Soil Biology & Biochemistry 43 (2011) 1023–1033

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Fungal and bacterial recolonisation of acid and alkaline forest soils following artificial heat treatments

Gema Bárcenas-Moreno [a,b,*], Johannes Rousk [c,d], Erland Bååth [c]

[a] GEA – Grupo de Edafología Ambiental – Environmental Soil Science Group, Department of Agrochemistry and Environment, Miguel Hernández University, Avda. de la Universidad s/n. 03202, Elche, Alicante, Spain
[b] MED Soil Research Group, Department of Crystallography, Mineralogy and Agricultural Chemistry, Chemistry Building, Seville University, C/Prof. García González 1, 41012 Sevilla, Spain
[c] Microbial Ecology, Department of Biology, Lund University, Ecology Building, SE-223 62 Lund, Sweden
[d] Environment Centre Wales, Bangor University, Bangor LL57 2UW, UK

ARTICLE INFO

Article history:
Received 29 October 2010
Received in revised form
14 December 2010
Accepted 16 January 2011
Available online 2 February 2011

Keywords:
Forest fire
Soil
Fungal growth
Bacterial growth
Respiration
pH

ABSTRACT

The direct response and the short-term recolonisation of soil by fungi and bacteria were studied after heat treatments of a humus soil with high carbon content and low pH, and a calcareous soil with lower carbon content and high pH. Heating was administered using a muffle furnace or an autoclave, with different temperatures and times of heat exposure, after which fresh soil (1%) was added as inoculum. Autoclaved soil showed more marked increases in bacterial growth during the recovery phase than oven-heated soil, and the bacterial growth response was more rapid in calcareous than in humus soil. Fungal growth recovered more rapid and reached values higher than the control in humus soil, while it remained low until the end of the study in calcareous soil. Respiration rate showed similar patterns in both soils. Fungal biomass (ergosterol and PLFA 18:2ω6,9) indicated that fungi benefited by autoclaving in humus soil, while they were disfavoured by this treatment in calcareous soil. The sum of bacterial PLFAs did not change due to heating, but some bacterial PLFAs (e.g. cy17:0) increased in both soils. We propose that the community assembly of the microbial communities after heating were mainly driven by pH, in that the high pH soil selected primarily for bacteria and the low pH soil for fungi.

1. Introduction

The most evident effect of fire on forest ecosystem is the general damage of vegetation and partial sterilisation of soil. After fire, soil appears as a complex mosaic of patches of severely affected as well as less affected areas (Rab, 1996), the pattern mainly due to the influence of fuel characteristics which condition the energy released by fire (DeBano et al., 1998). The effects of fire on soil microorganisms have been studied for decades in many habitats (Deka and Mishra, 1983; Dunn et al., 1985; Vázquez et al., 1993; Pietikäinen and Fritze, 1995; Acea and Carballas, 1996; Dumontet et al., 1996; D'Ascoli et al., 2005; Guerrero et al., 2005; Waldrop and Harden, 2008) due to the importance of microorganisms in ecosystem functioning. Microbial recolonisation processes after fire are governed by numerous interconnected factors that sometimes are difficult to isolate in field studies. Soil changes in organic matter quantity and quality have been identified as key factors in regulating the microbial response after fire (Vázquez et al., 1993; Bååth et al., 1995; Acea and Carballas, 1996; Díaz-Raviña et al., 1996). A common pattern is the proliferation of bacteria after fire, which is attributed to the increase in available carbon (as increased levels of dissolved organic carbon [DOC]) and/or to increased soil pH (Pietikäinen and Fritze, 1995; Mataix-Solera et al., 2002; Bárcenas-Moreno and Bååth, 2009; Ponder et al., 2009). The fungal response following fire-perturbation has been less clear, but the recolonisation of fungi has been found to be less rapid than that of bacteria, also in acid soils (Pietikäinen and Fritze, 1995). However, this could partly be explained by the considerable mycorrhizal part of fungi in acid forest soils. Although many ectomycorrhizal fungi have shown some saprotrophic ability, these symbionts would not be able to fully recolonise the soil before the reestablishment of their plant hosts.

Recently, Bárcenas-Moreno and Bååth (2009) found that bacterial growth was stimulated after soil heating (80–400 °C), irrespective of the severity of heating, while fungi recovered more

* Corresponding author. MED Soil Research Group, Department of Crystallography, Mineralogy and Agricultural Chemistry, Chemistry building, Seville University, C/Prof. García González 1, 41012 Sevilla, Spain. Tel.: +34 954556950; fax: +34 954557140.
E-mail address: gbarcenas@us.es (G. Bárcenas-Moreno).

0038-0717/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2011.01.019

slowly, corroborating earlier findings by Guerrero et al. (2005). It was proposed that favourable conditions to bacteria after heating (high pH soil and higher C-availability) could explain both the rapid bacterial proliferation and lower fungal recovery, due to competitive interactions between these decomposer groups. Rousk et al. (2009) demonstrated different pH-relationships for fungi and bacteria, showing that neutral or slightly alkaline conditions strongly favoured bacterial growth, while acid pH disfavoured it, and vice versa for fungal growth. Follow-up experiments (Rousk et al., 2010a) suggested that the competitive influence by bacteria at high pH negatively affected fungal growth. This interpretation is also consistent with earlier experimental studies based on specific inhibition of bacteria (Rousk et al., 2008). Nevertheless there are studies where fungi appeared to be less affected or even stimulated after fire (Mataix-Solera et al., 2002; Banning and Murphy, 2008; Kara and Bolat, 2009) emphasising that the factors controlling fungal growth following a fire-event still are elusive.

In order to elucidate some of the factors controlling microbial growth after fire, we performed an experiment with two different soils: One calcareous soil from a Mediterranean forest area with high pH and low organic carbon (C) content, and one humus soil from a Swedish spruce forest with low pH and high organic-C content. We applied heating to the soil in two different ways. One treatment was applied with a muffle furnace, with different temperatures and times of exposure. This treatment would include aspects of fire-related heating such as drying and partial combustion, although the control of homogeneity in exposure would be limited. To also isolate the temperature effect of fire-related heating, a second treatment was administered with an autoclave, which exposed the soil samples more homogeneously to the assigned temperature, and circumvented effects related to variable water content. We hypothesised that the recolonisation by fungi would be more favoured in low pH soil, while bacteria would be favoured in the high pH soil. Secondly, since prolonged wet-heating will decrease pH (Skipper and Westermann, 1973) and could also produce toxic substance (Salonius et al., 1967) we hypothesised that wet-heat administered in the autoclave would affect the microbial community more intensively than heat of the muffle furnace, especially in the case of bacteria in low pH soils.

2. Material and methods

2.1. Soils

Two forest soils with different organic-C content and pH were used. A calcareous soil was collected in a forest area located in Torremanzanas (Alicante Province, South-East of Spain). Mediterranean sclerophyllous vegetation characterises this area, with Pinus halepensis as the dominant tree species. Soil pH (H_2O) was 7.9 and organic-C 6.0%. The site is described in Bárcenas-Moreno and Bååth (2009). The low pH soil was the humus horizon from a Norway spruce forest in south Sweden. Soil pH (H_2O) was 3.9 and organic-C 33%. Soil samples were randomly collected from the first 5 cm after removing litter. The soils were sieved (2.8 mm mesh), thoroughly mixed and air-dried before the start of the experiment. The soils were then rewetted to 60% of water holding capacity (WHC), and incubated four days at room temperature before heating treatments started.

2.2. Soil heating and incubation of soils

The soil samples were subjected to 7 different treatments using two kinds of heating: muffle furnace and autoclave (Table 1). Unheated (UH) control samples were included and were used to normalise the data. For each treatment triplicate samples were used. Soil samples (about 65–85 g depending on the soil) were placed in a 2 cm thick layer before being heated in the furnace. Due to the decrease in temperature when the samples were placed in the furnace, temperature immediately after was recorded and the time taken for the furnace to re-establish the desired treatment temperature (120 and 160 °C) was measured. The soil samples were removed after keeping them in the furnace for the desired temperature (120 and 160 °C) during the corresponding time for each treatment (20 and 40 min).

Samples for autoclaving were placed in glass jars and autoclaved at the desired temperatures (100, 120, 140 °C) for 1 h, and then cooled to 80 °C, when the soil was removed. Triplicate samples of each soil were autoclaved at each temperature.

The heated soils were rewetted adding distilled water to achieve 60% WHC and inoculated with the original fresh soil (1 g per 100 g heated soil) the day after heating.

The soils were then incubated in plastic pots with closed lids in the dark at 22 °C. The total incubation period was 21 days and during this period 6 time-points were sampled. The first time-point was collected before inoculation but after rewetting to indicate the activity of the surviving microbial community after heating. The remaining time-points were collected 2, 4, 7, 14, 21 days after inoculation with fresh soil to monitor the dynamics of the short-term recovery of microbial activity, growth and biomass.

2.3. Microbial measurements

Bacterial growth was measured with the leucine (Leu) incorporation technique, which is a relative estimate of bacterial growth (Bååth, 1994; Bååth et al., 2001). Two gram of fresh soil were mixed

Table 1
Description of heating treatments. Loss of water expressed as percentage of water lost compared to the original content of the sample (60% WHC) (mean ± SE). Heating in an autoclave is denoted with A.

Soil type	Treatment designation	Treatment temperature (°C)	Time at treatment temperature (min)	Time to reach treatment temperature (min)	Total time from 80 to 80 °C (min)	Loss of water during heating (%)
Humus soil	120°-20 min	120	20	55		66.2 ± 5.4
	120°-40 min	120	40	55		83.8 ± 5.3
	160°-20 min	160	20	25		64.3 ± 3.8
	160°-40 min	160	40	25		92.7 ± 0.7
	A100°	100	60		85	−0.18 ± 0.3
	A120°	120	60		125	0.02 ± 1.4
	A140°	140	60		130	4.7 ± 0.9
Calcareous soil	120°-20 min	120	20	20		84.7 ± 2.7
	120°-40 min	120	40	20		95.6 ± 0.3
	160°-20 min	160	20	10		89.2 ± 3.1
	160°-40 min	160	40	10		93.6 ± 0.2
	A100°	100	60		85	−3.5 ± 2.3
	A120°	120	60		125	16.3 ± 0.8
	A140°	140	60		130	17.5 ± 3.7