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Native soil organic matter conditions the response of microbial communities to organic inputs with different stability

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ABSTRACT

The response of soil microbial communities from soils with different soil organic matter (SOM) content to organic inputs with different stability is still poorly understood. Thus, an incubation experiment was designed to study how the addition of pig slurry (PS), its manure (M) and its biochar (BC) affect soil microbial community and activity in three soils differing in SOM content (Regosol, Luvisol and Kastanozem). The evolution of different C and N fractions, microbial biomass C and N, enzyme activities and microbial community structure by the use of phospholipid fatty acid (PLFA) analysis was assessed for 60 days. Results showed that the different amendments had different effect on microbial properties depending on the soil type. The addition of M caused the highest increase in all microbial properties in the three soils, followed by PS. These changes were more intense in the soil with the lowest SOM (Regosol). The addition of M and PS caused changes in the microbial community structure in all soils, which were more related to the presence of available sources of N than to the labile fractions of C. The addition of BC was followed by increases in the proportions of fungi and Gram negative bacteria in the Regosol, while enhanced the proportion of actinobacteria in all soil types, related to increments in pH and soil C recalcitrance. Thus, native SOM determined the response of microbial communities to external inputs with different stability, soils with low SOM being more prone to increase microbial biomass and activity and change microbial community structure.

1. Introduction

Long-term carbon sequestration strategies which also enhance soil fertility are urgently being sought to counter the effects of rising levels of atmospheric CO_2, as highlighted by the 4‰ initiative (4p1000.org). The addition of organic wastes to soil may achieve this goal (Ros et al., 2003). Nonetheless, there is a need to find the best organic wastes treatment which once applied to soil, enhance soil fertility but also carbon sequestration. Thus, it has been proposed that organic wastes must be properly treated to avoid possible deleterious effects on soil properties and reduce environmental or health hazards associated with raw wastes (Fernández et al., 2012).

Soil microorganisms control ecosystem functioning as mediators of decomposition, C stabilization, and nutrient cycling (Coleman and Whitman, 2005; Schimel and Schaeffer, 2012). The C mineralization and stabilization processes are related to intrinsic resistance to degradation of organic compounds, mostly mediated by microorganisms (Marinari et al., 2010). Soil properties such as pH, texture and native soil organic matter (SOM) are main drivers that influence the abundance and diversity of microorganisms in soil after application of organic amendments (Zornoza et al., 2016a). To have a holistic approach of the effects of amendments on soil quality and fertility, it is essential to study the microbial community dynamics to really understand soil functioning and organic matter mineralization, stabilization and immobilization processes.

Beneficial effects of slurries, manure, compost and BC application on soil fertility and crop yield have been documented (Choudhary et al., 1996; Jeffery et al., 2011), but specific effects on soil microbial community biomass and structure are still poorly explored. Microbial biomass has been used as a sensitive indicator of change in soil organic matter due to its fast turnover compared to organic C and N (Babujia et al., 2010; Balota et al., 2003). Phospholipid fatty acid analysis (PLFAs) is a useful tool for monitoring the microbial community dynamics and hence can help our understanding of the succession of microbial populations (Klamer and Bååth, 1998). Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Makoi and Ndakidemi, 2008). They are essential in catalyzing reactions necessary for the life processes of microorganisms and the decomposition of organic wastes,

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organic matter stabilization and nutrient cycling (Dick, 1994). The enzymes β-glucosidase, β-galactosidase and arylesterase are involved in the soil C cycle. The enzymes β-glucosidase and β-galactosidase are glycosidases that catalyze the hydrolysis of glycosides to produce sugars. The β-glucosidase acts on D-glucopyranoside and cellobiose, while β-galactosidase hydrolyzes lactose (Eivazi and Tabatabai, 1988). Thus, these enzymes contribute the degradation of carbohydrates and so their products are energy sources for microorganisms. Arylesterase is a carboxylic ester hydrolase catalyzing the hydrolysis of phenolic esters, and so involved in the degradation of recalcitrant organic compounds (Zornoza et al., 2009). Protease and urease activities are involved in the N cycling in soil, regulating the amount of available N. Protease is responsible for the decomposition of proteins to peptides, and finally to amino-acids, acting on peptide bonds (Bonmatí et al., 1988). Urease is involved in the hydrolysis of urea-like substrates producing NH_4^+ (Bremner and Mulvaney, 1982). The enzyme b-glucosaminidase plays an important role in both C and N cycling in soil. This enzyme hydrolyzes *N*-acetyl-b-D-glucosamine residues from the terminal non-reducing ends of chitobiose, chitin and glycoproteins (Parham and Deng, 2000).

The current information on the effect of amendments on microbial community indicates that amending tends to alter microbial community composition and activity, but the effect is not univocal, with very diverse results in terms of the amendment, soil type and management practice. Bastida et al. (2015) observed that bacterial abundance was higher in sewage sludge-amended soils than in compost-treated soils. These authors also reported that the stabilized nature of compost in comparison to sludge promoted microbial communities with different physiology. Carrera et al. (2007) found that addition of compost and manure did not support different microbial community structure compared to inorganic fertilizers under agricultural soils with polyethylene mulch. Zornoza et al. (2016a) observed that addition of pig slurry to soils supported the highest microbial growth owing to high labile organic substrates, whereas biochar provoked the lowest microbial growth, with no significant increase in fungal biomass and no changes in microbial community structure with regard to unamended soils. Contrarily, O'Neill et al. (2009) observed a higher microbial taxonomic diversity in biochar-enriched soils as compared to adjacent unamended soils. Thus, the topic clearly demands more investigation to assess how different organic matter stability provided by organic amendments can affect microbial community abundance, diversity and activity in soils with different native SOM content. For example, animal slurries are mostly composed by soluble organic compounds, highly decomposable, and with high available N (Hernández et al., 2007; Plaza et al., 2004). Composting animal residues leads to the stabilization of the organic materials by humification processes, with increases in the C/N ratio (Fornes et al., 2012; Lhadi et al., 2006). Biochar (BC) can be produced through pyrolysis of different organic wastes, with presence of highly recalcitrant organic matter rich in aromatic compounds (Mukherjee and Lal, 2013; Zornoza et al., 2016b). As a consequence, addition of amendments with different organic matter stability may differently stimulate soil microbial communities (e.g. Bastida et al., 2013, 2015; Carrera et al., 2007; Gálvez et al., 2012; Lehman et al., 2011; Yanardag et al., 2015; Zornoza et al., 2016a). This may lead to the incorporation of most fractions of the organic materials as SOM (e.g. amendments with highly recalcitrant compounds such as biochar (Xu et al., 2016; Zavalloni et al., 2011; Zornoza et al., 2016a)), or to their practically complete decomposition and mineralization by microorganisms (e.g. amendments with labile organic compounds and high N availability, such as animal slurries (Hernández et al., 2007; Plaza et al., 2004, 2007)). In this context, native SOM may have an essential role since it controls soil microbial community size and structure (Ding et al., 2012; Stewart et al., 2008; Subedi et al., 2016).

According to the latter approaches, the objective of this study was to elucidate how the amendment of soils with pig slurry, its manure and its BC (amendments with the same organic C content but different stabilization grade) affect soil microbial community structure and activity in different soils differing in SOM content (Regosol, Luvisol and Kastanozem), and to investigate the extent to which microbial community structure respond to changes in soil physicochemical properties. In a previous study (Yanardag et al., 2015), we observed that different soil types behaved differently in response to the same amendments with regard to soil organic C (SOC) and N dynamics, being the Regosol more prone to increase SOC content and stability after applications. Thus, the hypothesis tested here was that the application of pig slurry and manure (with high proportion of labile organic compounds) to soil types with low organic matter content would promote more intense shifts in microbial community structure and activity. The addition of BC should have low impact on soil microorganisms in all soils owing to high C stabilization.

2. Materials and methods

2.1. Soils and amendments

The soils were sampled from the A horizon (0–20 cm) of a Haplic Regosol (37°52′1.29″ N, 1°31′20.10″ W), a Calcic Luvisol (37°52′37.91″ N, 1°29′29.85″ W), and a Calcic Kastanozem (37°51′56.65″ N,1°34′ 21.05″ W) (IUSS, 2014) from SE Spain, all developed over limestome parent material. The three soils mainly differed in their SOM content. In addition, Regosol has higher $CaCO_3$ content, and Luvisol has higher content of clay. Main soil characteristics are shown in Table 1. After sampling, soils were air dried and sieved <2 mm.

Three different amendments were used with different organic matter stability. Pig slurry (PS) was obtained from a pig farm located in Cartagena (SE Spain). The solid manure (M) was obtained after separation of the solid phase of the raw pig slurry from the liquid phase using a physical phase separator. The solid fraction was outdoor air-dried for one month and sieved <2 mm after sampling. Biochar (BC) was obtained by pyrolysis of the solid manure under oxygen-limited conditions in a muffle furnace. The temperature was ramped at 5 °C min^{-1} up to 420 °C, and maintained for 30 min at this temperature. At the end, the BC was cooled overnight. Main amendment characteristics are shown in Table 2.

2.2. Soil incubation

Laboratory incubations were carried out in triplicate for each treatment and soil type in the dark, at constant soil water content (55% of water holding capacity) and temperature (25 °C), under aerobic conditions for 60 days. PS, M and BC were thoroughly mixed with soil at a dose of 5 g C kg^{-1} soil. An unamended soil for each type was used as control (CT). Soil moisture levels were gravimetrically maintained adding deionized water if needed. Soil samples (300 g air-dry basis) were placed in 500 mL polypropylene containers. Soils were sampled in each container to monitor the evolution of soluble C and N (Csol and Nsol, respectively), microbial biomass C and N (MBC and MBN, respectively) and β-glucosidase, β-galactosidase, arylesterase, β-glucosaminidase, urease and protease enzyme activities at 0, 1, 3, 7, 15, 30, and 60 days of incubation (collection of 20 g of soil at each sampling time). Phospholipid fatty acid (PLFA) analysis was

Table 1
Main characteristics of the three different soils used in the experiment.

Soil type	pH	SOM	C/N	CEC	$CaCO_3$	Textural class
		%		$cmol_+ kg^{-1}$	%	
Regosol	7.91	2.34	16	6.7	34.9	Sandy clay loam
Luvisol	7.67	9.51	18	16.5	5.1	Clay loam
Kastanozem	7.72	16.08	19	21.1	6.2	Sandy clay loam

SOM: soil organic matter; CEC: cation exchange capacity.