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Microbial activity in pig slurry-amended soils under aerobic incubation

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Abstract

A 120-day aerobic incubation experiment was conducted to study the effects of pig slurry application on soil microbial activity. Pig slurry was added to soil at rates of 0 (control treatment), 150 and 300 m^3 ha^{-1}. Soil samples were taken after 0, 7, 14, 30, 45, 60, and 120 days of incubation and analyzed for total organic C and microbial biomass C contents, and basal respiration. Most of the organic C applied to soil with pig slurry was readily decomposed within 30 days. During the first phase (0 to 14–30 days), the addition of pig slurry to the soil, especially at the larger rate, increased microbial biomass C content, microbial biomass C/total organic C ratio, basal respiration, and metabolic quotient. The microbial growth and the increase of their activity that these results reflected were not persistent, since the initially measured values in pig slurry-amended soils decreased and reached those of the control soil in a relatively short time.

Abbreviations: PS – pig slurry; PS150 and PS300 – soils amended with either 150 or 300 m^3 ha^{-1} of PS, respectively; qCO_2 – metabolic quotient; TOC – total organic C

Introduction

The term microbial activity comprises all biochemical reactions catalyzed by microorganisms in soil (Alef & Nannipieri 1995). These reactions are very important in regulating soil properties (Dick 1992). Thus, nutrient cycles in soils are driven by the activities of different microbial communities, which will continuously influence physical structure and organic matter turnover of soil (Gregorich et al. 1996).

Since soil ecosystems display very complex dynamics, the simple determination of the overall number of microorganisms is not useful for evaluating the ecological significance of a given agricultural practice. Brookes et al. (1987) suggested that it is more important to obtain information regarding microbial activity. Together with the microbial biomass C, the following microbiological parameters have been proposed in order to achieve this objective (Pascual et al. 1997): basal respiration, microbial biomass C/total organic C (TOC) ratio, and metabolic quotient (qCO_2). Further, all these measurements have been suggested as potential indicators of soil quality because of their relationship to soil biology, ease of measurement, and rapid response to changes in soil management (Doran & Parkin 1994).

Agricultural soils in semiarid Spanish Mediterranean areas are characterized by low soil organic matter contents, mainly due to the warm and dry climate and to the cultivation system, and feature low fertility levels through extended exposure to erosion and degradation (García et al. 1994; García-Gil et al. 2000). Thus, recycling of animal manures, such as pig slurry (PS), in these agro-ecosystems can increase soil organic matter and nutrient contents and also helps to solve

environmental and economic problems related to the disposal of these waste materials.

The direct application of PS to soil may induce changes in the soil microbial activity. These effects need to be investigated to effectively evaluate this practice (Bandick & Dick 1999). The use of laboratory incubation experiments with soil-slurry mixtures under controlled conditions of moisture and temperature combined with the aforementioned microbiological determinations has been proven to be a valuable tool for providing information in this respect (Perucci 1992; Pascual et al. 1997).

The objectives of this study were to investigate the changes and evolution of microbial activity of a soil amended with PS during an aerobic incubation experiment and to evaluate the extent to which the rate can affect, by use of chemical and biological parameters including TOC, microbial biomass C, microbial biomass C/TOC ratio, basal respiration and qCO_2.

Materials and methods

Pig slurry and soil

The PS sample used in this work was collected from a pig-breeding farm located in the Toledo province (Spain), which employs a closed-cycle production system. The mean composition of PS was the following: dry matter content, 22.4 g l^{-1}; pH, 7.2; electrical conductivity, 19.5 dS m^{-1}; TOC, 11.0 g l^{-1}; total N, 4.9 g l^{-1}; NH_4^+—N, 3.2 g l^{-1}; NO_3^-—N, 42 mg l^{-1}; total P, 137 mg l^{-1}; and total K, 530 mg l^{-1}.

The soil sample used in this experiment was taken from the arable layer (Ap horizon, 0–20 cm depth) of a Typic Haploxeralf (Soil Survey Staff 1998) cropped to barley (*Hordeum vulgare* L.), which is placed in the experimental station ''La Higueruela'' (Toledo, Spain), very close to the pig farm. This soil is representative of the Spanish Mediterranean region and is characterized by a sandy loam texture (sand 59%, silt 22%, clay 19%), slightly acidic pH (5.8), a low electrical conductivity (0.06 dS m^{-1}), and a low content of TOC (12.9 g kg^{-1}) and nutrients (total N, 1.2 g kg^{-1}; available P, 41 mg kg^{-1}; available K, 487 mg kg^{-1}).

Incubation procedure and soil analysis

The effects of PS application to soil at two rates of 150 (PS150) and 300 m^3 ha^{-1} (PS300) in a laboratory aerobic incubation were compared to a control (soil without fertilization). The experiment was carried out in 500 ml open plastic containers into which 500 g of air-dried and 2 mm sieved soil was placed and mixed with either 0, 25, or 50 ml of PS. These loadings correspond to the studied rates, assuming a soil bulk density of 1.5 g ml^{-1} for the top 20 cm. There were three replicates for each treatment and, in order to prevent unnecessary disturbance of the soils by frequent sampling, the three treatments were replicated the number of times a sample was to be taken, i.e. seven. Then, water was added to an equivalent of 60% of the water holding capacity and the containers were incubated in the dark at 28 °C in order to create favorable conditions for microbiological processes in the soil. Moisture losses were monitored by daily weighing during the entire incubation period and corrected by the addition of deionized water.

Soil samples were taken after 0, 7, 14, 30, 45, 60 and 120 days of incubation and analysed for TOC and microbial biomass C contents, and basal respiration. Total organic C content was determined by dichromate oxidation and subsequent titration with ferrous ammonium sulphate (Yeomans & Bremner 1989). Microbial biomass C content was determined by the fumigation-extraction method as reported by Vance et al. (1987); prior to analysis, the samples were incubated for 48 h at 60% of field capacity. Basal respiration was determined by measuring the CO_2 given off in a 14 d incubation experiment at 28 °C, in which 50 g of each soil (at 65% of its field capacity) was placed in a hermetically sealed polyethylene flask with a vial containing 15 ml 0.1 M NaOH; the NaOH being titrated with 0.1 M HCl. The microbial biomass C/TOC ratio and metabolic quotient (qCO_2), i.e, CO_2-C released from the soil sample in 1 h per unit of microbial biomass C content, were also obtained.

The results were statistically analyzed by two-factor ANOVA (rate applied and incubation time are the factors) and mean separation was performed with the least significant difference (LSD) test when *F*-test was significant at a 0.05 probability level.

Results and discussion

Analysis of variance results show that incubation time and doses significantly affected the chemical and microbiological parameters measured, i.e., TOC content, microbial biomass C content, TOC/microbial biomass C ratio, basal respiration and qCO_2 (Table 1). Furthermore, a significant interaction between the factors (i.e. time and slurry dose) was found, which indicates that the evolution of these soil chemical and microbiological properties over time varies with the slurry application and with the rate added.

Figure 1 shows the TOC contents of the control and PS-amended soils during the incubation time. During the first 7 days of incubation, the TOC content decreased significantly in control soil as a consequence of the mineralization processes, which suggests that this soil is not capable of preserving part of the organic C under favorable conditions for microbiological processes. In the case of PS-amended soils, the decrease was greater and continues through the first 30 days, since it was in this first stage when the biodegradable C fractions incorporated with the PS were degraded by the microorganisms. After 45 days, the TOC contents of the three treatments were very similar to one another and almost constant with the incubation time. These results indicate the soft and ephemeral effect of the PS application on soil TOC, which may be ascribed to the small, mostly easily decomposable amount of organic C contained in this kind of manure.

Large variations were previously measured in decomposition rates of different slurries in laboratory incubation studies, and these differences were mainly ascribed to the variable composition of PS, which may result from various factors including animal type and storage, and handling conditions (Kirchmann & Lunvall 1993; Dendooven et al. 1998). For example, Bernal & Kirchmann (1992) found that about 75% of C added to soil with PS was mineralised after 70 days in the laboratory incubation, whereas Saviozzi et al. (1997) reported a value of 44% mineralization after 230 days under similar conditions.

According to several authors (García et al. 2000), the microbial biomass C can be used more effectively than the TOC content as an indicator of variations in soil fertility, since it responds more rapidly and with a greater degree of sensitivity to soil changes. Thus, short-term measurements of microbial biomass can reflect the long-term tendency of the organic matter (Powlson et al. 1987). The mean of 202 mg kg^{-1} measured in control soil (Figure 1) was very low compared to the values obtained by other authors in agricultural and natural soils (Dick 1992; García et al. 2000), which suggests a very low potential microbial activity of this soil (Nannipieri et al. 1990). During the first 30 days of incubation, PS-amended soils showed an enhancement of microbial biomass C with respect to the control soil. This increase, which coincides with the results of other authors (Saviozzi et al. 1997; Griffiths et al. 1998) and could reflect a greater potential microbial activity in these treatments, was greater when higher doses of PS were added. These results can be attributed to the incorporation of easily biodegradable organic matter, which stimulates the growth of the

Table 1. Analysis of variance (ANOVA) results for the data from total organic C (TOC) content, microbial biomass C content, TOC/microbial biomass C ratio, basal respiration, and metabolic quotient (qCO_2) of unamended and pig slurry-amended soils at a rate of either 150 or 300 m^3 ha^{-1}

Parameter	Source of variation								
	Dose			Time			Dose×Time		
	df	*F*	*p* value	df	*F*	*p* value	df	*F*	*p* value
TOC	2	39.78	0.0000***	6	46.11	0.0000***	12	2.22	0.0284*
Microbial biomass C	2	12.62	0.0001***	6	14.74	0.0000***	12	3.68	0.0008***
TOC/microbial biomass C	2	5.31	0.0088**	6	15.22	0.0000***	12	3.17	0.0028**
Basal respiration	2	172.11	0.0000***	6	1296.34	0.0000***	12	76.14	0.0000***
qCO_2	2	30.60	0.0000***	6	644.80	0.0000***	12	18.35	0.0000***

*, **, *** Significant at the probability level of 0.05, 0.01 and 0.001, respectively.

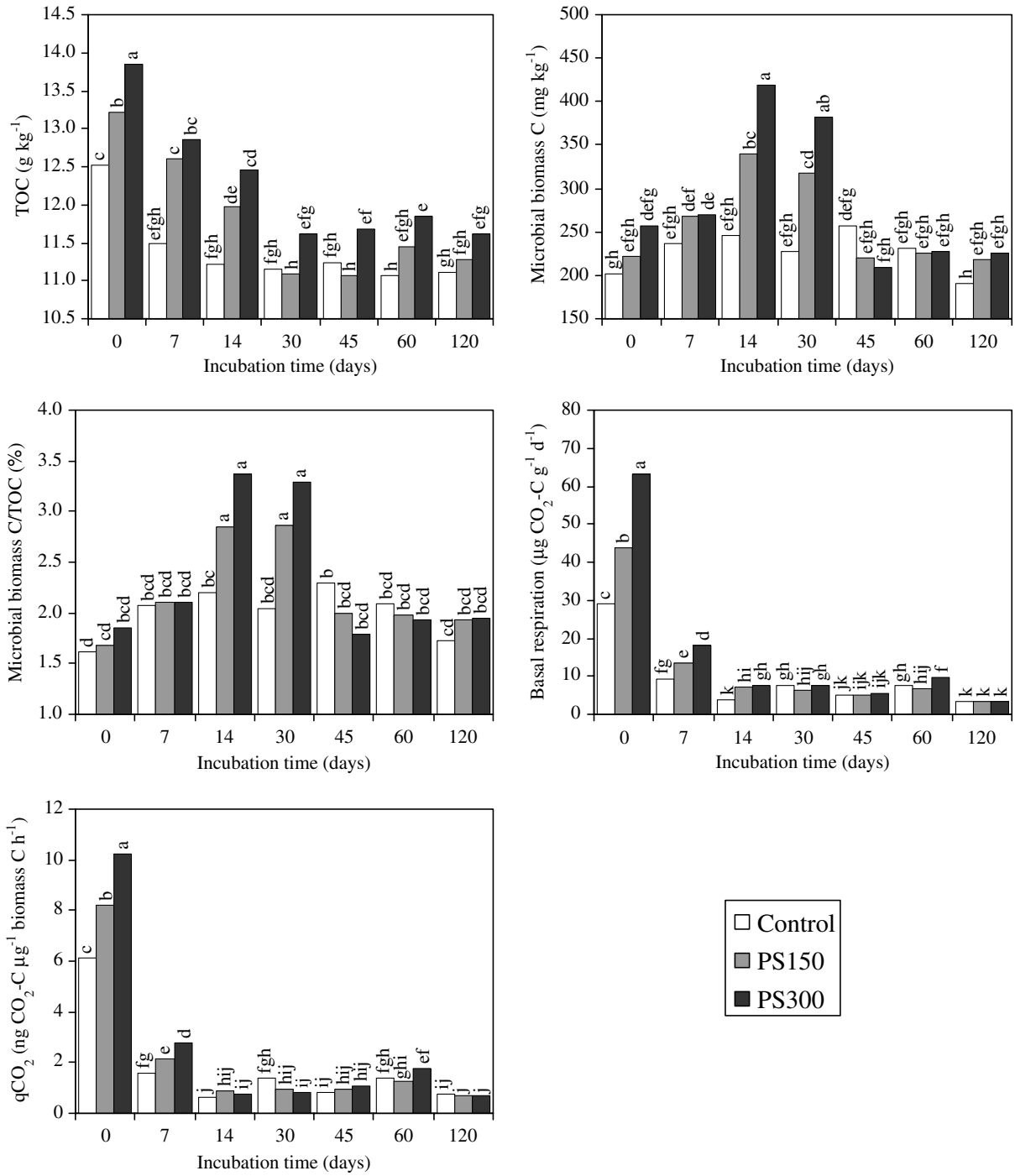

Figure 1. Total organic C (TOC) content, microbial biomass C content, TOC/microbial biomass C ratio, basal respiration, and metabolic quotient (qCO_2) of unamended (control) and pig slurry-amended soils at a rate of either 150 or 300 m^3 ha^{-1} (PS150 and PS300, respectively).

autochthonous microorganisms of the soil (Sakamoto & Oba 1994) and, secondarily, to the incorporation of exogenous microbes existing in the waste material (Perucci 1992).

In control soil, microbial biomass C varied very slightly with the incubation time, whereas in PS-amended soils, a very noticeable increase took place during the first 14 days, especially with the higher dose of PS. Similar increasing tendencies in the initial stages of incubation, paralleling the biodegradation of the more easily biodegradable organic compounds, were also observed previously by other authors with the addition of different organic wastes to soil (De Luca & Keeney 1993; Moreno et al. 1999). After 14 days, the microbial biomass C decreased with incubation time, possibly as a result of the exhaustion of labile organic substrates, which were unable to maintain the significant initial microbial development for a longer period. After 45 days of incubation, there were no significant differences between the values of PS-amended soils and those of the control soil, which indicates that amended soils rapidly recover their initial characteristics.

The microbial biomass C/TOC ratio has been proposed as a sensitive index of the changes undergone by the organic matter content of soil and it has also been used effectively to follow the state of soil organic matter after the addition of organic materials (Sparling 1992; Insam & Merschack 1997). According to Insam & Domsch (1988) and Sparling (1992), the higher the ratio the more active organic matter is present in the soil and the more susceptible the soil organic matter is to change. This could explain why the PS-amended soils, especially in higher doses, showed an increase with respect to the control soil in a first stage of the incubation and why the values were reduced considerably reaching those of the control soil after this initial phase, when TOC contents showed a higher degree of stability (Figure 1).

Although the control soil did not receive fresh organic matter, it showed a relatively high basal respiration at time 0 d (Figure 1). In agreement with Kieft et al. (1987), remoistened soils can show very high initial metabolic activities, possibly as a result of the release of easily biodegradable organic compounds caused by chemical and physical processes initiated by the moistening of dry soils. Besides, basal respiration was significantly raised with the PS addition and with the slurry rate, which indicates that this waste incorporates organic substrates able to increase soil microbial initial activity. Nevertheless, basal respiration decreased with time, and, after 30 days, the PS-amended soils showed values that were similar to those of the control soil.

According to Odum's theory on ecosystem development and the energetic optimization during succession, the respiration to microbial biomass ratio decreases during maturation of an ecosystem (Insam 2001). Anderson & Domsch (1985) adopted this concept for soils by means of the metabolic quotient, which is obtained by dividing the basal respiration by the microbial biomass C content. For many environmental studies the metabolic quotient has proven to be more sensitive than the measurement of microbial biomass or respiration alone (Insam 2001), since it estimates the efficiency of soil microbial populations in utilizing organic C compounds (Wardle & Ghani 1995). Soil ecosystems in a steady-state display qCO_2 values equivalent to the maintenance energy requirements of soil microorganisms (Nannipieri et al. 1997). Stress or soil disturbance cause a decrease in microbial efficiency, i.e., an enhancement of qCO_2, because the microbial population needs to spend more energy on maintenance, limiting the incorporation of added substrate into the cellular components (Leita et al. 1999). Moreover, the qCO_2 could be affected by a shift in the composition of the microbial population, the availability of substrates and diverse abiotic factors (Anderson 1994; Wardle & Ghani 1995; Insam et al. 1996).

PS-amended soils displayed qCO_2 values significantly higher than that of the control soil at the beginning of the incubation (Figure 1). This initial increase, which was greater in soil amended with the high dose, could be explained by a stress caused by the incorporation of fresh exogenous organic materials or some toxic substances with PS. Further, the increased amounts of easily biodegradable organic substrates could favor the prevalence of zymogenous (r-strategists) in contrast to autochthonous microbiota (K-strategists), since the growth strategy of the former is characterized by a higher energy consumption with a lower efficiency than the latter (Bradley & Fyles 1995). In opposition to these possible explications, Sparling (1997) suggested that a potential problem with the qCO_2-stress concept is that when a readily available C source is added to soil there is

inevitably an increase in basal respiration and qCO_2, which could be what happened in this study.

The qCO_2 decreased in all the soils, especially in that with the higher dose of PS, during the first 14 d, after which the values reached the same levels for the three treatments. This decrease could be explained by several, apparently interrelated factors: the progressive degradation of the compounds that initially induce stress and, therefore, the reduction of the adverse conditions imposed by PS; the adaptation of the soil microbiota to the changes introduced by the PS in the soil ecosystem; and the increase of the stabilization of the organic matter and, thus, reduction of the opportunistic microorganisms with respect to native communities characterized by a slower metabolism and able to obtain energy from a more stable organic matter.

Conclusions

The results obtained show that incubation time and PS dose significantly affect soil chemical and microbiological parameters, including TOC, microbial biomass C, microbial biomass C/TOC ratio, basal respiration and qCO_2. Further, the evolution of these soil chemical and microbiological properties over time varies with the rate of PS added. In particular, soil amendment with PS, especially at large rates, produces a significant increase of TOC and microbial biomass C contents, microbial biomass C/TOC ratio, basal respiration, and qCO_2. However, these effects are not persistent. In terms of its transient effects on soil TOC, basal respiration and microbial C dynamics, PS behave very similarly to easily degradable organic C compounds (e.g., glucose).

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