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Changes in microbial community structure and functioning of a semiarid soil due to the use of anaerobic digestate derived composts and rosemary plants

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ABSTRACT

We studied the changes in structure and functioning of the microbial community in a degraded agricultural soil after the addition of two composts, obtained from cattle manure or pig slurry anaerobic digestate, and the use of rosemary plants for restoring soil quality.

The composts were added at low or high doses to soil samples (30 or 60 t ha^{-1}, respectively), which were kept in microcosms for 6 months. Some soil microcosms were treated with inorganic fertiliser and other non-treated soils were used as microbiological controls. Rosemary plants, used both for their ability to grow in semi-arid regions and for the capacity of their root system to protect soil from erosion, were planted in half of the entire microcosm set up. At different times (0–180 days) microbial abundance and dehydrogenase activity were measured in the various experimental treatments. Total and water-soluble soil organic carbon and nitrogen contents were assessed at 0 and 180 days. With an increase in carbon and nitrogen soil content, a rise in microbial abundance was also observed in the presence of both composts. However, microbial activity was significantly influenced by the presence of rosemary, without considering the allochthonous carbon and nitrogen input. Microbial community structure and diversity were also assessed by Fluorescence *In Situ* Hybridization in the different treatments. The highest values for microbial community biodiversity were found in the co-presence of rosemary and at low concentrations of both composts.

The overall results suggest that the use of composts together with plant species suited to Mediterranean areas seems to be an appropriate strategy for restoring soil quality and the ecosystem services provided by microorganisms.

1. Introduction

The loss of organic matter and biodiversity are among the main threats to soil quality, as identified by the EU Soil Thematic Strategy COM 2006 (231) and in the Policy report on its implementation, COM 2012 (46). Soil Organic Matter (SOM) depletion and soil erosion are caused by inappropriate agricultural practices, clearing of vegetation, increased levels and frequency of drought or flooding and forest fires. It has been observed, for example, that land without vegetation can be eroded more than 120 times faster than land covered by vegetation, which can thus lose less than 0.1 tons of soil per ha $year^{-1}$ (Turbé et al., 2010). The activity and diversity of soil microorganisms are directly affected by the reduction of SOM content, and indirectly by the reduction in plant diversity and productivity. Microbial communities play a key role in organic matter decomposition (Lavelle and Spain, 2001; Chaudhry et al., 2012) and in biogeochemical cycles (Doran and Zeiss, 2000; Paul, 2007; Zhong et al., 2010) and enhance the efficiency of plant nutrient assimilation by promoting their growth and health (Gu et al., 2008; Güneş et al., 2014). Since most of the soil processes are microbially mediated, soil microorganisms are central to soil ecological functioning, providing several regulation ecosystem services (Millennium Ecosystem Assessment, 2005). Microbial activity and soil fertility are closely related and the soil microbiota adapts quickly to environmental constrains by adjusting its biomass, activity rates and community composition. Microbial structure and its functioning can represent accurate indicators of soil quality (Winding et al., 2005; Benedetti et al., 2006; Giacometti et al., 2013). Only recently has more attention been focused on the maintenance of the structural and functional diversity of soil bacterial communities and the ways in which they might respond to various natural or anthropogenic disturbances (Zhang and Xu, 2008; Bouasria et al., 2012).

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Studies on microbial biomass carbon and enzyme activities provide information on the biochemical processes occurring in soil and there is evidence that soil biological parameters are early and particularly accurate indicators of soil ecological stress and restoration of soil treated with organic amendments (Sciubba et al., 2014).

In Mediterranean areas, intensive agriculture practices, together with adverse climatic conditions (e.g. water scarcity), are among the main causes of soil degradation (Anderson, 2003; Ros et al., 2003; Guerrero et al., 2007) and organic carbon loss (Garcia et al., 1997). As it is an important factor influencing soil structure and nutrient availability for soil biota and plants, SOM depletion leads to a decrease in soil fertility with negative consequences for soil biodiversity and crop production (Pimentel and Wilson, 1997; Turbé et al., 2010).

Anaerobic digestion is an efficient biological method for the use of livestock and agro-industrial wastes for producing energy and it transforms organic wastes into two products: a source of renewable energy (biogas) and a potential fertiliser, the digested material (digestate) (Bernal et al., 2009; Poeschl et al., 2012). Composting, a treatment based on the aerobic biological decomposition and stabilisation of organic substrates, can prove a suitable method for improving the properties of the solid fraction of digestate and thus enhance its fertilising value (Bustamante et al., 2012; Bustamante et al., 2013).

The application of compost to degraded soils has been found to be a suitable environmental strategy for improving soil physical structure and increasing the amounts of soil organic carbon and other major nutrients such as nitrogen and phosphorous (Filcheva and Tsadilas, 2002; Bustamante et al., 2012).

Moreover, the incorporation of compost into soil provides macro- and micro-nutrients to plants, favouring their growth and the development of the rhizosphere; the latter generally promotes a further increase in soil carbon content through root exudation, which can in turn stimulate microbial growth (Walsh et al., 2012). In addition, the origin of the raw materials used in compost processing and the characteristics of compost can differently affect soil microbial communities and their use of the carbon contained in these amendments (Martens, 2000), favouring the activity of the microbial groups that are most suited to the amendments used (Bastida et al., 2008a).

Although the effects of long-term amendment with organic wastes on soil microbial characteristics have been reported (Tiquia et al., 2002; Crecchio et al., 2007; Bastida et al., 2008a; Chaudhry et al., 2012), very little is known about the changes in soil microbial structure after an amendment of an agricultural soil with compost during the restoration of degraded soils (Ros et al., 2003; Saison et al., 2006).

In this work we studied the changes in the structure (cell abundance, phylogenetic characterization) and functioning (dehydrogenase activity) of the microbial community in a degraded agricultural soil, in the presence of rosemary plants, after adding two different composts derived from livestock anaerobic digestates, using greenhouse microcosms. Microcosm experiments enabled the studying, under controlled conditions, of the effects of the different treatments (separately or together) on the natural microbial community in order to assess which treatment could be more suitable for restoring soil quality.

The microbial community structure was evaluated by using culture independent molecular methods suitable for identifying microbial populations in their natural environment (Grenni et al., 2009; Barra Caracciolo et al., 2010; Godoi et al., 2014). In particular, the Fluorescence *In Situ* Hybridization (FISH) method made it possible to characterize the active microbial community using specific fluorescent labelled 16s rRNA targeted oligonucleotide probes. The method detects the specific sequences of rRNA in single cells, which correspond to the classification of a bacterial population at different phylogenetic levels. Since only viable and highly active cells have a sufficient number of ribosomes for *in situ* hybridization to be applied successfully with a specific probe, FISH is a valuable tool for determining which microbial populations are really active in soil ecosystem functioning (Kirk et al., 2004; Godoi et al., 2014).

2. Materials and methods

2.1. Description of the soil and of the composts used

For this study soil samples were manually collected using a shovel from the surface layer (0–20 cm) of a semiarid agricultural area unused for ten years and located in Montelibretti, province of Rome, Lazio (Italy). There had been intensive agriculture there for more than 20 years previously. The soil was left to dry at room temperature and then sieved (<2 mm). It was highly calcareous (% $CaCO_3$ 33.4%), slightly alkaline, with an available phosphorous content of 15.7 mg kg^{-1}, low salinity and poor organic carbon content (7.5 g kg^{-1}). The soil texture was classified as clay loam on the basis of USDA (43% sand, 42% silt and 15% clay) and as Calcaric Cambisol on the basis of the FAO-UNESCO Classification (FAO-UNESCO, 1990). The main characteristics of the soil and the composts are shown in Table 1.

The two composts used consisted of the solid fraction of the anaerobic digestate of cattle slurry (CS) or pig slurry (PS) mixed with vine shoot pruning at the following rates (on a dry mass basis): compost CS, solid fraction of anaerobically digested cattle slurry (75%) + vine shoot pruning (25%); and compost PS, solid fraction of anaerobically digested pig slurry (75%) + vine shoot pruning (25%). A detailed description of the composting process has been reported elsewhere (Bustamante et al., 2012; Bustamante et al., 2013).

Both composts showed a suitable degree of maturity for use as soil amendments, on the basis of the different criteria suggested by various authors, such as: total organic carbon to total nitrogen ratio (C/N) (Mathur et al., 1993), with values <20 (11.9 and 11.4, in CS and PS, respectively); cation exchange capacity (CEC) >60 cmol kg^{-1} SOM (Harada and Inoko, 1980) (CS = 124 and PS = 171 cmol kg^{-1}); CEC/TOC > 1.9 (Iglesias-Jiménez and Pérez-García, 1992) with 2.47 and 3.36 for CS and PS, respectively; and the absence of phytotoxicity, in accordance with the germination index (GI) >50% as suggested by Zucconi et al. (1981) (99.6% and 79.8% in CS and PS, respectively).

2.2. Microcosm set up

Polyethylene pots were filled with 1 kg of soil (air-dried and passed through a 2 mm sieve) thoroughly mixed with cattle (CS) or pig (PS) anaerobic digestate derived compost at two different doses (expressed on a fresh mass basis): low dose (low), by adding 11.54 g compost per kg soil (corresponding to a dose of 30 t ha^{-1}) and high dose (high), by adding 23.08 g of compost per kg soil (equivalent to a dose of 60 t ha^{-1}). The doses employed were based on the nitrogen requirements of the rosemary crop, on the assumption that the availability of N to plants is low since most (>90%) of total compost N is bound to the organic N-pool (Amlinger et al., 2003). These doses were also similar to those used by other authors in rosemary cropping experiments using compost (Cala et al., 2005; Madejón et al., 2009).

The amended soils were compared with soils treated with an inorganic fertiliser (NPK, proportion 100:60:73 obtained adding 192 mg kg^{-1} soil of commercial fertiliser Nitrophoska top 20, 20:5:10, and 26 mg kg^{-1} of Monopotasic phosphate, 0:52:34) and with untreated soil samples, the latter used as microbiological controls. Each treatment was replicated three times.

Table 1
Main characteristics of the soil and of the cattle (CS) or pig (PS) anaerobic digestate derived composts.

Parameter	Soil	Compost CS	Compost PS
pH (H_2O)	7.6	6.88	6.53
EC ($dS \cdot m^{-1}$)	0.10	6.19	5.11
Total organic carbon (%)	0.75	34.6	34.5
Total nitrogen (%)	0.19	2.90	3.03
Water-soluble organic carbon (%)	0.0059	0.57	0.81
Water-soluble organic nitrogen (%)	0.0005	0.23	0.61