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Soil enzymatic response to addition of municipal solid-waste compost

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Abstract Modifications of soil microbiological activity by the addition of municipal solid-waste compost were studied in laboratory incubations. Three composts were compared, one lumbricompost and two classical composts with different maturation times. Organic C mineralization and nine enzyme activities (dehydrogenase, peroxidase, cellulase, β-glucosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, protease, amidase, and urease) were determined in the composts and the amended soil. Initial enzyme activities varied in the soil according to the sampling date (winter or summer) and were greater in the composts than in the soil, except for urease. Generally, the youngest compost exhibited greater activity than the oldest one. In the amended soil, the composts did not increase enzyme activity in an additive way. Dehydrogenase, the only strictly endocellular enzyme, was the only one for which the activity in the amended soil increased significantly in proportion to the addition of compost. During the incubations, C mineralization and dehydrogenase activity were significantly correlated, indicating that dehydrogenase was a reliable indicator of global microbial activity. Peroxidase activity in the soil remained constant, but increased in the composts and amended soil. Addition of the oldest compost had no effect on the activity of the C cycle enzymes, but the youngest compost increased soil activity at the higher application rate. Enzymes of the N cycle were stimulated by all compost amendments, but the increase was only transient for amidase and urease. Lumbricomposting had no marked effect on compost enzyme activity, either before or during the incubation.

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Introduction

Composting of municipal solid wastes is not only an alternative to landfilling or incineration, but also provides a useful organic amendment that can improve soil properties such as structure and nutrient status (Gallardo-Lara and Nogales 1987). Nevertheless, adverse effects of immature municipal solid-waste composts on crop yield have been reported. These have most frequently been attributed to the immobilization of soil N in the microbial biomass, to a decreasing O_2 concentration, or to the production of phytotoxic compounds including NH_3 and organic acids. Consequently, numerous studies have been carried out to assess compost maturity (Jimenez and Garcia 1989). Both physical parameters (temperature, odor, color) and chemical parameters (composition of the humified organic matter, Giusquiani et al. 1989; organic C to organic N ratio, Hirai et al. 1983; cation exchange capacity, Harada and Inoko 1980) have been proposed to characterize compost maturity. Biological methods, including a germination index of seeds in compost or compost extracts, have also been used (Zucconi et al. 1981). Microbial criteria include microbial counts (Kaiser 1983), respiration rates (Morel et al. 1986), ATP content (Garcia et al. 1992, 1993b), the organic C mineralization rate (Forster et al. 1993), microbial biomass measurements, and enzyme assays.

Enzyme activities in municipal solid wastes during composting have been studied by Garcia et al. (1992). They have also been measured to monitor soil microbial activity and fertility (Frankenberger and Dick 1983; Beyer et al. 1993). Thus enzyme activity can be used to monitor the effects of adding municipal solid-waste compost on soil microbial activity. Perucci (1990) showed that the addition of 2.5% compost increased some soil enzyme activities permanently during a 1-year incubation. Increases

in soil enzyme activities were also observed after the addition of compost in field experiments (Perucci 1992). The duration of the increase was related to the compost application rate; it was either transient or lasted until the next compost application.

Some studies have shown that undesirable materials, in particular heavy metals, affect enzyme activity in these composts (Garcia et al. 1992) or in soils amended with the composts (Perucci 1992). Home-sorting of municipal solid waste makes it possible to compost only the organic fraction of the waste and thus to avoid the negative effects of any heavy metals (Beyea et al. 1993).

In the work presented here, measurements of enzyme activity and organic C mineralization were used to study the effects of compost amendment on microbiological activity in a loamy soil. The composts were made from home-sorted municipal solid waste. The impacts of compost age and lumbricomposting were also investigated.

Materials and methods

Soil and composts

The soil (typic Eutrochrept) was sampled in the surface layer (0–20 cm) of a bare experimental plot at Grignon (France), in July 1992 (S1) and in February 1993 (S2). Its main characteristics were 22% clay, 73% loam, 25% water-holding capacity, a pH of 7.3, 1.08% organic C, 0.13% organic N, and a C:N ratio of 8.3. The composts used were formed from the organic fraction on the municipal solid household wastes of Bapaume (France) collected in summer, using windrow fermentation with complementary maturation. The waste material was collected in July 1991 and 1992; the fermentation period lasted 6 (C1 compost) and 10 (C2 compost) weeks, and was followed by 7 and 3 months of maturation, respectively. After fermentation, part of the younger C2 compost was lumbricomposted for 2 months with *Eiseinia andrei* Bouché, and was then subjected to 1 month of windrow maturation without earthworms (LC2 compost). The main characteristics of the three composts are summarized in Table 1.

Incubations experiments

Fresh soils (50 g), called "soil alone", were incubated in the dark, at 28±1 °C, in sealed 500-ml jars. Similar incubations were run with soil-compost mixtures containing 10 and 30% of compost (weight/weight) and with 25 g compost was added to 25 g inert sand to provide good aeration. The older compost was mixed with soil S1 and the two younger composts (C2 and LC2) with soil S2. The water content of each sample was adjusted to 95% water-holding capacity. CO_2 evolved was trapped in 10 ml 0.5 *N* NaOH. Three replicate bottles were opened for analysis after 0, 5, 14, 21, 35, 56, 70, 91, 119, 147, and 189 days of incubation for the older compost and 0, 7, 14, 28, 43, 56, 84, 114, 141, and 189 days for the two younger composts. The CO_2 traps were replaced in all the jars on each sampling dates. The CO_2 (trapped in NaOH) was measured by colorimetry (Chaussod et al. 1986) with a continuous flow-analyzer (Skalar, The Netherlands). The C mineralization rate between each sampling date was calculated as mg CO_2-C kg^{-1} dry substrate day^{-1}, substrate being either soil alone, soil plus compost (10 and 30% mixtures), or compost alone (compost without soil).

Table 1 Main characteristics of compost (results are expressed on a dry weight basis)

	C1 (7 months' maturation)	C2 (3 months' maturation)	LC2 (lumbricompost
pH (H_2O)	8.5	8.6	8.7
Organic C (%)	16.87	13.85	13.72
Organic N (%)	1.34	1.43	1.45
C:N ratio	11.3	9.7	9.5
Water-holding capacity (%)	119	113	122

Enzyme assays

Nine enzyme activities were followed during the incubations. Table 2 summarizes the assay procedures. All the methods listed were slightly modified. Dehydrogenase and peroxidase activities were measured on each sampling date, and the other enzymes were measured on days 0, 56, and 189 (soil+oldest compost) or days 0, 84, and 189 (soil+other composts). Enzyme activity was expressed in activity units (AU) per gram dry substrate. Relative enzyme activity was calculated as enzyme activity units per gram organic C in dry substrate.

Dehydrogenase

A sample (3 g) was incubated in 3 ml water and 3 ml 3% 2,3,5-triphenyl-tetrazolium chloride (TTC) at 37 °C for 24 h in darkness. Then 10 ml methanol was added. The suspension was homogenized and filtered through a glass fiber filter, and the filter was washed with methanol until the reddish colour caused by the reduced TTC (triphenyl formazan) disappeared, and the volume adjusted to 100 ml. The optical density at 485 nm was compared to those of triphenyl formazan standards.

Peroxidase

A sample (9 g) was agitated in 25 ml 0.2 *M* phosphate buffer (pH 6.0) for 5 min, then centrifuged at 8000 g for 10 min; the supernatant was filtered through 0.22-μm Durapore filters (Millipore) for sterilization. Then 0.3 ml 0.06% H_2O_2 in 0.05 *M* phosphate buffer (pH 6.0) and 0.05 ml 0.5% *o*-dianisidine in methanol were added to 2.7 ml supernatant in a spectrophotometric cuvette. The increase in optical density at 460 nm was measured every 30 s for 5 min. Reference data were obtained from purified horseradish peroxidase solutions (Sigma).

Cellulase

A sample (3 g) was incubated at 30 °C for 24 h in 10 ml 0.2 *M* acetate buffer (pH 5.9) and 10 ml 1% carboxymethyl cellulose in the acetate buffer, on a rotary shaker. After centrifugation at 17000 *g* for 10 min, the sugar content of the supernatant was determined by the colorimetric anthrone method.

Protease

A sample (3 g) was suspended in 10.8 ml 0.1 *M* Tris-HCl buffer and 12 ml 2 m*M* benzyloxycarbonyl-phenylalanyl-leucine in buffer. After 1 h of agitated incubation at 40 °C, sample was cooled to 20 °C, and enzyme activity was stopped by adding 1.2 ml 5 *N* HCl. After centrifugation at 8000 *g* for 10 min, 0.8 ml of the supernatant was

Table 2 Methods used for enzyme assays

Enzymes	Substrate	Incubation conditions				Product assay	
		Buffer	Temperature	Time	Reference	Product	Method[a]
Dehydrogenase	2,3,5-Triphenyltetrazolium chloride (TTC)	–	37 °C	24 h	Tabatabai (1982)	Triphenyl formazan (TPF)	A_{485}
Peroxidase	Hydrogen peroxide[b]	0.2 *M* phosphate buffer (pH 6.0)[c]	20°C	5 min	Bartha and Bordeleau (1969)	Oxidized *o*-Dianisidine	A_{460}
Cellulase	Carboxymethyl-cellulose	0.2 *M* acetate buffer (pH 5.9)	30 °C	24 h	Pancholy and Rice (1973)	Reducing sugars	Anthrone, Brink et al. (1960)
β-Glucosidase	*p*-Nitrophenyl-β-glucopyranoside	0.1 *M* acetate buffer (pH 5.0)	37 °C	1 h	Martens et al. (1992)	*p*-Nitrophenol	A_{410}
β-Galactosidase	*p*-Nitrophenyl-β-galactopyranoside	0.1 *M* acetate buffter (5.0)	37 °C	1 h	Martens et al. (1992)	*p*-Nitrophenol	A_{410}
N-acetyl-β-glucosaminidase	*p*-Nitrophenyl-*N*-acetyl-β-D-glucosaminide	0.1 *M* acetate buffer (pH 5.0)	37 °C	1 h	Martens et al. (1992)	*p*-Nitrophenol	A_{410}
Protease	Benzyloxycarbonyl-phenylalanyl-leucine	0.1 *M* Tris-HCl buffer (pH 8.1)	40 °C	1 h	Ladd and Butler (1972)	Amino acids	Ninhydrine, Moore and Stein (1954)
Amidase	Formamide	0.1 *M* Tris-H_2SO_4 (pH 8.5)	37 °C	2 h	Frankenberger and Tabatabai (1980)	Ammonium	Indophenol blue, Charlot (1961)
Urease	Urea	0.1 *M* Tris-H_2SO_4 (pH 8.5)	37 °C	2 h	Frankenberger and Tabatabai (1980)	Ammonium	Indophenol blue, Charlot (1961)

[a] Spectrophotometric measures by a lambda 5 Perkin-Elmer spectrophotometer
[b] Plus *o*-dianisidine as electron donnor
[c] Extraction buffer

neutralized with 0.2 ml 1 *N* NaOH and the amino acid contents determined with the ninhydrin reagent.

Amidase and urease

A sample (2 g) was suspended in 6 ml Tris-H_2SO_4 buffer (pH 8.5) and 3 ml 0.1 *M* formamide or urea, and incubated at 37 °C for 2 h. The suspension was then filtered through Whatman no. 41 paper and the NH_4^+ released was measured by colorimetry.

N-acetyl-β-glucosaminidase, β-glucosidase and β-galactosidase

A sample (3 g) was suspended in 8 ml 0.1 *M* acetate buffer (pH 5.0) and 2 ml *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (for *N*-acetyl-β-glucosaminidase activity), *p*-nitropenyl-β-glucopyranoside (for β-glucosidase activity) or *p*-nitrophenyl-β-galactopyranoside (for β-galactosidase activity). After 1 h of incubation at 37 °C, 2 ml 0.5 *M* $CaCl_2$ and 8 ml 0.5 *M* NaOH were added, and the suspension was filtered through a glass fiber filter. The *p*-nitrophenol released was measured at 410 nm.

Results and discussion

No bacteriostatic agent such as toluene was added during the enzyme assays. Toluene is often used to prevent the synthesis of enzymes and assimilation of the reactant products by living cells during enzyme analysis, but it is known to induce plasmolysis and to inhibit some enzyme activities (Tabatabai 1982). No bacteriostatic agent was necessary in the peroxidase assay as performed after extraction in a cell-free extract. For β-glucosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, protease, amidase, and urease, as the incubation time did not exceed 2 h, cell proliferation could be considered negligible and toluene was also unnecessary (Ladd and Butler 1972). Because bacteriostatic compounds inhibit dehydrogenase activity, toluene is never included in dehydrogenase assays (Tabatabai 1982). The cellulase assay lasted 24 h. Toluene was not added to this assay either, to avoid possible negative effects and to provide the same assay conditions as for the other enzymes.

Initial enzyme activities

Enzyme activities vary widely in different soils. In the two soils of the present study (Table 3), the enzyme activities measured were comparable to those reported by Ladd and Butler (1972) for protease, but differed from those found by Martens et al. (1992) for dehydrogenase, β-glucosidase, amidase, and urease. In soil, all enzymes were more active in February (S2) than in July (S1), except for protease. Variations in soil enzyme activity during the year have of-

Table 3 Enzyme activity (activity units g^{-1} dry substrate) soil and compost before incubation. Values in *parentheses* are expressed as activity units g^{-1} organic C (*TPF* triphenyl formazan, *PNP p*-nitrophenol)

Enzymes	S1 (soil sampled in July)	S2 (soil sampled in February)	C1 (7 months' matured compost)	C2 (3 months' matured compost)	LC2 (lumbricompost)
Dehydrogenase	100	72	1520	2315	2101
(μg TPF $24\,h^{-1}$)	(9292)	(6710)	(9667)	(16716)	(15314)
Peroxidase	0.4	0.9	14.4	12.2	19.3
(nmol H_2O_2 $30\,s^{-1}$)	(37)	(83)	(85)	(88)	(141)
Cellulase	6.7	14.4	5.4	47.3	46.7
(mg ~ glucose $24\,h^{-1}$)	(617)	(1337)	(32)	(342)	(340)
β-Glucosidase	9.7	16.8	29.3	111.6	109.5
(mg PNP h^{-1})	(893)	(1554)	(174)	(806)	(798)
β-Galactosidase	6.8	7.0	40.3	76.3	70.5
(mg PNP h^{-1})	(631)	(650)	(239)	(551)	(514)
N-acetyl-β-glucosaminidase	4.8	14.7	63.3	134.2	153.6
(mg PNP h^{-1})	(446)	(1359)	(375)	(969)	(1119)
Protease	4.0	2.1	14.0	8.3	5.6
(μmol ~ leucine $2\,h^{-1}$)	(373)	(196)	(83)	(60)	(41)
Amidase	0.31	1.08	0.35	3.37	3.68
(mg NH_4^+ h^{-1})	(28.9)	(92.7)	(2.1)	(24.3)	(26.8)
Urease	0.44	0.38	0.48	0.56	0.30
(mg NH_4^+ h^{-1})	(41.2)	(35.2)	(3.6)	(3.4)	(1.5)

ten been reported in field studies (Martens et al. 1992; Beyer et al. 1993). The activity is usually higher in summer than in winter. Nevertheless, smaller measures in summer than in winter have been reported (Martens et al. 1992; Pancholy and Rice 1973). Most enzyme activity decreased by about 50%. The largest variation was found in amidase activity, which decreased by 71%. β-Galactosidase and urease activity remained constant and protease activity was doubled.

Except for urease, which showed no difference between the soil and the composts, the activity of all enzymes, expressed per gram of dry substrate, was higher in the composts than in the soil (Table 3). Higher activity in organic amendments than in soils has been reported previously (Martens et al. 1992). In the present study the levels of enzyme activity in compost were within the range of those found by other authors, although the urease activity in all three composts and β-glucosidase in the two younger composts were 3- to 10-fold higher than those of a mature compost studied by Garcia et al. (1992).

Many enzyme activities are correlated with total organic C in soil (Frankenberger and Tabatabai 1981; Frankenberger and Dick 1983) and composts (Garcia et al. 1993a). If enzyme activity depended only on the organic matter content, the relative activity, expressed per gram of organic C, would be similar for different substrates and higher values would indicate organic substrate with a high level of enzyme activity. In fact, the present results showed that organic matter was not the only interacting parameter (Table 3), and three groups of enzymes were distinguished. The range of peroxidase, amidase and *N*-acetyl-β-glucosaminidase activity was similar in both soil and compost. Cellulase, β-glucosidase, β-galactosidase, protease, and urease activity was lower in compost than in soil. In contrast, compost dehydrogenase activity was higher than in soil. Moreover, relative enzyme activity appeared to be lower in compost than in soil, except for dehydrogenase. This enzyme is involved in the respiratory chain of microorganisms, and has often been used as a parameter to evaluate the overall microbial activity of soil (Nannipieri et al. 1990) and of composts (Forster et al. 1993). Even if the relative activity of the individually assayed enzymes is lower in compost than in soil, the overall microbiological activity, as indicated by dehydrogenase, is greater in compost.

Protease was the only enzyme with lower activity in the youngest compared to the oldest compost. Peroxidase and urease activities did not differ significantly between these two composts. The other six enzymes exhibited higher activity in the youngest compost, up to 10-fold higher for cellulase and amidase. The microbiological activity, as determined by dehydrogenase activity, was lower in the oldest than the youngest compost because of the difference in compost maturity (Garcia et al. 1992). This is in accord with Forster et al. (1993), who proposed dehydrogenase activity as an index of compost maturity.

The two younger composts, which were prepared from the same household refuse, presented no significantly different enzyme activities, indicating that the lumbricomposting process did not modify the enzyme activity. Nevertheless, earthworm faeces have displayed higher levels of enzyme activity than uningested municipal waste compost (Businelli et al. 1984). However, this increased activity, related to microbial activation during gut transit, is often temporary and decreases rapidly in ageing faeces (Martin and Marinissen 1993). Thus, as this compost had undergone 1 month of maturation after lumbricomposting, there were no longer any differences in enzyme activity between the lumbricompost and the compost prepared from the same refuse without earthworms.

Table 4 Enzyme activity losses in soil-compost mixtures before incubation compared to the theoretical additive activity (soil proportion·activity of soil alone + compost proportion·activity of compost alone); activity loss = [(theoretical additive activity − measured activity)/theoretical additive activity]·100. *ND* not determined

	Compost proportions in the soil-compost mixtures					
	C1 (7 months' matured compost)		C2 (3 months' matured compost)		LC2 (lumbricompost)	
	10%	30%	10%	30%	10%	30%
Dehydrogenase	22.8	35.9	26.3	15.5	33.5	23.4
Peroxidase	65.8	79.5	20.4	35.6	29.4	45.5
Cellulase	ND	ND	24.9	35.3	14.9	30.8
β-Glucosidase	7.1	11.5	42.3	65.9	50.4	31.0
β-Galactosidase	69.7	−77.1	53.5	99.7	30.2	23.3
N-acetyl-β-glucosaminidase	6.1	−20.8	64.8	63.3	42.2	48.1
Protease	ND	ND	39.1	19.7	23.9	20.6
Amidase	ND	ND	20.5	11.1	25.4	46.2
Urease	ND	ND	27.1	−6.0	22.0	74.7

In nearly all the soil-compost mixtures, there was less enzyme activity than expected from the sum of soil and compost activity (Table 4). The most important loss in activity, compared to the additive calculation, was found for the three enzymes β-glucosidase, β-galactosidase, and *N*-acetyl-β-glucosaminidase in the youngest soil + compost mixtures; nevertheless, for these same enzymes, the activity increased, but only slighly, as the proportion of compost increased in the oldest and the lumbricomposted soil mixtures. Dehydrogenase was the only enzyme that showed significantly increased activity in soil-compost mixtures as the proportion of compost increased. No significant modifications were observed for peroxidase, cellulase, protease, amidase, and urease in 10% compost mixtures. Soil protease, amidase, and urease activity increased only with 30% compost. Most often, the soil had a buffer effect on the modification to enzyme activity. However, no general trend in variation was found among the different enzymes and the different compost proportions. Martens et al. (1992) also found that field additions of organic amendments, with an application rate of only 1% however, sometimes increased the enzyme activity, without any additive effect.

Among the enzymes tested, dehydrogenase was the only endocellular enzyme, while the others can be also extracellular. As mentioned, dehydrogenase was the only enzyme to show significantly increased activity with a higher proportion of compost. Therefore, phenomena involving the extracellular fraction of enzymes could explain why enzyme activity in the soil-compost mixtures was not additive. The addition of soil to an enzyme solution has often produced a decrease in enzyme activity (Skujins 1967; Boyd and Mortland 1990). We have also observed this phenomenon by adding soil to horseradish peroxidase solution (data not shown); more than 70% of the initial enzyme activity was lost and about 50% could not be recovered by extraction in a phosphate buffer (0.2 *M*, pH 6.0). Less activity after adsorption on clays has also been reported for cellulase (Alexander 1977), urease (Pink and Allison 1961), protease (Loll and Bollag 1983), and peroxidase (Skujins 1967). Soil enzymes are likely to be adsorbed on clays, soil organic matter, or clay-organic matter complexes (Boyd and Mortland 1990). Usually, enzymes immobilized on soil particles exhibit less activity than free enzymes. However, this residual activity is more persistent because the enzymes are protected against degradation by proteolytic microorganisms (Burns 1982). Thus the adsorption of the soluble fraction of compost enzymes on soil with consequent partial inactivation could explain enzyme activity losses when compost was added to soil. The adsorption must have been very rapid because loss of activity was observed immediately after soil and compost was mixed. A transfer of compost enzymes to soil constituents probably occurred during the incubation period of the enzyme assays. Nevertheless, no relationship was found between incubation time (1−24 h, Table 3) and activity loss. There may also have been indirect effects on enzyme activity. Mixing soil and compost induced immediate changes in pH, salinity, and other parameters that severely affect soil and compost microflora. Microorganisms containing specific enzymes, like ureolytic organisms, may have been destroyed or inhibited, inducing further losses in enzyme activity.

Variations in enzyme activity during incubation

Global microbiological activity

C mineralization rates, calculated from respirometric measurements of the CO_2 evolved between two sampling dates (Fig. 1), and dehydrogenase activity (Fig. 2) varied similarly during the incubations. Similar results were reported from a comparison of dehydrogenase activity with CO_2 production (Frankenberger and Dick 1983). However, many studies have shown no relationship between dehydrogenase activity and respiration parameters (Nannipieri et al. 1990). One explanation for these contradictory results is the lower competitive ability of TTC com-

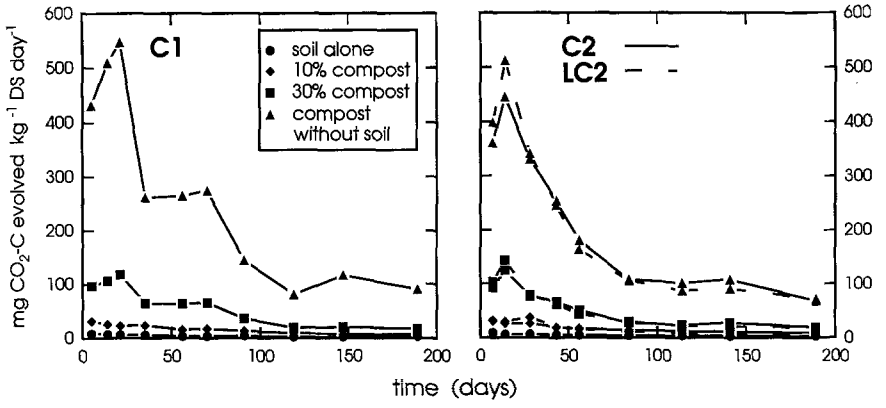

Fig. 1 CO_2 evolution during the incubation of soil-compost mixtures. *DS* dry substrate, *C1* 7 months' matured compost, *C2* 3 months' matured compost, *LC2* lumbricompost

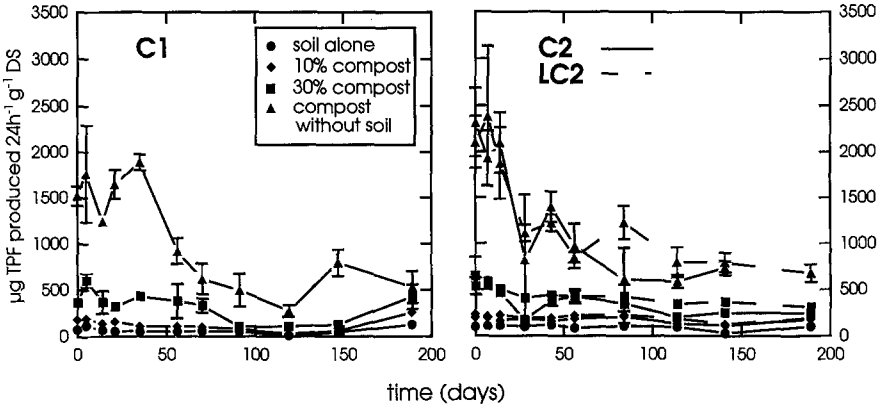

Fig. 2 Dehydrogenase activity during incubation of soil-compost mixtures (*TPF* triphenyl formazan; see Fig. 1 for other explanations)

pared with O_2 in capturing electrons; since electrons removed by dehydrogenases reduce O_2 rather than TTC (Nannipieri et al. 1990). Therefore, any interpretation of dehydrogenase measurements in terms of respiration requires caution. In the present experiment, the correlation coefficient between these parameters was calculated using all sampling dates, the different compost proportions (soil alone, 10 and 30% compost, and compost without soil) and the three types of compost. The significant correlation ($r = 0.92$, $P = 0.01$) we found confirmed that under these experimental conditions, both parameters can be used as microbiological indices.

Three periods were observed in the kinetics of compost mineralization (composts without soil). First, a flush of mineralization (550, 450, and 500 mg CO_2-C kg^{-1} dry substrate day^{-1}, respectively, for the oldest, youngest, and lumbricompost) with maximum dehydrogenase activity (1600, 2200, and 2200 AU for the three composts, respectively). This first period lasted 14–35 days. Then, microbiological activity decreased continually until about day 119 for the oldest compost and day 84 for the two younger composts. Finally, dehydrogenase activity and mineralization rates remained stable until the end of the incubation, representing 30% of the initial enzyme activity and 20% of the mineralization flush in all the composts. At the end of the incubation period, 19.7% of total compost organic C was mineralized in the oldest compost and 20.8% with the two younger composts. These mineralization rates were relatively high for matured composts.

The highest level of dehydrogenase activity coincided with the mineralization flush at the beginning of the incubation and both were related to intense activity of the microflora in degrading easily metabolizable compounds. Thereafter, dehydrogenase activity and microbial respiration decreased as the easily biodegradable substances decreased. Higher dehydrogenase activities at the beginning of the incubation indicated the youth of the two younger composts, since this enzyme activity is related, as mentioned before, to compost maturity. Differences disappeared during the incubation, suggesting that mineralization of the easily metabolizable compounds led to uniform microbial activity. Garcia et al. (1993c) observed that mineralization of different municipal wastes during composting led to homogeneous composition of organic matter, irrespective of the starting material.

Similar results were observed in the soil and the soil-compost incubations. The mineralization rate (Fig. 1) and dehydrogenase activity (Fig. 2) increased with the proportion of compost in the mixtures, during the early weeks of incubation. But after 3 months of incubation, the compost impact on soil dehydrogenase activity and respiration disappeared and no differences remained between the soil and the mixtures with 10 and 30% of compost. This was also observed in a field trial and in incubation

experiments where soil had been amended with compost (Perucci 1990; Perucci 1992); 5 months after application of the compost, dehydrogenase activity was no longer significantly different between the different treatments. We assume that at the end of the soil-compost incubations, the easily available organic matter was exhausted and the soil microbial activity was restored, because homeostatic mechanisms maintain a stable biological composition in the soil (Nannipieri et al. 1983).

Peroxidase activity

Soil peroxidase activity remained constant at between 1 and 2 AU during incubation (Fig. 3). In contrast, in the incubation of compost (without soil), it regularly increased to 77 AU in the oldest compost and 40 AU in the two younger composts. These different results were probably related to compost age.

Peroxidases oxidize phenols, aromatic amines, and various other compounds in the presence of H_2O_2. During the reaction, one electron is removed from subunits of phenolic polymers like lignin, with production of phenoxy radicals, which are then involved in oxidative coupling reactions, resulting in polymerization or in ring hydroxylation followed by ring-fission. This step is necessary for phenolic C to be used as a substrate by the microorganisms. The increase in peroxidase activity during compost incubation could indicate the development of lignolytic microorganisms. The decrease in easily degradable substances during incubation of the compost could have promoted the development of a microflora able to degrade less easily metabolizable constituents, such as lignin. A larger induction of peroxidase in the oldest compost during incubation is likely to have been the result of a higher residual percentage of lignin than in the two younger composts, in which the proportion of easily metabolizable compounds is likely to have been larger. Additional analysis is required to characterize the compost organic matter.

Soil peroxidase activity was significantly increased by the addition of 10 and 30% of compost. However, no additivity was observed. This activity grew during incubation, similarly to that in the compost, but remained much lower. Maxima of 0.6 and 1.8 AU for 10 and 30% of the oldest compost and 2.3 and 5.0 AU for 10% and 30% of the youngest were reached. This indicates that an input of compost to soil stimulated the lignolytic population.

Activity of C cycle enzymes

The other enzymes were monitored less often. Only three measures were taken, (1) at the beginning of the incubation, corresponding to the period of increasing activity, defined previously, (2) during the decreasing phase, and (3) at the end of incubation, representing the final equilibrium.

Cellulases catalyse the conversion of insoluble cellulose into water-soluble mono- or disaccharides and consist of three distinct classes of hydrolytic enzymes, including β-glucosidase which, at the end of cellulose degradation, hydrolyses cellobiose to glucose (Alexander 1977).

Cellulase activity remained much higher in compost (without soil) than in soil throughout the incubations, increasing by up to 3.7-fold (Fig. 4). In both soils and the three composts, cellulase activity increased during incubation, with an intermediate maximum for soil S2 and the two younger composts. The activity was more important in S2 (maximum of 29.2 AU), which was sampled in February, than in S1, which was sampled in July (maximum of 12.4 AU). In the same way, cellulase activity was more important in the two younger composts (up to 100 AU) than with the oldest one (up to 35 AU).

β-Glucosidase activity followed a similar pattern to cellulase. It increased in the soils and in the oldest compost during incubation, reaching 25.4 and 28.2 AU in S1 and S2, respectively, and 62 AU in the oldest compost. In the two younger composts (without soil), β-glucosidase activity remained nearly constant, at around 100 AU. In these composts, both enzymes had a high level of activity compared to the oldest compost and the soils. Therefore, cellulase and β-glucosidase appeared to be closely linked, probably because β-glucosidase is included in the cellulase system.

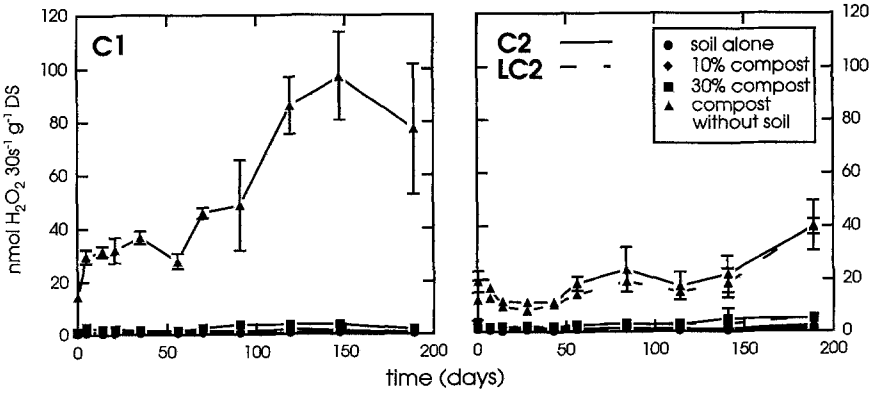

Fig. 3 Peroxidase activity during incubation of soil-compost mixtures (see Fig. 1 for further explanations)

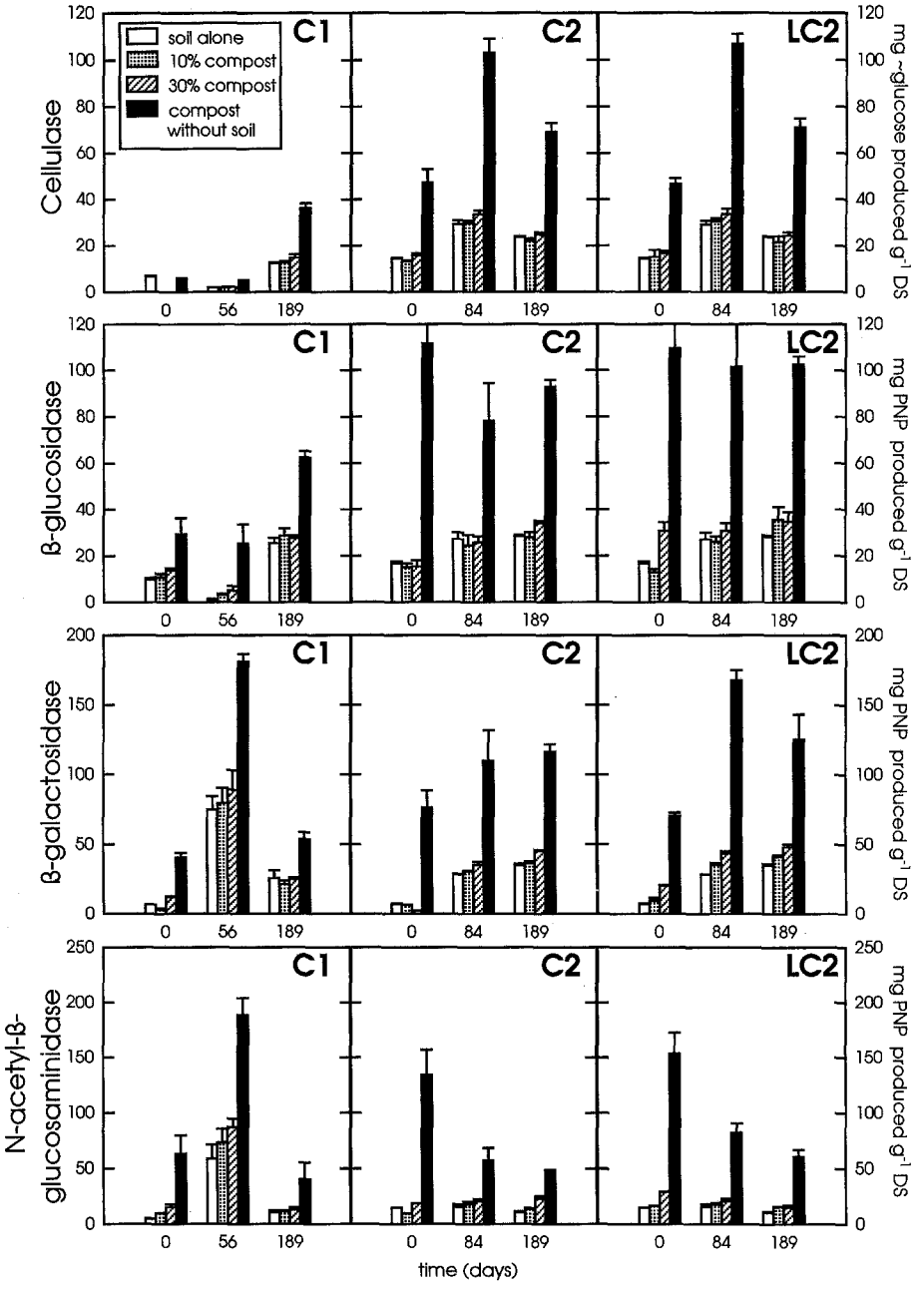

Fig. 4 Activity of enzymes involved in C cycle during incubation of soil-compost mixtures (*PNP p*-nitrophenol; see Fig. 1 for further explanations)

The cellulase system can be induced in many microorganisms and can be synthesized in the presence of cellulose or other carbohydrates (Alexander 1977). The increase in cellulase activity in the present study may have been related to the release of cellulose from lignocellulolyse, after degradation of lignin promoted by the increase in peroxidase activity. In this way, Crawford et al. (1993) found that increased soil peroxidase activity increased the development of the cellulolytic population.

β-Galactosidase activity also increased during all incubations. With the February soil (S1), the maximum (74.8 AU) was reached after 56 days, and then the activity decreased. With S2 (July), the activity increased until day 189 (34.8 AU). Again, the enzyme was more active in compost (without soil) than in soil. In the oldest compost, β-galactosidase activity followed the same kinetics as in S1, but with higher maximum values (181.0 AU). Activity in the two younger composts increased 116.5 (C2 compost) and 167.5 (lumbricompost) AU.

N-acetyl-β-glucosaminidase activity remained fairly constant during S2 incubation (10–15 AU). With S1, a maximum was observed after 56 days (58.8 AU). The activity decreased by about 60% during incubation of the two younger composts (without soil). A maximum was observed after 56 days in the oldest compost (188.5 AU), and then the activity decreased below the value found at the start of the incubations.

Addition of the oldest compost to soil (10 or 30%) had no effect on cellulase, β-glucosidase, β-galactosidase and *N*-acetyl-β-glucosaminidase activity. The two younger composts did not modify the soil cellulase activity, but after 6 months of incubation, the youngest compost (C2

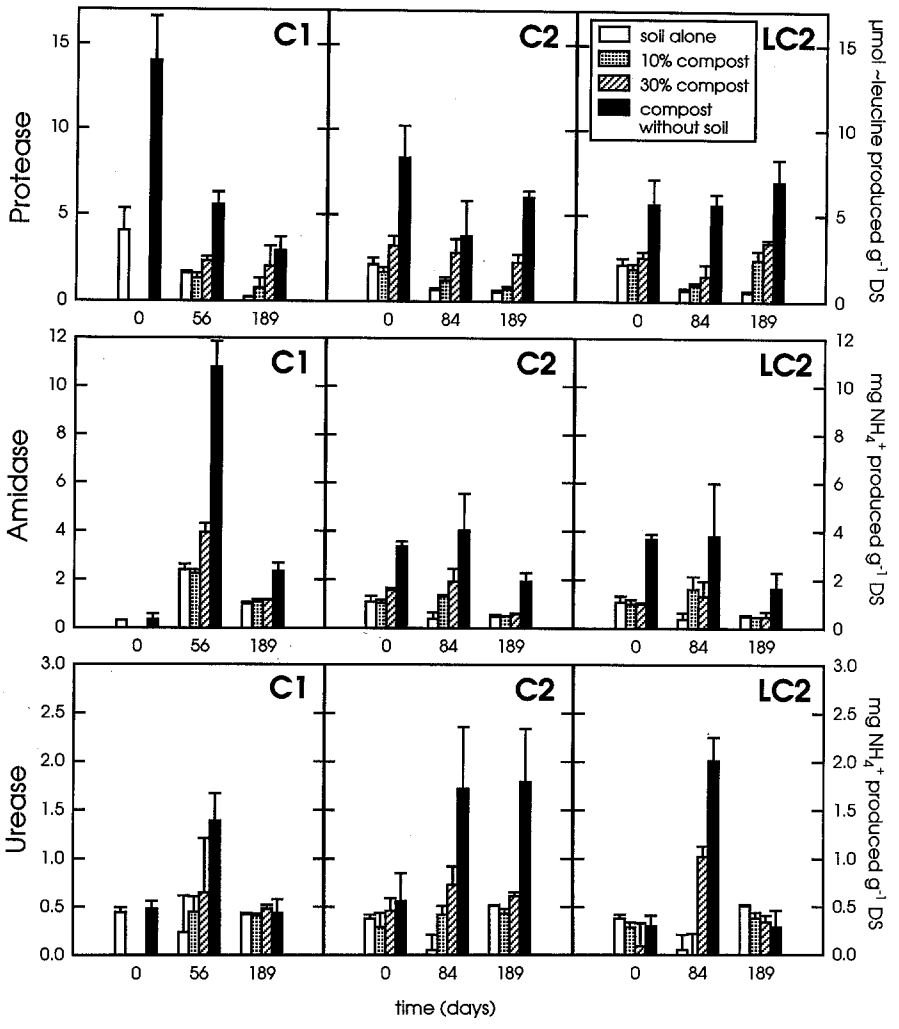

Fig. 5 Activity of enzymes involved in N cycle during incubation of soil-compost mixtures (see Fig. 1 for further explanations)

compost) consistently produced a slight but significant increase in β-glucosidase, β-galactosidase, and *N*-acetyl-β-glucosaminidase soil activity with a 30% addition, and sometimes with only 10%. Again, the differences are likely to be a direct result of age; the older compost was biologically less active and therefore unable to produce a lasting effect on soil enzyme activity.

Activity of N cycle enzymes

Protease activity (Fig. 5) decreased during soil incubation. As previously, the composts (without soil) displayed more activity than the soils. Protease activity decreased strongly in the oldest compost during incubation, but remained fairly constant in the two younger composts. Amidase increased in S1 but decreased in S2. Urease activity in both soils fell to a minimum during the incubations but then rose to similar values as those found at the start. Amidase and urease in the composts followed an approximately similar pattern, with a maximum during the incubations; this maximum was marked for amidase in the oldest compost. Both enzymes are involved in the ultimate stage of N compound degradation by hydrolysing C-N bonds (other than peptide bonds) of amides and urea. A large portion of these enzymes was probably of microbial origin and came from the same group of microorganisms (Frankenberger and Tabatabai 1981), explaining the similar pattern during incubation. The greater urease activity in the youngest C2 compost compared with the lumbricompost after 189 days was the only important difference observed between these two composts.

Several authors reported a strong correlation between urease activity and the size of the microbial biomass in amended soil (Skujins 1967; Nannipieri et al. 1983; Garcia et al. 1992). However, Frankenberger and Dick (1983) found that urease activity was not significantly correlated with microbial respiration. In the present experiment, compost urease activity was higher in the middle of incubation than at the beginning, whereas microbial activity, estimated by the C mineralization rate and dehydrogenase activity, decreased strongly after a short early period of increase. This may be explained by a decline in microbial activity, related to death of some of the microbial population, which led to an increase in available proteins and stimulated the production of N-cycling enzymes (Nannipieri et al. 1979). Probably, the increase in protease activity, necessary to hydrolyse proteins, was not observed

because it happened earlier than the increase in amidase and urease activity, and was not recorded because of the long periods between measurements.

During the incubations, all N cycle enzymes were stimulated in soil by the compost amendments. But protease was the only enzyme that showed increased activity in the 10 and 30% mixtures at the end of incubation, with all three composts. Therefore, after 6 months of incubation, the composts no longer had any effect on amidase and urease activity whereas they still increased protease activity. Perucci (1990) also found that in a soil amended with 2.5% compost, protease activity was still higher than in the control soil after 6 months of incubation. In contrast, in the same study, urease was quite as active in the amended soil as in the unamended soil, indicating that compost had less effect on urease activity in soil than on protease.

No enzymes activities were related to the microbial activity measurements, except dehydrogenase, which is used to estimate global microbiological activity. The enzyme activities measured, with the exception of dehydrogenase, were all substrate-specific. These individual enzyme measurements gave informations about specific nutrient cycles, but could not reflect the total microbiological status of the soil (Skujins 1978). In addition, all enzymes measured, except dehydrogenase, might also have been extracellular, so that they might have been active in soil or composts, independently of the living microorganisms, in a free or bound form, and not directly related to microbiological activity. The present study has thus confirmed the results reported by Engels et al. (1993) who found, during a 27-month field study, a strong correlation between dehydrogenase and microbial biomass in the soil, but not between extracellular enzymes and the biomass.

In summary, measurements of enzyme activity in soil, compost, and soil-compost mixtures, before and during incubation, gave information about microbiological activity in the different substrates. Initial differences in enzyme activity in S1 and S2 clearly explained the different patterns of activity observed during incubation and confirmed the influence of soil sampling date on enzyme activity and, more generally, microbial activity. All composts were initially far more active than soils and this greater activity persisted throughout the incubation, excepted for urease in the oldest compost and the lumbricompost incubations. The influence of compost age on enzyme activity was marked, as the older compost was less active than the younger one. No clear effect of lumbricomposting on compost enzyme activity was demonstrated. The addition of compost to soil increased global microbial activity, as reflected by dehydrogenase and respiration measurements, but this effect was only transient and disappeared in the course of incubation. The other enzymes assayed were little affected by compost amendment. Finally, dehydrogenase proved to be a very reliable indicator of microbiological activity in soil and composts, before as well as during incubation.

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