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Metabolic and genetic patterns of soil microbial communities in response to different amendments under organic farming system

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ABSTRACT

In the organic farming system plant production mostly depends on the decomposition of soil organic matter through the activity of the microbial biomass, which is able to provide significant quantities of essential nutrients for plant growth. The aim of this work was to compare the persistence of microbial heterotrophic metabolism along decimal dilutions of soil treated with different organic amendments, by using Biolog EcoPlate™. The amount of the different amendments was adjusted in order to meet the N requirement of tomato crop. The Biolog results were used to draw a binomial matrix of data by setting all the positive results to 1 and all the negative results to 0. The occurrence of the microbial oxidation of each Biolog Ecoplates™ C source was calculated as probability '*p*' on the binomial set of data for each dilution. In terms of persistence of C sources utilization by soil microflora, along decimal soil dilutions, the treatments can be roughly divided in 3 different categories: the worst performing (control), the intermediate performing (biochar), and the best performing (biochar added to an organic fertilizer, the organic fertilizer alone and 3 composts). Biolog positive wells at the dilution 10^{-4} were used to carry out a molecular characterization of bacterial communities by 16S fingerprinting, through the H′ Shannon diversity index. Microbial communities utilizing cellulose and hemicelluloses as C source changed their species composition in response to the different amendments. In particular, amendments with biochar, regardless of the application of organic fertilizers, brought to the highest diversity of cellulose degrading bacteria.

1. Introduction

The use of organic soil amendments is a critical issue in organic agriculture, where plant production depends on the supply of essential nutrients for plant growth through the microbial decomposition of soil organic matter (SOM). Farming systems under organic management are known to improve both chemical and biological soil quality (Fließbach et al., 2007).

Although a great deal of research focused on the comparison between organic and conventional systems, little attention has been paid to the analysis of different management practices within organic cropping systems (Hartmann, 2006) in terms of soil quality.

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The biodiversity and the biochemical activities of soil microflora are sensitive tools for assessing soil quality, since they respond quickly to physical and chemical changes, including nutrient availability (Anderson et al., 2002; Giacometti et al., 2013). The loss of any microbial biochemical activity is an indicator of soil quality decrease (Chapman et al., 2007; Chiurazzi, 2008; Zak et al., 1994).

The beneficial effect of organic amendments on soil biota is well known (Jilani et al., 2007; Yao et al., 2000; Zhang et al., 2012) and their effect well documented through the analysis of different parameters, as soil respiration, metabolic quotient, enzyme activity, microbial biomass C and N, Biolog and DNA analysis (Garcia-Gil et al., 2000; Intanon et al., 2015; Pascual et al., 2000; Zhang et al., 2012; Zhen et al., 2014).

Studies on the effects of soil organic amendment, both in microcosms and in field, show that compost amendment improves soil structure, enhances SOM content and strongly influences activity, biomass and species composition of soil microflora (Crecchio et al., 2001). Furthermore, chemical and organic fertilizers, as well as plant rhizodepositions, influence biochemical activities and species composition of soil bacterial communities (Acosta-Martínez et al., 2010; Bulluck et al., 2002; Marschner et al., 2001; Pérez-Piqueres et al., 2006; Wang et al., 2012; Zhang et al., 2008). De Fede et al. (2001) and Graystone et al. (2001) pointed out that the type and amount of available organic substrates strongly influence the abundance of microbial groups and their functional and genetic diversity in soil ecosystems.

The pioneering work of Garland and Mills (1991) opened new frontiers to the assessment of soil quality through the measurement of the Community Level Physiological Profile (CLPP) of soil microflora. This assessment is based on the utilization of 31 sole carbon sources (Biolog EcoPlate™) and has been successfully used as a tool for the characterization of microbial communities (Arias et al., 2005; Garland, 1999; Gomez et al., 2006) and to detect short- (Classen et al., 2003) and long-term (Rutgers et al., 2016) changes of functional diversity of soil microflora.

In this work, we used Biolog EcoPlate™ in order to compare the microbial heterotrophic metabolic activities along decimal dilutions in soil treated with different organic amendments (biochar, an organic fertilizer made by a mixture of guano, milled bones and manure and 3 different composts) Our aim was to evaluate the biochemical shift of microbial activities across parcels treated with amendments and fertilizers allowed in organic farming. The scope was to identify the treatments able to preserve the highest possible oxidizing ability of C substrates, along serial dilutions. In addition, a molecular analysis of the microbial DNA was carried out in the Biolog EcoPlate™ wells in order to compare the genetic diversity of the culturable microflora pertaining to the different treatments by using the Shannon's diversity index (H′).

2. Materials and methods

2.1. Experimental site

The experimental site was located in Valenzano (Italy, 41° 80′ 30″ N, 16° 85′ 20″ E) at an altitude of 72 m above the sea level. The experiment was carried out from February to April 2011. The site was characterized by a daily mean temperature ranging from 7 to 17 °C and a total rainfall of 177.6 mm. Physical and chemical characteristics of the soil at the beginning of experiment, in the 0–25 cm layer, are reported in Table 1. Experimental field was left uncultivated and was not amended for 3 years prior the sampling.

Table 1
Physical and chemical characteristics of soil and amendments.

Characteristics	Units	Soil	Amendments			
			MVC	DVC	SVC	BCH
Stones and gravel	g kg^{-1}	22	–	–	–	–
Sand (>0.005 mm)	g kg^{-1}	180	–	–	–	–
Silt (0.005–0.002 mm)	g kg^{-1}	592	–	–	–	–
Clay (<0.002 mm)	g kg^{-1}	228	–	–	–	–
Textural class (USDA)		Silt loam	–	–	–	–
pH (H_2O)		8.0[a]	8.4[b]	7.6[b]	7.6[b]	9.8[b]
pH ($CaCl_2$)		7.5[a]	–	–	–	–
EC 25 °C	dS m^{-1}	0.14[c]	4.3[d]	2.7[d]	1.5[d]	2.29[d]
Total carbonates	g kg^{-1}	57	–	–	–	–
Organic carbon	g kg^{-1}	12	205	268	418	753
Organic matter	g kg^{-1}	20	410	536	836	–
Total nitrogen	g kg^{-1}	1.2	21	19	37	16
C/N		9.5	9.7	14.1	11.3	48
Total phosphorus (as P_2O_5)	g kg^{-1}	–	13.5	4.6	20.6	–
Available P	mg kg^{-1}	6.5	–	–	–	–
Total potassium (as K_2O)	g kg^{-1}	–	24.2	6	14.5	–
Exchangeable K	mg kg^{-1}	309	–	–	–	–

MVC: cattle manure and vineyard pruning compost; DVC: dairy industry waste and vineyard pruning compost; SVC: solid poultry slaughterhouse waste and vineyard pruning compost; BCH: biochar.
[a] Dilution 1:2.5.
[b] Dilution 3:50.
[c] Dilution 1:2.
[d] Dilution 1:10.

2.2. Soil physical and chemical characteristics

The soil physical and chemical characteristics were determined according to the following methods. The particles size analysis was performed by pipette method according to Gee and Bauder (1986). Soil pH was determined both in water and in $CaCl_2$ 0.01 M solution with a soil/solution ratio 1:2.5 (w/v). The soil salinity was assessed by determination of the electrical conductivity (EC) on an aqueous soil extract (ratio 1:2 w/v). Total carbonates were measured using the Dietrich-Fruhling calcimeter (gas volumetric method). Soil organic carbon (OC) content was determined according to the Walkley and Black method; the organic matter (OM) content was calculated (Nelson and Sommers, 1996) by multiplying the OC by 1.724. Total nitrogen was determined according to Bremner (1996). Available phosphorus was determined by Olsen method (Olsen and Sommers, 1982). Phosphorus content was determined colorimetrically at 650 nm absorbance using the modified ascorbic acid method (Watanabe and Olsen, 1965). Exchangeable potassium was determined by means of Inductively Coupled Plasma – Optical Emission Spectroscopy (iCAP 6300, Thermo Scientific, UK), after soil extraction by a solution of $BaCl_2$ and triethanolamine buffered at pH 8.2.

2.3. Amendments and fertilizers

Biochar (BCH) and 3 different composts have been used in this experiment. The BCH was produced from olive mill waste by steam gasification process at 1100–1200 °C for 30 min using an apparatus supplied by Advanced Gasification Technology (Cremona, Italy). Composts have been produced from a) cattle manure and vineyard pruning (MVC), b) dairy industry waste and vineyard pruning (DVC) and c) solid poultry slaughterhouse waste and vineyard pruning (SVC) by windrow method at Mediterranean Agronomic Institute (Valenzano, southern Italy).

The Organic fertilizer (OF) was made by a mixture of fertilizers (guano, milled bones and manure), as approved for use in organic farming (Council Regulation (EC) No 834/2007; Commission Regulation (EC) No 889/2008). Italian standard methods were used for composts analysis (Decreto Ministeriale 15 marzo, 2006).

Composts were air dried, grounded with mixer mill, and passed through a 1 mm sieve. The pH was measured by a glass electrode in distilled water at a ratio 3:50 (w/v). EC was determined by a conductometer on filtrates at a ratio 1:10 (w/v). Humidity was determined at 105 °C. OC for composts was determined according to Ciavatta et al. (1989) and OM was calculated by multiplying OC value by 2.0. Total nitrogen for composts was determined according to Kjeldahl method. Total potassium and phosphorus were determined after digestion by microwave digester (CEM model, MARS Xpress) of air dried sample by using concentrated 1 ml H_2O_2, 1 ml HCl, and 5 ml HNO_3 for 20 min at 190 °C. The concentration of potassium in the digested sample was measured by means of an Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES), total phosphorus was measured colorimetrically by a spectrophotometer (Megatech SP-930) at 650 nm according to the modified ascorbic acid method. EC and pH of biochar were determined by the same procedures used for the composts, while carbon and nitrogen were determined by dry oxidation using a C, H, N, S analyzer (FLASH 2000 Series CHNS/O Analyzer, Thermo Scientific, UK). The instrument was calibrated by using 2,5-bis-(5-*tert*-butylbenzoxazol-2-yl)-thiophene standard. All the analysis were done in triplicates. Table 1 shows the chemical characteristics of amendments.

2.4. Experimental design

The following 7 treatments were set according to a randomized block design with four field replicates: unamend control soil (CNT); biochar (BCH); organic fertilizer (OF); biochar and organic fertilizer (BCH + OF); cattle manure and vineyard pruning compost (MVC);