Crushed cotton gin compost on soil biological properties and rice yield

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Abstract

This study presents an account of soil quality parameters and rice (*Oriza sativa* cv. Puntal) yields as influenced by applying an organic waste (crushed cotton gin compost, CC). Such information is desirable for determining the suitability of renewable energy resources such as organic wastes as replacements for synthetic fertilizers. However, CC has low N levels. For this reason, some authors suggest co-applying mineral fertilizers to provide the nutrients plant requires in the early stages of development. The main objective of this work was to study the effect of incorporating CC at rates of 10, 15 and 20 t ha^{-1} with and without inorganic fertilizers on soil biological properties (soil microbial biomass, soil respiration and soil enzymatic activities), nutrition (pigments and leaf soluble carbohydrate concentrations) and yield parameters of rice (*O. sativa* cv. Puntal) crop for three years on an Aquic Xerofluvent located near Sevilla (Spain). Soil biological properties increased when CC was applied with inorganic fertilizers. Since soil enzymatic activities measured are responsible for important cycles such as C, N, P and S, an increase of leaf soluble carbohydrate contents and pigments were observed, and better rice yield parameters were obtained for soils treated with CC + inorganic fertilizers. Yield parameters of the third experimental season were better than those of the second and first experimental season, due to the residual effect of the organic matter after their application in the first season. The application of CC + inorganic fertilizers in soils increased the grain protein concentration (18%), the grain starch concentration (7%), the percentage of full grains (3%) and the rice yield (5%) with respect to the application of CC without inorganic fertilizers in soils.
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1. Introduction

Soil organic matter constitutes an important source of macro- and micronutrients for plants and microorganisms, contributes largely to the acid–base buffering capacity of soils, plays an important role in metal speciation in soil, interacts with organic xenobiotics, extensively affects the soil microbiological activity and is able to bind mineral particles together promoting a good soil structure and improving aeration and moisture retention (Stevenson, 1994; Plaza et al., 2004).

Compost may improve the stability of soil aggregates and applied as a mulch may reduce the risk of erosion (Pinamonti and Zorzi, 1996). It may increase soil porosity and water holding capacity (McConell et al., 1993; Giusquiani et al., 1995), decrease soil acidification (Bengtons and Cornette, 1973) and it releases nutrients (Sikora and Enkiri, 1999; Tejada et al., 2001; Benitez et al., 2003). However, compared with chemical fertilizer most composts contain relatively low levels of nutrients (1–2% N, less than 1% P) (Sikora and Enkiri, 1999). In addition, low mineralization rates from composts require high application rates to satisfy the complete N or P requirement of a crop (Sikora and Enkiri, 1999).

Combining compost with sufficient N fertilizer to meet crop requirements is an appealing possibility because it could: (i) utilize composts at lower rates than when applied as the soil fertilizer, (ii) reduces the amount of N-inorganic fertilizer that need to be applied to soils and (iii) reduces the accumulation of non-nutrient compost constituents in soils (Sikora and Enkiri, 1999).

Also, the organic matter added to soil, while greatly improving the physical properties of the soil, needs a certain time to mineralize and supply the nutrients needed by the crops. Moreover a large quantity of product is needed to fulfil the nutritional requirements of the crops. This is the reason why some authors

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suggest the addition of mineral fertilizers at the same time, to supply the nutritional nutrients that the plant requires in the early stages of development (Tejada and Gonzalez, 2003b).

Since many enzymes respond immediately to changes in soil fertility status, they can be used as potential indicators of soil quality for sustainable management (Garcia et al., 2000). Enzymes may react to changes in soil management more quickly than other variables and therefore may be useful as early indicators of biological changes (Bandick and Dick, 1999; Masciandaro et al., 2004). In fact, they may also indicate the soil's potential to sustain microbiological activity (Paul and Clarck, 1989).

Oxidoreductases and hydrolases enzymes act on the basic processes of organic matter decomposition. In this respect, dehydrogenase activity is an oxidoreductase enzyme which has been used as a measurement of overall microbial activity (Garcia et al., 1997; Pascual et al., 1998; Masciandaro et al., 2004), since it is an intracellular enzyme related to the oxidative phosphorylation process (Trevors, 1986). Other hydrolytic enzymes involved in the cycling of principal nutrients such as β-glucosidase, urease, phosphates, arylsulfatase linked to C, N, P and S, are sensitive indicators of management induced changes in soil properties due to their strong relationship with soil organic matter content and quality (Pascual et al., 1998; Masciandaro and Ceccanti, 1999; Masciandaro et al., 2004). These parameters are the most sensitive to the changes which occur in soil, and provide rapid and accurate information on changes in soil quality, and will help decide the best ways of maintaining sustainable productivity.

The objective of this study then, was to evaluate the effects of a crushed cotton gin compost at different rates with and without inorganic fertilizer on some biological soil properties, such as soil microbial biomass, soil respiration and soil enzymatic activities (dehydrogenase, protease-BBA, β-glucosidase, arylsulfatase and alkaline phosphates) and their repercussion in the nutrition and yield of rice grown in a semiarid Mediterranean agro-ecosystem. The study was carried out in field conditions over a period of three consecutive years.

2. Materials and methods

2.1. Site and properties of compost

The study was conducted during three seasons (April–October 2000, April–October 2001 and April–October 2002) near Sevilla (Guadalquivir Valley, Andalusia, Spain). The soil of the experimental field is an Aquic Xerofluvent. The main soil characteristics (0–25 cm) are shown in Table 1. The general properties of the CC are shown in Table 2 and the composting process of crushed cotton gin is described in Tejada et al. (2001).

Organic matter was determined by dry combustion method. To determine humic and fulvic acids-C, beet vinasse were extracted with 0.1 M sodium pyrophosphate and 0.1 M sodium hydroxide. The supernatant was acidified to pH 2 with HCl and allowed to stand 24 h at room temperature. To separate humic acids from fulvic acids, the solution was centrifuged and the precipitate containing humic acids was dissolved with sodium

Table 1
Main soil characteristics (data are the means of six samples)

pH	8.0
Electric conductivity ($dS\,m^{-1}$)	2.07
Clay ($g\,kg^{-1}$)	543
Silt ($g\,kg^{-1}$)	348
Sand ($g\,kg^{-1}$)	109
Textural class	Clay
Total N ($g\,kg^{-1}$)	0.5
Total C ($g\,kg^{-1}$)	3.8
C/N	7.6
SMB [biomass-C ($\mu g\,C\,g^{-1}$ dry soil)]	93
Dehydrogenase activity ($\mu g\,INTF\,g^{-1}\,h^{-1}$)	19.1
BBA protease activity ($\mu mol\,NH_4\,g^{-1}\,h^{-1}$)	0.09
β-glucosidase activity ($\mu mol\,PNP\,g^{-1}\,h^{-1}$)	3.6
Arylsulfatase activity ($\mu g\,PNF\,g^{-1}\,h^{-1}$)	5.3
Alkaline phosphatase activity ($\mu mol\,PNP\,g^{-1}\,h^{-1}$)	20.1

SMB: soil microbial biomass; INTF: 2-*p*-iodo-3-nitrophenyl; PNF: *p*-nitrophenyl; PNP: *p*-nitrophenol.

hydroxide (Yeomans and Bremner, 1988). The carbon of humic acid and fulvic acids was determined by the method of Sims and Haby (1971). After nitric and perchloric digestion, inorganic soluble P ($PO_4H_2^-$ principally) was determined by Willians and Stewart method, described by Guitian and Carballas (1976). This method consists of phosphomolibdic complex formation and posterior colorimetric determination. K and Na were determined by atomic emission spectrometer after nitric and perchloric digestion, and Ca, Mg, Fe, Cu, Mn, Zn, Cd, Pb, Ni, Cr and Hg were determined by atomic absorption spectrometer after nitric and perchloric digestion.

2.2. Experimental layout and treatments

The experimental layout was a split plot in a randomized complete block with a total amount of 24 plots, with each plot measuring 5 m × 6 m. Six treatments were used (four replicates

Table 2
Characteristics of CC (data are the means of six samples)

pH (H_2O)	7.0
Dry matter ($g\,kg^{-1}$)	630
Organic matter ($g\,kg^{-1}$)	356
Humic acid-C ($g\,kg^{-1}$)	89.8
Fulvic acid-C ($g\,kg^{-1}$)	0.96
Total N ($g\,kg^{-1}$)	13.2
P ($g\,kg^{-1}$)	6.3
Ca ($mg\,kg^{-1}$)	275
Mg ($mg\,kg^{-1}$)	30
Na ($g\,kg^{-1}$)	<0.1
K ($g\,kg^{-1}$)	126
Fe ($mg\,kg^{-1}$)	3.9
Cu ($mg\,kg^{-1}$)	0.1
Mn ($mg\,kg^{-1}$)	0.6
Zn ($mg\,kg^{-1}$)	0.5
Cd ($mg\,kg^{-1}$)	<0.1
Pb ($mg\,kg^{-1}$)	<0.1
Ni ($mg\,kg^{-1}$)	<0.1
Cr ($mg\,kg^{-1}$)	<0.01
Hg ($mg\,kg^{-1}$)	<0.001

per treatment): (1) control soil, non-fertilized control plot, (2) CC10, fertilized with $10 \, t \, ha^{-1}$ of CC (dry weight), (3) CC15, fertilized with $15 \, t \, ha^{-1}$ of CC (dry weight), (4) CC20, fertilized with $20 \, t \, ha^{-1}$ of CC (dry weight), (5) CCM10, fertilized with $250 \, kg \, N \, ha^{-1}$ (as urea) plus $10 \, t \, ha^{-1}$ of CC (dry weight), (6) CCM15, fertilized with $250 \, kg \, N \, ha^{-1}$ (as urea) plus $15 \, t \, ha^{-1}$ of CC (dry weight) and (7) CCM20, fertilized with $250 \, kg \, N \, ha^{-1}$ (as urea) plus $20 \, t \, ha^{-1}$ of CC (dry weight).

The mineral fertilizers were incorporated on 27 April 2000, 28 April 2001 and 27 April 2002, respectively, to a 25-cm depth. The compost was surface broadcasted on 29 April 2000, 1 May 2001 and 1 May 2002, respectively, and incorporated to a 25-cm depth by chisel plowing and disking the day after application. The quality of the compost was the same for the three experimental seasons. In this respect, the compost was kept in refrigeration camera at $0 \, ^{\circ}C$ after its application in the first experimental season, so that there were no problems of mineralization of the organic compounds of this product.

As test crop, rice (*Oriza sativa* cv. Puntal) was seeded at a rate $180 \, kg \, ha^{-1}$, which is common practice in the area. The sowing dates were 18 May 2000, 19 May 2001 and 17 May 2002, respectively.

2.3. Soil and plant sampling and analysis

Soil samples (0–25 cm) were collected from each plot (five replicates per plot) with a gauge auger (30-mm diameter) at 115 days after seeding for each experimental year, from June to September.

After drying at $20 \, ^{\circ}C$, the soil samples were ground to pass through a 2-mm sieve and stored in sealed polyethylene bags at $4 \, ^{\circ}C$ until analysis.

Soil pH was determined in distilled water with a glass electrode (soil:H_2O ratio 1:1). Soil electric conductivity was determined in distilled water with a glass electrode (soil:H_2O ratio 1:5). Soil texture was determined by the Robinson's pipette method (SSEW, 1982). Soil organic carbon was determined by oxidizing organic matter in soil samples with $K_2Cr_2O_7$ in sulfuric acid (96%) for 30 min, and measuring the concentration of Cr^{3+} formed (Sims and Haby, 1971). Soil total N was determined by the Kjeldahl method (Hesse, 1971). Analysis of biological biomass and enzymatic activities of soil were determined at 115 days after seeding for each experimental year. Soil microbial biomass was determined using the $CHCl_3$ fumigation–extraction method (Vance et al., 1987). The levels of five enzymatic activities in soil were measured. Dehydrogenase activity was measured by reduction of 2-*p*-iodo-3-nitrophenyl-5-phenyl tetrazolium chloride to iodonitrophenylformazan (Garcia et al., 1993). Protease activity (BBA protease) was measured using *N*-α-benzoyl-L-argininamide as substrate (Nannipieri et al., 1980). β-Glucosidase activity was measured using *p*-nitrophenyl-β-D-glucopyranoside as substrate (Masciandaro et al., 1994). Arylsulfatase activity was measured using *p*-nitrophenylsulfate (*p*NPS) as substrate (Tabatabai and Bremner, 1970). Alkaline phosphatase activity was measured using *p*-nitrophenyl phosphate disodium as substrate (Tabatabai and Bremner, 1969).

Soil respiration was measured in the laboratory and in samples at the end of year. For all treatments, soil respiration was measured by incubation for 3, 15, 45, 90 and 120 days. Total C-CO_2 collected in the NaOH flasks was determined by the addition of an excess of $1.5 \, M \, BaCl_2$ followed by tritation with standardized HCl using a phenolphthalein indicator (Zibilske, 1994).

Soil C/N ratio evolution was determined at 115 days after seeding for all treatments and for each experimental season with the intention of observing the organic matter mineralization of organic wastes compounds added to the soil.

Leaf samples were collected for each experimental season at 115 days after seeding in all plots to observe the influence of the fertilization with CC and with and without inorganic fertilizers in plant nutrition. Leaves samples consisting of 40 uppermost fully expanded leaves were randomly collected at the four growth stages from plots of seven treatments. Leaf samples were washed, lyophilized and frozen in a liquid N_2 freezer at $-20 \, ^{\circ}C$ until analyzed. Chlorophylls and total carotenoids in the leaf samples were measured by means of extraction with solvent organic in the lyophilized samples and quantifying the extract using the Lichtenthaler method (1987).

Soluble carbohydrate concentrations in the leaf samples were measured using the anthrone method (Yemm and Willis, 1954). About 50 g samples were collected from each plot. Dried leaf samples were extracted in $5 \, cm^3$ 80% (v/v) ethanol (30 min, $30 \, ^{\circ}C$). The extract was centrifuged (10 min, $2650 \times g$) and the pellet was extracted again with ethanol. After centrifugation, chlorophyll was removed from the combined supernatants by chloroform extraction. The samples were analyzed colorimetrically for soluble carbohydrates using the anthrone method.

Percentage of full grains, protein concentration in the grain, starch concentration in the grain and crop yield ($kg \, ha^{-1}$) were determined in each experimental season on samples collected in each plots on 18 October (143 days after seeding). Grain mineral composition was characterized by analyzing N (by the Kjeldahl method, Hesse, 1971) in fresh matter, and P (by the Williams and Stewart method, described by Guitian and Carballas, 1976) and K, Ca, Mg, Fe, Cu, Mn, Zn (by atomic absorption spectrophotometry). Like leaf samples, grain samples were washed, lyophilized and frozen in a liquid N_2 freezer at $-20 \, ^{\circ}C$ until analyzed.

For starch concentration in the grain in a 15 ml centrifuge tube, 100 mg of sample was added with 10 ml ethanol (density at $630 \, g \, l^{-1}$) and kept in a water bath at $80 \, ^{\circ}C$ for 30 min. The tube was then centrifuged at $2000 \times g$ for 20 min after cooling. The residue in the centrifuge tube was dried at $80 \, ^{\circ}C$ for starch extraction, 2 ml of distilled water was added to the tube which was then shaken in a boiling water bath for 15 min and 2 ml of $9.36 \, M \, HClO_4$ added after cooling. The solution was shaken for 15 min, made up to about 10 ml and centrifuged at $2000 \times g$ for 20 min. The supernatant was collected and a further 2 ml of $4.68 \, M \, HClO_4$ was added to the residue. The extraction was repeated as above. The supernatants were combined and made up to 50 ml with distilled water. The starch was analyzed by the method of Pucher et al. (1948).

Table 3
Soil microbial biomass and dehydrogenase and BBA-protease activities at 115 days after seeding during the three experimental seasons

	Soil microbial biomass (μg C g^{-1} dry soil)			Dehydrogenase activity (μg INTF g^{-1} h^{-1})			BBA protease activity (μg NH$_4$ g^{-1} h^{-1})		
	2000	2001	2002	2000	2001	2002	2000	2001	2002
Control soil	92a	80a	83a	18.0a	15.9a	13.8a	0.07a	0.05a	0.03a
CC10	112a	153ab	216b	33.8ab	50.4ab	74.8b	1.77b	2.56b	3.64b
CC15	148ab	220b	301bc	45.2ab	73.4b	102.3bc	2.21b	3.29b	5.09bc
CC20	199b	330bc	432c	56.3ab	108.9bc	145.4bc	3.02b	4.80bc	7.35c
CCM10	150ab	251b	331bc	52.2ab	86.3b	113.9bc	2.31b	3.58b	5.62bc
CCM15	238b	294bc	392c	68.3b	94.6bc	135.5bc	3.70b	4.18bc	6.61c
CCM20	294bc	400c	539cd	88.6b	120.6bc	195.5c	4.49bc	5.79c	9.12c

INTF: 2-*p*-iodo-3-nitrophenyl. Different letters (a–d) following the figures indicate a significant difference at $P < 0.05$.

Table 4
β-glucosidase, arylsulfatase and alkaline phosphatase activities at 115 days after seeding during the three experimental seasons

	β-Glucosidase activity (μmol PNP g^{-1} h^{-1})			Arylsulfatase activity (μmol PNF g^{-1} h^{-1})			Alkaline phosphatase activity (μmol PNP g^{-1} h^{-1})		
	2000	2001	2002	2000	2001	2002	2000	2001	2002
Control soil	2.3ab	1.4a	0.7a	4.7a	4.0a	3.3a	18.7a	17.3a	15.a
CC10	11.1b	23.2b	36.2bc	10.6ab	15.7ab	25.4b	28.1ab	36.2ab	43.1b
CC15	17.4b	33.4bc	59.5c	13.7ab	21.8b	37.6b	35.1ab	39.9ab	49.9b
CC20	31.2bc	60.1	94.4cd	17.6ab	26.4b	45.6bc	39.7ab	48.6b	56.8b
CCM10	18.4b	37.9bc	66.8c	16.4ab	25.5b	39.8b	37.6ab	45.8b	54.8b
CCM15	39.6bc	49.9bc	83.6c	19.7ab	29.8b	51.3bc	42.3b	50.1b	64.6bc
CCM20	51.8c	77.6c	123.1cd	23.8b	34.6b	61.3bc	48.8b	55.8b	72.9bc

PNP: *p*-nitrophenol; PNF: *p*-nitrophenyl. Different letters (a–d) following the figures indicate a significant difference at $P < 0.05$.

2.4. Statistical analysis

The results obtained were analyzed by ANOVA using the Statgraphics v. 5.0 software package (Statistical Graphics Corporation, 1991) and considering the treatment as the independent variable. The means were separated by the Tukey's test, considering a significance level of $P < 0.05$ throughout the study.

3. Results

3.1. Soil properties

Tables 3–5 show the soil biochemical analysis for all treatments during the experimental period.

Soil microbial biomass (Table 3) was significantly higher for CC with inorganic fertilizers amended soils than that for CC without inorganic fertilizers amended soils. The highest values of soil microbial biomass were observed for the third experimental season followed by the second and the first experimental season. Control soil has the lowest values of soil microbial biomass. Also, cumulative C-CO$_2$ (Table 5) in soils was significantly higher for CC with inorganic fertilizers amended soils than that for CC without inorganic fertilizers amended soils. Control soil has the lowest values of soil respiration.

With respect to the soil enzymatic activities (Tables 3 and 4), the lowest values are presented for the control soil. The highest values were observed for CC with inorganic fertilizers amended soils than that for CC without inorganic fertilizers amended soils. Also, the highest values were observed for the third experimen-

Table 5
Cumulative C-CO$_2$ (mg kg soil^{-1}) during incubation in soils affected by application of CC

	Incubation days				
	3	15	45	90	120
2000					
Control soil	128	380	804	814	867a
CC10	151	463	703	874	961ab
CC15	238	931	1463	1798	1995b
CC20	269	1106	1586	1894	2089b
CCM10	219	889	1375	1687	1886b
CCM15	244	1006	1499	1793	1948b
CCM20	276	1155	1679	2003	2201b
2001					
Control soil	132	399	610	789	855a
CC10	183	496	756	899	943ab
CC15	251	639	1512	1877	2103b
CC20	282	703	1643	2022	2204b
CCM10	238	622	1402	1748	2005b
CCM15	269	691	1567	1856	2116bc
CCM20	293	745	1788	1994	2312bc
2002					
Control soil	140	411	623	801	851a
CC10	206	530	811	903	997ab
CC15	273	686	1593	1964	2207b
CC20	296	749	1722	2106	2310bc
CCM10	255	688	1492	1808	2105b
CCM15	283	726	1629	1949	2208bc
CCM20	316	799	1906	2131	2420bc

Different letters (a–c) following the figures indicate a significant difference at $P < 0.05$.

Table 6
Soil C/N ratio in soils during the three experimental seasons

	2000	2001	2002
Control soil	7.5a	7.4a	7.3a
CC10	8.6a	10.5ab	12.6ab
CC15	9.1a	11.5ab	13.1ab
CC20	10.0ab	12.9ab	14.4ab
CCM10	7.8a	9.0a	9.9ab
CCM15	8.7a	9.3a	10.7ab
CCM20	9.3a	9.8ab	11.9ab

Different letters (a and b) following the figures indicate a significant difference at $P < 0.05$.

tal season than that for the second and the first experimental season, respectively. This increase in soil enzymatic activities is in line with the increase of soil microbial biomass and soil respiration.

Table 6 shows soil C/N ratio evolution during the three experimental seasons for all treatments. The lowest values were observed for control soil, followed by CC with inorganic fertilizers amended soils and CC without inorganic fertilizers amended soils. Optimum C/N ratio (10–12) was observed for CC + inorganic fertilizers amended soils, however for CC without inorganic fertilizers amended soils C/N ratio had slightly highest values.

3.2. Plant analysis

Table 7 shows the leaf pigments and soluble carbohydrate contents in rice crop and for the three experimental seasons. The statistical analysis indicated significant differences of leaf pigments and soluble carbohydrate contents with respect to fertilizer treatments. The highest values of chlorophyll A and B, carotenoids and soluble carbohydrate contents were obtained for CC with inorganic fertilizers amended soils than that for CC without inorganic fertilizers amended soils. The lowest values are presented for control soil. These results are in line with the data obtained for soil microbial biomass, soil respiration and soil enzymatic activities. Also, the highest values were observed for the third experimental season than that for the second and the first experimental season, respectively.

Table 8 shows the chemical analysis of the rice grain from the different treatments. Again, the highest concentrations of the macro- and micronutrients analyzed were obtained for CC with inorganic fertilizers amended soils followed for CC without inorganic fertilizers amended soils and the control soil. The highest values were observed for the third experimental season than that for the second and the first experimental season, respectively. With respect to macronutrients analyzed, significant differences were found only in N. The P, K, Ca and Mg levels did not show any significant differences with the fertilizer treatments. The P levels were lower and the N, K, Ca and Mg levels higher than the values previously reported by Tejada and Gonzalez (2004) for the same rice variety and similar pedoclimatic conditions. With respect to the analyzed micronutrients, the statistical analysis indicated that significant differences were observed within fertilizer treatments.

Table 9 shows the grain protein content and crop yield parameters for the different treatments. The highest protein content and grain starch was for CC with inorganic fertilizers amended soils than that for CC without inorganic fertilizers amended soils. Control soil had the lowest grain protein content and crop yield parameters. The statistical analysis indicated significant differences within fertilizer treatments, highlighting the highest values for the third experimental season than that for the second and the first experimental season, respectively. In this respect, the application of CC with inorganic fertilizers in soils increased the grain protein concentration of about 18% with respect to the application of CC without inorganic fertilizers in soils, and increased the grain starch concentration of about 7% with respect to the application of CC without inorganic fertilizers in soils. These values were higher than those reported by Tejada and Gonzalez (2004) for the same rice cultivar fertilized with a byproduct of the two-step olive oil mill process applied to soil and similar pedoclimatic conditions.

The percentage of full grains were similar to those reported by Tejada and Gonzalez (2004) for the same rice cultivar in the Guadalquivir Valley and similar pedoclimatic conditions. The statistical treatment indicated significant differences between fertilizer treatments, highlighting the highest values for CC with inorganic fertilizers amended soils than that for CC without inorganic fertilizers amended soils and the highest values for the third

Table 7
Leaf pigments and soluble carbohydrate contents at 115 days after seeding in rice crop for each experimental season

	2000				2001				2002			
	Chl A ($mg\,kg^{-1}$)	Chl B ($mg\,kg^{-1}$)	Carot. ($mg\,kg^{-1}$)	SCC ($mg\,kg^{-1}$)	Chl A ($mg\,kg^{-1}$)	Chl B ($mg\,kg^{-1}$)	Carot. ($mg\,kg^{-1}$)	SCC ($mg\,kg^{-1}$)	Chl A ($mg\,kg^{-1}$)	Chl B ($mg\,kg^{-1}$)	Carot. ($mg\,kg^{-1}$)	SCC ($mg\,kg^{-1}$)
Control soil	2699a	1889a	891a	182a	2678a	1923a	911a	184a	2725a	1948a	931a	191a
CC10	2940a	2045a	952a	195a	3002ab	2105a	976a	199a	3091ab	2166a	1043a	206a
CC15	3099ab	2160a	993a	204a	3175ab	2193a	1046a	209a	3231ab	2244a	1119a	214ab
CC20	3200ab	2200a	1102a	213ab	3289ab	2248a	1162a	218ab	3312ab	2297a	1211a	223ab
CCM10	3110ab	2173a	1079a	209a	3199ab	2225a	1122a	215ab	3269ab	2350ab	1193a	219ab
CCM15	3256ab	2256a	1178a	216ab	3302ab	2271a	1199a	223ab	3395ab	2388ab	1264a	229ab
CCM20	3300ab	2303ab	1265a	221ab	3398ab	2308ab	1273a	229ab	3470b	2444b	1312ab	234ab

Chl A: chlorophyll A; Chl B: chlorophyll B; Carot.: carotenoids; SCC: soluble carbohydrate contents. Different letters (a and b) following the figures indicate a significant difference at $P < 0.05$.

Table 8
Chemical analysis of the grains during the experimental period

	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
2000									
Control soil	8.1a	1.8a	2.2a	0.1a	0.4a	16.3a	73.4a	2.1a	18.3a
CC10	12.1ab	2.2a	3.3a	0.1a	0.5a	21.8a	61.2a	2.2a	21.3a
CC15	12.7ab	2.3a	3.5a	0.1a	0.5a	23.4a	59.6ab	2.4a	21.6a
CC20	13.1ab	2.4a	3.5a	0.2a	0.6a	24.1a	57.3ab	2.6a	22.0a
CCM10	12.9ab	2.3a	3.4a	0.1a	0.5a	23.6a	58.0ab	2.7a	21.8a
CCM15	13.3ab	2.4a	3.6a	0.2a	0.6a	24.9a	55.5ab	2.9a	22.2a
CCM20	13.6ab	2.5a	3.7ab	0.2a	0.6a	25.5ab	53.4ab	3.2ab	22.4ab
2001									
Control soil	9.2a	1.9a	2.1a	0.1a	0.4a	18.5a	70.1a	2.2a	19.0a
CC10	12.7ab	2.3a	3.8ab	0.1a	0.5a	28.6ab	50.2ab	2.4a	21.6a
CC15	13.2ab	2.3a	4.1ab	0.2a	0.6a	29.9ab	49.1ab	2.7a	22.0a
CC20	13.6ab	2.5a	4.3ab	0.2a	0.6a	32.5ab	47.3ab	2.9a	22.4a
CCM10	13.5ab	2.4a	4.2ab	0.2a	0.6a	31.8ab	46.4ab	3.0a	22.3a
CCM15	13.8ab	2.5a	4.5ab	0.2a	0.7a	35.9ab	42.1ab	3.3ab	22.8ab
CCM20	14.3b	2.6a	5.0ab	0.3a	0.7a	40.4ab	38.2b	3.5ab	23.5ab
2002									
Control soil	8.7a	1.9a	2.4a	0.1a	0.5a	17.7a	72.2a	2.2a	18.5a
CC10	13.1ab	2.3a	4.2ab	0.2a	0.6a	39.8ab	40.0ab	2.7a	22.2a
CC15	13.9ab	2.5a	4.6ab	0.3a	0.6a	41.2ab	37.1b	2.9a	23.2a
CC20	14.1ab	2.6a	5.2ab	0.3a	0.7a	48.9b	33.2b	3.2ab	24.3ab
CCM10	14.0ab	2.5a	5.0ab	0.3a	0.6a	46.6b	32.8b	3.1ab	24.1ab
CCM15	14.4b	2.6a	5.5ab	0.4a	0.7a	53.8b	30.5b	3.5ab	25.8ab
CCM20	15.1b	2.6a	6.1b	0.4a	0.8a	68.9b	28.8b	3.6ab	26.7ab

Different letters (a and b) following the figures indicate a significant difference at $P < 0.05$.

experimental season than that for the second and the first experimental season, respectively. In this respect, the application of CC with inorganic fertilizers in soils increased the percentage of full grains of about 3% with respect to the application of CC without inorganic fertilizers in soils.

Finally, rice yield shows significant differences regarding the fertilizer treatment. Also, the highest values were for CC with inorganic fertilizers amended soils than that for CC without inorganic fertilizers amended soils and for the third experimental season than that for the second and the first experimental season, respectively. The application of CC with inorganic fertilizers in soils increased the rice yield of about 5% with respect to the application of CC without inorganic fertilizers in soils.

4. Discussion

The supply of readily metabolisable C in the organic waste is likely to have been the most influential factor contributing to the biomass-C increases. In this respect and according to De Neve and Hofman (2000), Schaffers (2000), and Tejada and Gonzalez (2003a,b), soil microbial biomass and soil respiration responds rapidly, in terms of activity, to additions of readily available C.

For same authors, the application of organic wastes decreased soil microbial biomass. In this respect, Brendecke et al. (1993), Fließbach et al. (1994) and Filip and Bielek (2002) reported a decrease of soil microbial biomass after a 10 year application of 5 and 15 t ha^{-1} year of sewage sludges. These authors indicated that the presence of high amounts of heavy metals (Cd, Cr, Hg, Pb, etc.) in this byproduct may counterbalance the posi-

tive effects of organic matter in soil microbial biomass. The CC analyses (Table 2) indicate very low concentrations of Cd, Cr, Hg and Pb.

Incorporation of CC influences soil enzymatic activities because the added material may contain intra- and extracellular enzymes and may also stimulate microbial activity in the soil (Goyal et al., 1993; Pascual et al., 1998). According to Garcia et al. (1994) and Pascual et al. (1998), the organic amendment had a positive effect on the activity of these enzymes, particularly when the amendment was at the high dose, probably due to the higher microbial biomass produced in response.

The greater dehydrogenase activity noted at the high dosage suggests either that the added CC did not include compounds which were toxic for this activity (Pascual et al., 1998). During each experimental season, dehydrogenase activity decreased. This may be due to microbial death because substrates were no longer available to sustain microbial biomass or that extracellular enzyme complexes were degraded by microorganisms inhabiting amended soils (Pascual et al., 1998).

The highest values of soil protease activity (BBA protease) and soil β-glucosidase activity for CC-amended soils, are in agreement with Garcia et al. (1994) and Pascual et al. (1998), who indicated that the organic amendment had a positive effect on the activity of these enzymes, particularly when the amendment was at the high dose, probably due to the higher microbial biomass produced in response. Also, soil arylsulfatase activity and soil alkaline phosphatase activity was higher in the CC with inorganic fertilizers amended soils than for CC without inorganic fertilizers amended soils.

Table 9
Grain protein concentration, grain starch and crop yield parameters

	2000				2001				2002			
	Protein concentration (mg g^{-1})	Grain starch (mg g^{-1})	Full grains (%)	Yield (kg ha^{-1})	Protein concentration (mg g^{-1})	Grain starch (mg g^{-1})	Full grains (%)	Yield (kg ha^{-1})	Protein concentration (mg g^{-1})	Grain starch (mg g^{-1})	Full grains (%)	Yield (kg ha^{-1})
Control soil	51.1a	689a	82a	9023a	58.0a	703a	80a	8833a	53.4a	695a	83a	9194a
CC10	76.4ab	785.2ab	91.3a	9609b	79.3ab	791.2ab	91.7a	9694b	81.1ab	806.7ab	92.0ab	9761b
CC15	81.6ab	799.4ab	92.4a	9698b	84.6ab	814.4ab	92.8a	9786b	85.9ab	820.9ab	93.1ab	9877bc
CC20	84.9ab	812.6ab	93.5a	9784b	88.5b	822.6ab	93.9ab	9864bc	90.7b	826.7b	94.2b	9956bc
CCM10	82.2ab	806.6ab	92.8a	9766b	86.2ab	819.8ab	93.1a	9855bc	88.3b	826.4b	93.4ab	9922bc
CCM15	87.8ab	824.1ab	94.0ab	9851b	91.3b	835.4b	94.4ab	9948bc	94.1b	846.9b	94.8b	10097bc
CCM20	90.1b	836.7b	94.3ab	9946bc	95.5b	847.1b	94.7ab	10106bc	99.7b	860.1b	95.0b	10269bc

Different letters (a–c) following the figures indicate a significant difference at $P < 0.05$.

However, under field conditions, the decomposition of compost is complex, and is controlled by numerous factors such as availability of carbon and nitrogen, the biochemical nature of the compost, contact between soil and compost and soil and climatic factors (Hadas and Portnoy, 1994). Moreover the N content of the compost and its C/N ratio have a considerable effect on the dynamics of mineral N in the soil. The C/N ratio of the compost will largely determine the balance between mineralization and immobilization.

CCM20, CCM15 and CCM10 treatments show the highest values in soil microbial biomass, soil respiration and soil enzymatic activities. For this reason, we think that in the treatments where organic matter and mineral fertilizers provide supply to the soil mineralization prevails over the immobilization, while in the treatments where there was supply of organic matter alone soil immobilization can exist. This characteristic is also corroborated by the best values of the C/N ratio in the CCM20, CCM15 and CCM10 treatments that in the other treatments.

Since soil enzymatic activities measured are responsible for important cycles such as C, N, P and S, leaf pigments and soluble carbohydrate contents during the rice growth cycle increased significantly during each experimental year for CC with inorganic fertilizers amended soils than for CC without inorganic fertilizers amended soils. This may be due to better mineralization of organic matter for CC with inorganic fertilizers amended soils than for CC without inorganic fertilizers amended soils. These results are of great importance, because the photosynthesis could be increased over a longer period of time as the levels of pigments in the leaf increase, resulting in a higher production of soluble carbohydrates and thereby increased grain quality and crop yield. These parameters of the third experimental season were better than those of the second and first experimental season, due to the residual effect of the organic matter after their application in the first season.

Since the increase in microbial diversity may increase soil microbial functionality and therefore increase the N, P and S available levels by plants, rice yield parameters increased significantly when a higher dose of CC with inorganic fertilizers was applied to the soil with respect to the same dose of CC without inorganic fertilizers.

5. Conclusions

Compared to compost alone, application of compost + inorganic fertilizer resulted in increased soil biological activity, plant nutrient uptake and crop yield as well as improved grain quality. This is due to the fact that when compost alone was applied, immobilization of N resulted but with the addition of mineral N, net mineralization occurred. This increase in mineralization decreased the time necessary for organic matter in the compost to break down and supply nutrients to the plant in the early stages of development.

References

Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzymes activities. Soil Biol. Biochem. 31, 1471–1479.

Bengtons, G.W., Cornette, J.J., 1973. Disposal of composted municipal waste in a plantation of young slash pine: effects on soil and trees. J. Environ. Qual. 2, 441–444.

Benitez, C., Tejada, M., Gonzalez, J.L., 2003. Kinetics of the mineralization of the nitrogen in a pig slurry compost. Compost Sci. Util. 11, 72–80.

Brendecke, J.W., Axelson, R.D., Pepper, I.L., 1993. Soil microbial activity as an indicator of soil fertility: long-term effects of municipal sewage sludge on an arid soil. Soil Biol. Biochem. 25, 751–758.

De Neve, S., Hofman, G., 2000. Influence of soil compaction on carbon and nitrogen mineralization of soil organic matter and crop residues. Biol. Fertil. Soils 30, 544–549.

Filip, Z., Bielek, P., 2002. Susceptibility of humic acids from soils with various contents of metals to microbial utilization and transformation. Biol. Fertil. Soils 36, 426–433.

Fließbach, A., Martens, R., Reber, H.H., 1994. Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol. Biochem. 26, 1201–1205.

Garcia, C., Hernandez, T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Comm. Soil Sci. Plant Anal. 1–2, 123–134.

Garcia, C., Hernandez, T., Costa, F., Ceccanti, B., Masciandro, G., 1993. XI International Symposium Environmental Biogeochemistry, Salamanca, Spain, pp. 89–100.

Garcia, C., Hernandez, T., Costa, F., Ceccanti, C., 1994. Biochemical parameters in soils regenerated by the addition of organic wastes. Waste Manage. Res. 12, 457–466.

Garcia, C., Hernandez, T., Pascual, J.A., Moreno, J.L., Ros, M., 2000. Microbial activity in soils of SE Spain exposed to degradation and desertification processes. Strategies for their rehabilitation. In: Garcia, C., Hernandez, M.T. (Eds.), Research and Perspectives of Soil Enzymology in Spain. CEBAS-CSIC, Spain, pp. 93–143.

Giusquiani, P.L., Pagliai, M., Gigliotti, G., Businelli, D., Benetti, A., 1995. Urban waste compost: effects on physical, chemical and biochemical soil properties. J. Environ. Qual. 24, 175–182.

Goyal, S., Mishra, M.M., Dhankar, S.S., Kapoor, K.K., Batra, R., 1993. Microbial biomass turnover and enzyme activities following the application of farmyard manure to field soils with and without previous long-term applications. Biol. Fertil. Soils 15, 60–64.

Guitian, F., Carballas, T., 1976. In: Sacro, P. (Ed.), Técnicas de análisis de suelos. Santiago de Compostela, España.

Hadas, A., Portnoy, R., 1994. Nitrogen and carbon mineralization rates of composted manures incubated in soil. J. Environ. Qual. 23, 1184–1189.

Hesse, P.R., 1971. A Textbook of Soil Chemical Analysis. Muray, London.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosyntesis biomembranes. Methods Enzymol. 148, 350–383.

Masciandaro, G., Ceccanti, B., 1999. Assessing soil quality in different agro-ecosystems through biochemical and chemico-structural properties of humic substances. Soil Till. Res. 51, 129–137.

Masciandaro, G., Ceccanti, B., Garcia, C., 1994. Anaerobic digestion of straw and piggery waste waters. II. Optimization of the process. Agrochimica 38, 195–203.

Masciandaro, G., Ceccanti, B., Benedicto, S., Lee, H.C., Cook, F., 2004. Enzyme activity and C and N pools in soil following application of mulches. Can. J. Soil Sci. 84, 19–30.

McConell, D.B., Shiralipour, A., Smith, W.H., 1993. Compost application improves soil properties. Biocycle 34, 61–63.

Nannipieri, P., Ceccanti, C., Ceverlli, S., Matarese, E., 1980. Extraction of phosphatase, urease, protease, organic carbon and nitrogen from soil. Soil Sci. Soc. Am. J. 44, 1011–1016.

Pascual, J.A., Hernandez, T., Garcia, C., Ayuso, M., 1998. Enzymatic activities in an arid soil amended with urban organic wastes: laboratoty experiment. Bioresour. Technol. 64, 131–138.

Paul, E.A., Clarck, F.E., 1989. Reduction and transport of nitrate. Soil Microbiology and Biochemistry, vol. 9. Academic press, New York, NY, pp. 81–85.

Pinamonti, F., Zorzi, G., 1996. Experiencies of compost use in agriculture and in land reclamation projects. In: deBertoldi, M., Sequi, P., Lemmes, B., Papi, T. (Eds.), The Science of Composting. Academic and Professional, London, pp. 515–527.

Plaza, C., Hernandez, D., Garcia-Gil, J.C., Polo, A., 2004. Microbial activity in pig slurry-amended soils under semiarid conditions. Soil Biol. Biochem. 36, 1577–1585.

Pucher, G.W., Leavenwarth, C.S., Vikery, H.B., 1948. Determination of starch in plant tissues. Anal. Chem. 20, 850–853.

Schaffers, A.P., 2000. In situ annual nitrogen mineralization predicted by simple soil properties and short-period field incubation. Plant Soil 221, 205–219.

Sikora, L.J., Enkiri, N.K., 1999. Growth of tall fescue in compost/fertilizer blends. Soil Sci. 56, 125–137.

Sims, J.R., Haby, V.A., 1971. Simplified colorimetric determination of soil organic matter. Soil Sci. 112, 137–141.

Soil Survey of England Wales [SSEW], 1982. Soil Survey Laboratory methods. Tech. Monogr. 6. SSEW, Harpenden, UK.

Statistical Graphics Corporation, 1991. Statgraphics 5.0. Statistical Graphics System. Educational Institution Edition, USA, pp. 105.

Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions. Wiley-Interscience, New York.

Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenol phosphate in assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.

Tabatabai, M.A., Bremner, J.M., 1970. Arylsulfatase activity of soils. Soil Sci. Soc. Am. Proc. 34, 225–229.

Tejada, M., Dobao, M.M., Benitez, C., Gonzalez, J., 2001. Study of composting of cotton residues. Bioresour. Technol. 79, 199–202.

Tejada, M., Gonzalez, J.L., 2003a. Effects of foliar application of a byproduct of the two-step olive oil mill process on maize yield. Agronomie 23, 617–623.

Tejada, M., Gonzalez, J.L., 2003b. Effects of the application of a compost originating from crushed cotton gin residues on wheat yield under dryland conditions. Eur. J. Agron. 19, 357–368.

Tejada, M., Gonzalez, J.L., 2004. Effects of foliar application of a byproduct of the two-step olive oil mill process on rice yield. Eur. J. Agron. 21, 31–40.

Trevors, J.T., 1986. Electron transport system: activity in soil, sediment and pure culture. Crit. Rev. Microbiol. 11, 83–99.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Yemm, E.W., Willis, A.J., 1954. The estimation of carbohydrate in plant extracts by anthrone. J. Biochem. 75, 508–514.

Yeomans, J.C., Bremner, J.M., 1988. A rapid and precise method for routine determination of organic carbon in soi. Comm. Soil Sci. Plant Anal. 19, 1467–1476.

Zibilske, L.M., 1994. Carbon mineralization. In: Weaver, R.W. (Ed.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. SSSA Book Series 5. SSSA, Madison, WI, pp. 835–863.