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A microcosm approach to assessing the effects of earthworm inoculation and oat cover cropping on CO_2 fluxes and biological properties in an amended semiarid soil

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Abstract

We designed a microcosm experiment to assess the influence of inoculation with *Eisenia foetida* earthworms and the establishment of an *Avena sativa* cover crop on biological (enzyme activities and labile carbon fractions) soil quality indicators in a soil treated with a composted organic residue, and to determine the contribution of these treatments to carbon dioxide emissions from the soil to the atmosphere of the microcosm. The microcosms were incubated for 53 days under 28 °C/18 °C day/night temperatures. The addition of earthworms and the planting of *A. sativa* increased dehydrogenase activity of compost amended soil by about 44% after 23 days of incubation. The metabolic potential, calculated as the ratio dehydrogenase activity/water soluble C, was higher in the compost amended soil planted with *A. sativa*. The highest total amount of CO_2–C evolved occurred in the soil treated with composted residue and earthworms (about 40% of the total amount of CO_2 evolved came from earthworm activity). The planting of *A. sativa* increased the decomposition rate constant of organic matter in the amended soil but decreased the potentially mineralizable C pool. In conclusion, the establishment of an *A. sativa* cover crop and the addition of *E. foetida* to a degraded agricultural soil treated with composted residue were effective treatments for improving the biological and biochemical quality and the metabolic potential of the soil.

Keywords: Dehydrogenase activity; *Eisenia foetida*; Mineralization potential; Soil respiration; Water soluble carbon

1. Introduction

The soils of Mediterranean agroecosystems are subject to progressive degradation and desertification, leading to a decrease in soil productivity and fertility (Caravaca et al., 2002). The addition of organic amendments, particularly composted residues is a proven method for improving the quality of semiarid disturbed soils and an acceptable strategy for their disposal (Roldán et al., 1996). 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 (Caravaca et al., 2003). Another

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management practice with the potential to increase soil carbon is the return of agricultural soils to grassland. Likewise, annual grass species such as *Avena sativa*, used as a cover crop, have been proposed also as an agroecological alternative for improving or maintaining soil structure in degraded ecosystems (Carter et al., 1994).

Information on the influence of management on soil CO_2 fluxes is required to identify practices that maintain soil productivity and retard the conversion of soil C into atmospheric CO_2. Soils contribute C to the atmosphere through plant root respiration and decomposition of soil organic matter by soil microorganisms. The release of CO_2 by these processes can be measured in controlled conditions, to assess the potential effect of soil management on atmospheric CO_2. In the field, partitioning of CO_2 according to source can be complicated because of confounding effects related to temperature and moisture differences (Curtin et al., 1998).

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 crop 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). Studies on the effect of earthworms on soil microbial biomass have given contradictory results. Thus, decreased (Bohlen et al., 2002), increased (Scheu, 1992) or unchanged (Daniel and Anderson, 1992) microbial biomass has been reported by different authors. Moreover, information concerning the relative contribution of earthworms to CO_2 efflux is scarce.

The objectives of this study were: (i) to assess the influence of inoculation with *Eisenia foetida* earthworms and the establishment of an *A. sativa* cover crop on biological (enzyme activities and labile carbon fractions) soil quality indicators in a soil treated with a composted organic residue, and (ii) to determine the contribution of these treatments to carbon dioxide emissions from the soil to the atmosphere of a microcosm.

2. Materials and methods

2.1. Materials

A Cambic Arenosol (FAO, 1988) soil was collected from the A horizon of a grape vineyard located along the Italian Mediterranean coast. This sandy soil (98% sand, 1% silt and 1% clay) had a low organic matter content and a poorly developed structure. Soil characteristics are shown in Table 1. The soil was formed under a semiarid, Mediterranean type climate (Rivas-Martinez, 1987), with 538 mm/year average rainfall, 1124 mm/year evapotranspiration and an average annual temperature of 14.0 °C.

Table 1
Physico-chemical, chemical and biochemical characteristics of the soil and composted residue used in the incubation experiment

	Soil	Compost
Texture	Sandy	–
Electrical conductivity (1/10) ($\mu S\ cm^{-1}$)	46	580
pH (1/10)	6.7	7.8
Total organic C (%)	0.32	29
Water-soluble C ($\mu g\ g^{-1}$)	127	6873
Total carbohydrates C ($\mu g\ g^{-1}$)	270	9764
Water-soluble carbohydrates C ($\mu g\ g^{-1}$)	14	524
Total N (%)	0.035	1.65
$N–NH_3$ ($\mu g\ g^{-1}$)	0.368	33
$N–NO_3^-$ ($\mu g\ g^{-1}$)	27	10
Total P ($\mu g\ g^{-1}$)	102	588
Available P ($\mu g\ g^{-1}$)	12	111
Protease-BAA[a] ($\mu g\ NH_4^+\ g^{-1}\ h^{-1}$)	30	123
Urease ($\mu g\ NH_4^+\ g^{-1}\ h^{-1}$)	19	235
Dehydrogenase (μg INTF[b] $g^{-1}\ h^{-1}$)	0.5	284

[a] BAA: *N*-α-benzoyl-L-argininamide.
[b] INTF: iodo-nitrotetrazolium formazan.

The compost used in this experiment was produced from a mixture of cereal residues and a small proportion (10%) of aerobically digested sewage sludge, using 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.

2.2. Experimental design

The experiment was a microcosm assay, conducted as a completely randomised design with five replicates, making a total of 20 microcosms. Thus, four treatments were established: untreated soil (control soil, C), soil treated with composted residue (R), soil treated with composted residue and incubated with *E. foetida* worms (RW) and soil treated with composted residue and planted with *A. sativa* (RP).

The experiments were performed in closed 8-l chambers. In each chamber (microcosm), 1500 g of sieved (0–2 mm) dry soil were placed. To establish the system, 300 g of gravel were used at the base of the chamber and an inert membrane was placed between the gravel and the soil. Composted residue was incorporated into the soil at a rate of 58.2 g compost kg^{-1} soil, sufficient to raise the soil total organic C content by 1.68%. Three weeks after the addition of the compost, about 40 seeds of *A. sativa* were sown in five chambers. At the same

time, 10 adult *E. foetida* earthworms were added to five other chambers. The total number of earthworms added was equivalent to a field population density of about 104 worms m^{-2}. The chambers were kept at 28 °C/18 °C, day/night, respectively, and at 60% of the soil water-holding capacity (24% WHC), using deionised water. To maintain soil moisture, the chambers were weighed every 5 days and supplied with deionised water. No fertiliser was added.

2.3. Sampling and analysis procedures

Carbon dioxide emissions from incubation chambers were sweeped across the soil, removing CO_2 and replenishing O_2 in the head space above the soil, every 2 days for 53 days of the experiment. The trapped CO_2 in NaOH trap was precipitated as carbonate with excess $BaCl_2$ and the excess NaOH was titrated with HCl (Zibilske, 1994).

Soil samples of the chambers were taken for analysis 0, 23 and 53 days after incubation. Soil samples were divided into two subsamples. One soil subsample was stored at 2 °C for biochemical analysis and another soil subsample was allowed to dry at room temperature for physical–chemical and chemical analysis. The following parameters were determined using an aqueous extract of soils 1:10 (w/v): electrical conductivity, water-soluble C by dichromate oxidation and measurement of absorbance at 590 nm (Sims and Haby, 1971), NH_4^+ with ammonia-selective electrode (ORION), and NO_3^- with a DIONEX chromatograph.

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolysing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates, respectively. Two millilitres of buffer and 0.5 ml of substrate were added to 0.5 g of sample, which was incubated at 30 °C (for urease) or 39 °C (for protease) for 90 min. Both activities were determined as the NH_4^+ released in the hydrolysis reaction (Nannipieri et al., 1980).

Dehydrogenase activity (DH-ase) was determined by the method of García (1997) using 2-*p*-iodo-3-nitrophenyl-phenyltetrazolium chloride (INT) as substrate. The product iodo-nitro-tetrazolium formazan (INTF) of the reaction, was determined spectrophotometrically at 490 nm. Analyses are reported on an air dry weight basis.

2.4. Statistical analysis

All data were subjected to an 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 with the software package SPSS 10.0 for Windows.

3. Results and discussion

3.1. Changes in soil physico-chemical and chemical properties

Before incubation, the addition of composted residue increased the electrical conductivity in the soil (Table 2). In the treated soils (R, RW and RP), the electrical conductivity increased with incubation time, particularly in the soil treated with composted residue and earthworms. However, the increases observed in soil electrical conductivity were not sufficient to limit plant growth or soil biological activity. Available mineral N (constituted by nitrate and ammonia) was significantly lower in the control soil than in the soil treated with composted residue (Table 2). Variations in ammonia during the experiment were relatively low, ranging from 2 to 9 μg NH_3 kg^{-1} soil, so that mineral N in the soil during the incubation period (53 days) increased with time as nitrate, which was the major component, indicating the high nitrification potential of the soil. The similarity between the nitrate values of the control soil and the soil treated with composted residue at the end of incubation period could be due to its immobilization by microorganisms during the decomposition of added residue. The highest nitrate values were observed in the soil treated with composted residue and earthworms, reaching a nitrate

Table 2
Electrical conductivity (EC), nitrate and ammonia in response to the addition of composted residue and *E. foetida* earthworms and to the planting of *A. sativa* previous incubation, 23 and 53 days after incubation

	Days of incubation		
	0	23	53
EC (1:10) (μS cm^{-1})			
C	46a	45a	42a
R	103b	110b	123b
RW	103b	311d	306d
RP	103b	188c	185c
NH_3 (μg g^{-1})			
C	2a	3a	5a
R	5b	6b	8b
RW	5b	8b	9b
RP	5b	7b	7b
NO_3^- (μg g^{-1})			
C	30a	39a	68a
R	39b	43a	67a
RW	39b	252b	490c
RP	39b	217b	249b

For each sampling date, treatment means followed by the same letter are not significantly different (LSD test, $p < 0.05$).
C: control soil; R: composted residue addition; RW: composted residue and worms addition; RP: composted residue addition and planting of *A. sativa*.

concentration of 490 μg kg^{-1} soil at the end of the incubation period. This supports Bohlen and Edwards (1995) suggestion that earthworms convert microbial biomass-N to extractable N, increasing the amount of soil nitrate.

3.2. Changes in soil labile carbon and biochemical properties

Water soluble C represents the most labile fraction of soil organic matter because it is susceptible to microbial attack. This fraction can be used as carbon and energy sources by soil microflora and can be related positively to microbial activity (Ghani et al., 2003). The concentration of soluble C was significantly higher in the soil treated with composted residue than in the control soil (about 4.2 times greater), as shown in Table 3. The water-soluble carbon content of the treated soils decreased during the incubation period, particularly in the soil treated with composted residue and planted with *A. sativa*.

Dehydrogenase is an oxidoreductase which is only present in viable cells. This enzyme has been considered as a sensitive indicator of soil quality in degraded soils (García, 1997) and it has been proposed as a valid biomarker to indicate changes in total microbial activity due to changes in soil management, under different agronomic practices and climates (Ceccanti et al., 1994). Compost addition to soil significantly increased the dehydrogenase activity (Table 3). The labile organic matter incorporated with the compost contributed to the significant increase in dehydrogenase activity and hence in microbial metabolism. For the incubation period, untreated soil showed the lowest value (on average about 1.1 μg INTF g^{-1} h^{-1}), followed by the soil treated with compost, while the highest values were observed in the amended soil treated with earthworms or planted with *A. sativa* after 23 days of incubation. The metabolic potential (Masciandaro et al., 1998), calculated as the ratio between the size and activity of the viable microbial community (dehydrogenase activity) and the sources of energy for microorganisms (water soluble carbon concentration) was highest in the soil treated with composted residue and planted with *A. sativa*, which indicates the presence of more active microorganisms in this soil.

Measurement of soil hydrolases provides an early indication of changes in soil fertility, since they are related to the mineralisation of such important nutrient elements as N, P and C (Ceccanti et al., 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 stabilised by soil colloids in the extracellular soil environment. We also found that urease and protease-BAA activities were higher in the soil treated with composted residue than in the control soil (Table 3). The increases in urease activity with incubation time observed in the amended soil treated with earthworms may be related to the addition and/or selection of ureolytic microorganisms. With the exception of the amended soil treated with earthworms, the protease activity did not vary with incubation time. The cause of the reduction in protease activity by the addition of *E. foetida* is unknown. One possibility is that protease-active microorganisms were degraded in the gut of the earthworm.

Table 3
Water soluble C, dehydrogenase, metabolic potential, urease and protease-BAA activities in response to the addition of composted residue and *E. foetida* earthworms and to the planting of *A. sativa* previous incubation, 23 and 53 days after incubation

	Days of incubation		
	0	23	53
WSC[a] *(μg g^{-1})*			
C	127a	134a	120a
R	530b	454c	429c
RW	530b	401c	400c
RP	530b	334b	273b
Dehydrogenase (g INTF[b] *g^{-1} h^{-1})*			
C	0.8a	1.5a	1.1a
R	2.7b	3.9b	3.8b
RW	2.7b	5.4c	4.6c
RP	2.7b	5.8c	5.1c
Dehydrogenase/WSC (μg INTF mg^{-1}C h^{-1})			
C	6.30b	11.19b	9.17ab
R	5.09a	8.59a	8.86a
RW	5.09a	13.47c	11.50b
RP	5.09a	17.37d	18.68c
Urease (μg NH_4^+ g^{-1} h^{-1})			
C	19a	15a	17a
R	30b	29b	38b
RW	30b	52c	68c
RP	30b	36b	63c
Protease-BAA[c] *(μg NH_4^+ g^{-1} h^{-1})*			
C	30a	33a	30a
R	51b	52b	53b
RW	51b	53b	31a
RP	51b	49b	50b

For each sampling date, treatment means followed by the same letter are not significantly different (LSD test, $p < 0.05$).
C: control soil; R: composted residue addition; RW: composted residue and worms addition; RP: composted residue addition and planting of *A. sativa*.
[a] WSC: Water soluble C.
[b] INTF: iodo-nitrotetrazolium formazan.
[c] BAA: *N*-α-benzoyl-L-argininamide.

3.3. Fluxes of carbon dioxide

Carbon dioxide emissions from the soil responded rapidly to the addition of composted residue and

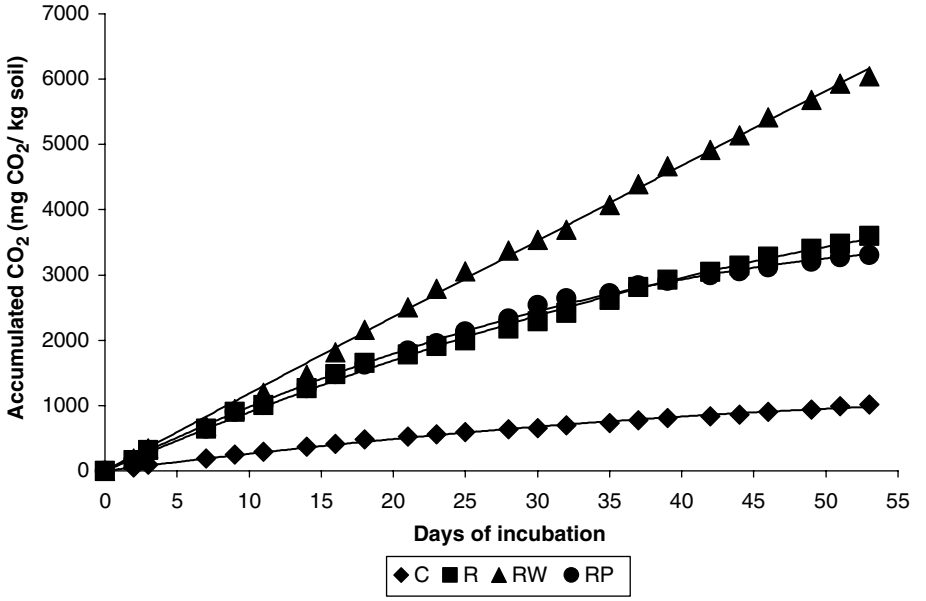

Fig. 1. Cumulative amount of CO_2–C evolved from soil in the microcosms, as influenced by the addition of composted residue and *E. foetida* earthworms and by the planting of *A. sativa* (C: control soil; R: composted residue addition; RW: composted residue and worms addition; RP: composted residue addition and planting of *A. sativa*).

E. foetida earthworms and to the planting of *A. sativa*. The first measurements (second day of incubation) showed emissions from treated soils three to four times greater than those of the untreated soil (Fig. 1). The absence of an initial lag or delay phase for C mineralisation in untreated and treated soils indicates that microbial activity was not limited by substrate availability (Ajwa et al., 1998). In the untreated soil, with a low index of overall microbial activity (dehydrogenase activity about 1 μg INTF g^{-1} h^{-1}), the CO_2 flux remained constantly low during incubation. The increased CO_2 flux in treated soils was due either to the input of additional available C substrates to the soil, as found by Rochette and Gregorich (1998) and/or mainly to the addition of microflora, which has significantly modified the soil microbial activity. The highest levels of CO_2 production were reached in the soil treated with composted residue and earthworms. During the incubation period, the accumulated CO_2 of the soil treated with composted residue was similar to that of the soil treated with composted residue and planted with *A. sativa*.

The total evolution of CO_2–C in 53 days ranged from 277 to 1649 mg kg^{-1} soil, depending on the applied treatment (Table 4). The total amount of CO_2–C evolved for the untreated sandy soil (277 mg CO_2–C per kg soil) is in agreement with data reported by Biederbeck et al. (1994) and Curtin et al. (1998) for a silt-loam soil.

The ratio of the CO_2 evolved during the whole experiment divided by the total organic C content can be used to express the contribution of total organic C to mineralisation. This ratio in the soil amended was lower than that in the control soil. Hence, the mineralisation capacity of organic C added as compost is less than that of soil native organic C despite increased concentration of soluble C in the amended soil. This could result from the presence of recalcitrant forms of soluble C that are subsequently decomposed. Other authors have also found that not all of the soluble C is readily decomposable and that the recalcitrant fraction increases with incubation time (Rochette and Gregorich, 1998).

Table 4
Total amount of CO_2 evolved in 53 days as influenced by the addition of composted residue and *E. foetida* earthworms and by the planting of *A. sativa*

C	R	RW	RP
mg CO_2–C kg^{-1} soil			
277c[a]	982b	1649a	902b

C: control soil; R: composted residue addition; RW: composted residue and worms addition; RP: composted residue addition and planting of *A. sativa*.

[a] Means followed by the same letter within a row are not significantly different at $p < 0.05$.

The CO_2–C evolved from earthworms in 53 days (estimated by subtracting the C evolved from the soil treated with compost-earthworm from that produced by the compost-treated soil) was 667 mg CO_2–C kg^{-1} soil. Similar results were found by Hendrix et al.

Table 5
Mathematical equations describing the cumulative CO_2–C evolution data from soil in the systems assayed, as influenced by the addition of composted residue and *E. foetida* earthworms and by the planting of *A. sativa*

	First-order equation $C = C_1 + C_0*(1 - e^{-K \cdot T})$	R^2	VC (%)		
			C_1	C_0	K
C	$C = 0.30 + 43.5*(1 - e^{-0.018 \cdot T})$	0.997	109.3	5.9	10.2
R	$C = 1.12 + 181.1*(1 - e^{-0.014 \cdot T})$	0.998	84.1	6.5	10.0
RP	$C = -3.0 + 123.2*(1 - e^{-0.028 \cdot T})$	0.997	37.9	2.7	6.3
	Linear equation $C = K \cdot T$				
RW	$C = 3.19*T$	0.998			0.5

VC: variation coefficient; C_1: the most easily and rapidly mineralizable carbon subfraction (mg·100 g^{-1} soil); C_0: potentially mineralisable organic C (mg·100 g^{-1} soil); K: rate constant of CO_2–C production (day^{-1}); T: the time of incubation in days. C: control soil; R: composted residue addition; RW: composted residue and worms addition; RP: composted residue addition and planting of *A. sativa*.

(1987), regarding the contribution of earthworms to carbon dioxide emissions from soil under field conditions (about 40% of the total amount of CO_2 evolved).

3.4. Kinetic models of C mineralisation

The cumulative CO_2–C evolution data from the control soil, the soil treated with composted residue and the soil treated with composted residue and planted with *A. sativa* followed the pseudo-first order rate model (Table 5) proposed by Jones (1984) with high correlation coefficients (correlation coefficients of about 0.998):

$$C = C_1 + C_0 * (1 - e^{-K \cdot T})$$

where C is the cumulative amount of CO_2–C mineralised after time t (mg · 100 g^{-1} soil), C_1 the most easily and rapidly mineralizable carbon subfraction (mg · 100 g^{-1} soil), C_0 is the potentially mineralisable organic C (mg · 100 g^{-1} soil), T is the time of incubation in days and K is the rate constant of CO_2–C production (day^{-1}). The C mineralisation occurred in two distinct phases: a first, rapid phase (corresponding to the decomposition of the most labile products by the microorganisms) and a second, slower phase, during which the most resistant organic compounds started to mineralise. The potentially mineralisable C from the soil treated with composted residue alone was greater than in the control soil and the amended soil planted with *A. sativa*. However, the decomposition rate constant was substantially higher in the amended soil planted with *A. sativa*, which may be related to the rapid microbial assimilation of labile carbon observed in this soil. Only in the amended soil planted with *A. sativa* the C_1 value was negative, which reveals the existence of a lag phase in the mineralisation process (Jones, 1984). The plant influence on CO_2 flux is complicated by the fact that CO_2 can be fixed by the plant and evolved from root activity.

The CO_2 flux from the soil treated with composted residue and earthworms increased linearly with incubation time (Table 5). The difference in CO_2 flux between the soil treated with composted residue and earthworms and the other treated soils could be explained in terms of an alteration in the microbial community and size, in addition to worm respiratory activity (Devliegher and Verstraete, 1995). In fact, earthworms selectively consume soil fractions with high concentrations of microorganisms, and not all the ingested microorganisms are killed during passage through the earthworm intestine (Zhang et al., 2000).

In conclusion, our results show that the establishment of an *A. sativa* cover crop and the addition of *E. foetida* to a degraded agricultural soil treated with composted residue produced changes in the fluxes of carbon dioxide, the labile C fraction and enzyme activities (dehydrogenase, urease and protease). Both treatments were effective for improving the biological and biochemical quality and the metabolic potential of the soil. However, when a management aim is the retention of maximum C in the soil, then the addition of *E. foetida* earthworms appears to be less appropriate, because it increases CO_2 fluxes.

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