Catena 135 (2015) 419–429

Contents lists available at ScienceDirect

Catena

journal homepage: www.elsevier.com/locate/catena

Using phospholipid fatty acid and community level physiological profiling techniques to characterize soil microbial communities following an experimental fire and different stabilization treatments

A. Barreiro [a,*], M.T. Fontúrbel [b], A. Lombao [a], A. Martín [a], J.A. Vega [b], C. Fernández [b], T. Carballas [a], M. Díaz-Raviña [a]

[a] Departamento de Bioquímica del Suelo, Instituto de Investigaciones Agrobiológicas de Galicia (IIAG-CSIC), P.O. Box 122. Avda. Vigo s/n, 15780 Santiago de Compostela, Spain
[b] Centro de Investigación Forestal-Lourizán, Consellería de Medio Rural e do Mar., P.O. Box 127, 3680 Pontevedra, Spain

ARTICLE INFO

Article history:
Received 4 February 2014
Received in revised form 18 July 2014
Accepted 20 July 2014
Available online 2 August 2014

Keywords:
Experimental fire
Soil stabilization treatments
Phospholipids fatty acids
Metabolic profiling

ABSTRACT

The phospholipid fatty acid (PLFA pattern) and community level physiological profiling (CLPP) techniques were simultaneously used to evaluate the short- and medium-term effects produced by an experimental fire and two different stabilization treatments on the soil microbial communities. The study was performed in a scrubland ecosystem located in Galicia (N.W. of Spain). The measurements were made in soil samples collected from the top layer (0–5 cm) immediately and 90, 180 and 365 days after the fire and application of seeding and mulching treatments. Regardless of the technique used (PLFA, CLPP), the results indicated that the experimental fire caused marked changes in the soil microbial community, which persisted even 1 year after the fire, whereas the post-fire treatments induced no changes or slight changes on the microorganisms of this burned soil. In addition, a significant effect of the sampling time on the functional diversity and the soil microbial community structure, particularly on the latter, was observed. The relative importance of the two main factors (experimental fire and intra-annual variation) in determining the microbial community composition of the studied soils varied notably depending on the technique used; the experimental fire had a greater impact on the functional diversity (as evidenced by CLPP) than on the microbial community structure (as evidenced by PLFA). The results support the convenience of using both methodological approaches (PLFA pattern and CLPP) to gain more insight into the microbial communities of this degraded burned soil.

1. Introduction

Soil microorganisms live in complex communities and are responsible for 80–90% of the soil processes and reactions (e.g. decomposition, mineralization and immobilization) that recycle important nutrients and degrade environmental pollutants (Nannipieri et al., 2003). Microorganisms react quickly to changes in their environment and variations in their biomass, metabolic activity or community structure may be an early signal of favorable or adverse alterations in the whole ecosystem. Numerous parameters such as microbial C, respiration, N mineralization potential, and enzyme activities based on the mass and activity of soil microorganisms are often used to detect soil quality changes induced by soil management and land use change (Dalal and Moloney, 2000; Doran and Zeiss, 2000; Doran et al., 1999). However, studies on the microbial community composition are less frequent probably due to the laborious and time consuming methods used by traditional microbiology as plate count and most probable number. Furthermore, less than 1–5% of the microorganisms in the environment can be usually quantified because the culture techniques fail to reproduce in artificial media the niches of many microorganisms found in high diversity and complex environments such as soils (Olsen and Bakken, 1987).

The recent development of biochemical and molecular biology techniques, which do not rely on cultivation methods, allows microbial ecologists to detect inhabitants of the natural microbial communities that have not yet been cultured. As a result, methods such as phospholipid fatty acid (PLFA) and community level physiological profiling (CLPP) are now widely applied to characterize the microbial community structure in different environments. The PLFA analysis is used as an indicator of the microbial biomass and the community structure since certain groups of microorganisms have different signature fatty acid and therefore, changes in the PLFA profile represent changes in the total soil microbial community (Frostegård et al., 1993). The CLPP analysis is based on the premise that microorganisms vary in the pattern and the rate at which they utilize carbon sources; therefore, carbon utilization patterns can be used as a measure of the microbial community structure and the functional potential (Garland and Mills, 1991; Insam, 1997). Although the CLPP technique considers only viable microorganisms, it

* Corresponding author. Tel.: +34 981590958; fax: +34 981592504.
E-mail address: anabarreiro@iiag.csic.es (A. Barreiro).

http://dx.doi.org/10.1016/j.catena.2014.07.011
0341-8162/

has been highlighted that it is not the utilization of single substrates but the change in the substrate utilization pattern that is important (Insam and Goberna, 2004), and thus the method has been successfully used to differentiate soils subjected to different disturbances such as fire, harvesting and restoration treatments (D'Ascoli et al., 2005; Giai and Boerner, 2007; Guénon et al., 2011). Since each of these approaches offers a focus on specific aspects of the soil microbiological characteristics, they represent an independent analysis of the differences or changes in the soil microbial community structure and function. Although both PLFA and CLPP have been used to measure community responses, few studies have compared both techniques together.

Fire has become the major disturbance agent of forest ecosystems; thus, the interest in quantifying disturbance impacts on the biotic component of soil ecosystems has increased with the concern for the sustainability of our forest systems. It is well-known that soil microorganisms play a major role in ecosystems function and therefore they can be used as indicators of soil quality; however, fire effects on microbiological properties have received less attention than its effects on physical and chemical properties (Certini, 2005). Furthermore, most investigations concerning the effects of fires on soil microorganisms have focused on changes in total microbial biomass and activity (Dooley and Treseder, 2012; Mataix-Solera et al., 2009). The structure of the microbial communities following the fire, that is, the different kinds of organisms and their abundances, is also important for ecosystems functioning. Research within the past decades has shown that there are fire-induced changes in physiological and taxonomical microbial groups determined by plate counting and most probable number techniques, the effect being dependent on the burning temperature and the time passed after fire (Acea and Carballas, 1996; Ahlgren, 1974; Badía and Martí, 2003; Fontúrbel et al., 1995; Vázquez et al., 1993). During the last decade, several studies using PLFA, CLPP and DNA/RNA analysis have reported differences between burned and unburned soils in genetic and physiological diversity following wildfires and prescribed fires (Bárcenas-Moreno et al., 2011; Barreiro et al., 2010; Campbell et al., 2008; Dangi et al., 2010; Díaz-Raviña et al., 2006; Goberna et al., 2012; Guénon et al., 2011; Hamman et al., 2007; Holden et al., 2013; Smith et al., 2008; Weber et al., 2014); however, since natural intra-annual variation (spatial, seasonal) is not taken into account in most investigations, there are still uncertainties about the consequences of these fire induced microbial changes in the soil functionality.

More recently experimental fires have been conducted in shrubland ecosystems for research purposes such as to evaluate the efficacy of soil stabilization treatments for controlling soil erosion as well as their effects on soil-plant systems (Díaz-Raviña et al., 2012; Fernández et al., 2011, 2012; Fontúrbel et al., 2012; Gómez-Rey et al., 2013a,b). The aim of the present study is to characterize, by means of phospholipid fatty acid (PLFA) and community level physiological profiling (CLPP) techniques, the soil microbial community structure and functional diversity of a Galician shrubland (N.W. Spain) affected by an experimental fire combined or not with two different soil stabilization treatments (seeding and mulching) and to determine the short- and medium-term microbial changes induced by these forest management practices. This is one of the few field studies that analyze shifts in microbial structure induced by intra-annual natural variation in the soil environment as well as by forest management practices and hence allows us to determine the relative importance of the fire and the post-fire stabilization treatments as disturbance agents in this shrubland ecosystem.

2. Material and methods

2.1. Experimental design

The study was conducted in an experimental fence field located at an altitude of 660 m a.s.l., in Cabalar (A Estrada, 42° 38′ 58″ N; 8° 29′ 31″ W; N.W. Spain) with temperate and rainy climate. The soil, developed over granite and with a slope of 38–54% oriented to the north, has vegetation representative of many oceanic-climate shrublands in Galicia dominated by gorse *Ulex europaeus* L. and some *Pteridium aquilinum* (L.) Kuhn., *Ulex gallii* Planch., *Daboecia cantabrica* (Huds.) K. Koch and *Pseudoarrenhaterum longifolium* Rouy, with a height of 123 cm on average and 100% ground cover. This area has a long history of repeated fires (the last fire occurred 5 years before the study start) and is representative of Galician shrublands subjected to high fire recurrence.

Sixteen experimental plots (30 × 10 m each) were established with the longest dimension parallel to the maximum slope. Twelve plots were burned and the remaining four plots were used as unburned control. In June 2009, the shrub was cut and laid down directly on the ground to favor a more homogeneous burn and higher fuel combustion, particularly in the litter and duff layers. The rate of fire spread was slow (0.30–0.33 m/min) and the soil temperature reached, monitored with 10 chromel alumel thermocouples per plot, was moderate at the mineral soil surface (153 °C) and low at 2 cm soil depth (34 °C) (Fontúrbel et al., 2012). The fire totally destroyed the aerial part of the vegetation but the root systems were not affected.

Four treatments were randomly applied to the experimental plots in quadruplicate: (a) unburned soil (U); (b) burned soil (B); (c) burned soil with a mixture of seeds at a rate of 45 g m^{-2} (*Lolium multiflorum*, 35%; *Trifolium repens*, 25%; *Dactylis glomerata*, 20%; *Festuca arundinacea*, 10%; *Festuca rubra*, 5%, *Agrotis tenuis*, 5%) (B + S); (d) burned soil with 230 g m^{-2} of straw mulch (B + M). The soil stabilization treatments were applied by hand to minimize soil perturbation immediately after the fire. Soil samples were taken from the A horizon (0–5 cm depth) after removing the litter layer, if present, immediately before and 1, 90, 180 and 365 days after the fire and treatments application. Ten 10 × 10 cm squares, uniformly distributed around each plot, were sampled, mixed and thoroughly homogenized after sieved at 2 mm. All samples were stored at 4 °C for no longer than 2 weeks until analyses of biochemical and microbiological properties.

2.2. Microbial community structure

The total biomass and the biomass of specific microbial groups as well as the microbial community structure were estimated by phospholipid fatty acid (PLFA) analysis using the procedure described by Frostegård et al. (1993). Briefly, lipids were extracted from the 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) from their relative retention times, using methyl nondecanoate (19:0) as internal standard. 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 (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:1ω8, i17:0, a17.0, 17:0, cy17:0, 18:1ω7 and cy19:0), was used as an index of the bacterial biomass (bactPLFAs), and the quantity of the 18:2ω6 and 18:1ω9 PLFAs was used as an indicator of the fungal biomass (fungPLFAs) (Frostegård and Bååth, 1996; Frostegård et al., 2011). 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 characterize 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).

2.3. Microbial community diversity

Laboratory assays on the community level physiological profiles (CLPPs) of the soil microflora utilizing various carbon substrates were