values calculated with the Shannon-Weaver index (H) in A Estrada soils samples (n=16, p<0.001).

Data were analyzed by ANOVA1 for the same sampling time and area. The Tukey-B test was used to compare the means and to establish the different groups in case of significant differences. Correlations coefficients between microbial taxonomic and functional diversity calculated by these two independent measurements, PLFA pattern and Biolog technique, were calculated by simple regression using the average values obtained with the Shannon-Weaver index (H).

4 RESULTS AND CONCLUSIONS

The values of the diversity indices obtained for both study soils with different treatment immediately and 90, 180 and 365 days after the fire are shown in Table 1.

The richness values ranged from 21.45 to 23.25 in the unburned soils and from 24.25 to 27.75 in the burned samples; therefore, the microbial communities in the burned soils were able to use more C substrates. The H values ranged from 2.88 to 3.15 and E values from 0.83 to 0.96, the values obtained by Biolog being slightly higher than those calculated by PLFA analyses. However, the H values calculated with the PLFA technique were strongly correlated with those obtained with the Biolog technique ($r=0.7503$; $n=14$; $p<0.001$) (Fig. 1). This indicated that the information concerning taxonomic diversity given by H-PLFA is consistent with that provided by H-Biolog, metabolic diversity.

The values for the H and E indices tended to be higher in the burned soils than in the respective unburned control, indicating that the diversity was slightly higher and the microbial groups were distributed more equitably following the fire. It should be noticed that the magnitude of these microbial diversity induced changes seems to depend on fire severity since they were small and not significant in A Estrada (low severity fire) and significant and more marked in Laza (high severity wildfire), where they persisted even 1 year after the fire. The same tendency was found by others studies showing scarce and slight changes in functional diversity after the fire (Staddon et al., 1997; D'Ascoli et al., 2005). A possible explanation for these findings is that more favourable soil conditions after the fire (pH increase and higher carbohydrate availability) (Díaz-Raviña et al., 1992) may stimulate the proliferation of different microbial taxonomic groups and hence also increase the use of C-substrates. Zornoza et al. (2009) also found higher values in both H and E index on altered environment (agricultural soil). Differences in the functional evenness as well as a more marked effect of a high-compared to low-severity fire were also reported by D'Ascoli (2005).

Independently of the method used (Biolog, PLFA) there was no evidence of any significant difference in the diversity indices (R, H, E) among the two different post-fire treatments (B+S, B+M) and the corresponding burned treatment (B) on both sampling areas. The lack of any response of the microbial diversity 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).

We can conclude that, despite the reduction in its biomass and activity (Díaz-Raviña et al., 2006 and 2012; Barreiro et al., 2010; Fontúrbel et al., 2012) and the change in microbial community structure (Díaz-Raviña et al., 2006; Barreiro et al., 2010) produced as a consequence of prescribed or wildland fire, the microbial community was able not only to maintain but also to increase slightly its functional and taxonomic diversity.

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