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ORIGINAL PAPER

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Effect of *Eisenia foetida* earthworms on mineralization kinetics, microbial biomass, enzyme activities, respiration and labile C fractions of three soils treated with a composted organic residue

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Abstract The aim of this work was to assess and compare the influence of *Eisenia foetida* Savigny earthworms on C mineralization rate, labile C fractions (water-soluble C and water-soluble carbohydrates), microbial biomass C, and enzyme activities (dehydrogenase, urease, phosphatase and β-glucosidase) in three soils of varying texture treated with a composted organic residue and cropped with *Avena sativa* L. Mineralization decreased with the addition of earthworms to the sandy and clay-loam soils, especially in sandy soil (by about 4 μg CO_2-C g^{-1} day^{-1}). There were no significant effects on the amount of CO_2 evolved from clay soil due to the addition of *E. foetida*. The addition of *E. foetida* to sandy soil significantly decreased microbial biomass C and increased microbial metabolic quotient the qCO_2 (CO_2-C to biomass C ratio). The addition of *E. foetida* did not affect the microbial biomass or the qCO_2 of the clay-loam and clay soils.

Keywords Biomass C · C mineralization · Clay soil · Microbial metabolic quotient · Sandy soil

Introduction

Intensive arable farming and semiarid climatological conditions cause a progressive decline in soil organic matter levels and subsequently contribute to degradation of soil quality (Caravaca et al. 2002b). In coarse-textured soils from arid and semiarid environments, the risk of soil fertility loss with cultivation is particularly high, due to their low levels of organic matter, low water-holding capacity and excessive deep percolation losses. Moreover, long-term monoculture on these degraded soils leads to a decrease in the density of the soil macrofauna. The methods commonly used for rehabilitating the quality and productivity of degraded soils are based on the addition of organic amendments to soil (Caravaca et al. 2002a). The beneficial effects of organic amendments include decreased soil bulk density and increased water-holding capacity, aggregate stability, saturated hydraulic conductivity, water infiltration rate, and biochemical activity (Zebarth et al. 1999). Stabilized and composted organic residues are used preferentially because they constitute a source of available nutrients for plants and of microflora, and their use leads to fewer problems related to toxic substances, which are eliminated during the composting process (Pascual et al. 1999). The extent to which organic matter contributes to soil quality depends not only on organic matter quality but also on soil fauna activity and environmental conditions, in particular temperature and humidity (Ouédraogo et al. 2001). Likewise, soil texture may affect the capacity of soils to stabilize organic matter. Fine-textured soils have higher organic C and N contents than coarse-textured soils when supplied with a similar input of organic residue (Hassink 1997). Organic additions typically decompose more rapidly in sandy soils than in clay soils.

The abundance of soil macroinvertebrates such as earthworms can be considered as an index of soil quality in agroecosystems (Boyer et al. 1999). A positive correlation between earthworm abundance and the productivity of cropped plants has been shown (Pashanasi et al. 1992). Field and laboratory studies have indicated that interactions between earthworms and microorganisms increase soil C turnover, soil nutrient availability and microbial activity (Schindler-Wessells et al. 1997). However, studies on the effect of earthworms on soil microbial biomass have yielded contradictory results (Bohlen et al. 2002; Daniel and Anderson 1992; Scheu 1992). There is little information concerning the effects of soil texture on the ability of earthworms to degrade organic matter added to soil. The aim of this work was to assess and compare the influence of *Eisenia foetida* Savigny earthworms on C mineralization rate, labile C fractions (water-soluble C and water-soluble carbohydrates), microbial biomass C, and enzyme activities

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(dehydrogenase, urease, phosphatase and β-glucosidase) in three soils of varying texture treated with a composted organic residue and cropped with *Avena sativa* L. Annual grass species such as *Avena sativa*, used as a cover crop, have been proposed as an agroecological alternative for improving or maintaining soil structure in degraded ecosystems (Carter et al. 1994).

Materials and methods

Materials

Soils of central-western Italy with similar agricultural management, consisting of fallow–corn crop rotations for the past 20 years, were selected in order to provide a range of textures. A clay (9% sand, 31% silt and 60% clay) and a clay-loam texture (31% sand, 32% silt and 37% clay) soil were sampled near Putignano (Pisa). The third soil was sampled near Castagneto Carducci (Livorno) and had a sandy texture (98% sand, 1% silt and 1% clay). All soils were sampled from the A horizon (0–20 cm depth), air-dried and sieved (<2 mm). The climate is semiarid, Mediterranean type (Rivas-Martinez 1987), with 538 mm year^{-1} average rainfall, 1,124 mm year^{-1} evapotranspiration and 14.0°C mean annual temperature.

The compost used in this experiment was produced from a mixture of cereal residues and a small portion (10%) of aerobically digested sewage sludge, according the Beltsville Aerated Pile Method (Willson et al. 1980). The analytical characteristics of the composted residue, determined by standard methods (Page et al. 1982), are shown in Table 1.

Adult specimens of the earthworm *Eisenia foetida* (fresh weight varying from 0.6 to 0.7 g) were purchased from the laboratory stock. *E. foetida* is an epigeic species which feeds mainly on plant litter.

Experimental design

The experiment was a mesocosm assay, conducted as a completely randomized factorial design with two factors. The first factor had three levels (sandy, clay-loam and clay soil) and the second factor had two levels (addition or not of *E. foetida* earthworms). Five replicates per treatment were carried out, making a total of 30 pots.

Soil treatments

Subsamples (1.5 kg air-dried soil, sieved to <2 mm) of the three soils were placed in 2-l pots. Composted residue was incorporated into the soil at a rate of 30 t ha^{-1}, which is sufficient to raise the soil total organic C content by 1.5%. Three weeks after the addition of the compost, about 40 seeds of *A. sativa* were sown in each pot. Two weeks after sowing *A. sativa*, ten adult *E. foetida* earthworms were added to half of the pots. The total number of earthworms added (10) was equivalent to a field population density of 333 worms m^{-2}, approximately the same as the population density of earthworms at the site from which the soils were collected. Pots were kept at 28/18°C, day/night respectively, and at 60% of the soil water-holding capacity. To maintain soil moisture, the pots were weighed every 5 days and supplied with deionized water. The experiment was carried out without any fertilizer treatment. Sixty days after sowing *A. sativa*, soil samples of the pots were taken and stored at 2°C prior to analysis.

Chemical analysis

The total organic C (TOC) was determined by oxidation with potassium dichromate in a concentrated sulphuric medium, with measurement of the excess dichromate using Mohr's salt (Yeomans and Bremner 1988). Total N (TN) was determined by Kjeldahl digestion. Humic substances were extracted with 0.1 M sodium pyrophosphate at pH 9.8 (soil-solution ratio of 1:10 w/v, at 37°C, for 4 h under mechanical shaking). The extract was centrifuged at 30,476 *g* for 15 min and filtered (0.45 μm) before oxidation with sodium dichromate.

Water-soluble C, extracted with distilled water (solid:liquid ratio, 1:5), and C extracted with pyrophosphate were determined by oxidation with potassium dichromate and measurement of absorbance at 590 nm (Sims and Haby 1971). Water-soluble carbohydrates was measured using the method of Brink et al. (1960).

Table 1 Chemical and biochemical characteristics of the composted residue. Mean ± standard error (n=8)

Characteristic (units)	Value
pH (1:10)	7.8±0.2
Electrical conductivity EC (1:10, μS cm^{-1})	580±40
Total organic C (g kg^{-1})	290±15
C extractable in pyrophosphate (g kg^{-1})	63±10
Water-soluble C (μg g^{-1})	2,680±204
Total carbohydrates (μg g^{-1})	9,764±350
Water-soluble carbohydrates (μg g^{-1})	524±33
Total N (g kg^{-1})	16.5±0.9
NH_4^+ (μg g^{-1})	40±9
NO_3^- (μg g^{-1})	43±8
Total P (μg g^{-1})	588±43
Available P (μg g^{-1})	111±12
Dehydrogenase (μg INTF g^{-1})	5,680±321
Urease (μg NH_4^+ g^{-1} h^{-1})	235±50
Protease-BAA (μg NH_4^+ g^{-1} h^{-1})	23±2
Phosphatase (μg PNP g^{-1} h^{-1})	398±24
β-Glucosidase (μg PNP g^{-1} h^{-1})	135±13

Biochemical and biological analysis

Dehydrogenase activity was determined by the reduction of 2-*p*-iodo-nitrophenyl-phenyltetrazolium chloride (INT) to iodo-nitrophenyl formazan (INTF), using 1 g of soil, at 60% field capacity, treated with 0.2 ml of 0.4% INT in distilled water for 20 h, at 22°C in darkness (García et al. 1997). The INTF formed was extracted with 7 ml of a mixture of 1:1.5 tetrachloroethylene/acetone, by shaking vigorously for 1 min. INTF was measured spectrophotometrically at 490 nm.

Urease activity was determined in 0.1 M phosphate buffer at pH 7; 1 M urea was used as substrate. Two millilitres of buffer and 0.5 ml of substrate were added to 0.5 g of sample, which was incubated at 30°C. Activity was determined as the NH_4^+ released in the hydrolysis reaction (Nannipieri et al. 1980).

Phosphatase and β-glucosidase activities were determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) and *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M; Masciandaro et al. 1994) as substrates, respectively. These assays are based on the release and detection of PNP. Two millilitres of 0.1 M maleate buffer at pH 6.5 and 0.5 ml of substrate were added to 0.5 g soil sample and incubated at 37°C for 90 min. The reaction was stopped by cooling at 2°C for 15 min; 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were then added and the mixture centrifuged at 2,287 *g* for 5 min. To stop the reaction of β-glucosidase activity, tris-hydroxymethyl aminomethane (THAM) was used according to Tabatabai (1982). The amount of PNP was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner 1969).

For all enzyme assays, controls were included with each soil analysed. The same procedure as for the enzyme assay was followed for the controls but the substrate was added to the soil after incubation but prior to stopping the reaction. All data were expressed on the oven-dry weight of soil.

Microbial biomass C was determined by the fumigation-extraction method (Vance et al. 1987).

Carbon mineralization was determined using 50 g dry soil, moistened to 60% of its water-holding capacity, placed in hermetically-sealed polythene tubes containing a vial of 10 ml 2 N NaOH and incubated for 49 days at 28°C. Every 2 days for 49 days, the trapped CO_2 was precipitated as carbonate with excess $BaCl_2$ and the excess NaOH was titrated with 1 M HCl (Zibilske 1994).

Statistical analysis

Soil texture, the addition of earthworms and their interaction effects on all parameters were tested by a two-way analysis of variance and comparisons among means were made using the least significant difference (LSD) test calculated at $P<0.05$. Statistical procedures were carried out using the software package Statgraphics for Windows 7.0.

Results and discussion

Initial soil characteristics

The sandy soil had lower total organic C and total N concentrations than the clay-loam and clay soils (Table 2). Pyrophosphate-extractable C was higher in the clay-loam soil than in the sandy and clay soils (Table 2). However, the percentage of pyrophosphate-extractable C with respect to total organic C was higher in the sandy soil, representing about 59% of the total organic C. The highest water-soluble C and water-soluble carbohydrate concentrations were recorded in the sandy soil, followed by the clay-loam and clay soils (Table 2).

Dehydrogenase, phosphatase and β-glucosidase activities, total amount of CO_2-C evolved, and metabolic quotient (qCO_2) were higher in the clay-loam soil than in the sandy and clay soils (Table 2). Likewise, the values of dehydrogenase, phosphatase and β-glucosidase activities per unit of microbial biomass were higher in the clay-loam soil than in the sandy and clay soils. However, the highest urease activity per unit of microbial biomass was recorded in the sandy soil. Landi et al. (2000) introduced the enzyme activity/microbial biomass ratio in order to be able to monitor changes in extracellular enzyme activity and/or intracellular enzyme activity of soil.

Organic matter quantity and quality

The addition of *E. foetida* earthworms to the sandy and clay soils had no significant effect on soil total organic C, but decreased the organic C of the clay-loam soil (Table 3). The application of composted residue and

Table 2 Chemical, biochemical and microbiological characteristics of the sandy, clay-loam and clay soils. Mean ± standard error (n=6)

Soil characteristic (units)	Sandy	Clay-loam	Clay
Total organic C (g kg^{-1})	3.2±0.1	11.5±0.0	6.5±0.0
Total N (g kg^{-1})	0.30±0.01	1.45±0.03	0.67±0.02
C/N	11±0	8±0	10±0
C extractable in pyrophosphate (g kg^{-1})	1.9±0.1	2.9±0.0	1.5±0.1
Water-soluble C (μg g^{-1})	127±6	90±4	71±3
Water-soluble carbohydrates (μg g^{-1})	14±1	10±1	3±0
Dehydrogenase (μg INTF g^{-1})	7±0	56±1	11±0
Urease (μg NH_4^+ g^{-1} h^{-1})	62±1	22±0	16±0
Phosphatase (μg PNP g^{-1} h^{-1})	922±52	1207±57	960±67
β-Glucosidase (μg PNP g^{-1} h^{-1})	340±4	624±2	323±9
Total CO_2 (μg CO_2-C $g^{-1}$$day^{-1}$)	6.0±0.2	12.0±0.1	6.4±0.3
Microbial biomass C (μg g^{-1})	334±5	275±8	311±5
Dehydrogenase:C_{mic} (μg INTF μg C_{mic}^{-1})[a]	0.02±0.00	0.20±0.00	0.04±0.01
Urease:C_{mic} (μg NH_4^+ μg C_{mic}^{-1} h^{-1})[a]	0.19±0.00	0.08±0.00	0.05±0.00
Phosphatase:C_{mic} (μg PNP g^{-1} μg C_{mic}^{-1} h^{-1})[a]	2.76±0.04	4.39±0.05	3.09±0.24
β-Glucosidase:C_{mic} (μg PNP g^{-1} μg C_{mic}^{-1} h^{-1})[a]	1.02±0.01	2.27±0.07	1.04±0.01
qCO_2 (ng CO_2-C μg^{-1} biomass C day^{-1})	18±1	44±1	21±1

[a] C_{mic} = microbial biomass C

Table 3 Changes in chemical properties of sandy, clay-loam and clay soils in the systems assayed as influenced by the addition of *E. foetida* earthworms. For each property, values with the same letter are not significantly different (LSD, $P<0.05$)

Treatments	Total organic C (g kg−1)	Total N (g kg−1)	Pyrophosphate-extractable C (g kg−1)	Water-soluble C (μg g−1)	Water-soluble CH (μg g−1)
C1	17.2a	1.39a	5.5a	239b	13b
C1 + *E. foetida*	16.7a	1.33a	5.3a	314c	21c
C2	27.8c	1.90c	8.7b	247b	16b
C2 + *E. foetida*	24.7b	1.87c	8.2b	238b	22c
C3	18.8a	1.61b	5.0a	153a	7a
C3 + *E. foetida*	19.1a	1.57b	5.6a	166a	9a

CH = carbohydrates
C1 = sandy soil treated with composted residue and cropped with *A. sativa*
C2 = clay-loam soil treated with composted residue and cropped with *A. sativa*
C3 = clay soil treated with composted residue and cropped with *A. sativa*

planting of *A. sativa* increased the pyrophosphate-extractable C concentration of the sandy, clay-loam and clay soils (Tables 2 and 3). In this case, the pyrophosphate-extractable C of all three soils represented about 30% of total organic C. No changes induced by *E. foetida* in the pyrophosphate-extractable C of all three soils were recorded (Table 3), suggesting that this type of organic substance is not degraded by *E. foetida*. It is likely that the pyrophosphate-extractable C contains recalcitrant humic acid and fulvic acid fractions and is thus resistant to being degraded by microorganisms. Total N followed a similar pattern to extractable C (Table 3).

The water-soluble organic matter fraction consists of a heterogeneous mixture of components of varying molecular weight, such as mono- and polysaccharides, polyphenols, proteins, and low-molecular-weight organic acids (Kuiters and Dennenman 1987). This fraction can be used as a C and energy source by soil microflora (Roldán et al. 1994) and represents a short-term reservoir of plant nutrients (Gregorich et al. 1994). Moreover, the water-soluble organic matter fraction may indicate the potential microbial activity in soil (Ceccanti and García 1994) and it only represents a small proportion of the total organic C. In general, the addition of *E. foetida* to the soil increased water-soluble C and water-soluble carbohydrate concentrations of the sandy and clay-loam soils (Table 3). The gut of *E. foetida* is characterized by high cellulase activity (Zhang et al. 2000), and it is probable that this earthworm may have increased the degradation of the cellulose of the composted residue, causing an increase in the water-soluble C and water-soluble carbohydrate concentrations in the sandy and clay-loam soils. However, both water-soluble C and water-soluble carbohydrate concentrations of the clay soil were unaffected by the addition of *E. foetida* earthworms. These substances are also important as binding agents of soil aggregates (Lu et al. 1998), which are expected to be more stable in clay soil than in sandy soil. The labile fractions of soil organic matter in the clay soil could be associated with clay particles and involved in soil aggregates, thus being inaccessible to microbes.

C mineralization rate

Clay-loam soil treated with composted residue and cropped with *A. sativa* evolved higher levels of CO_2 than clay and sandy soils (Fig. 1). The absence of an initial lag or delay phase in C mineralization from sandy, clay-loam and clay soils supports the fact that microbial activity was not limited by substrate availability (Ajwa et al. 1998). The amount of composted residue- and *A. sativa* cropping-derived C can be calculated roughly by subtracting the amount of CO_2 evolved by untreated soil from that of the treated soil, assuming that the composted residue and *A. sativa* plants did not affect the decomposition of native organic matter in soil. This value was significantly higher in clay-loam and sandy soils, on average by about 20 μg CO_2-C g^{-1} day^{-1} than in the clay soil. Probably clays interacted with the added organic matter, protecting it from degradation.

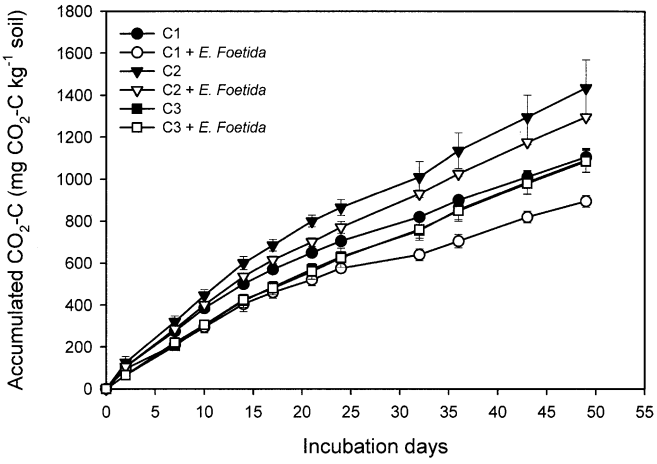

Fig. 1 Cumulative amount of CO_2-C evolved from sandy, clay-loam and clay soils in the systems assayed, as influenced by the addition of *E. foetida* earthworms (*C1* sandy soil treated with composted residue and cropped with *A. sativa*, *C2* clay-loam soil treated with composted residue and cropped with *A. sativa*, *C3* clay soil treated with composted residue and cropped with *A. sativa*). *Error bars* represent standard error

The cumulative CO_2-C evolution data from sandy, clay-loam and clay soils, as influenced by the addition of *E. foetida* earthworms (Fig. 1), followed the first-order rate model:

$$C = C_o^*\left(1 - e^{-kt}\right)$$

where C is the cumulative amount of CO_2-C mineralized after time t (mg kg^{-1}), C_o is the potentially mineralizable organic C (mg kg^{-1}), t is the incubation days after

Table 4 Effect of the addition of *E. foetida* earthworms on the cumulative CO_2-C evolution from sandy, clay-loam and clay soils in the systems assayed

Treatments	Cumulative CO_2-C production (mg CO_2-C kg^{-1} soil)	r^2
C1	$C = 1{,}374.8\ (1 - e^{-0.031t})$	0.996
C1 + *E. foetida*	$C = 1{,}126.9\ (1 - e^{-0.030t})$	0.993
C2	$C = 2{,}030.9\ (1 - e^{-0.024t})$	0.996
C2 + *E. foetida*	$C = 1{,}907.9\ (1 - e^{-0.022t})$	0.997
C3	$C = 1{,}930.0\ (1 - e^{-0.017t})$	0.998
C3 + *E. foetida*	$C = 1{,}890.5\ (1 - e^{-0.017t})$	0.998

C1 = sandy soil treated with composted residue and cropped with *A. sativa*
C2 = clay-loam soil treated with composted residue and cropped with *A. sativa*
C3 = clay soil treated with composted residue and cropped with *A. sativa*

Table 5 Changes in biochemical and microbiological properties of sandy, clay-loam and clay soils in the systems assayed as influenced by the addition of *E. foetida* earthworms. For each property, values with the same letter are not significantly different (LSD, $P<0.05$)

Treatments	Dehydrogenase activity (μg INTF g^{-1} h^{-1})[a]	Urease activity (μg NH_4^+ g^{-1} h^{-1})	Phosphatase activity (μg PNP g^{-1} h^{-1})[b]	β-Glucosidase activity (μg PNP g^{-1} h^{-1})[b]	Total CO_2 (μg CO_2-C g^{-1}day^{-1})	Microbial biomass C (μg g^{-1})	qCO_2 (ng CO_2-C μg^{-1} biomass C day^{-1})
C1	130d	100c	3,269b	745a	22.6b	511d	44a
C1 + *E. foetida*	103c	99c	2,448a	689a	18.3a	264a	69bc
C2	88bc	94bc	4,656d	1,298b	29.3d	389bc	75cd
C2 + *E. foetida*	75b	95bc	4,578d	1,158b	26.4c	348ab	76d
C3	26a	87ab	2,749ab	614a	22.2b	378bc	59b
C3 + *E. foetida*	26a	83a	3,971c	760a	22.2b	401c	55ab

[a] INTF: iodo-nitrotetrazolium formazan
[b] PNP: *p*-nitrophenol
C1 = sandy soil treated with composted residue and cropped with *A. sativa*
C2 = clay-loam soil treated with composted residue and cropped with *A. sativa*
C3 = clay soil treated with composted residue and cropped with *A. sativa*

treatments, and k is the rate constant of CO_2-C production (day^{-1}). Equations that best described the evolution data (correlation coefficients ranging from 0.993 to 0.998) are presented in Table 4. The potentially mineralizable C from the clay-loam and clay soils was higher than in the sandy soil. However, the decomposition rate constant of added organic C was significantly higher in the sandy soil, followed by the clay-loam and clay soils. This is due to the greater physical protection of soil organic matter and microbial biomass in fine-textured soils. It is well established that organic matter adsorbed by clay and silt particles is physically protected against microbial degradation in soil (Hassink 1997).

The total amounts of CO_2-C evolved from all three soils in 49 days depended on soil texture and on the addition of *E. foetida*. Moreover, there was a very significant interaction ($P<0.001$) between soil texture and *E. foetida* addition. Mineralization decreased with the addition of earthworms to the sandy and clay-loam soils, mainly in the sandy soil (by about 4 μg CO_2-C g^{-1} day^{-1}), as shown in Table 5. This could be due to higher ingestion of microorganisms by the earthworm in the heavier textured soils (Zhang et al. 2000).

Biochemical properties

Dehydrogenase activity has been considered as an indicator of microbial activity in soil (Nannipieri 1994) and it has been proposed as a valid biomarker to indicate changes in microbial activity due to changes in soil management under different agronomic practices and climates (Ceccanti et al. 1994). Dehydrogenase activity decreased when *E. foetida* was applied to the sandy and clay loam soils (Table 5). As the relative amount of large pores is usually higher in coarse-texture soils than in fine-textured soils (Hassink et al. 1993), predation of microbes by *E. foetida* is expected to be more intense in the sandy soil than in the clay soil. Presumably, the presence of habitable small pores in aggregates of the clay soil could preserve soil microorganisms from *E. foetida*. Thus the addition of *E. foetida* would affect more soil microorganisms and thus dehydrogenases in the sandy soil than in the clay and clay-loam soils because the latter soil microorganisms are inaccessible to the earthworms.

Measurement of soil hydrolases provides an early indication of changes in soil fertility, since they are related to the mineralization of such important nutrient elements as N, P and C (Ceccanti and García 1994). Many researchers have found that soil hydrolase activities are enhanced by the addition of organic materials (García et al. 1998), probably because these enzymes are stabilized by soil colloids in the extracellular soil environment. We also found that urease, β-glucosidase and phosphatase activities were higher in the three soils treated with composted residue and cropped with *A. sativa* (Tables 2 and 5). With the exception of the sandy soil, the highest increase was observed for the urease activity, which may be related to the addition and/or selection of ureolytic

Table 6 Two-factor ANOVA (soil texture and earthworm addition) for all parameters studied. *P* significance values

Soil characteristic	Soil texture (T)	Earthworms (E)	Interaction (T×E)
Total organic C	<0.001	0.004	0.008
Total N	<0.001	0.073	0.877
Pyrophosphate-extractable C	<0.001	0.812	0.009
Water-soluble C	<0.001	0.010	0.004
Water-soluble carbohydrates	<0.001	<0.001	0.002
Dehydrogenase activity	<0.001	<0.001	0.010
Urease activity	<0.001	0.234	0.317
Phosphatase activity	<0.001	0.227	<0.001
β-glucosidase activity	<0.001	0.467	<0.001
Total CO_2	<0.001	<0.001	<0.001
Microbial biomass C	0.122	<0.001	<0.001
qCO_2	<0.001	<0.001	<0.001

microorganisms. There were no changes in urease and β-glucosidase activities due to the addition of *E. foetida* earthworms, for all three soils (Tables 5 and 6). On the other hand, the addition of *E. foetida* reduced phosphatase activity in the sandy soil, probably because phosphatase-active microorganisms were degraded in the gut of the earthworm. The increased level of phosphatase activity in the clay soil following the addition of *E. foetida* might be due to the increase in the active enzymes immobilized by colloids.

Microbiological properties

Microbial biomass C generally followed a trend similar to dehydrogenase activity and the total amount of CO_2-C evolved from sandy, clay-loam and clay soils (Table 5). The addition of composted residue and planting of *A. sativa* significantly increased microbial biomass C of all three soils, especially that of the sandy soil (Tables 2 and 5). However, the addition of *E. foetida* to sandy soil significantly decreased microbial biomass, which indicates that this earthworm may consume soil microorganisms, in accordance with results reported by Zhang et al. (2000). For all enzyme activities, the enzyme activity/microbial biomass ratio was increased by the addition of *E. foetida* in major proportion in the sandy soil than in the clay-loam and clay soils, which indicates the presence of more active microorganisms in the sandy soil (Landi et al. 2000). Indeed, it is unreasonable to hypothesize the formation of enzyme organic complexes in the sandy soil. The presence of more active microorganisms in the sandy soil was confirmed by the increase of the metabolic quotient qCO_2 (CO_2-C to biomass C ratio). Indeed, the qCO_2 quotients of "young" microorganisms are frequently higher than those of "aged" ones (Anderson and Domsch 1993). This is also indicative of a less-efficient metabolism with less C converted to the synthesis of microbial biomass. Thus, *E. foetida* seems to promote a less stable and more dynamic microflora in the sandy soil. In the clay-loam and clay soils, the addition of *E. foetida* only decreased the amount of CO_2-C evolved from the clay-loam soil, but did not affect the microbial biomass content or the qCO_2 of either soil.

In conclusion, our results confirm that the addition of *E. foetida* to three soils treated with composted residue and cropped with *A. sativa* produced changes in the C mineralization rate, labile C fractions (water-soluble C and water-soluble carbohydrates), microbial biomass C, and enzyme activities (dehydrogenase, urease, phosphatase and β-glucosidase) and that the changes depended on the texture of the soil. In particular, the addition of *E. foetida* earthworms could have detrimental effects on the chemical and microbiological quality of sandy soils. Hence, soil texture may be of great importance in the selection of appropriate management practices for improving the chemical and microbiological quality of degraded agricultural soils from a semiarid Mediterranean environment.

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References

Ajwa HA, Rice CW, Sotomayor D (1998) Carbon and nitrogen mineralization in tallgrass prairie and agricultural soil profiles. Soil Sci Soc Am J 62:942–951

Anderson TH, Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol Biochem 25:393–395

Bohlen PJ, Edwards CA, Zhang Q, Parmelee RW, Allen M (2002) Indirect effects of earthworms on microbial assimilation of labile carbon. Appl Soil Ecol 20:255–261

Boyer J, Michellon R, Chabanne A, Reversat G, Tibere R (1999) Effects of trefoil cover crop and earthworm inoculation on maize crop and soil organisms in Reunion Island. Biol Fertil Soils 28:364–370

Brink RH Jr, Dubach P, Lynch DL (1960) Measurement of carbohydrates in soil hydrolysates with anthrone. Soil Sci 89:157–166

Caravaca F, Hernández MT, García C, Roldán A (2002a) Improvement of rhizosphere aggregates stability of afforested semi-arid plant species subjected to mycorrhizal inoculation and compost addition. Geoderma 108:133–144

Caravaca F, Masciandaro G, Ceccanti B (2002b) Land use in relation to soil chemical and biochemical properties in semi-arid Mediterranean environment. Soil Till Res 68:23–30

Carter MR, Angers DA, Kunelius HT (1994) Soil structural form and stability, and organic matter under cool-season perennial grasses. Soil Sci Soc Am J 58:1194–1199

Ceccanti B, Garcia C (1994) Coupled chemical and biochemical methodologies to characterize a composting process and the humic substances. In: Senesi N, Miano T (eds) Humic substances in the global environment and its implication on human health. Elsevier, New York, pp 1279–1285

Ceccanti B, Pezzarossa B, Gallardo-Lancho FJ, Masciandaro G (1994) Bio-tests as markers of soil utilization and fertility. Geomicrobiol J 11:309–316

Daniel O, Anderson JM (1992) Microbial biomass and activity in contrasting soil materials after passage through the gut of earthworm *Lumbricus rubellus* Hoffmeister. Soil Biol Biochem 24:465–470

García C, Hernández MT, Costa F (1997) Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun Soil Sci Plant Anal 28:123–134

García C, Hernández MT, Albaladejo J, Castillo V, Roldán A (1998) Revegetation in semiarid zones: influence of terracing and organic refuse on microbial activity. Soil Sci Soc Am J 62:670–676

Gregorich EG, Carter MR, Angers DA, Monreal CM, Ellert BH (1994) Towards a minimum data set to assess soil organic matter quality in agricultural soils. Can J Soil Sci 74:367–385

Hassink J (1997) The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant Soil 191:77–87

Hassink J, Bouwman LA, Zwart KB, Bloem J, Brussaard L (1993) Relationships between soil texture, physical protection of organic matter, soil biota, and C and N mineralization in grassland soils. Geoderma 57:105–128

Kuiters AT, Dennenman CAJ (1987) Water soluble phenolic substances in soils under several coniferous and deciduous tree species. Soil Biol Biochem 19:765–769

Landi L, Renella G, Moreno JL, Falchini L, Nannipieri P (2000) Influence of cadmium on the metabolic quotient, L-: D-glutamic acid respiration ratio and enzyme activity: microbial biomass ratio under laboratory conditions. Biol Fertil Soils 32:8–16

Lu G, Sakagami K, Tanaka H, Hamada R (1998) Role of soil organic matter in stabilization of water-stable aggregates in soils under different types of land use. Soil Sci Plant Nutr 44:147–155

Masciandaro G, Ceccanti B, García C (1994) Anaerobic digestion of straw and piggery wastewater. II. Optimization of the process. Agrochimica 38:195–203

Nannipieri P (1994) The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Pankhurst CE, Doube BM, Gupta VVSR, Grace PR (eds) Soil biota: management in sustainable farming systems. CSIRO Australia, pp 238–244

Nannipieri P, Ceccanti B, Cervelli S, Matarese E (1980) Extraction of phosphatase, urease, proteases, organic carbon and nitrogen from soil. Soil Sci Soc Am J 44:1011–1016

Ouédraogo E, Mando A, Zombré NP (2001) Use of compost to improve soil properties and crop productivity under low input agricultural system in West Africa. Agric Ecosyst Environ 84:259–266

Page AL, Miller RH, Keeny OR (1982) Methods of soil analysis, part 2. American Society of Agronomy, Madison, Wis.

Pascual JA, García C, Hernández MT (1999) Comparison of fresh and composted organic waste in their efficacy for the improvement of arid soil quality. Bioresour Technol 68:255–264

Pashanasi B, Melendez G, Szott L, Lavelle P (1992) Effect of inoculation with the endogeic earthworm *Pontoscolex corethrurus* (Glossoscolecidae) on N availability, soil microbial biomass and the growth of three tropical fruit tree seedlings in a pot experiment. Soil Biol Biochem 24:1655–1659

Rivas-Martinez S (1987) Memoria del mapa de series de vegetación de España. ICONA, Spain

Roldán A, García-Orenes F, Lax A (1994) An incubation experiment to determine factors involving aggregation changes in an arid soil receiving urban refuse. Soil Biol Biochem 26:1699–1707

Scheu S (1992) Automated measurement of the respiratory response of soil micro-compartments: active microbial biomass in earthworm faeces. Soil Biol Biochem 24:1113–1118

Schindler-Wessells M, Bohlen PJ, McCartney DA, Edwards CA (1997) The effect of earthworms on soil respiration in corn agroecosystems with different nutrient treatments. Soil Biol Biochem 29:409–412

Sims JR, Haby VA (1971) Simplified colorimetric determination of soil organic matter. Soil Sci 112:137–141

Tabatabai MA (1982) Soil enzymes. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, Part 2, 2nd edn. (Agronomy monograph 9) ASA and SSSA, Madison, Wis., pp 501–538

Tabatabai MA, Bremner JM (1969) Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1:301–307

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring microbial biomass C. Soil Biol Biochem 19:703–707

Willson GB, Parr JF, Epstein E, March PB, Chaney RL, Colacicco D, Burge WD, Sikora LJ, Tester CF, Hornick SB (1980) Manual for composting sewage sludge by the Beltsville Aerated Pile Method. Joint USDA/EPA Spec Rep EPA-600/8-80-022, US Government Printing Office, Washington, D.C.

Yeomans JC, Bremner JM (1988) A rapid and precise method for routine determination of organic carbon in soil. Commun Soil Sci Plant Anal 19:1467–1476

Zebarth BJ, Neilsen GH, Hogue E, Neilsen D (1999) Influence of organic waste amendments on selected soil physical and chemical properties. Can J Soil Sci 79:501–504

Zhang BG, Li GT, Shen TS, Wang JK, Sun Z (2000) Changes in microbial biomass C, N, and P and enzyme activities in soil incubated with the earthworms *Metaphire guillelmi* or *Eisenia foetida*. Soil Biol Biochem 32:2055–2062

Zibilske LM (1994) Carbon mineralization. In: Weaver RW, Angle JS, Bottomley PS (eds) Methods of soil analysis. II. Microbiological and biochemical properties. Soil Science Society of America, Madison, Wis., pp 835–863