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Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure

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ABSTRACT

Cattle manure is produced in large quantities in industrial breeding facilities and the storage and/or spreading of this waste on land may cause contamination of the atmosphere, soil and water. The aim of the present study was to evaluate the effectiveness of the active phases of composting, vermicomposting, and also a combination of composting and vermicomposting for reducing the polluting potential and for stabilizing cattle manure in the short-term. For this, the degree of decomposition as well as the microbial activity and microbial composition of the resulting products after the active phase of composting and vermicomposting were analysed. None of the treatments significantly reduced the dissolved organic carbon and dissolved organic nitrogen contents relative to the control, and therefore more time may be required for stabilization. Nevertheless, the lowest values of microbial biomass and activity corresponded to the earthworm-worked substrates, in which fungal growth was also promoted; the combined treatment (composting + vermicomposting) was the most effective in terms of stabilizing the cattle manure. Moreover, earthworms promoted the retention of nitrogen and gradual release of P, as well as a reduction in electrical conductivity, thereby producing improved substrates for agricultural use.

1. Introduction

Cattle manure is a valuable resource as a soil fertilizer, as it provides high contents of macro- and micro-nutrients for crop growth and is a low-cost alternative to mineral fertilizers. However, overproduction of this waste substance has led to inappropriate disposal practices such as the indiscriminate and inappropriately-timed application to agricultural fields. Such practices can cause serious environmental problems, including an excessive input of potentially harmful trace metals, inorganic salts and pathogens, increased nutrient loss from soils through leaching, erosion and runoff – caused by lack of consideration of the nutrient requirements of crops – and the emission of hydrogen sulphide, ammonia and other toxic gases (Hutchison et al., 2005).

Animal wastes pose health and environmental risks similar to those of human wastes and should be treated accordingly. Stabilization involves the decomposition of a waste substance to the extent where the hazards are eliminated, and is normally reflected by decreases in microbial activity and concentrations of labile compounds (Benito et al., 2003). Stabilization therefore reduces the environmental problems associated with the management of manure by transforming it into a safer and more stabilized material suitable for application to soil (Carr et al., 1995). Furthermore, depending on the characteristics of the waste, high quality mulches can be obtained for agricultural use, and with further maturation and elimination of phytotoxic compounds, high quality organic fertilizers.

Composting and vermicomposting are two of the best-known processes for the biological stabilization of solid organic wastes. Composting involves the accelerated degradation of organic matter by microorganisms under controlled conditions, in which the organic material undergoes a characteristic thermophilic stage that allows sanitization of the waste by the elimination of pathogenic microorganisms (Lung et al., 2001). Two phases can be distinguished in composting: (i) the thermophilic stage, where decomposition takes place more intensively and which therefore constitutes the active phase of composting; and (ii) a maturing stage which is marked by the decrease of the temperature to the mesophilic range and where the remaining organic compounds are degraded at a slower rate. The duration of the active phase depends on the characteristics of the waste (amount of easily decomposable substances) and on the management of the controlling parameters (aeration and watering). The extent of the maturation phase is also variable and it is normally marked by the disappearance of the phytotoxical compounds. Composting is well established at the industrial scale for solid organic waste treatment, although the loss of nitrogen through volatilization of NH_3 during the thermophilic stage of the process is one of the major drawbacks of the process (Eghball et al., 1997).

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Vermicomposting involves the bio-oxidation and stabilization of organic material by the joint action of earthworms and microorganisms. Although it is the microorganisms that biochemically degrade the organic matter, earthworms are the crucial drivers of the process, as they aerate, condition and fragment the substrate, thereby drastically altering the microbial activity. Earthworms act as mechanical blenders and by comminuting the organic matter they modify its physical and chemical status by gradually reducing the ratio of C:N and increasing the surface area exposed to microorganisms – thus making it much more favourable for microbial activity and further decomposition (Domínguez et al., 1997). Therefore two phases can also be distinguished here, (i) an active phase where the earthworms process the waste modifying its physical state and microbial composition (Lores et al., 2006), and (ii) a maturation-like phase marked by the displacement of the earthworms towards fresher layers of undigested waste, where the microbes take over in the decomposition of the waste. Like in composting, the duration of the active phase is not fixed, and it will depend on the species and density of earthworms, the main drivers of the process, and their ability to ingest the waste (ingestion rate). Vermicomposting is not fully adapted to the industrial scale (Domínguez et al., 1997) and since the temperature is always in the mesophilic range, pathogen removal is not ensured, although some studies have provided evidence of suppression of pathogens (Monroy et al., 2008). In some cases, organic residues require pretreatment before being vermicomposted as they may contain substances that are toxic for earthworms, such as acidic compounds (Nair et al., 2006).

The combination of composting and vermicomposting has recently been considered as a way of achieving stabilized substrates (Tognetti et al., 2007). Composting enables sanitization of the waste and elimination of toxic compounds, and the subsequent vermicomposting reduces particle size and increases nutrient availability; in addition, inoculation of the material resulting from the thermophilic phase of composting with earthworms reduces the expense and duration of the treatment process (Ndegwa and Thompson, 2001).

Although several studies have addressed the optimization of either composting, vermicomposting or composting with subsequent vermicomposting (Domínguez et al., 1997; Frederickson et al., 1997; Ndegwa and Thompson, 2001; Tognetti et al., 2005; Tognetti et al., 2007), there are no studies concerning the efficiency of these three processes together to stabilize a specific organic waste.

To obtain high quality organic fertilizers, it is necessary to understand the changes that the material undergoes during the biological stabilization process. The stability of the final products is essential for its successful application to crops. Thermophilic stage in composting largely conditions microbial communities (Klamer and Bååth, 1998), and so do the earthworms by ingesting the waste (Lores et al., 2006). Since microorganisms are responsible for chemical degradation in the last term, the changes occurred on microbial communities in each process might also condition the further decomposition of the waste, as well as the establishment and survival of beneficial (plant growth promoting bacteria) or deleterious (fecal coliforms) microorganisms for land application. In the present study we evaluated the effectiveness of the active phases of composting, vermicomposting, and a combination of composting and vermicomposting, for the short-term stabilization of cattle manure, by analysing the physicochemical, biochemical and microbiological characteristics of the final products.

2. Material and methods

2.1. Source materials and biostabilization processes

Cattle manure, consisting of a mixture of faeces, urine and straw was obtained from the agricultural cattle complex "Energía Viva, S.A." in León, Spain; the main physicochemical and microbiological characteristics of the manure are summarized in Table 1.

Composting was carried out in five trenches of 42 m long, 4.5 m wide and 1.8 m deep, each of which contained approximately 300 m^3 of material. Throughout the process, the trenches were aerated from the bottom with forced air (through a blower) in order to induce movement of air into the material and deliver oxygen to microorganisms. The functioning of the air blower varied depending on the temperature: (a) continuous aeration when the temperature of the composting mass exceeded 60 °C; (b) intermittent aeration according to a preset cycle of 5 min aeration and 5 min pause when the temperature was between 55 and 60 °C; and (c) intermittent aeration according to a preset cycle of 5 min aeration followed by a pause of 10 min when the temperature was below 55 °C. In addition to the forced ventilation, the compost was turned daily in order to homogenize the mass, and to avoid compaction of the substrate and subsequent low porosity and poor air distribution. The composting material was watered and the moisture content was monitored daily and maintained within 55–65%. At the end of the active phase (15d), ten sub-samples were randomly collected within each trench and composited into single samples, each of 10 l (Gómez-Brandón et al., 2008).

Vermicomposting was carried out in a 1 m^3 vermireactor containing a stable and very active population of the earthworm *Eisenia andrei*. The reactor was fed with different animal manures and mixed agricultural wastes, and supported a population density of 250 g of earthworms kg^{-1} in the top layers. The upper surface of the vermireactor was divided into four independent compartments

Table 1
Mean values ± standard error of the physicochemical and biochemical properties in the initial raw cattle manure and the substrates produced by the different treatments: incubation under field conditions for 15d (control); active phase of composting (composting); vermicomposting, and composting with subsequent vermicomposting (Composting + vermicomposting)

	Raw cattle manure	Control	Composting	Vermicomposting	Composting + vermicomposting
pH	7.70–8.94	8.89–8.78[a]	8.86–8.07[a]	7.73–7.51[b]	7.85–7.14[b]
EC (dS m^{-1})	1.25 ± 0.08	1.32 ± 0.08[a]	2.13 ± 10[b]	0.78 ± 0.02[c]	0.72 ± 0.04[c]
C to N ratio	17.0 ± 0.74	15.7 ± 1.09[a]	17.5 ± 0.33[a]	11.1 ± 0.24[b]	11.3 ± 0.16[b]
Total C (g kg^{-1} dw)	399.2 ± 2.8	395.7 ± 3.2[a]	384.9 ± 2.7[a]	314.0 ± 5.4[b]	309.0 ± 8.6[b]
Total N (g kg^{-1} dw)	23.6 ± 0.9	25.6 ± 1.7[ab]	22.0 ± 0.3[a]	28.3 ± 0.2[b]	27.4 ± 0.8[b]
DON (mg kg^{-1} dw)	2190 ± 380	2260 ± 244[a]	2571 ± 896[a]	3726 ± 153[a]	2165 ± 198[a]
NH_4^+–N (mg kg^{-1} dw)	610 ± 92	534 ± 128[a]	1235 ± 291[b]	276 ± 24[a]	191 ± 30[a]
NO_3^-–N (mg kg^{-1} dw)	19 ± 15	0 ± 0[a]	721 ± 184[b]	917±113[b]	829 ± 110[b]
DOC (mg kg^{-1} dw)	4406 ± 704	6819 ± 772[a]	9338 ± 2103[a]	5249 ± 302[a]	4825 ± 387[a]
Available P (mg kg^{-1} dw)	211 ± 6	175 ± 7[a]	342 ± 22[b]	111 ± 3[c]	109 ± 6[c]

Results of the Tukey HSD test for the different treatments are shown; the data corresponding to the raw manure were not included in the statistical comparisons.
dw: Dry weight.
[a,b,c] Means with the same letter were not statistically different (Tukey HSD test, $\alpha = 0.05$).

and 15 kg of cattle manure were placed in three successive layers (5 kg each) added to each compartment as the waste was processed by the earthworms. The moisture content of the cattle manure in the vermireactor was maintained at 75–80% and the samples were collected from the last layer (40d of earthworm processing) of the reactor once the manure was processed by the earthworms.

Composting plus subsequent vermicomposting was carried out by first composting the manure for 15d, as described above, and then vermicomposting in the 1 m^3 vermireactor for 40d with the earthworm *E. andrei*, as described for the vermicomposting treatment. Samples were collected from the vermireactor 40d after the addition of the third layer of composted manure.

As controls (no treatment), five manure heaps (15 kg each) were maintained under field conditions and moistened twice a week for 15d. All samples were placed in sealed plastic containers and stored at 5 °C until analysis.

The aim of this study was to compare stabilization treatments and the duration of the treatments differed as it depended on the time necessary for the completion of the active phase in each process: 15d for the active or thermophilic phase of composting, 40d for processing of the manure by the earthworms and 55d for the combined treatment. The duration of these processes could not be modified without altering the processes themselves.

2.2. Chemical analyses

The moisture and organic matter contents of the samples were determined after drying at 105 °C for 24 h and ashing at 550 °C for 4 h, respectively. The pH and electrical conductivity were determined in water extracts (1:20, w/v). Total C and N were measured in oven-dried (60 °C) and ball-milled sub-samples, with a Carlo Erba NA 1500 C/N analyzer. Inorganic nitrogen (NH_4^+ and NO_3^-) was determined in 0.5 M K_2SO_4 extracts (1:10 w/v) by the modified indophenol blue technique (Sims et al., 1995), with a microplate reader (Bio-Rad Model 550). Total extractable N was determined after oxidation with $K_2S_2O_8$ as described by Cabrera and Beare (1993) and the dissolved organic nitrogen (DON) content was calculated as (total extractable N)–(NH_4^+–N +NO_3^-–N). Dissolved organic carbon (DOC) in the cattle manure and the final products were determined colorimetrically at 590 nm after moist digestion ($K_2Cr_2O_7$ and H_2SO_4) of aliquots of 0.5 M K_2SO_4 extracts (1:10 w/v) of the samples.

Available P was analyzed in ammonium bicarbonate–diethylene triaminepentaacetic acid extracts of oven-dried and ball-milled samples (1:6, w/v) by induced coupled plasma optical emission spectrometry (Soltanpour and Schwab, 1977).

2.3. Microbiological and biochemical analyses

Microbial activity and biomass were assessed by measurement of the rate of CO_2 evolution from the samples during 6 and 12-h incubation, for basal and substrate-induced respiration (SIR), respectively. Prior to incubation, 0.75 ml of glucose solution (equivalent to 100 mg glucose g^{-1} dw sample) was added to samples for the SIR assay. The evolved CO_2 was trapped in 0.02 and 0.04 M NaOH (basal and SIR, respectively) and then measured by titration with HCl to a phenolphthalein endpoint, after addition of excess $BaCl_2$ (Anderson, 1982). Incubation times, NaOH and glucose concentrations were adjusted in order to obtain the most accurate response for this type of organic samples as shown in Aira et al. (2006, 2007a).

Fungal biomass was determined by quantification of ergosterol, a membrane-bound molecule commonly used as a fungal biomarker. The ergosterol content of the samples was extracted by the microwave assisted extraction method and determined by HPLC analysis. Briefly, samples (500 mg fresh weight) were digested with 2 ml of methanol and 0.5 ml of 2 M NaOH in a scientific microwave oven (CEM Corporation MDS-2000), processed at 2450 MHz and 630 W maximum output and irradiated at medium power (60% of maximum power output, manufacturer's setting) for 20 s three times, with 1 min of cooling between each time. The contents were extracted with pentane (3 × ca. 2 ml), the pentane extracts were then evaporated to dryness under a stream of N_2 gas and then redissolved with 1 ml of methanol and filtered through a 0.2 μm syringe filter (MFS) prior to HPLC analysis (Young, 1995). Dehydrogenase enzyme activity was measured by estimation of the rate of reduction of triphenyltetrazolium chloride (TTC) (1.5%) to triphenylformazan (TPF), after incubation at 30 °C for 24 h, in a microplate reader (Bio-Rad Model 550), at 545 nm (Casida et al., 1964). Protease activity was measured by determination of the amino acids released, after incubation of the samples (1 g fresh weight) with sodium caseinate (2%) for 2 h at 50 °C and with Folin–Ciocalteu reagent, in a microplate reader (Bio-Rad Model 550), at 700 nm (Ladd and Butler, 1972). β-Glucosidase activity was assessed by determination of the *p*-nitrophenol (PNP) released, after incubation of the samples (1 g fresh weight) with β-D-glucopyranoside (0.025 M) for 1 h at 37 °C, in a microplate reader (Bio-Rad Model 550), at 400 nm (Eivazi and Tabatabai, 1988). Alkaline phosphomonoesterase activity was estimated by determinatio of the PNP released, after incubation of the samples (1 g fresh weight) with *p*-nitrophenyl phosphate (0.025 M) for 1 h at 37 °C, with a microplate reader (Bio-Rad Model 550), at 400 nm (Eivazi and Tabatabai, 1972).

For quantification of actinomycetes, samples were homogenized and added to a tris-buffered saline solution, and then serially diluted and incubated at room temperature for 2 h. Dilutions were plated on actinomycetes agar and incubated at 30 ° C. The number of colony forming units (CFU) was counted after 72 h.

2.4. Statistical analyses

Results are means of either five replicates, for the composting and control treatments, or of four replicates, for the vermicomposting and composting plus vermicomposting treatments. One-way analysis of variance and comparison of means based on the Tukey honestly significance difference test (HSD, $P < 0.05$) were used to determine significant differences between treatments. Differences between the raw manure and the substrates after the treatments were analysed by *t*-test. All statistical tests were evaluated at the 95% confidence level. The relationships between variables were defined by regression analysis. Statistical analyses were carried out with SPSS 11.0 for Windows.

3. Results and discussion

Although several physical, chemical, and biological parameters have been suggested as indicators of compost stability, and some of them, such as respiration rates, constitute widely-used, rapid and reliable measurements, it is not easy to establish the stability of an organic amendment based on just one parameter, and the threshold values may not be applicable to all composts, given the variety of parent wastes and feedstock as well as the composting processes (more or less controlled) from which they are originated. In this sense, an integrated approach is recommended for a more accurate determination.

3.1. Evaluation of the chemical changes

The main chemical properties of the cattle manure processed by composting, vermicomposting, composting plus vermicomposting and the control treatment are summarized in Table 1. The control

treatment and the active phase of the composting process did not significantly change the pH of the initial raw waste (*t*-test: $P = 0.262$, $P = 0.859$, respectively), whereas vermicomposting and composting plus vermicomposting significantly decreased the pH (*t*-test: $P = 0.009$, $P = 0.017$, respectively). Other authors have found similar results in vermicomposting experiments, and have suggested that the mineralization of N and P compounds, the release of CO_2 and organic acids from microbial metabolism, and the production of humic and fulvic acids, as possible causes of the decrease in pH during vermicomposting (Ndegwa and Thompson, 2001; Kaushik and Garg, 2004).

The electrical conductivity (EC) reflects the salinity of an organic amendment. High salt concentration may cause phytotoxicity problems and therefore EC is a good indicator of the suitability and safety of a compost or vermicompost for agricultural purposes. EC was affected in different ways by the application of the different treatments. The value of this parameter increased after the active phase of composting, relative to the control, whereas it decreased after vermicomposting and the combined treatment of composting and vermicomposting (Table 1). A sharp increase due to the release of soluble salts like ammonium and phosphate after the degradation of the most labile compounds in the thermophilic stage of composting has also been reported by several authors (Villar et al., 1993). During vermicomposting the minor production of soluble metabolites such as ammonium (NH_4^+), as well as precipitation of the dissolved salts may lead to lower EC values (Mitchell, 1997). The EC of the cattle manure after the different treatments did not exceed the threshold value of 3 dS m^{-1} that indicates a material that can be safely applied to soil (Soumaré et al., 2002).

The C to N ratio indicates the degree of decomposition of a waste, as carbon is lost as CO_2 during biooxidation, whereas N is lost at a lower rate, and therefore the more decomposed a waste, the lower the C to N ratio. In the present study, total C was significantly reduced after the vermicomposting and combined treatment, whereas it remained high after the active phase of composting. In contrast, total N content was higher after vermicomposting and the combined treatment than after the active phase of composting. Consequently, the C to N ratio was significantly lower in the treatments involving vermicomposting, which indicates that they underwent more intense decomposition (Table 1).

The concentration of DON was still high after stabilization, with no differences between the treatments, indicating that in this respect, stabilization was not sufficient, as established by Hue and Liu (1995). The concentration of mineral N (NH_4^+ and especially NO_3^-) was significantly higher following the three treatments than in the raw cattle manure, indicating an important degree of mineralization. The concentration of NH_4^+ only increased after the active phase of the composting process, with significantly higher values than in the other treatments (Table 1).

There were no significant differences in the DOC contents corresponding to the different treatments. The content of labile carbon was higher following the active phase of composting, vermicomposting and the control treatment, than in the initial manure. An organic amendment with a high DOC can cause serious damage to crops, since it will continue to degrade in the soil consuming oxygen, hampering root respiration and leading to the production of phytotoxical compounds such as SH_2 (Mathur et al, 1993). Although DOC consistently decreases during a complete composting process, the initial degradation of solid polymeric material after the thermophilic stage in the composting substrate may lead to the formation of soluble organic matter, which would increase the DOC concentration. Gómez-Brandón et al. (2008) observed that the DOC content decreased sharply within the first two weeks of composting, to values of less than 4000 mg kg^{-1} dw by the end of the process, which would guarantee safe plant growth. This pattern was not observed in the present study, in which the active phase of composting increased the DOC relative to that in the raw manure. The increase was minor following the vermicomposting and the combined treatments, but nevertheless shows an incomplete and active degradation that mobilises the insoluble carbon from the organic matter to the soluble phase. We expect that if the processing time was longer, the DOC would decrease as shown by, for example, Gómez-Brandón et al. (2008) for composting, and Aira et al. (2007a) for vermicomposting.

3.2. Evaluation of the microbiological and biochemical changes

From the stabilization treatments assayed, only vermicomposting and composting with subsequent vermicomposting reduced significantly the microbial biomass of the raw manure (*t*-test, $P = 0.029$, $P = 0.007$ respectively). These treatments were also significantly different from the control (Fig. 1a). On the contrary, the active phase of composting did not reduce the microbial biomass of the manure and was not significantly different from the control (Fig. 1a). Similar results were reported by Tognetti et al. (2005) for comparison between composting and vermicomposting. It is known from incubation experiments that the passage of microorganisms through the earthworm gut has a negative effect and leads to a short-term decrease in microbial biomass through feeding and digestion (Aira et al., 2006).

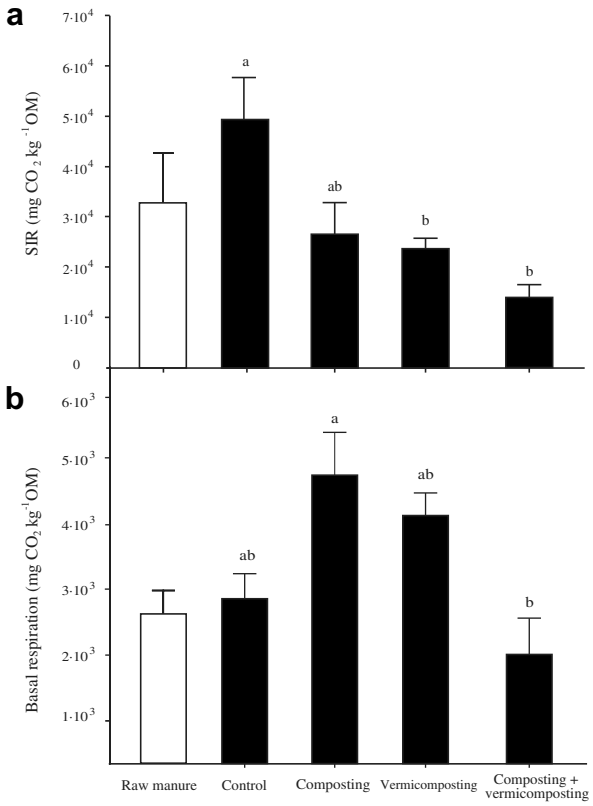

Fig. 1. Microbial biomass (a) and microbial activity (b) in the raw cattle manure and the substrates produced by the different treatments: incubation under field conditions for 15d (control); active phase of composting (composting); vermicomposting, and composting until the end of the active phase plus subsequent vermicomposting (composting + vermicomposting). Values are means ± standard error (control and composting: $n = 5$; vermicomposting and composting plus vermicomposting: $n = 4$). Results of the Tukey HSD test for the different treatments are shown; the data corresponding to the raw manure were not included in the statistical comparisons. Different letters indicate significant differences at $P < 0.05$.

The stabilization treatments resulted in substrates with similar (*t*-test, control and combined treatments, $P = 0.614$, $P = 0.322$, respectively) or even higher microbial activity (*t*-test, active phase of composting, vermicomposting, $P = 0.018$, $P = 0.010$, respectively) than in the raw manure (Fig. 1b); after the stabilization treatments none of the substrates differed significantly from the control, which indicates that decomposition was incomplete and total stabilization was not achieved. The high activity registered after the composting treatment may be due to the short duration of the process. Gómez-Brandón et al. (2008) reported high metabolic rates within the first weeks in composting of cattle manure, which then tended to decrease during the maturation phase. Despite the above-mentioned reduction in microbial biomass by the earthworms, microbial activity was not affected, and it is possible that the passage through the earthworms' gut favoured the appearance of a reduced but more catabolically active microflora (Aira et al., 2007a). Nevertheless, this pattern was not observed after the earthworms had digested the compost from the active phase, when a significant reduction in microbial activity was produced.

Fungal biomass was not affected by the active phase of composting in comparison with the control (Fig. 2) and the ergosterol content remained at approximately the same levels as in the raw manure (*t*-test, $P = 0.652$). This was not true for vermicomposting and the combined treatment, as these had a marked effect on fungal growth, and the ergosterol content of the raw manure was increased by 28 and 41 times respectively following these treatments (*t*-test, $P < 0.001$ and $P = 0.001$ respectively); this indicates significantly higher fungal contents than both the control and the compost. Enhanced fungal growth in degrading organic matter has been attributed to the depletion of easily degradable organic compounds and the subsequent decrease in bacteria. Nevertheless, this was not the cause of the increase in fungal abundance in both vermicomposts since DOC and DON contents were still high in these substrates. The higher ergosterol content in both kind of vermicompost shows that earthworms enhanced fungal growth in the short-term, either indirectly through the modification of the substrate, or directly through their feeding activity. Enhanced fungal biomass after gut transit was also found by Aira et al. (2006) after vermicomposting of pig slurry with *Eisenia fetida*, and Pizl and Nováková, 2004, who found that the density of microfungi was higher in the earthworm gut and vermicompost than in fresh substrate in vermicomposting facilities with the earthworm *E. andrei*.

The number of actinomycetes was also significantly affected by the treatment applied (Fig. 3); it was very high in the raw manure and the active phase of composting increased the abundance slightly. The most remarkable result was that both vermicomposting treatments showed abundances of these microorganisms 100 times lower than the active phase of composting. Actinomycetes compete rather ineffectively when nutrient levels are high, develop far more slowly than most bacteria and fungi, and are typical during the final stages of decomposition (Alexander, 1980). Thus their abundance in the fresh waste and in the control treatment is difficult to explain. Actinomycetes are tolerant to high temperatures, some of them being facultative thermophilic microorganisms, and thus they may be expected to survive the thermophilic stage of the composting treatment. Nakasaki et al. (1985) studied the changes in the different microbial groups in composting of sewage sludge and reported that the increase in actinomycetes was typical of the final stage of the thermophilic phase. The significantly lower numbers of these microorganisms in both vermicompost treatments showed that the earthworms may have some effect. Earthworms have been found to cause changes in the microbial communities in several organic wastes during vermicomposting (Lores et al., 2006), moreover, these microbial communities have been found to be more efficient metabolically (Aira et al., 2007a). Development of actinomycetes was probably not favoured during vermicomposting of the cattle manure because their ineffective competition with the more active microbial communities promoted by the earthworms.

The changes in dehydrogenase, protease, glucosidase, and phosphatase (key enzymes involved in aerobic metabolism, as well as degradation of polypeptides, polysaccharides and phosphate esters, respectively) are summarized in Fig. 4. Dehydrogenase activity was significantly lower after both vermicomposting treatments than after the active phase of composting (Fig. 4a). However, the activity was not significantly lower in any of the substrates than in the control probably due to the high DOC content which correlated positively with this parameter ($R^2 = 0.235$; $P = 0.019$). This indicates that the substrates were not sufficiently stabilized. Dehydrogenase activity is used as a measure of overall microbial

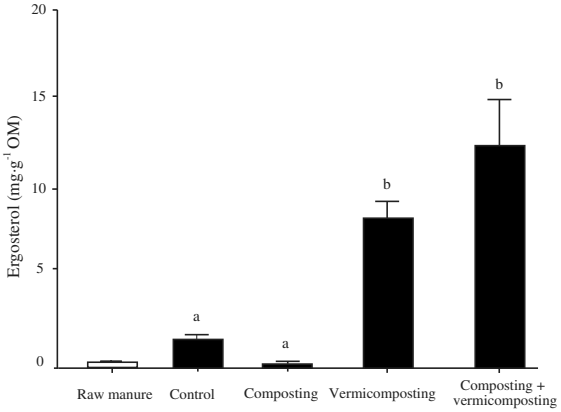

Fig. 2. Ergosterol content of the raw cattle manure and the substrates produced by the different treatments: incubation under field conditions for 15d (control); active phase of composting (composting); vermicomposting, and composting until the active phase plus subsequent vermicomposting (composting + vermicomposting). Values are means ± standard error (control and composting: $n = 5$; vermicomposting and composting plus vermicomposting: $n = 4$). Results of the Tukey HSD test for the different treatments are shown; the data corresponding to the raw manure were not included in the statistical comparisons. Different letters indicate significant differences at $P < 0.05$.

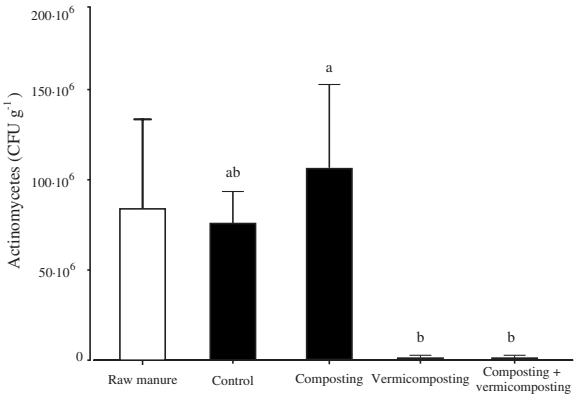

Fig. 3. Abundance of actinomycetes in the raw cattle manure and the substrates produced by the different treatments: incubation under field conditions for 15d (control); active phase of composting (composting); vermicomposting, and composting until the end of the active phase plus subsequent vermicomposting (composting + vermicomposting). Values are means ± standard error (control and composting: $n = 5$; vermicomposting and composting plus vermicomposting: $n = 4$). Results of the Tukey HSD test for the different treatments are shown; the data corresponding to the raw manure were not included in the statistical comparisons. Different letters indicate significant differences at $P < 0.05$.

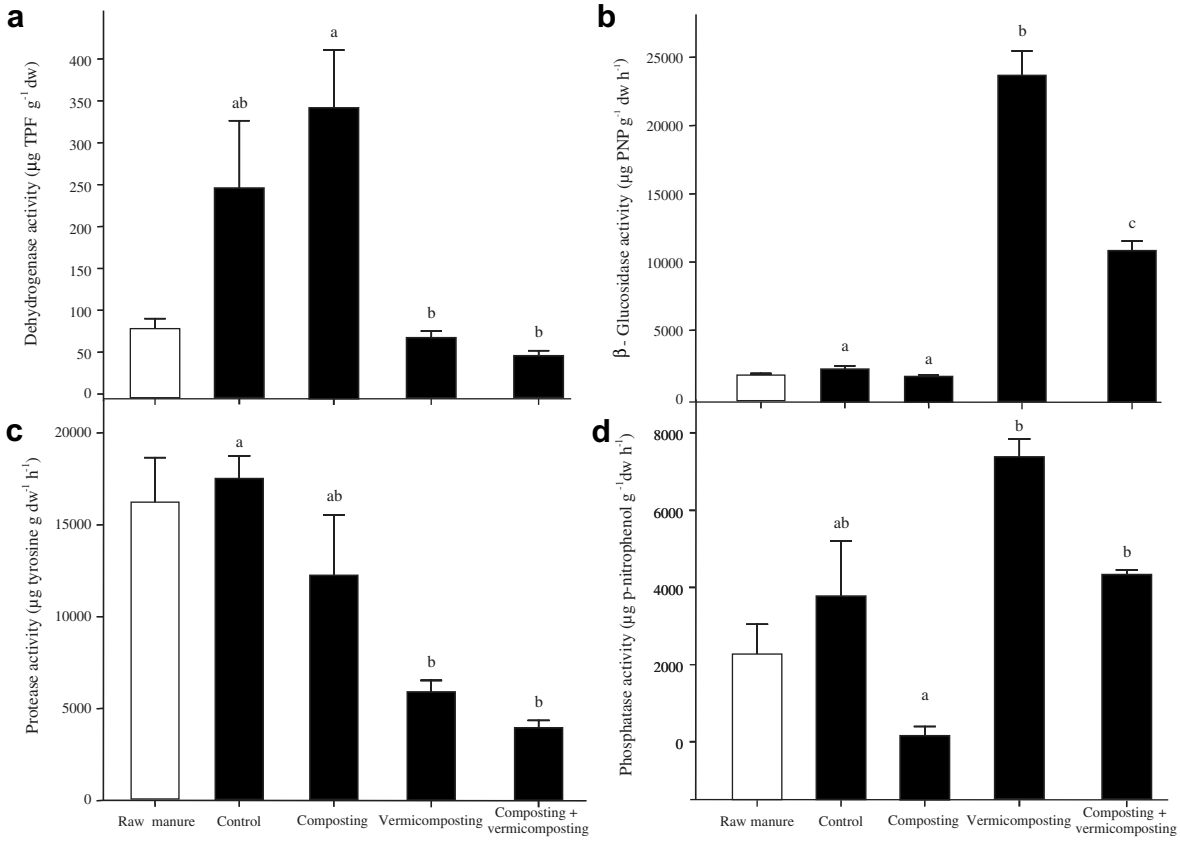

Fig. 4. Dehydrogenase (a), protease (b), β-glucosidase (c), and phosphatase (d) activities in the raw cattle manure and the substrates produced by the different treatments: incubation under field conditions for 15d (control); active phase of composting (composting); vermicomposting, and composting until the end of the active phase plus subsequent vermicomposting (composting + vermicomposting). Values are means ± standard error (control and composting: $n = 5$; vermicomposting and composting plus vermicomposting: $n = 4$). Results of the Tukey HSD test for the different treatments are shown; the data corresponding to the raw manure were not included in the statistical comparisons. Different letters indicate significant differences at $P < 0.05$.

activity; and the levels therefore indicated that both vermicomposting treatments produced more stabilized substrates than the active phase of composting, in accordance with the basal respiration levels obtained. Nevertheless, it should be noted that this analysis only accounts for a limited percentage of respiration since oxygen is a better electron acceptor than the TTC used in our assay (Nannipieri et al., 1990). The active phase of the composting process exhibited the highest degree of protease activity, and differed significantly from the control; this high activity explains the high N–NH_4^+ concentration found after both treatments. On the contrary, treatments with earthworms resulted in significantly lower protease activity than in the control (three times lower following vermicomposting, and 4.4 times lower following the combined treatment: Fig. 4c). Significant reductions in the activity of this enzyme were also observed by Aira et al. (2007b) in vermicomposting experiments with pig slurry. Protease activity is highly dependent on substrate availability (Aira et al., 2007b), and therefore it is a very good indicator of the level of decomposition of a substrate. According to this criterion, the vermicomposted materials were significantly more stabilized than the compost. β-Glucosidase activity of the manure was significantly affected by the treatments applied. The active phase of composting resulted in the lowest values, which were not significantly different from the control values, whilst both vermicomposting treatments resulted in significantly higher activity, the highest corresponding to the vermicomposting treatment (21 times higher than the control). β-Glucosidase catalyzes the breakdown of glucosides to glucose, one of the last steps in the degradation of cellulose, and it is assumed that in soils this enzyme is mainly produced by fungi (Hayano and Tubaki, 1985). In the present study, a significant correlation between the ergosterol content of the substrates and their ß-glucosidase activity ($R^2 = 0.406$, $P = 0.004$) was observed, which is consistent with the results of previous studies (Aira et al 2006). The higher activity detected after both vermicomposting treatments therefore corresponded to the higher fungal abundance than observed after composting. None of the assayed treatments showed significant differences in alkaline phosphatase activity in comparison with the control; nevertheless, the substrate produced after the active phase of composting showed significantly lower activity than both kinds of vermicompost (22 and 13 times lower) (Fig. 4d). Phosphatase catalyzes hydrolysis of the phosphoric esters to inorganic P, which inhibits the activity of the enzyme when present at high quantities in the substrate. In the present study we found a significant negative correlation between phosphatase activity and available P ($R^2 = 0.532$; $P < 0.0001$). This explains the low activity after the active phase of composting, as a higher concentration of soluble P than in the rest of substrates was detected (Table 1). The high activity following vermicomposting and the combined treatment shows that there were still sufficient amounts of phosphate esters available and that the release of P was not sufficient to produce enzyme inhibition. Benítez et al. (2005) observed that phosphatase activity increased gradually throughout the vermicomposting process, first reaching stability and then decreasing slightly. The recovery of the phosphatase activity after vermicomposting the compost from the active phase was remarkable and was probably due to a more gradual decomposition by the earthworms.

4. Conclusions

The treatments considered here (active phase of composting, vermicomposting and composting with subsequent vermicomposting) exhibited important differences in efficiency in terms of the short-term stabilization of cattle manure. Although the reduction of easily metabolizable compounds was not sufficient for complete stabilization in any of the treatments and longer processing times appear to be necessary, there were clear differences in the microbial composition – and consequently the degrading metabolism of the different substrates. While the cattle manure subjected to the active phase of composting did not differ significantly from the control, both vermicomposts exhibited lower levels of actinomycetes, enhanced fungal growth and had low concentrations of total microbial biomass. In addition, earthworms appeared to modify the degrading activity of the manure to a much greater extent than the active phase of composting. This was reflected by the lower EC, C to N ratio and pH, as well as by a more gradual release of P, which made the vermicomposts more suitable substrates for agronomic purposes.

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