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Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass

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Abstract

A long-term field experiment utilising barley received four different treatments prior to sowing: municipal solid waste (MSW) compost at either 20 t ha^{-1} (C20) or 80 t ha^{-1} (C80); cow manure (MA) at 20 t ha^{-1}; mineral fertilizer (MIN) or NPK (400 kg ha^{-1}); and NH_4NO_3 (150 kg ha^{-1}). The effects of these applications on soil enzyme activities and microbial biomass at crop harvest were measured after nine years. In comparison with the control (no amendment) MSW addition increased biomass C by 10 and 46% at application rates of 20 and 80 t ha^{-1}, respectively, while MA treatment increased microbial biomass C by 29%. The ratio of soil microbial C to soil organic C was the lowest at the high rate of MSW application. Oxidoreductase enzymes, such as dehydrogenase and catalase, were higher in the MSW treatments by 730 (C20) and 200% (C80), respectively, and by 993 and 140% in MA treatments than in the unamended soil, indicating an increase in the microbial metabolism in the soil as a result of the mineralization of biodegradable C fractions contained in the amendments. The addition of MSW and MA caused different responses in hydrolase enzymes. Phosphatase activity decreased with MSW (±62% at both rates) and MA (±73%), to less than those in the mineral fertilization and the control treatments. Urease activity decreased by 21% (C20) and 28% (C80), possibly being affected by the heavy metals contained in the MSW. However, β-glucosidase and protease-BAA increased in all the organic treatments, especially with MA (by 214 and 177%, respectively). This is attributed to the microbial stimulation by the organic C and is correlated with the increase in dehydrogenase ($r^2 = 0.882$) and catalase ($r^2 = 0.654$) activities.

Keywords: Soil biomass; Enzyme activities; Municipal solid waste compost; Heavy metal pollution

1. Introduction

One of the characteristics of soils in the south of Spain is their low organic matter content. The decrease in soil organic matter is paralleled by declines in soil fertility, as many authors have demonstrated (Clapp et al., 1986; Tate, 1987). One method to reverse this degradation in soil quality is the addition of organic matter (Bastian and Ryan, 1986).

In recent years, composted urban waste has been added to agricultural land for both waste disposal and to improve soil fertility. Compost is rich in organic matter and an important source of nutrients for plants (Gallardo-Lara and Nogales, 1987) even though it may increase the level of potentially harmful trace metals and various persistent organic toxins (Giusquiani et al., 1995).

Incorporation of organic materials, such as municipal solid waste (MSW) compost, into soil promotes microbiological activity. Microbial activity and soil fertility are generally closely related because it is through the biomass that the mineralization of the important organic elements (C, N, P and S) occur (Frankenberger and Dick, 1983). Studies of microbial biomass C and enzyme activities provide information on the biochemical processes occurring in the soil and there is growing evidence that soil biological parameters may have a potential as early and sensitive indicators of soil ecological stress and restoration (Dick and Tabatabai, 1992).

In many arable agricultural soils, the soil microbial biomass is related to the soil organic matter content (Houot and Chaussod, 1995) and biomass C generally represents 2–3% of soil organic C (Anderson and Domsch, 1989). Soils in semiarid areas have a very low microbial activity (García et al., 1994a), low levels of microbial biomass and a low organic matter content. The last mentioned is due to the increased oxidation after cultivation, tillage operations that cause physical disruption on the soil surface, and erosion of top soil rich in organic matter (Smith et al., 1993). Thus, microbial biomass, being the living part of soil organic matter, can be a good index for comparing

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Table 1
Characteristics of soil, compost and manure used in the experiment

	Soil	Compost	Manure
% Ash	ND[a]	66.7	64.1
pH	6.4	7.9	8.8
E.C.$_{25°C}$ (dS m^{-1})	0.1	7.0	7.0
C (g kg^{-1})	8.0	124.0	158.0
N (g kg^{-1})	0.7	14.0	9.0
C/N	10.0	8.7	15.9
Macronutrients (total (g kg^{-1}))			
P	0.03	5.0	1.6
K	0.2	4.1	15.0
Ca	1.5	39.3	9.8
Mg	0.2	3.8	3.7
Na	0.01	6.4	1.4
Heavy metals (total (mg kg^{-1}))			
Fe	9685	11662	4288
Mn	111	175	86
Zn	18	1325	28
Cu	6	548	<3
Cr	4	83	8
Ni	4	81	3
Pb	<3	681	<3
Cd	<0.2	<0.2	<0.2

[a] ND(not determined).
[b] E.C.$_{25°C}$(electrical conductivity).

natural (Ross et al., 1982) and degraded (Sparling et al., 1981) ecosystems.

The aim of the present research was to evaluate the changes in microbial activity that took place in an agricultural soil that has been amended with MSW compost at two different rates over nine years and to compare a manure treatment, a mineral fertilization and a non-amended control. The specific activity of some enzymes (dehydrogenase, catalase, phosphatase, urease and protease) and the microbial biomass C content were measured.

2. Materials and methods

2.1. Sampling sites

The study was carried out in the experimental field "La Higueruela" situated in Santa Olalla (Toledo) in Central Spain. The environmental conditions define the area as continental semiarid with an average annual rainfall of 487 mm and a mean annual temperature of 14°C. Urban waste compost was obtained from the Valdemingomez Municipal Waste Treatment Plant in Madrid, Spain. It was applied to a soil (*Typic haploxeralf*) with a sandy texture and low organic matter content (Díaz-Marcote and Polo, 1996). The main characteristics of soil, compost and cow manure are listed in Table 1. Both organic residues have a high electrical conductivity, alkaline pH and a large concentration of nutrients and organic compounds. Heavy metals, such as Zn, Cu, Ni and Pb, had higher concentrations in MSW compost than in manure.

2.2. Experimental design

The field experiment consisted of plots in four blocks of five different treatments, arranged randomly. The treatments were: control without fertilization (CONT), compost applied at two rates of 20 t ha^{-1} (C20) and 80 t ha^{-1} (C80), cow manure (MA) with 20 t ha^{-1} and mineral fertilization (MIN) consisting of 400 kg ha^{-1} of NPK 15–15–15 and 150 kg ha^{-1} of NH_4NO_3. All the treatments were applied before the barley seeds were sown.

One year after treatment, each plot was divided into two sub-plots of 10 × 3 m. The effects of annual application of the treatments were studied in one half and the longer term effects of the first application in the other half. There were three consecutive years of compost treatment at the mentioned rates for all plots, followed by three years of no application and two successive years repeating the initial treatments. In the last year of the experiment at harvest time, soil samples were collected and sieved (2 mm) field-moist. Each sample consisted of a mixture of 20 soil cores (3 cm dia) taken randomly from the arable layer (0–20 cm) of each sub-plot. The samples were stored at 4°C until analysis.

2.3. Chemical analysis

Soil pH and electrical conductivity in the residues were measured in a 1:5 sample:water extract after shaking for 30 min. Organic carbon (OC) was analysed by dichromate oxidation and titration with ferrous ammonium sulphate (Walkley and Black, 1934). Total nitrogen in all samples was determined by the Kjeldahl method and using a an autoanalyser (Bran-Lubbe Technicon AAII, USA) (Hinds and Lowe, 1980). The total content of macronutrients and heavy metals in soil and both organic wastes, samples (<0.5 mm) were determined following digestion with nitric and perchloric acids and then analysed in an atomic absorption spectrophotometer (Perkin Elmer HGA500, USA)

2.4. Biological and biochemical analysis

Biomass C was determined by fumigation of the sample with ethanol-free $CHCl_3$ and extraction with 0.5 M K_2SO_4, according to Vance et al. (1987). Prior to analysis, samples were incubated for 12 h at 25°C.

Dehydrogenase activity was determined by the reduction of 2-*p*-iodo-nitrophenyl-phenyltetrazolium chloride (INT) to iodo-nitrophenyl formazan (INTF) using the method of Skujins (1976) as modified by García et al. (1993). Dehydrogenase activity was measured in 1 g soil, following incubation in the dark with 0.2 ml of 0.4% INT in distilled water for 20 h at 22°C. The INTF was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through a Whatman no. 5 filter paper. The INTF was measured spectrophotometrically at 490 nm.

Table 2
Soil microbial biomass C (mg C kg^{-1} soil) and its ratio to the organic carbon (OC) in soil. (Values within the same column followed by the same letter are not significantly different ($P \leq 0.05$))

	Biomass-C		Biomass-C/OC	
	Residual	Accumulative	Residual	Accumulative
Manure	194 b	265 c	3.0 ab	2.7 bc
Mineral fertilization	147 a	204 a	2.0 a	3.0 b
Compost 20 t ha^{-1}	165 ab	226 b	2.1 a	2.0 ab
Compost 80 t ha^{-1}	262 c	301 d	2.9 ab	1.2 a
Control	202 b	205 a	3.4 b	3.5 b

Catalase activity was determined by measuring the O_2 absorbed by $KMnO_4$ after addition of H_2O_2 to the samples (Rodríguez-Kábana and Truelove, 1982).

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

Phosphatase and β-glucosidase activities were determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) or *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrates, respectively (Masciandaro et al., 1994). These assays are based on the release and detection

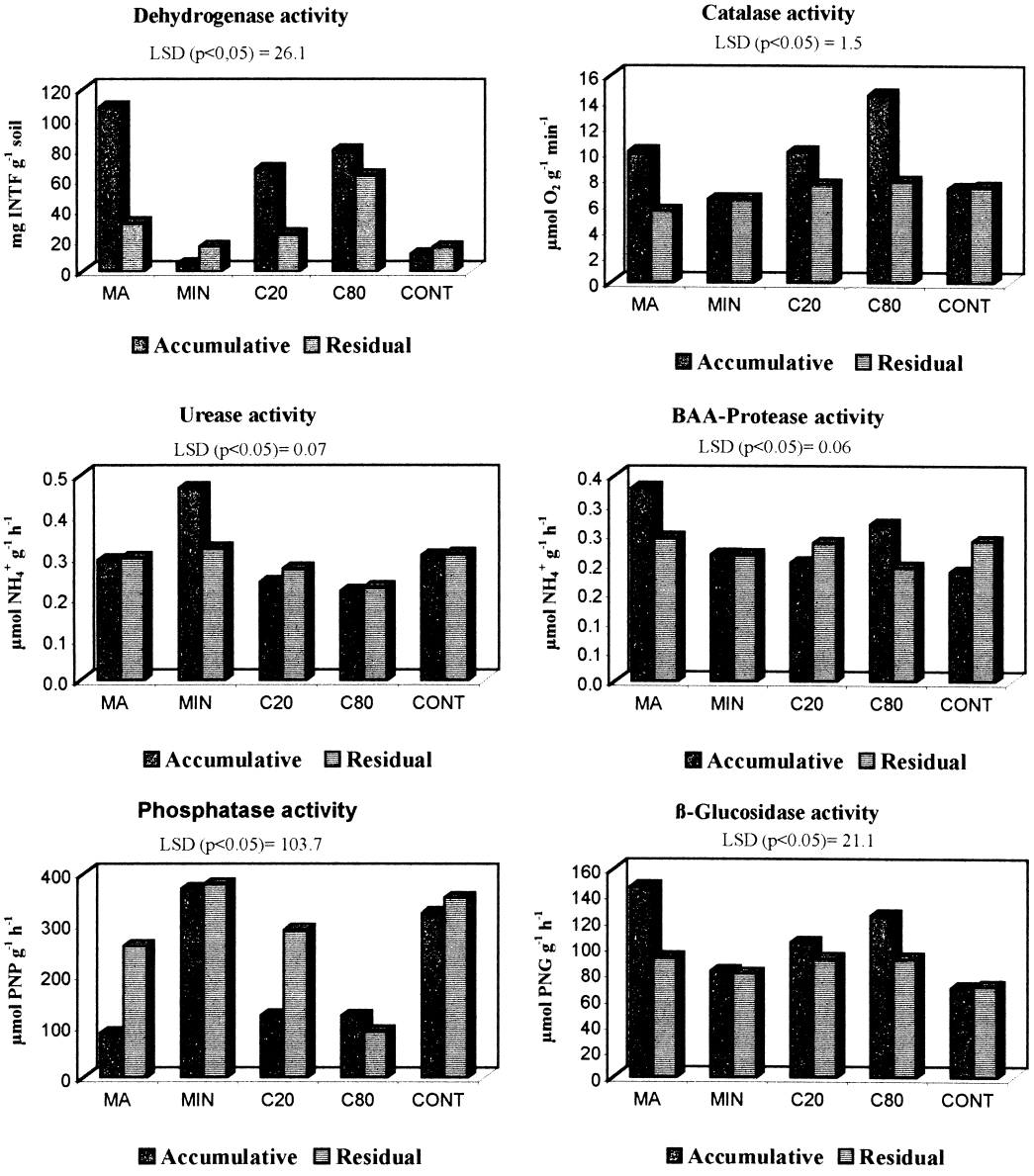

Fig. 1. Enzyme activities in soil with different treatments. MA(manure), MIN(mineral fertilization), C20(compost 20 t ha^{-1}), C80(compost 80 t ha^{-1}), CONT(control).

Table 3
Correlation coefficients, (*, **, *** Indicate significance at the 5, 1, and 0.1% level, respectively. Cbio = biomass C; DH = dehydrogenase; Cat = catalase, Ure = Urease, Prot = protease, Phos = phosphatase, β-Gl = β-glucosidase)

	Cbio	DH	Cat	Ure	Prot	Phos	β-Gl
Accumulative plots							
Cbio		0.406*	0.473*	−0.082	0.073	−0.257	0.420
DH			0.680***	−0.501*	0.600*	−0.791***	0.882***
Cat				−0.596**	0.308	−0.655**	0.654**
Ure					−0.253	0.720***	−0.208
Prot						−0.510*	0.557*
Phos							−0.685***
β-Gl							
Residual plots							
Cbio		0.521*	−0.043	−0.250	−0.255	−0.560**	−0.0584
DH			0.358	−0.223	−0.162	−0.720***	0.531*
Cat				−0.225	0.086	−0.301	0.350
Ure					0.214	0.490*	−0.188
Prot						0.323	0.225
Phos							−0.324
β-Gl							

of *p*-nitrophenol (PNP). Two ml of 0.1 M maleate buffer (pH 6.5 for both phosphatase and β-glucosidase activities) and 0.5 ml of substrate were added to 0.5 g of sample and incubated at 37°C for 90 min. The reaction was stopped by cooling rapidly to 2°C for 15 min; 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were then added and the mixture centrifuged at 2000*g* for 5 min. To stop the β-glucosidase assay, trishydroxymethyl aminomethane (THAM) was used according to Tabatabai (1982). The amount of PNP was determined using a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

The same procedure as for the enzyme assay was followed for the controls but the substrate was added to the soil after incubation and immediately prior to stopping the reaction.

2.5. Statistical analysis

All data was subjected to an analysis of variance using the least significant difference test and comparing the differences between specific treatments. The correlation matrix between biological and biochemical parameters was made with the Statgraphics Plus 2.0.

3. Results

3.1. Soil microbial biomass

The highest contents of microbial biomass C were observed in the organic treatments of accumulative plots, particularly in the C80, followed by the MA and C20 treatments (Table 2). The C80 treatment with the highest organic C (2.58%) had the lowest proportion (1.2%) of organic C in the microbial biomass. The other treatments had intermediate proportions (2–3.5%). In the residual plots, and nine years from the first application of all treatments, the highest rate of compost had the greatest biomass C.

3.2. Soil enzyme activities

Enzyme activities varied widely among the treatments studied. The treatment in which the highest activity occurred was found to vary depending on the enzyme (Fig. 1). Basically, enzymes involved in intracellular microbial metabolism, such as dehydrogenase and catalase, increased with the organic amendments. The highest values for dehydrogenase, protease and β-glucosidase activities were found in the MA, followed by the MSW treatments in plots that received accumulative additions of these amendments. Catalase activity increased with the highest rate of compost, but urease and phosphatase activities were significantly inhibited ($P \leq 0.05$) in the organic amended soils.

A correlation matrix (Table 3) shows some significant relationships between the enzyme activities studied in the residual and accumulative plots. There was a strong positive correlation in the accumulative plots between oxidoreductase enzymes and β-glucosidase activity, which are involved in soil C mineralization. Urease showed a positive correlation with phosphatase, and both enzymes had a negative correlation with dehydrogenase and catalase activities. In the residual plots, phosphatase maintained a negative correlation with dehydrogenase.

4. Discussion

4.1. Microbial biomass

The increase of microbial biomass in this long-term experiment with the organic amendments is mainly due to the microbial biomass contained in the organic residues and

Table 4
Heavy metals content in soils under different treatment (mg kg^{-1})

	Accumulative						Residual					
	Zn	Cu	Ni	Cd	Cr	Pb	Zn	Cu	Ni	Cd	Cr	Pb
Manure	25	6	7	0.2	3	3	14	5	4	0.2	3	4
Mineral fertilization	23	6	5	0.2	7	3	13	5	4	0.2	3	4
Compost 20 t ha^{-1}	67	22	8	0.2	6	31	26	8	5	0.2	3	5
Compost 80 t ha^{-1}	159	61	13	0.2	12	116	45	18	6	0.2	5	6
Control	18	6	4	0.2	4	3	18	6	4	0.2	3	4

the addition of substrate-C, which stimulates the indigenous soil microbiota, as confirmed by prior analyses. Other authors have reported a similar dual influence on biomass (Perucci, 1993; Diaz et al., 1994; García et al., 1998).

Microbial biomass is a much more sensitive indicator of changing soil conditions than is the total organic matter content. There is accumulating evidence that heavy metals decrease the proportion of microbial biomass C in total soil organic matter (Brookes and McGrath, 1984) and the ratio of soil microbial C to soil organic C has been proposed as a useful measure of soil pollution by heavy metals (Brookes, 1995) and a reduction in this ratio as a result of metal pollution has been reported from other studies (Chander and Brookes, 1991b; Fliessbach and Reber, 1992). In semiarid conditions, soil biomass is subject to seasonal variations and has an influence on this ratio. Our data show that the highest rate of MSW compost had the lowest ratio of biomass C to soil C (Table 2), indicating a low biomass C content in comparison with the organic C in soil. Heavy metals content in soil under different treatments (Table 4) showed an increase in Zn, Cu and Pb with compost addition. This low ratio could be attributed to heavy metal that had been added with the amendment in the high rate, but also to a high condensation and humification of organic matter that is resistant to microbial attack (Tate, 1987). This may account for the results, particularly the low microbial biomass content in the soils amended with MSW, compared to the MA treatment, which is a labile source of organic C for soil biota.

4.2. Correlation among enzyme activities

Dehydrogenase and catalase are intracellular enzymes that are involved in microbial oxidoreductase metabolism. The activity of such enzymes basically depends on the metabolic state of the soil biota. Dehydrogenase activity was significantly correlated with soil biomass C in both accumulative and residual plots ($P < 0.05$). This means that dehydrogenase activity could be a good indicator of soil microbial activity in semiarid areas (García et al., 1994b). A significant increase in dehydrogenase activity occurred in the accumulative plots with organic treatments, especially with MA. Both rates of MSW had smaller activities than MA, presumably due to the humified organic matter added with compost, which is more resistant to microbial mineralization. Other authors have reported that dehydrogenase activity was inhibited by the toxic effect of heavy metals added with an organic amendment, particularly Pb (Marzadori et al., 1996) and Cu (Chander and Brookes, 1991a), but the levels of these heavy metals in soil (Table 4) were much lower than those reported by previous investigations and, in any case, the C80 treatment had a greater dehydrogenase activity than C20. Consequently, this enzyme was not affected by the heavy metal concentrations reached in soil with the highest rate of compost. In the residual plots, there was an increase in dehydrogenase activity following C80 treatment for nine years, but the other treatments showed no significant differences.

Catalase is an oxidoreductase associated with aerobic microbial activity (Rodríguez-Kábana and Truelove, 1982) and its activity was significantly and positively correlated with dehydrogenase ($P < 0.001$), microbial biomass ($P < 0.05$) and β-glucosidase ($P < 0.01$) in the accumulative plots. Residual plots showed no significant differences between treatments. Catalase activity was higher in the organic treatments being stimulated the synthesis of this enzyme by the addition of organic residues. This result may be explained by the improved soil aeration in the organic amended soils as a consequence of an increase in soil porosity (Giusquiani et al., 1995).

Urease and protease hydrolyse nitrogen compounds to ammonium, using urea and low molecular weight protein substrates, respectively. Urease activity was negatively correlated with dehydrogenase ($P < 0.05$) and catalase ($P < 0.01$). Mineral fertilization plots had the greatest urease activity, while MSW compost treatments inhibited this activity. The inhibition of urease may be due to the higher concentration of heavy metals (Tabatabai, 1977) in MSW compost, to the constituents of organic matter (Bremner and Douglas, 1971), or because there was a high concentration of metabolites such as NH_4^+ (Konig et al., 1966) as a consequence of the mineralization process in soil. The rates of protease activity were higher with the organic amendments than in the other treatments, being correlated with dehydrogenase activity ($P < 0.05$) and remaining active probably because of their substrates were added with the organic wastes and root exudates after cropping. Other authors (Ladd, 1978; Burns, 1982; Nannipieri, 1994) suggested that strong bonding of enzymes to soil colloids may protect the enzyme from denaturation.

Changes in soil phosphatase activities, which play an essential role in the mineralization of organic phosphorus, were also observed between treatments. Both phosphatase and urease activities were significantly correlated in the accumulative plots ($P < 0.001$). Phosphatase was inhibited in organic amended soils while the mineral fertilization and the control treatments exhibited an increase in this enzyme activity. In the residual plots, phosphatase increased in all treatments except for the highest rate of compost. Generally, this enzyme is activated when there is low P availability in soils. Phosphatase can be inhibited not only by heavy metals such as Cu and Zn (Tyler, 1974) that are added with MSW compost to the soil, but also by inorganic phosphate, which produces a feedback inhibition of this enzyme (Nannipieri et al., 1979). This may explain why available P can be found in these soils amended with organic residues.

The additions of organic residues increased β-glucosidase activity in soil. This activity was greater in MA than in MSW treatments, due to the labile C continued in the fresh organic matter. There was a positive correlation between β-glucosidase and oxidoreductase enzymes ($P < 0.01$) in the accumulative plots. Consequently, mineralization of organic matter may provide to substrates for β-glucosidase (García et al., 1998) and microbial growth and therefore dehydrogenase and catalase activities are enhanced due to this process.

Overall, our results have shown that the addition of organic residues to an agricultural soil has a variety of effects on microbial biomass C and enzyme activities. These two important soil properties respond to soil perturbation or restoration over a relatively short time. Soils amended with MSW compost can improve soil quality, increasing the organic matter content of degraded soils and improving soil biological and biochemical properties. However, the wide variety of substances such as heavy metals and other potential pollutants in municipal solid wastes limits the use of these residues in compost. Consequently, there must be a quality control of these organic amendments in order to minimise the risk of inhibiting essential biogeochemical processes as well as contributing to environmental pollution.

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