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The impacts of organic amendments: Do they confer stability against drought on the soil microbial community?

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ABSTRACT

The application of organic residues has been presented as an adequate strategy against soil degradation in semiarid environments. However, the interactions between organic amendments and drought are not fully known. Here, we evaluate whether sludge and compost amendment in semiarid areas influences the stability of the soil microbial community and microbially-mediated processes against drought. Sludge-amended, compost-amended and control (without amendment) soils were collected from a long-term restoration trial in southeastern Spain. A drought-induction model was initiated by first pre-incubating soil samples from each treatment at an optimum water-holding capacity (WHC) of 60%. One subset of samples was then partly dried and subsequently maintained at 20% of the WHC (induced-drought samples) and another subset was maintained at 60% of the WHC. The responses of the microbial biomass (through the analysis of phospholipid fatty acids, PLFAs), enzyme activities related to the C, N and P cycles, microbial diversity and microbial populations (through 16S rRNA and ITS amplicon sequencing) were analysed after 2, 9, and 45 days of incubation. In parallel samples, the mineralisation of soil organic matter (SOM) and fresh-organic matter (FOM) were evaluated by tracking the isotope signature of CO_2 after addition of ^{13}C-enriched plant tissue (97 atom %). Overall, we found that: i) the soil microbial biomass was greater in amended soils at 20% WHC than in the corresponding soils at 60% WHC after 45 days of incubation; ii) changes in the soil microbial biomass were accompanied by changes in the relative abundances of microbial populations; iii) the release of CO_2 from SOM and FOM was diminished in soils at 20% WHC, in comparison to soils at 60% WHC, while β-glucosidase and urease activities were higher in amended soils at 20% WHC than in soils at 60% WHC; and iv) bacterial and fungal diversity did not change as a consequence of drought. A multi-level characterisation of the soil microbial community provided a better understanding of the responses of amended soils to drought.

1. Introduction

Degradation processes in arid and semiarid environments are often linked to the low precipitation -usually below 300 mm per year in southeastern Spain- and scant plant growth that limits organic matter inputs into the soil (García et al., 1994; Bastida et al., 2006). In this scenario, organic amendments have been shown to improve soil chemical, physical and biological properties, and to increase soil fertility (Ros et al., 2003; Bastida et al., 2008; Diacono and Montemurro, 2010). Several studies have demonstrated the benefits of organic amendments (sewage sludges, prunings composts, etc.) with regard to physical and chemical soil properties and the development and activity of the soil microbial biomass (Bastida et al., 2008; Tejada et al., 2009; Torres et al., 2015; Luna et al., 2016a, 2016b). Organic amendments provide a source of energy and nutrients that favours microbial colonisation (Griffiths and Philippot, 2013) and, hence, can influence the response to climate change. This is important since microbes are responsible for the cycling of elements, including processes such as organic matter stabilisation and CO_2 release to the atmosphere (Bardgett et al., 2008; Schmidt et al., 2011). Thus, microbially-mediated processes are vital for the cycling of soil organic matter (SOM), which is very limited in semiarid areas of southeastern Spain but a fundamental component of soil sustainability (Bastida et al., 2006).

Mediterranean ecosystems are expected to be vulnerable to the

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impacts of climate change, particularly because a reduction in precipitation is expected according to climate change models for these (IPCC, 2013), as well as many other areas (Canarini and Dijkstra, 2015; Canarini et al., 2016). Limited water availability may reduce soil organic C levels through the diminished formation of organo-mineral complexes, as a consequence of the alteration of the microbial biomass and its activity (Canarini et al., 2016). Indeed, several studies have found that reduced soil water contents promote the abundance of fungi and Gram-positive bacteria (Manzoni et al., 2012; Barnard et al., 2013; Fuchslueger et al., 2014); these microbial groups are generally considered more resistant to drought. However, other studies did not find consistent impacts of drought on the composition of microbial communities (Rousk et al., 2013; de Vries and Shade, 2013; Canarini et al., 2016).

It has been hypothesised that soil practices which enhance soil C content may also increase soil microbial diversity (Thiele-Bruhn et al., 2012) and thereby improve the stability of microbial communities in response to stresses such as those derived from climate change. However, the addition of organic residues to soil, and the resulting increase in SOM, are not always associated with changes in microbial diversity in the long-term (Bastida et al., 2013). Further research is needed to evaluate how past organic amendments have influenced the responses of soil microbial communities to factors related to climate change. In fact, the interactions between soil organic amendment and climate change are not fully known. For instance, the dynamics of soil microbial communities have not been characterised using genomic tools (Hueso et al., 2012; Canarini et al., 2016). This is critical since detailed knowledge of community composition and diversity is fundamental to properly understand the connections between climate change, soil restoration and microbially-mediated processes such as C storage (Kaisermann et al., 2013).

Previous studies have shown that organic amendments increase the soil microbial biomass and its activity and enhance soil physical structure, water retention and plant development (Hargreaves et al., 2008; Bastida et al., 2008). Therefore, it has been proposed that organic amendments can mitigate the impacts of drought on the soil microbial community (Hueso et al., 2012). However, Ng et al. (2015) found little interaction between drought and compost application and concluded that soil microbial communities were, overall, resistant to water fluctuations regardless of compost amendment, and that water rather than nutrients was the limiting factor in an Australian grassland ecosystem.

Here, we aimed to determine if long-term restoration with organic amendments (11 years) influences the response of the soil microbial community to drought in terms of biomass, diversity, composition and functionality. We expected that the higher organic matter content of amended soils would enhance the retention of water; hence, the microbial community in amended soils would be less affected by drought than that of unamended soils. Specifically, we hypothesised that: i) the responses of bacterial and fungal populations to drought, in terms of their abundance, would be shaped by organic amendments; ii) changes in community composition in response to drought, but not the Shannon-Wiener diversity index, would be influenced by organic amendments; and iii) the responses of soil functionality to drought would be influenced by the greater organic matter content in restored soils.

The impacts of soil restoration with sewage sludge and compost amendments, with regard to the drought resistance of soil microbial communities, was evaluated by a suite of methods including the analysis of chemical pools, phospholipid fatty acids, CO_2 fluxes, enzyme activities and 16S rRNA and ITS amplicons.

2. Materials and methods

2.1. Study area, experimental design and soil sampling

The soil restoration experimental area is located in Murcia, southeastern Spain (38°1′N 1°12′W). The studied soil has a sandy clay loam texture and is classified as an Aridic alcisol (Soil Survey Staff, 2014). The area is affected by a semiarid climate with a mean annual rainfall of less than 300 mm and a mean annual temperature of 18 °C.

Agricultural use ceased more than 25 years ago, and the soil had no vegetation when organic amendments were applied. This abandonment together with the climatic conditions had triggered significant soil degradation. The pH of this soil was 8.6, its electrical conductivity was 250 μS cm^{-1} and the bulk density was 2.57 g cm^{-3}. In March 2005, nine plots (20 m^2 each) were established in the experimental area. Each plot was separated from the others by a corridor (2 m wide). In three randomly-selected plots, sewage sludge (SS) from a depuration plant in Murcia was added at a rate of 12 kg m^{-2}. The SS had been anaerobically digested for stabilisation and hygienisation. Compost made from this material, with straw as the bulking agent, was added to another three plots (CM) at 12 kg m^{-2}. The composting process was carried out as follows: piles of about 3 m^3 of sludge mixed with cereal straw at a ratio of 3:1 (v:v) were prepared and turned periodically (every 4–5 days) for three months, to maintain adequate aeration and homogenise the composting mass which passed through a thermophilic phase. The remaining three plots received no amendment and acted as the control. The compost and sewage sludge were incorporated into the top 15 cm of the soil using a rotovator. The control plots were also subjected to rotovation. The heavy metals contents in both the sludge and compost were below the limits established by the EU (Directive 86/278(CEE)) for sludges and soils. The plots were then left in natural conditions.

Since 2005, several studies have been carried out that have demonstrated the successful improvement of this soil in terms of physical, chemical, plant and microbial properties (Bastida et al., 2008, 2009, 2015; Nicolás et al., 2014). The mean organic C concentration of samples of the control, sludge-amended and compost-amended soils was 2.10, 3.10 and 5.55 g 100 g^{-1}, respectively; the total N was 0.15, 0.31 and 0.52 g 100 g^{-1}, respectively; and the water-holding capacity (WHC) was 40.2, 47.6 and 55.3 g 100 g^{-1}, respectively. The chemical, biochemical and microbial characterisation of the collected field samples is presented in Supporting Information.

2.2. Induced-drought experiment

In April 2016 (11 years after sludge and compost application), six subsamples from each plot were randomly collected to a depth of 15 cm and mixed to form a single sample per plot. In order to study the influence of soil restoration with organic amendments on the microbial community of soils subjected to drought, an incubation experiment was performed under controlled laboratory conditions. A total of 54 microcosm incubations were prepared in plastic containers, each with 50 g soil. A total of 6 incubations were prepared per field plot. All samples were pre-incubated at WHC of 60%, achieved by adding distilled water, for 15 days at 25 °C. Once the soil samples had adapted to this optimal water content, 27 containers [3 treatments (control, sludge-amended soil, compost-amended soil) x 3 replicates x 3 independent incubation times] were maintained at 60% WHC during the following incubation. The other 27 soil samples were dried and maintained at 20% WHC for the subsequent incubation (induced-drought samples). Both the optimally-watered and induced-drought samples were incubated