Soil Biology & Biochemistry 50 (2012) 167–173

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils

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ARTICLE INFO

Article history:
Received 19 September 2011
Received in revised form
19 March 2012
Accepted 29 March 2012
Available online 16 April 2012

Keywords:
Drought
Organic amendments
Semiarid soils
Microbiological properties
Soil enzymes
CLPPs
Phospholipid fatty acids

ABSTRACT

Biological activity could be affected severely by the impact on soil quality of drought, which can be very severe in Southern areas of Europe. The objective of this work was to assess, under controlled laboratory conditions, whether a long period of severe drought (six months) can affect the structure, size and activity of the microbial community of a semiarid soil, as well as the influence of organic amendments on these effects. The soil was incubated for 180 days under controlled conditions (25 °C and 60/80% day/night relative humidity), with two treatments: unamended (US) and amended (AS) with manure compost (100 t ha^{-1}). Two levels of irrigation were imposed: (1) well-watered (MUS and MAS), the soil being maintained at 60% of its water-holding capacity (WHC), and (2) dry soils, without irrigation (DUS and DAS). The drought conditions caused a significant inhibition of C and N mineralisation, and affected negatively the size and activity of the soil microbial biomass. Thus, after 180 days under drought conditions, the non-watered soils showed higher organic carbon content than the well-watered soils. Likewise, the stressed soils showed significantly lower values of water-soluble N, ATP content, microbial biomass C, alkaline phosphomonoesterase activity and total functional diversity than the well-watered soils. There was a significant decrease in the total amount of each fatty acid in DUS and DAS with respect to MUS and MAS after 180 days under drought. The physiology of the microbial community was affected more strongly by water stress than was the microbial community structure, changes in the structure caused by drought being less pronounced in amended than in unamended soils. Furthermore, the organic amendments increased the soil organic matter content, hence improving the size and activity of the soil microbial biomass and helping soil to retain moisture.

1. Introduction

In Southern areas of Europe, a future decrease in rainfall is predicted, which could be exacerbated by increased temperatures, resulting in a greater incidence of drought (Sumner et al., 2003). Biological activity, for which water availability is the primary limiting factor in these areas, could be affected severely, since the impacts of drought on soil quality are very severe (Sowerby et al., 2005). There is increasing evidence that microbial activity has a direct influence on the stability and fertility of ecosystems, (Hu et al., 2011) microbiological parameters being sensitive indicators of the response of ecosystems to stresses, such as drought (Zornoza et al., 2007). Bacteria are the most-abundant soil microbes and, as a result, are intrinsic to soil functioning. Soil moisture directly influences the physiological status of bacteria and may limit their capacity to decompose certain compounds (e.g. organic substrates). Water availability will also regulate substrate availability and soil properties, which can also influence the microbial populations and their overall activity. Periods of moisture limitation may affect bacterial communities through starvation, induced osmotic stress and resource competition, eliciting a strong selective pressure on the structure and functioning of soil bacterial communities (Griffiths et al., 2003).

Soil microorganisms synthesise and secrete extracellular enzymes, which constitute an important part of the soil matrix (Sinsabaugh et al., 1993). Enzymes play an important role in soil nutrient cycles and, consequently, factors influencing soil microbial activity will affect the production of the enzymes which control nutrient availability and soil fertility. Therefore, enzyme activities decreased in Mediterranean ecosystems due to more-severe drought conditions might have a negative effect on nutrient availability, compromising the current structure of enzymes. Soil

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0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2012.03.026

enzymatic activities have been suggested as potential sensitive indicators of changes in soil quality (Bastida et al., 2008; Hu et al., 2011). Therefore, soil microbiological and biochemical properties, such as microbial biomass, community composition, metabolic activity and functional diversity and various enzymatic activities, are often measured in order to provide immediate and accurate information about small changes in soils (He et al., 2008; Hu et al., 2011).

Remediation of semiarid, degraded soils, to reverse degradation, involves the addition of organic matter to improve soil quality (Ros et al., 2003). Organic amendments increase the soil organic matter content, improving the soil water-holding capacity and microbial activity and affecting several critical soil functions, such as plant nutrient availability and the diversity and activity of soil organisms (Tejada et al., 2006). The amount and type of organic matter applied to soils can influence the microbial community composition as measured by analysis of microbial lipids. Differences in the PLFA composition of microbial communities have been observed in farming systems which receive different amounts of organic inputs. (Bossio and Scow, 1998; Lundquist et al., 1999).

Microbial community level physiological profiles (CLPP) (BIOLOG) are based on the assessment of the ability of soil microbial communities to metabolize a range of organic C substrates that vary in structural complexity. This technique has the potential to produce a rich data set that is ideal for the detection of site-specific differences in soil bacteria and the evaluation of the relationship between biodiversity and site conditions (Li et al., 2004).

The objective of this work was to assess, under controlled laboratory conditions, whether a long period of severe drought (six months) can affect the structure, size and activity of the microbial community of a semiarid soil, as well as the influence of organic amendments on these effects. We hypothesized that soil drought stress would lead to changes in both the structure of the microbial community and its functional diversity, and that this effect would be less pronounced in presence of organic amendments. In order to achieve the objective, different physicochemical (pH and EC), chemical (such as total organic C, water-soluble C and water-soluble N) and general biological and biochemical (such as microbial biomass carbon, basal respiration and ATP) parameters, as well as specific biochemical properties (such as hydrolytic soil enzymes related to the C, N and P cycles), were evaluated in stressed and well-watered soils. The use of these biological and biochemical properties is often suggested because they are very responsive and provide immediate and precise information on small changes occurring in soil (Ros et al., 2003). FAME profiles have been used as indicators of community structure and BIOLOG assays to assess the potential of soil microbial communities to utilise a diverse range of C substrates. The relationships between the soil chemical properties and microbial indices were tested by regression and multivariate analyses.

2. Materials and methods

2.1. Soil and compost

A Calcic Kastanozem (FAO- ISRIC and ISSS, 1998) semiarid agricultural soil was used in this work. It was sampled in an experimental area (38°06′29.84″N 1°02′13.09″W) located in Santomera (SE Spain). This is an area abandoned from agriculture 10 years ago which is affected by soil degradation processes such as hydrological erosion. The climate is semiarid Mediterranean with mean annual temperature of 17 °C. The mean annual rainfall is 333 mm irregularly distributed throughout the year with two maxima (in October and April). The vegetation in the area is scarce (about 5% of vegetal cover) and it is an open Mediterranean scrub with species such as *Beta maritima*, *Salsola genistoides*, *Piptatherum miliaceum* and *Stipa capensis*. The soil was sampled in the upper layer (0–15 cm), air-dried and sieved to 2 mm. The compost applied originated from a mixture of sheep manure and straw composted in static horizontal reactors in which the material was ventilated mechanically. During the process composting mass temperature reached 60 °C assuring the sanitization of the obtained compost as well as organic matter stabilization. The main characteristics of the soil and compost were, respectively, the following: pH_{H2O}, 8.56 and 8.99; electrical conductivity, 0.33 and 2.46 dS m^{-1}; total organic carbon, 21.50 and 302.00 g kg^{-1}; total nitrogen, 1.40 and 20.50 g kg^{-1}, and available phosphorus, 74.15 and 623.00 mg kg^{-1}.

2.2. Experimental design

Four hundred of the sieved soil were placed in 500 mL pots (6 cm high and 11 cm diameter) and amended with manure compost at the rate of 100 t ha^{-1} (3.3 g compost/100 g soil). Two batches, including the unamended (US) and amended (AS) soil, were set out, moistened to 60% of the soil water-holding capacity (WHC) and incubated for 180 days. One of the batches was watered periodically, to maintain the WHC at 40–60% (watered soils, MUS and MAS). The other batch was left to dry without watering (non-watered soils, DUS and DAS). The pots were placed randomly in an incubation chamber with controlled temperature (25 °C) and humidity (60/80% day/night), with three replicates per treatment. The soil moisture was determined throughout the experimental period. The soils were sampled destructively at the start of the experiment and after 180 days. After sampling, the soil samples from both batches were homogenised thoroughly, sieved and stored at 4 °C for analysis of chemical, microbial and biochemical parameters. BIOLOG and PLFA analysis were performed immediately after sampling.

2.3. Chemical, biochemical and microbiological analyses

Organic C (OC) content was assessed by oxidation with $K_2Cr_2O_7$ in concentrated H_2SO_4 (Walkley and Blanck, 1934). Water-soluble carbon (WSC) and water-soluble nitrogen (WSN) were measured in a Shimadzu TOC5050A Total Organic Carbon Analyzer (Walkley and Blanck, 1934).

Microbial biomass C (Cmic) was determined by the fumigation-extraction method (Vance et al., 1987). Basal respiration (BR) was analysed by incubating 30 g of each soil sample, moistened to 50–60% of the WHC, in a hermetically-sealed flask at 28 °C for 22 days, measuring the CO_2 at given time intervals, with an infrared gas analyzer (Toray PG 100, Toray Engineering Co. Ltd., Japan) (Hernández and García, 2003). Adenosine triphosphate (ATP) was extracted from the soil using the Webster procedure (Webster et al., 1984) and measured as recommended by Ciardi and Nannipieri (1990). Soil dehydrogenase activity (DHA) was determined by the reduction of p-iodonitrotetrazolium chloride (INT) to p-iodonitrotetrazolium formazan, according to the method of Mersi and Schinner (1991) as modified by García et al. (1997).

Alkaline phosphomonoesterase (APA) and β-glucosidase (β-GA) activities were determined using the methods of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1988) and soil urease activity (UA) was determined by the buffered method of Kandeler and Gerber (1988).

2.4. Community level physiological profiles (CLPPs)

The community level physiological profiles were determined with BIOLOG Ecoplates containing 31 different C sources and a water well. To obtain the bacterial extracts, soil suspensions in