Catena 158 (2017) 82–88

Contents lists available at ScienceDirect

Catena

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

Wildfire effects on the microbial activity and diversity in a Mediterranean forest soil

Juana Rodríguez[a], José A. González-Pérez[b,*], Adriana Turmero[a], Manuel Hernández[a], Andrew S. Ball[c], Francisco J. González-Vila[b], M. Enriqueta Arias[a]

[a] Departamento de Biomedicina y Biotecnología, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain
[b] Grupo de Materia orgánica en Suelos y Sedimentos (MOSS), Instituto de Recursos Naturales y Agrobiología (IRNAS-CSIC), Avda. Reina Mercedes, 10, 41012 Sevilla, Spain
[c] Centre for Environmental Sustainability and Remediation, School of Science, RMIT University, Bundoora, VIC 3083, Australia

ARTICLE INFO

Keywords:
Wildfires
Soil microbial activity
Soil microbial diversity
DGGE
Clone libraries

ABSTRACT

A set of chemical, physical and microbial characteristics of different burnt soils from Sierra de Aznalcóllar (Sevilla, Spain) affected by one or two sequential fires, were analysed and compared with those of their respective control soils. A decrease in total organic carbon was observed in burnt soils, which could be attributed to the impact of the fires on vegetation cover. Biomass (estimated as viable and culturable microorganisms), substrate-induced respiration (SIR) and activity of different soil enzymes involved in carbon, nitrogen and phosphorus cycles were determined to assess the effect of fire on total microbial populations and on soil activity. An increase in both bacterial and fungal biomass as well as respiratory activity was detected in most burnt soils. In terms of enzyme activity, no common pattern of behaviour was observed, except for the alkaline phosphatase activity, which showed increased levels in all the burnt soils. The effect of fire on microbial diversity was estimated for *Bacteria* and *Archaea* domains from DNA band patterns obtained in denaturing gradient gel electrophoresis (DGGE), as well as using 16S rRNA cloned sequences for *Bacteria*. Shannon index values obtained from the DGGE profiles showed higher diversity for both *Bacteria* and *Archaea* domains in burnt soils compared with the control ones. Variations in the number of different phyla present in burnt and control soils were inferred from the analysis of the 16S rRNA cloned sequences. However, in all areas the most important groups identified belonged to the *Proteobacteria*, *Acidobacteria* and *Actinobacteria* phyla. No differences between microbial communities present in burnt soils at the genus level were detected.

1. Introduction

Wildfires are considered one of the main disturbances in Mediterranean forest ecosystems (González-Pérez et al., 2008). They exert non-desirable effects on soil health and quality because of the destruction of vegetation cover and important changes in physical, chemical and biological properties of soil (González-Pérez et al., 2004; Gómez-Rey et al., 2013). The soil degradation induced by fire favours the occurrence of erosive processes and nutrient losses causing alterations in the normal biological cycling of nutrients (Certini, 2005; Fernández et al., 2007). However, it is difficult to generalise about the effects of wildfires on soil due to the variability of factors involved (i.e. severity and frequency of fires, pre- and post-fire climatic conditions, topography of site, amount and nature of live and dead fuel). In some cases fire causes dramatic alterations in soil structure and function but in others the effect is reduced to a slight burning of the aerial parts of vegetation, resulting in "fertilisation" of the soil and a change in the trophic status of certain elements captured in vegetation. The study of the effects of wildfires on soils using different approaches is required to design effective rehabilitation strategies after the fire event (Mataix-Solera and Cerdá, 2009b).

Biochemical and microbiological soil properties are most responsive to disturbances caused by fire (Xu et al., 2012) and their analysis can be very useful in assessing the health of soils affected by fire (Mataix-Solera and Cerdá, 2009a; Kara and Bolat, 2009; Mataix-Solera et al., 2009). Microorganisms are central for ecosystem functioning; any reduction in microbial biodiversity not only reduces genetic resources, but also ecosystem productivity and alters its ability to buffer against disturbances. In addition, soil microbial communities mediate the decomposition of organic matter and nutrient cycling, playing an important role in the regeneration of degraded ecosystems. Therefore, the study of microbial soil communities allows greater understanding of

* Corresponding author.
E-mail address: jag@irnase.csic.es (J.A. González-Pérez).

http://dx.doi.org/10.1016/j.catena.2017.06.018
Received 25 January 2017; Received in revised form 12 June 2017; Accepted 17 June 2017
Available online 22 June 2017

soil health and thus provides valuable information that enables the effective restoration of degraded ecosystems (Arias et al., 2005). In addition, the understanding of the resistance and resilience of soil microbial communities to climate change and associated disturbances, such as wildfire, currently represents a subject of increasing interest.

Although several researchers have reported data about total microbial biomass and activity in burnt soils, there is confusion concerning the real impact of fire on microbial populations. While some studies demonstrate a decline in total soil microbial biomass after a wildfire (Dooley and Treseder, 2012) others reported an increase in microbial biomass post-fire (Goberna et al., 2012) or no significant differences when comparing non-burnt and burnt soils (Hamman et al., 2007). However, most research about the effects of fire on microbial communities has focused on soil mineral horizons, not the organic horizons (Mikita-Barbato et al., 2015).

The main objective of this study was to evaluate the effect of wildfires on soil biological properties (microbial activity and diversity) from forest soils using both culture-dependent as well as molecular DNA-based techniques in order to assess the effect of fire on the distribution of specific bacterial communities within a complex ecosystem such as soil.

We hypothesised that the impact of wildfire on soil microbial communities would produce important changes in their activity and diversity allowing the use of these parameters as markers of soil health.

2. Materials and methods

2.1. Studied site and sampling

The selected study site was located in Sierra de Aznalcóllar, Seville province, Southern Spain. The area is under a Mediterranean climate where forest fires are frequent during the summer time. The soils are Dystric Cambisols on schists (ISSS Working Group, 1998) developed under cork (*Quercus suber*) and pine (*Pinus pinea*) forests and with scarce Mediterranean bush vegetation. The climate in the area is Mediterranean; the average annual temperature ranges from 16 to 18 °C and annual rainfall is 559 mm. Precipitation occurs mainly from October to May with a very dry and warm period between June and September (< 10 mm and > 25 °C) that favours the occurrence of wildfires.

Soils affected by high severity wildfires and nearby unburned soils with the same physiographic characteristics were selected for sampling. Two samples QQ1 and QQ2 (N 37° 34′ 21″, W 6° 22′ 20.3″ and N 37° 35′ 32.9″, W 6° 22′ 16″ respectively) correspond to burnt soils affected by the same sequential wildfire that occurred in August 1997 and in July 2004; the sample Q3 (N 37° 34′ 18.3″, W 6° 18′ 30.3″) corresponds to a soil burnt only once during the July 2004 wildfire event. A nearby soil with no recent history of forest fire (N 37° 34′ 12.9″, W 6° 23′ 34.1″) was used as control (C). Sampling was carried out one year after the last fire by taking three sub-samples within an area of approximately 20 m^2. The soil samples were taken from the A horizon (0–15 cm) after removal of the litter layer, collected in sterile flasks and transported to the laboratory on ice. The three sub-samples from each site were dried at room temperature and sieved to fine earth (2 mm mesh size). Samples were maintained at room temperature for physical and chemical analysis, at 4 °C for biological analysis and at − 20 °C for genetic analysis.

2.2. Soil physical and chemical characterization

Soil pH values were measured in a water slurry (1:2.5 solid:liquid ratio). Water holding capacity (WHC), total carbon and organic matter were determined following the standard methods described by Pérez-Leblic et al. (2012). The total nitrogen content was estimated by the Kjeldahl method (Kjeldahl, 1883).

2.3. Soil biological characterization

2.3.1. Viable microorganism's quantification

For this study 10 g dry weight soil samples were vigorously mixed with 95 mL of phosphate buffer 0.1 M, pH 7. Aliquots of this slurry were inoculated on 1:10 diluted Tryptic Soy Agar (TSA) and on solid Oxytetracycline-Glucose-Yeast Extract (OGYE) media for colony-forming units (cfu) determination of bacteria and fungi, respectively (Elsas and Smalla, 1997; Tanner, 1997). Three plates per dilution were incubated for 7 days at 28 °C and plates showing 30–300 colonies were counted. Counts were estimated as the means of three determinations and expressed as colony forming units per gram of oven-dried soil (cfu/g dwt).

2.3.2. Soil substrate-induced respiration (SIR)

Soil respiration was determined in sealed 250 mL sterile flasks containing 15 g soil, 15 mL sterile distilled water and 0.35 g talcum and glucose (4 mg/g soil). The flasks were incubated at 28 °C for 6 h and the CO_2 evolved was measured in a CO_2 detector 1440 Gas Analyser at 0, 3 and 6 h. The results were expressed as % CO_2/h/100 g of soil (Hernández and García, 2003).

2.3.3. Soil enzyme activities

Acid and alkaline phosphatases, β-glucosidase and β-*N*-acetyl-glucosaminidase activities were determined following the methods described in Tabatabai (1982). Invertase and cellulase activities were determined according to Hoffmann and Pallauf method (1965) modified by García-Álvarez and Ibáñez (1994) and urease activity as described by Kandeler and Gerber (1988).

2.4. Soil microbial community analysis

2.4.1. Bacteria and Archaea fingerprinting

DNA was extracted from two sets of 0.5 g of soil samples using the MoBio Powersoil DNA extraction kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Extracted DNA concentration was determined in a spectrophotometer ND-100 Nanodrop (Thermo Fischer Scientific, USA). *Bacteria* and *Archaea* 16S rRNA genes were amplified from DNA samples by PCR and then subjected to analysis by DGGE. Primers 341F + GC clamp and 907R and 344F + GC clamp and 915R were used to study *Bacteria* and *Archaea* community profiles, respectively. The Taq polymerase (FideliTaq PCR Master Mix) used for all PCR amplification was from Invitrogen (USA). The PCR thermal cycle for *Bacteria* comprised a hot start at 94 °C for 7 min, followed of 32 cycles of 45 s at 94 °C, 45 s at 49 °C and 1 min and 30 s at 72 °C, and a final extension of 10 min at 72 °C. The *Archaea* PCR reaction was carried out with an initial denaturation step at 94 °C for 5 min, followed of 32 cycles of 45 s at 94 °C, 1 min at 54 °C and 1 min at 72 °C, and a final extension step of 10 min at 72 °C.

Denaturing Gradient Gel Electrophoresis (DGGE) was performed with a D-code Universal Mutation Detection System (Bio Rad laboratories, Hercules, CA, USA). PCR products (between 800 and 1000 ng) were loaded into 6% (wt/vol) polyacrylamide gels with a linear gradient of 55 to 60% or 50 to 65% denaturant for *Bacteria* and *Archaea*, respectively in 1 X TAE. The 100% denaturant gradient was defined as 7 M urea and 40% (v/v) deionised formamide. After electrophoresis at 60 V and 60 °C for 18 h, bands were visualised by staining the gels with ethidium bromide (50 μg/mL) for 20 min and destaining in deionised water for 40 min. The gels were exposed to UV light to visualise the bands and digitalised in a Gel Doc 2000 (BioRad laboratories, Hercules, CA, USA).

UPGM cluster analysis using the PAST program (Hammer et al., 2001) was carried out using the DGGE banding profiles. The Jaccard's similarity measure was obtained from the absence-presence of bands. Similarities between the banding profiles were also displayed