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Long-term effects of organic amendments on bacterial and fungal communities in a degraded Mediterranean soil

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ABSTRACT

Addition of organic amendments is a common practice to restore fertility and to increase productivity of degraded soils. Long-term effects of this practice on Mediterranean soils are controversial, with previous works showing contrasting results about the durability of the organic material added and its effects on the structure of microbial communities. This article presents results from a long-term soil remediation experiment, where a range of soil chemical and biochemical indicators, as well as indices of microbial diversity and community structure, were analysed 13 years after the first application of two organic amendments (leonardite and biosolid compost) at different doses, in an area contaminated by trace elements. In general, differences in chemical and biochemical properties and trace element availability between control and treated soils were still very evident, mainly in those soils treated with the highest amendment dose. The structure and composition of the soil microbial community was significantly affected by the type of management. The addition of both amendments favoured the increase of the fungal/bacterial ratio in the soil community, although a correlation with the C/N ratio of amendments was not found. The abiotic factors that acted as main drivers of the belowground communities differed between bacteria (more sensitive to Zn and Cd contamination) and fungi (soil pH and nitrogen content). Organic amendments had a direct positive effect on these abiotic factors, especially on the soil pH, a key factor in achieving long-term remediation. The results revealed that the effect of both amendments on the soil is maintained years after their application, although it is necessary to repeat their application to maintain soil pH within appropriate ranges and achieve a long-lasting recovery of soil functions.

1. Introduction

Addition of organic amendments is a common practice to restore fertility and to increase productivity of degraded soils. In trace element (TE) contaminated soils, incorporation of organic amendments is frequently the first step of the reclamation practice (Kidd et al., 2015), since it promotes the establishment of a plant community that can be used for phytostabilization or phytoextraction of soil contaminants (Álvarez-López et al., 2016; Nawab et al., 2016). Ameliorating effects of organic amendments in contaminated soils include the improvement of physical, chemical and biological parameters (Park et al., 2011), resulting mainly from increases in soil water holding capacity and nutrient availability to plants and soil microorganisms (Asensio et al., 2013; Alvarenga et al., 2014), and from immobilization of TEs in soil (Bolan et al., 2014), due to the high cation sorption capacity of the organic material added (Violante et al., 2010).

The effect provided by the soil amendment might decrease as the added material is mineralized by soil microorganisms, leading to a released of those TE previously immobilized in the organic material. Therefore, long-term monitoring of the remediated soil is needed to prevent this chemical time-bomb effect (Stigliani et al., 1991). This monitoring scheme should include periodical analysis of those soil factors that drive the mobility of the TE in the soil, such as pH, as well as of biochemical indicators of the recovery of soil ecological functions (Moreno et al., 2011). Soil enzyme activities can be used as reliable indicators of the recovery of microbial activity and soil functioning (Pulleman et al., 2012), given their ability to respond quickly to environmental changes (Epelde et al., 2009) and their important role in

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soil nutrient cycling.

Input of nutrients and organic C added with the amendments has an immediate effect on soil microbial community, which is normally C-limited. Nutrient release and plant nutrient uptake and productivity are usually boosted shortly after the application of the organic amendment (Moreno et al., 2017), and it normally promotes the colonization of the contaminated soil by new plant species (Madejón et al., 2006).

Organic amendments can also induce a significant increase of the diversity of soil microbial communities on the long term, particularly in C-depleted soils (Thiele-Bruhn et al., 2012). However, mid-term or long-term responses to amendment addition are still controversial, as previous works have reported both limited or no effects of the amendments on microbial diversity (Renella et al., 2008; Ascher et al., 2009; Bastida et al., 2013), or significant increases in the genetic and functional diversity of the microbial communities (Bastida et al., 2008, 2013, 2016) in response to C inputs. In contaminated and remediated soils, the interactions between factors that might shape the structure of the microbial community (e.g. pollutant type and concentration, background soil properties and type and dose of amendment) are complex, and thus changes in microbial diversity of remediated soils are often site- and taxonomic-group specific (Touceda-Gonzalez et al., 2017). Given their important role in sustaining plant growth, changes in the relative abundances of bacteria and fungi can determine the success of remediation practices. In this regard, the quality of the used amendments has important implications because, for example, materials with a high C:N ratio or containing recalcitrant C compounds entail an increase in fungal dominance (Heijboer et al., 2016) and limit bacterial growth due to N availability (Waring et al., 2013).

We evaluated different microbiological and biochemical indicators of quality of a contaminated Mediterranean soil, which was remediated using leonardite and biosolid compost as soil amendments, added in single or repeated doses. We used a long-term (13-year) field experiment established after the Aznalcóllar mine spill (Andalusia, Southern Spain) occurred in 1998, which caused both soil acidification and TEs contamination. The evolution over time of TEs availability and main soil chemical properties (Madejón et al., 2010; Xiong et al., 2015), soil C pools (Montiel-Rozas et al., 2016a), and plant composition and TE accumulation at the site has been studied in previous works. In this work, fungal and bacterial community structure was analysed in amended and unamended soils. The relationships between the structure of these communities (richness, diversity and composition), the soil chemical properties (nutrients and contamination) and soil functioning indicators (enzyme activities) were also determined. We hypothesized that: 1) the positive effects of amendment addition on soil chemical and biochemical indicators persisted for 13 years after initial application, with soils that received two doses over time showing a greater recovery of pH conditions and biochemical indicators; 2) changes in the composition of the soil communities could be related to TE availability and soil fertility; 3) the addition of soil amendments resulted in a long-term increase in the diversity of bacteria and fungi, in comparison to unamended soils; and 4) the structure of fungal and bacterial communities is shaped by the quality of the material added, and thus communities from soils amended with different materials could be differentiated. This study is one of the longest field-scale experiments evaluating the effectiveness of organic amendments for long-term TEs stabilization and recovery of microbial ecological functions in contaminated soils.

2. Material and methods

2.1. Field site, experimental design and sampling

The study area is situated in the Guadiamar River Valley (SW of Spain), with a Mediterranean climate characterized by a mean annual temperature of 17 °C (with a maximum of 33.5 °C in July and a minimum of 5.2 °C in January) and about 500 mm mean annual rainfall (Domínguez et al., 2008). The soil at the site is classified as Protocalcic Fluvisol (WRB, 2014).

The area was affected by the toxic spill of the Aznalcóllar mine, that in 1998 released ca. 6 hm^3 of TE-contaminated waters and sludge into the Guadiamar River (Domínguez et al., 2008). Soils of the area were contaminated by several TEs, in particular As, Cu, Cd, Pb and Zn (Cabrera et al., 1999). The remediation trial plots analysed in this study were located 10 km downstream from the Aznalcóllar mine (N 37° 26′ 21″, W 06° 12′ 59″). The experiment was initiated in October 2002 and was set up in a completely randomized block design (Madejón et al., 2010). Initially, nine sampling plots (7 m × 8 m each) were delimited within the experimental area and assigned to three treatments, with three replicates per treatment: unamended plots (control), amended with biosolid compost (BC) and amended with leonardite (LEO). The application rates of biosolid compost (with a C:N ratio of 15) and leonardite (with a C:N ratio of 25), were 30 Mg ha^{-1} $year^{-1}$ and 25 Mg ha^{-1} $year^{-1}$, respectively (amendment application in 2002 and 2003, doses D1). In October 2005 each amended plot was further divided into two; one half of each plot received a second dose of amendment (application in 2005 and 2006, doses D2), while in the other half application of the amendment was not repeated, thus bringing the experimental trial to five treatments: unamended plots (control), biosolid compost (high dose (BC-D2) - low dose (BC-D1)) and leonardite (high dose (LEO-D2) - low dose (LEO-D1)). The most relevant characteristics of both amendments are reported in Madejón et al. (2006).

For the present study, soil was sampled in September 2015 from the 0–10 cm layer, with a soil corer, in four randomly chosen sampling points in each plot, and subsequently pooled, resulting in three independent replicates per treatment. For each sample, one subsample was stored at 4 °C for biochemical analysis, another subsample was frozen at −20 °C for PLFA and DNA analyses, and another subsample was air-dried and sieved (< 2 mm) for chemical analysis.

2.2. Chemical and biochemical analysis

Soil pH value was measured in 1 M KCl extracts (1:2.5, m/v) according to Hesse (1971). The available concentrations of Cd, Cu, Mn, and Zn in soils were determined in 0.01 M $CaCl_2$ extracts as described by Houba et al. (2000). The TE concentrations in all extracts were determined by ICP-OES (Varian ICP720-ES). Soil total organic C (TOC) was determined with the Walkley and Black (1934) method. Available P and K were determined as reported by Olsen et al. (1954) and Dewis and Freitas (1970), respectively. Total Kjeldahl-N (TKN) was determined following the method described by Hesse (1971).

Soil dehydrogenase activity was determined by the method of Trevors (1984). Soil acid phosphomonoesterase activity was assayed according to Tabatabai and Bremner (1969), and soil phosphodiesterase activity according to Browman and Tabatabai (1978). Arylsuphatase and arylesterase activities were determined as described by Tabatabai and Bremner (1970) and Zornoza et al. (2009), respectively. Urease activity was measured with the method of Nannipieri et al. (1978), β-glucosidase activity according to Tabatabai (1982) and protease activity by hydrolysis of N-benzoylargininamide (N-BAA) as reported by Ladd and Butler (1972). Glucosaminidase activity was determined by the method of Parham and Deng (2000). Phenol oxidase activity was estimated with the method of Hendel et al. (2005), using 50 mM acetate buffer at pH 5, and incubated with 5 mM dihydroxy phenylalanine (L-DOPA) for 3 h. Phenol oxidase activity was calculated from measurements of absorbance at 460 nm, with a molar absorption coefficient for the L-DOPA product 3-dihydroindole-5,6-quinone-2-carboxylate (diqc) of 3.7×10^4 (Mason, 1948). Microbial biomass C (MBC) was estimated using the chloroform fumigation-extraction method (Vance et al., 1987). The ATP content of soil samples was measured by the phosphoric acid method (Ciardi and Nannipieri, 1990).