

## MICROBIAL BIOMASS AND ACTIVITY OF AN AGRICULTURAL SOIL AMENDED WITH THE SOLID PHASE OF PIG SLURRY

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### ABSTRACT

The objective of this work was to study the effects of the application of the solid phase of pig slurry in an agricultural soil on the microbial biomass and activity in amended soils. Samples of this soil were mixed with two manures (at two rates, adding 7 and 14 g organic C kg<sup>-1</sup> soil) derived from the solid phase of pig slurry (composted, CSP, and non-composted, NSP), and were incubated during 163 days. Amended samples showed higher rates of respiration than the control ones, being higher for the NSP-amended samples. In addition to this, the increment of the application rate of the manure also increased the soil respiration. The maximum microbial biomass increments were not immediately recorded after amendments. The  $q\text{CO}_2$  values were increased by the organic amendments, due to the presence of easy decomposable compounds, and clearly tended to decrease throughout the incubation. Carbon mineralised from manures can be fitted to first-order kinetic model. In relation to nitrogen mineralisation, different patterns were observed between samples amended with CSP and NSP manure type. Inorganic N was lower in CSP-manure amended soils, probably due to the presence of more recalcitrant organic matter as consequence of composting. The use of the solid phase of pig slurries, composted or not, could be a feasible practice to enhance in a short-term the microbial biomass and activity of agricultural soils.

### INTRODUCTION

Intensive agriculture is one of the main causes of soil degradation, mainly due to the loss of organic matter. The addition of organic wastes has been proposed not only as one method of maintaining levels of nutrients in agricultural soils, but also, for increasing the biological activity. Microorganisms play important roles in soils, as biochemical transformations of mayor elements, being source and sink of nutrient for plants, or in physical properties of soils (Anderson & Domsch, 1980). In this study, the effect of the solid phase of pig slurry on the restoration of the microbial activity in a degraded soil by intense horticulture was evaluated.

### MATERIALS AND METHODS

For this study, a randomized 2 x 2 x 6 factorial arrangement, representing two manure types (CSP and NSP), two application rates, and 6 samplings (time) were established. The surface layer (0-15 cm) of a calcareous soil (Typic Xerofluvent) affected by degradation due to intensive horticulture practices were collected at Orihuela (SE Spain). This clayey-loam soil had 18.3 g organic C kg<sup>-1</sup>, 1.33 g organic N kg<sup>-1</sup>, with an electrical conductivity of 0.47 dS m<sup>-1</sup>. Samples of this soil were mixed with two manures derived from the solid phase of pig slurry (composted, CSP, and non-composted, NSP). The NSP and CSP manures have 320 and 329 g organic C kg<sup>-1</sup> respectively, 25.3 and 22.4 g organic N kg<sup>-1</sup> respectively. Total P was 15.8 g kg<sup>-1</sup> and 9.8 g kg<sup>-1</sup> for NSP and CSP respectively. The electrical conductivity was 2.33 and 2.85 dS m<sup>-1</sup> for NSP and CSP manure.

The manures were applied in soil samples at two rates, adding 7 and 14 g organic C kg<sup>-1</sup> soil, respectively, in 300g soil-pots, being five treatments established: 1) Control: soil without manu-

re; 2) NSP1: soil + NSP at rate 1; 3) NSP2: soil + NSP at rate 2; 4) CSP1: soil + CSP at rate 1; 5) CSP2: soil + CSP at rate 2. Then, soil samples were incubated (aerobically, non-leached conditions) during 163 days at 25°C (moisture content of 60% WHC). Four pots per treatment were collected and analysed at 3, 20, 41, 62, 90 and 163 days of incubation period.

In these soil samples were analysed: microbial biomass carbon ( $C_{mic}$ ); basal respiration rates (measured as  $CO_2-C$ );  $qCO_2$  (metabolic quotient); organic carbon ( $C_{org}$ ); ammonium ( $NH_4^+-N$ ), nitrates ( $NO_3^-N$ ),  $C_{mic} \cdot C_{org}^{-1}$  and the respiration normalized to  $C_{org}$  content. For each sampling date, the mean value (of 4 replications) of the control treatment were obtained, and then subtracted to the values (four values per manure-treatments) of the amended soils. These data (increment values with respect to the control: amended minus control;  $\Delta$  data) were analysed using F-ANOVA test (manure type, application rate and time), at  $P < 0.05$ . To calculate the  $CO_2-C$  evolved from manures mineralisation, the values of the untreated (control) soil were subtracted. Then, cumulative values were fitted to different kinetics functions. The best fits were using this first-order kinetic model  $C_{miner} = C_r(1 - e^{-K \cdot t})$ . All statistical analyses were performed using the SPSS 11.5 package (© SPSS Inc, 1989).

## RESULTS AND DISCUSSION

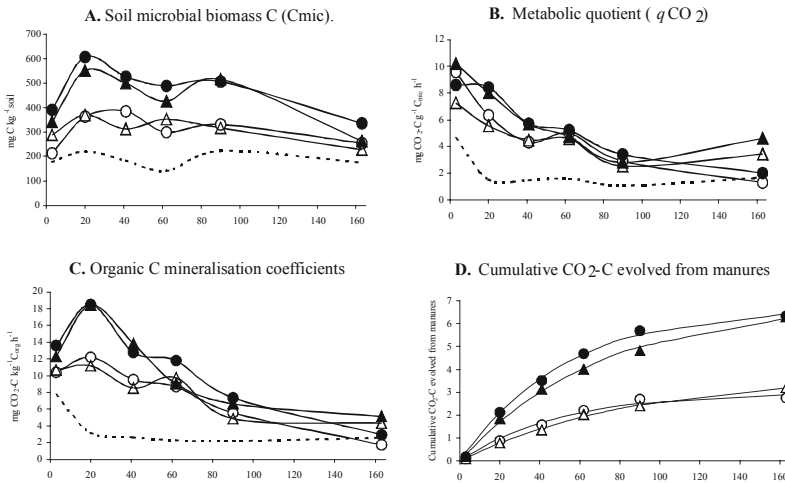
The microbial biomass C ( $C_{mic}$ ) was increased in the soils by the organic amendments. These increments were closely related to the application rate (Table 1). The  $\Delta C_{mic}$  (treated minus control) was slightly higher for the NSP treatments, probably due to a lower microbial stimulation in soil amended with composted manure, where most of the easy biodegradable organic compounds were mineralised in the composting process. The  $C_{mic}$  in treated samples have reached a maximum at 20 days after manure application, except in NSP1 treatment (41 days). A lag phase is usually observed before the growth of microorganisms. Then, a trend to decline with time is observed, and at the end of incubation (163 days), only the treatment NSCP-2 maintains significant higher  $C_{mic}$  values than the control samples.

**Table 1.** F-ANOVA results for the effect of treatments ( data: treated minus untreated values) on the parameters studied.

Source	df	$\Delta C_{org}$		$\Delta C_{mic}$		$\Delta CO_2 g^{-1} soil h^{-1}$		$\Delta qCO_2$	
		F	P <sup>a</sup>	F	P	F	P	F	P
Manure (M)	1	28.5	***	4.8	*	8.3	**	0.3	ns
Rate (R)	1	1192.5	***	263.7	***	911.4	***	39.5	***
Time (T)	5	57.6	***	38.9	***	367.2	***	65.5	***
M x R	1	0.1	ns	6.8	*	5.3	*	4.9	*
M x T	5	10.9	***	1.4	ns	11.4	***	8.2	***
R x T	5	7.1	***	6.4	***	67.9	***	3.5	**
Source	df	$\Delta NH_4^+-N$		$\Delta NO_3^-N$		$\Delta \% C_{mic} \cdot C_{org}^{-1}$		$\Delta CO_2 g^{-1} C_{org} h^{-1}$	
		F	P	F	P	F	P	F	P
Manure (M)	1	43.7	***	1585.6	***	0.2	ns	0.0	ns
Rate (R)	1	778.2	***	908.8	***	15.4	***	243.0	***
Time (T)	5	613	***	121.6	***	40.4	***	295.7	***
M x R	1	13.7	***	29.9	***	3.1	ns	1.6	ns
M x T	5	14.5	***	63.7	***	0.8	ns	7.2	***
R x T	5	401.9	***	75.4	***	1.1	ns	23.8	***

<sup>a</sup> Probability levels of F-ANOVA test: \*\*\* ( $P < 0.001$ ), \*\* ( $P < 0.01$ ), \* ( $P < 0.05$ ), ns ( $P > 0.05$ ).

The values of  $C_{mic}$  in soils usually were between 0.2 and 6% of the total organic C. In our experiment,  $C_{mic}$  in control samples represented 1.8% of the total organic C (on average of incubation period). The application of manure has not caused a clear effect on this percentage, and manured-soils values ranging from 1.1 to 2.5%. Significant effects of the factors “time” and “rate” were observed on the effect of treatments ( $\Delta$ : treated minus control) in this parameter (Table 1). In addition to this, the non-significant effect of the type of manure on  $\Delta\%C_{mic} \cdot C_{org}^{-1}$  indicates that the organic carbon in these manures presents similar characteristics in order to enhance the microbial biomass in amended soils.



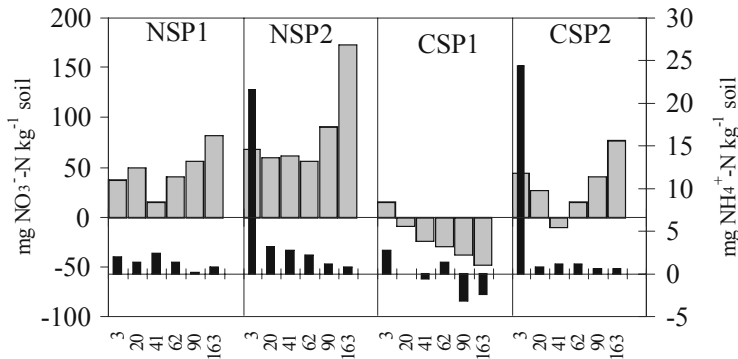
**Figure 1.** Evolution (time in days, in X-axis) of  $C_{mic}$  (A),  $qCO_2$  (B), and respiration normalized to  $C_{org}$  content (C). Line without symbol: Control treatment; white circles: NSP1; black circles: NSP2; white triangles: CSP1; black triangles: CSP2. (D) Cumulative  $CO_2$ -C mineralised from manures ( $g\ CO_2\text{-}C\ kg^{-1}\ soil+manure$ ) (experimental data: symbols; lines: the fittings of the model  $C_{miner} = C_r \cdot (1 - e^{-K_r t})$ ).

The values of basal respiration in control (un-amended) samples range from 0.23 to 0.81  $mg\ CO_2\text{-}C\ kg^{-1}\ soil\ h^{-1}$ , following a trend to decrease during the incubation. Higher rates of respiration at first stages of soil incubations are typical due to the sieve disturbance, the rewetting and the presence of easy decomposable substrates. The addition of solid pig slurry has caused a significant increase in the respiration rates of amended soils (ranging from 0.32 to 5.11  $mg\ CO_2\text{-}C\ kg^{-1}\ soil\ h^{-1}$ ), similar to those observed in soils treated with another organic wastes (Bernal & Kirchmann, 1992). The highest respiration rate in amended soils were recorded at 20 days, when maximum  $C_{mic}$  content were also observed and easy degradable compounds were not yet depleted. The manure type has a significant effect ( $P < 0.01$ ) on basal respiration, being higher for NSP due to the mineralisation of easy biodegradable compounds during composting. As expected, the application rate and the time of incubation have the higher significant effects (both  $P < 0.001$ ).

The increment of  $qCO_2$  is usually found after adding fresh substrate in a soil (Leifeld et al., 2002). The addition of organic compounds stimulate the activity of microorganisms, increases the zymogenous microbiota (*r*-type, less efficient), and the relative size of active versus latent biomass. During incubation,  $qCO_2$  trend to decrease due to the depletion of easily-decomposable compounds and the colonization of more efficient microorganisms.

The organic carbon mineralisation coefficients (respiration normalized to  $C_{org}$ ; Fig. 1.C)

were higher in amended than control samples, showing that organic C of manures is more biodegradable than native soil organic C. The rate 2 showed unexpected higher values than rate 1. This effect of application rate on the  $C_{org}$  mineralisation coefficient ( $P < 0.001$ ) could be due to the improvement of other parameters that affected mineralisation (such as nutrient increase or physical properties), enhancing the conditions for microbial activity. A positive priming effect could be also considered in rate 2. Cumulative  $CO_2$ -C evolved from manures mineralisation (Fig 1.D) can be fitted to a first-order kinetic model, explaining from 98.3 to 99.7% of the experimental data. Potentially mineralisable C (Cr, in  $g\ C\ kg^{-1}$ ) estimated with the model were 3.0, 6.7, 3.8 and 6.9 for NSP1, NSP2, CSP1 and CSP2, respectively.



**Figure 2.** Effects of treatments (treated minus control values:  $\Delta$  data) on inorganic N ( $\Delta NH_4^+ - N$  in black, right Y-axis;  $\Delta NO_3^- - N$  in grey, left Y-axis). All data are expressed in  $mg\ N\ kg^{-1}\ soil$ . The numbers in X-axis indicate the days after the organic amendments.

Inorganic nitrogen in amended soils was principally as  $NO_3^- - N$ , around 92% on average in this experiment. Inorganic N values were 86, 134, 176, 63 and 123  $mg\ N\ kg^{-1}\ soil$ , in C, NSP1, NSP2, CSP1 and CSP2 treatments, respectively. Lower nitrogen mineralisation was observed in the CSP manured-soils. In this experiment,  $NH_4^+ - N$  quickly decreased; in the samples treated with the non-composted manure (NSP) most of this  $NH_4^+ - N$  have been immobilised by microbes, or nitrified, especially in the last phase of the incubation. In samples treated with composted manure (CSP), most of this decrease in  $NH_4^+ - N$  were not recovered as  $NO_3^- - N$ , or immobilised by the microbial biomass (N stored in biomass were estimated from  $C_{mic}$  and the coefficients provided by Anderson & Domsch, 1980). Losses of N due to volatilization (of  $NH_4^+ - N$ ) and denitrification ( $NO_3^- - N$ ) processes have been usually observed in soils after organic amendments (Bernal and Kirchmann, 1992).

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