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Corresponding Author: M. Gómez-Brandón

Corresponding Author´s Institution: Universidad de Vigo

Order of Authors: M. Gómez-Brandón; C. Lazcano; J. Domínguez

Abstract: We examined chemical, microbiological and biochemical parameters in order to assess their effectiveness as stability and maturity indicators during the composting process of cattle manure. The composting material obtained after 15 d in trenches and at different times during the maturation phase (i.e. 80, 180 and 270 d) were analyzed. We found that the material collected at the end of the active phase was inadequate to be applied to soil as organic amendment due to its high content of NHþ, its high level of phytotoxicity and the low degree of organic matter stability. After a maturation period of 80 d, the stability of the sample increased. This was shown by a reduction in the dissolved organic carbon (DOC) content and NH4+ concentration and also by a reduction in the microbial activity and biomass; however, 180 d of composting were not sufficient to reduce the phytotoxicity to levels consistent for a safe soil application. Among the various parameters studied, the change in DOC with composting time gave a good indication of stability.

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The evaluation of stability and maturity during the composting of cattle manure

María Gómez-Brandón*, Cristina Lazcano, Jorge Domínguez

Departamento de Ecología y Biología Animal. Universidad de Vigo. Vigo E-36310. Spain

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*Corresponding author:

mariagomez@uvigo.es (M. Gómez-Brandón)

Introduction

Cattle manure is a valuable resource as a soil fertilizer, providing a high content of macro- and micronutrients for crop growth, and represents a low-cost alternative to mineral fertilizers (Sharpley and Smith, 1995). However, the overproduction of organic wastes by cattle breeding has led to inappropriate disposal practices; for example, their indiscriminate application to agricultural fields and their improper timing of application, that is, they are not applied when it would be most beneficial for crops. These practices could cause serious environmental problems that could include an excessive input of potentially harmful trace metals, inorganic salts and pathogens (Hutchison et al., 2005); an increase in nutrient loss from soils through leaching, erosion and runoff due to not considering the nutrient requirements of crops (Vervoort et al., 1998); and the emission of hydrogen sulphide, ammonia and other toxic gases (Salazar et al., 2005).

The composting process may significantly reduce the environmental problems associated with the management of manures by transforming them into a safer and more stabilized material for application to soil (Carr et al., 1995). To obtain high quality compost it is necessary to understand the changes that the material undergoes with the composting process. The stability and maturity of the compost are essential for its successful application, particularly for composts used in high value horticultural crops (Wang et al., 2004). The terms stability and maturity are usually used interchangeably to describe the degree of decomposition and transformation of the organic matter in compost (Zmora- Nahum et al., 2005), despite the fact they describe different properties of the composting substrate. Stability is strongly related to the rate of microbial activity in compost, and is evaluated by different respirometric measurements (Lasaridi and Stentiford, 1998) and/or by studying the transformations in the chemical characteristics of compost organic matter (Pichler and Kögel-Knabner, 2000). Respirometric tests have

been shown to be adequate for assessing compost stability because they are able to measure the extent of which readily biodegradable organic matter has decomposed during the composting process (Adani et al., 2004). Compost maturity generally refers to the degree of decomposition of phytotoxic organic substances produced during the active composting phase and to the absence of pathogens and viable weed seeds (Wu et al., 2000). Both these properties are critical for the quality and marketability of the final product.

The application of unstable compost to soil may produce a competition for oxygen between microbial biomass and plant roots/seeds. This fact can deprive plant roots/ seeds of oxygen, and lead to the production of H_2S and NO_2^- (Mathur et al., 1993). Another problem is nitrogen starvation of plants as microorganisms scavenge soil N to make up for the deficit resulting from the application of unstable compost with a high C to N ratio. The phytotoxicity of unstable composts represents another major problem; this is due to the emission of ammonia and the presence of other phytotoxic substances like phenolic compounds and ethylene oxide that is synthesized during the decomposition of unstable compost in soil. Low-molecular weight organic acids (i.e. acetic, propionic and butyric acids) produced by the anaerobic digestion of the organic matrix are also responsible for compost phytotoxicity (Fuchs, 2002).

Management of the composting process must consider the potential agronomic value of the end product and its suitability for plant crops by evaluating its degree of maturity. Biological methods involving seed germination tests and plant growth bioassays have been used to evaluate the maturity of compost (Cooperband et al., 2003). This is a tedious work and there are disagreements regarding the ability of these tests to determine compost maturity (Brewer and Sullivan, 2003). A large variety of techniques have been reported for the determination of compost stability (Wang et al., 2004).

Chemical parameters such as pH, electrical conductivity (EC), cation exchange capacity, dissolved organic carbon (DOC) and the ratios of C to N and NH_4^+ to NO_3^- have been applied as indicators of stability. Since stabilization implies the formation of humic-like substances, humification indexes are generally accepted as a criterion of stability, but their absolute values vary greatly among composts of different source materials. Moreover, their determination requires proper separation of the non-humic fraction from the fulvic acid fraction because other compounds with similar structure to humic substances but different biological meaning (i.e. lignin residues, quinones, polyphenols, fats, etc.) can be extracted (Sánchez-Monedero et al., 1999). Stability indicators based on the study of microbial biomass and its activity have also been proposed. Mondini et al. (2006) reported that microbial biomass can be used as a stability parameter in ligno-cellulosic waste composts because it clearly reflects the transformation of organic matter during the composting process. Respiration (CO_2 evolution rate and/or O_2 uptake rate) is a general measure of microbial activity, and it has been widely used to evaluate the stability of compost (Gómez et al., 2006). The ATP content and enzyme activities are also useful as indicators of compost stability (Tiquia et al., 2002; Boulter-Bitzer et al., 2006).

The use of different parameters appropriate to determine the maturity and/or stability of composts will allow us to broaden our knowledge about the composting process. Therefore, the two major objectives of this study were (a) to describe the chemical, microbiological and biochemical changes during the industrial composting of cattle manure and (b) to compare different parameters with respect to their ability to evaluate compost stability and maturity during the industrial composting of cattle manure.

1 **Material and Methods**

2 Source materials and composting process

3 This study followed the composting process of fresh cattle manure obtained from the
4 agricultural cattle complex ‘‘Energía Viva, S.A.’’ in León, Spain. The researchers did
5 not control the composting operation or attempt to influence the course of the
6 composting process, which involved an active phase of 15 d, followed by a maturation
7 stage in piles for 270 d.

8 Cattle manure subject to the active phase in five trenches with approximate
9 dimensions of 42 m long, 1.8 m wide and 4.5 m high where each contained
10 approximately 300 m3 of material. Throughout the process, these trenches were aerated
11 from the bottom with forced air through a blower in order to induce air convection
12 movement into the material and deliver oxygen to microorganisms. The functioning of
13 the air blower varied as a function of the temperature: (i) continuous aeration when the
14 temperature of the composting mass overcame the value of 60 ºC; (ii) intermittent
15 aeration according to a preset cycle of 5 min aeration and 5 min pause when the
16 temperature was found between 55 ºC and 60 ºC; and (iii) intermittent aeration
17 according to a preset cycle of 5 min aeration followed by 10 min pause when the
18 temperature was below 55 ºC. The forced ventilation was combined with daily turnings
19 in order to homogenize the composting mass and to avoid the substrate compaction and
20 the subsequent low porosity and deficient air distribution. The composting material was
21 watered with water and the moisture content was controlled daily and kept within the
22 range of recommended values (45–65%; Miller, 1993).

23 During the curing phase, the composting mixture from each trench was pilled up
24 and left to mature in maturation piles (50 m long, 2 m wide and 2 m high) up to 270 d in
25 a space covered on top by a ceiling with the sides opened. These piles were turned for

1 aeration twice a month and sporadically watered with leachates from the cattle farm.
2 Samples were collected at 10 random locations at 15, 80, 180 and 270 d and thoroughly
3 mixed to generate composite samples. All the samples were stored in sealed plastic
4 containers that were kept at 5 ºC until they were analyzed. The initial fresh cattle
5 manure was also analyzed for comparison.
6
7 Chemical analyses
8 EC and pH were analyzed in water extracts (1:10, w/v). Total C and N contents were
9 analyzed on a Carlo Erba 1500C/N analyzer on dried samples. DOC was determined in
10 0.5 M K2SO4 extracts (1:50, w/v) by heat digestion (150 ºC, 30 min) with sulphuric
11 acid and potassium dichromate and read in a Bio-Rad Microplate Reader 550 at 590 nm.
12 Inorganic nitrogen (NH_4^+ and NO_3^-) was determined in 0.5 M K2SO4 extracts (1:5,
13 w/v) using the modified indophenol blue technique (Sims et al., 1995) with a Bio-Rad
14 Microplate Reader 550. The content of P was analyzed in ammonium bicarbonate-
15 diethylenetriaminepentaacetic acid (AB-DTPA) extracts (1:6, w/v) by atomic absorption
16 spectrophotometry (Soltanpour and Schwab, 1977).
17
18 Microbiological and chemical analyses
19 Microbial biomass carbon (Cmic) was determined by the chloroform fumigation–
20 extraction method (Vance et al., 1987) on moist samples (5 g fresh weight). The filtered
21 extracts of both fumigated and non-fumigated samples were analyzed for soluble
22 organic C using a Microplate Reader (Bio-Rad Microplate Reader 550, 590 nm). Cmic
23 was estimated as the difference between the organic C extracted from the fumigated and
24 the nonfumigated sample, multiplied by the K_2SO_4 extraction efficiency factor for
25 microbial C (Kc = 2.64). Ergosterol is a membrane-bound molecule commonly used as

1 a fungal biomarker (Bååth and Anderson, 2003). The ergosterol content of the samples
2 was extracted by the microwave assisted extraction method and determined by HPLC
3 analysis (Young, 1995). Microbial activity was assessed by measuring the rate of CO_2
4 evolution from the samples (5 g fresh weight) after 6 h of incubation. The evolved CO2
5 was trapped in 0.02 M NaOH and then measured by titration with HCl to a
6 phenolphthalein endpoint, after adding excess $BaCl_2$ (Anderson, 1982). Alkaline
7 phosphomonoesterase activity was estimated by determining the p-nitrophenol (PNP)
8 released, after incubating the samples (1 g fresh weight) with p-nitrophenyl phosphate
9 (0.025M) for 1 h at 37 ºC, in a Bio-Rad Microplate Reader 550 at 400 nm (Eivazi and
10 Tabatabai, 1972). β-glucosidase activity was assessed by determining the PNP released,
11 after incubating the samples (1 g fresh weight) with β -D-glucopyranoside (0.025 M) for
12 1 h at 37 ºC, in a Bio-Rad Microplate Reader 550 at 400 nm (Eivazi and Tabatabai,
13 1988). Protease activity was measured by determining the amino acids released, after
14 incubating the samples (1 g fresh weight) with sodium caseinate (2%) for 2 h at 50 ºC,
15 using Folin–Ciocalteu reagent, in a Bio-Rad Microplate Reader 550 at 700 nm (Ladd
16 and Butler, 1972). Cellulase activity was estimated by determining the reducing sugars
17 released after incubating the samples (5 g fresh weight) with carboxymethyl cellulose
18 sodium salt (0.7%) for 24 h at 50 ºC, in a Bio-Rad Microplate Reader at 690 nm
19 (Schinner and Von Mersi, 1990).
20
21 Phytotoxicity assay
22 The phytotoxicity of the samples was determined in distilled water extracts (1:5, w/v)
23 following the method of Zucconi and de Bertoldi (1987). The extracts were agitated
24 vigorously for 1 h and then centrifuged for 15 min at 10000 rpm in order to separate the
25 phases. The supernatant was collected and filtered through a 0.45 lm membrane filter.

The extracts were diluted to 30% and used as germination media. One milliliter of the germination solution was pipetted into a sterilized Petri dish lined with Whatman #1 filter paper. Ten seeds of garden cress (*Lepidium sativum* L.), which is one of the most sensitive test species for evaluating the phytotoxicity (Gehringer et al., 2003), were evenly distributed on the filter paper and incubated 24 h at 20–25 ºC in the dark. Then, the germination was stopped by adding 1 ml of ethanol. The seed germination percentage and root elongation of *L. sativum* were also measured in distilled water and used as control. Finally, the germination index (GI), expressed as percentage of control, was calculated based on relative seed germination percentage and relative root elongation.

Statistical analyses

The statistical analysis of data was carried out using the SPSS 11.0 program for Windows. A normality test was made for all the parameters prior to analyzing the variance. The results were submitted to an ANOVA test in order to determine changes in the variables with the composting time. A Tukey's test was used for testing significant statistical differences among composting times. The relationships between variables were defined by regression analysis. A principal components analysis was carried out to summarize the results obtained with the chemical, biochemical and microbiological parameters. Two principal components (PC1 and PC2) were used for this analysis.

1 Results and Discussion

2 Chemical changes

3 The main chemical properties of the initial cattle manure and the composting mixture
4 after 15, 80, 180 and 270 d are shown in Table 1. An increase in the pH values was
5 recorded during the active phase, suggesting the alkalinization of the manure as a
6 consequence of the release of ammonia from the degradation and mineralization of
7 organic compounds. During the maturation stage, pH reached higher levels than in the
8 initial cattle manure and the sample collected at the end of the active phase. This
9 increase is not typical in the maturation phase, where it is expected that the pH drops to
10 neutral values and then stabilizes. The watering of the maturation piles with the
11 leachates from the cattle farm, and the turnings of these piles could be responsible of
12 these results. The pH values reached during the maturation are rather high and this fact
13 could have important implications on the fertility and productivity of soils subjected to
14 compost amendment, as well as on the development of pH-sensitive plants (Boulter-
15 Bitzer et al., 2000). Moreover, acceptable pH ranges should be within tolerable levels to
16 microorganisms, i.e. bacteria generally need a pH range of 6–7.5; fungi can tolerate a
17 wider range, 5.5–8.0; and actinomycetes 5.0–9.0. Our composting material collected at
18 different times during the maturation phase presented pH ranges that exceeded the
19 previous ones; thus, the addition of these materials to soil may also strongly influence
20 the soil microflora.

21 The EC also affects the quality of composts in a large way because it reflects
22 their salinity and suitability for crop growth. This parameter increased after the active
23 phase probably due to the release of soluble salts like ammonium and phosphate
24 resulting from the decomposition of easily biodegradable organic substrates. As in the
25 case of the pH, higher levels of EC were found during the maturation stage. The

1 addition of liquid animal wastes to the maturation piles and the turning of these piles
2 could have been responsible for the increase in EC. In spite of this increase, the EC of
3 compost obtained after 270 d of maturation did not exceed the limit value of 3 mS cm^{-1}
4 indicating a material that could be safely applied to soil (Soumare´ et al., 2002).

5 When an organic waste is composted, the C to N ratio generally decreases
6 throughout the composting process due to the C losses as CO_2 and then stabilizes in the
7 range of 10–15 (Chefetz et al., 1996). As the total N content changed slightly with the
8 composting time, in the present study, a significant reduction of this ratio during the
9 maturation phase was mainly due to the depletion of easily degradable carbon
10 compounds as outlined by DOC dynamics (Table 1). The rate of decrease in DOC
11 concentration depends on the source material and the composting technique utilized.
12 The amount of DOC during the composting process is related to the equilibrium
13 between various reactions which increase or decrease its concentration. The degradation
14 of solid polymeric material in the composting substrate may lead to the formation of
15 soluble organic matter, which would increase the DOC concentration. On the other
16 hand, the reduction in DOC depends on the continuous mineralization of soluble
17 organic compounds, and the repolymerization and condensation pathways that lead to
18 the formation of complex organic substrates with low solubility in water which tend to
19 flocculate out the solution (Said-Pullicino and Gigliotti, 2007).

20 Mineral N (NH_4^+ and NO_3^-) increased significantly during the active phase
21 which indicates an intense mineralization. On the contrary, at maturation stage, the
22 concentration of NH_4^+ greatly decreased due to its volatilisation as NH3 as a result of
23 the high pH observed during this phase and most likely because of the frequent turning.
24 Low levels of NO_3^- were also found during the maturation phase. This fact may have
25 been the result of leaching of NO_3^- with the watering of the maturation piles. Brewer

1 and Sullivan (2003) reported a decrease in NO_3^- in yard trimmings compost obtained
2 after 133 d of maturation probably due to the leaching from saturated compost or
3 denitrification. Compared to the initial cattle manure, the NH_4^+ to NO_3^- ratio greatly
4 decreased during the active phase due to the high levels of NO_3^- detected in this stage.
5 After 80 d of maturation, this ratio increased with respect to the sample collected at the
6 end of the active phase. However, lower values of this ratio were reported in the
7 samples collected after 180 and 270 d of maturation mainly due to the low content of
8 NH_4^+.
9
10 Microbiological and biochemical changes
11 The decreasing trend of microbial biomass throughout the composting process (Fig. 1a)
12 was in agreement with other works (García et al., 1992; Insam et al., 1996; Klamer
13 and Bååth, 1998), reporting results obtained with different biomass quantification
14 methods (fumigation–extraction, substrate-induced respiration, ATP content, total
15 phospholipid fatty acids content). The fungal biomass measured as ergosterol content
16 also decreased during the active phase and maturation stage compared to the initial
17 cattle manure (Fig. 1b).
18 The microbial activity measured as basal respiration reached a maximum value
19 of 5000 mg CO_2 kg^{-1} organic matter during the active phase, which could be attributed
20 to the presence of easily degradable materials that stimulate the microbial community of
21 the initial cattle manure. Moreover, the release of labile compounds resulting from the
22 oxidative biodegradation of the matrix during the composting process could also explain
23 the higher value obtained on day 15 with respect to the initial feedstock (Said-Pullicino
24 et al., 2007). However, lower rates of respiration indicative of minor microbial activity
25 were recorded after the maturation period of 80 d (Fig. 1c). The decrease of microbial

1 activity was corroborated by the reduction in the microbial biomass as indicated by the
2 low levels of Cmic during the maturation period.

3 Enzymatic parameters also reflect the activity of the microbial community and
4 indicate the ability of composting to degrade a wide range of common organic
5 substrates (Mondini et al., 2004). Important enzymes involved in the composting
6 process include cellulases, which depolymerize cellulose; b-glucosidases which
7 hydrolyse glucosides; amidohydrolases, proteases and ureases involved in N
8 mineralization; and phosphomonoesterases and arylsulphatases that remove phosphate
9 and sulphate groups from organic compounds.

10 In our study, the alkaline phosphomonoesterase activity greatly decreased during
11 the active phase probably due to the feedback inhibition of this enzyme by inorganic
12 phosphate (Ayuso et al., 1996). The high levels of extractable P found in the sample
13 collected at the end of the active phase support this hypothesis (Table 1). However,
14 compared to this sample, this enzyme activity was significantly higher after 180 and
15 270 d of maturation (Fig. 2a). This could be due to the decrease in extractable P content
16 during this period, as well as due to the formation of an enzyme–humus complex, which
17 would make this enzyme more resistant to denaturation (Mondini et al., 2004). Protease
18 activity decreased throughout the process (Fig. 2b), which is indicative of a reduction in
19 the substrate for protease capable of activating the synthesis of this enzyme (Diaz
20 Burgos and Polo, 1991). Compared to the initial cattle manure, lower levels of cellulase
21 activity were reported during the active and maturation stages (Fig. 2c). On the other
22 hand there were no significant differences in b-glucosidase activity with the composting
23 time (Fig. 2d). It is assumed that soil cellulases and β-glucosidases are mainly produced
24 by fungi (Hayano, 1986). In the present study, a reduction in the fungal biomarker
25 ergosterol was observed during the active phase and the maturation stage (Fig. 1b). We

1 found a significant correlation between ergosterol and cellulose activity (R^2 = 0.50, P =
2 0.0001), but not with the β-glucosidase activity (R^2 = 0.05, P = 0.164). One possible
3 explanation is the protection of β-glucosidase through the formation of complexes with
4 the humic substances, which made the enzyme more resistant to physical and microbial
5 degradation.
6
7 Summary of chemical, microbiological and biochemical changes using a principal
8 component analysis
9 PC1 explained 60% of the variance and PC2 22% for a total explained variance of 82%.
10 The variables responsible for the changes along PC1 included pH, EC, total C and N,
11 C to N ratio, DOC, NH_4^+, NO_3^-, NH_4^+ to NO_3^- ratio, extractable P, Cmic, basal
12 respiration and protease activity. All of them were positively correlated with this
13 function, except pH, EC and total N. The variables responsible for the changes along
14 PC2 included the phosphomonoesterase and cellulase activities and ergosterol, which
15 were positively correlated with this function. The sample collected at the end of the
16 active phase separated from the initial cattle manure along PC2 (Fig. 3). This change
17 was highlighted by a decrease in the concentration of ergosterol and
18 phosphomonoesterase and cellulose activities during the active phase. However, the
19 composting material collected at different times during the maturation phase separated
20 from the initial cattle manure and the sample collected at the end of the active phase
21 mainly along PC1. In this case, the change was characterized by an increase in the pH,
22 EC and total N and a decrease in the total C, C to N ratio, DOC, NH_4^+, NO_3^-, extractable
23 P, Cmic, basal respiration and protease activity during the maturation period.
24
25

1 Phytotoxicity bioassay

2 The germination index was 0% in the initial cattle manure, and reached a value of 24%
3 during the active phase. Then, at maturation stage, a GI of 87% was recorded after 270
4 d of maturation (Fig. 4). Thus, more than 180 d were needed to overcome the threshold
5 limit of 60% stated by Zucconi and de Bertoldi (1987) to reduce the phytotoxicity to
6 levels consistent for a safe soil application.

7

8 Evaluation of stability and maturity parameters

9 The greatest concern regarding the evaluation of maturity is that this parameter does not
10 only depend on the type of material and composting process utilized but also on the
11 target use of the final product. As the composting of animal manures increases, due to
12 its practical characteristic as a method of recycling, it becomes more and more
13 important to determine the appropriate use of each method for the evaluation of the
14 stability and maturity of the compost.

15 The application of chemical parameters to assess the stability of compost is a
16 common practise in the research on composting. To be a good indicator, a chemical
17 parameter should fulfil the following requirements: (a) follow a consistent trend during
18 the composting process; (b) provide reference, critical or threshold values; (c) require
19 relatively rapid and cheap analytical procedures and (d) be easily interpretable. Some of
20 the parameters frequently referred to in the literature regarding the evaluation of
21 compost stability are discussed in this study.

22 C to N ratio may not be a good indicator of compos stability because it can level
23 off before the compost stabilize (Namkoong et al., 1999). For example, when waste rich
24 in nitrogen are used as source material for composting like sewage sledges or manures,
25 the C to N ratio can b within the values of a stable compost even though it may still be

1 unstable. Zmora-Nahum et al. (2005) reported C to N ratio lower than the cutoff value
2 of 15 very early during the composting of cattle manure, while important stabilization
3 processes were still taking place.

4 DOC generally contains organic compounds having different susceptibilities to
5 microbial degradation and different phytotoxic properties. For this reason DOC
6 composition may have an important role in determining the stabilization process. In the
7 present study, DOC concentrations during the maturation phase were lower than the
8 threshold value of 4000 mg kg^{-1} suggested by Zmora- Nahum et al. (2005) for a stable
9 compost showing that the composting material was stabilized after 80 d from the point
10 of view of DOC content (Table 1). In addition, we found a significant correlation
11 between DOC and respiration ($R^2 = 0.60$, $P = 0.0001$). Chica et al. (2003) also showed
12 DOC to be highly correlated to respiration.

13 Compost stability can be determined in terms of nitrification, which mainly
14 takes place during the maturation stage when temperatures are close to the ambient. At
15 the end of the composting process the content of NO_3^- should be higher than that of
16 NH_4^+, indicating that the process has been performed under adequate aeration
17 conditions (Bernal et al., 1998). Our composting material collected at different times
18 during the maturation phase did not exceed the limit value of 400 mg kg^{-1} suggested by
19 Zucconi and de Bertoldi (1987) for the concentration of NH_4^+ in stable composts; but,
20 the levels of NO_3^- were lower than expected during the maturation phase (Table 1). The
21 NH_4^+ to NO_3^- ratio has also been used to estimate the compost stability (Bernal et al.,
22 1998), giving a limit value of 0.16 for stable composts. In our case, this value was
23 exceeded during the maturation phase due to the low content of NO_3^- detected during
24 this stage (Table 1). As mineral nitrogen forms changed irregularly with the composting
25 time, they cannot be reliable indicators.

After a maturation period of 80 d, similarly to DOC, the stability assessed by the rate of microbial respiration increased, because lower rates of respiration were reported in the composting material collected at different times during the maturation stage (Fig. 1c). Wang et al. (2004) also reported low rates of respiration, indicative of highly stabilized composts, towards the end of the composting of dairy and pig manures. Adani et al. (2004) proposed a dynamic respiration index as an accurate indicator to measure the stability of composts resulting from different starting materials. They reported threshold values of 1000 and 500 mg O2 kg^{-1} organic matter h^{-1} to indicate medium and high stability, respectively. Other studies have established that the measurements of microbial respiration can be problematic as they are very sensitive to changes of moisture, temperature, and oxygen and nitrogen availability (Herrmann and Shann, 1993).

Wu et al. (2000) reported that the the low CO_2 evolution is not always an indicator of a non-phytotoxic compost. Therefore, the evaluation of compost stability based on CO_2 evolution, and the maturity based on seed germination, are two different parameters of compost quality. In our study, despite of the fact that low rates of respiration, indicative of highly stabilized materials, were reported after 80 d of maturation, more than 180 d were needed to overcome the threshold limit of 60% stated by Zucconi and de Bertoldi (1987) for a compost to be considered phytotoxinfree (Fig. 4). The source material and the composting condition as well as the watering of the maturation piles with leachates from the cattle farm could have required more time to break down the phytotoxic substances.

Considering the key roles of microorganisms in the composting process, the use of microbiological properties as stability indicators is not surprising. Enzymatic activities play an important role during the composting process, as they are implicated

in the biological and biochemical processes through which the initial organic substrates are transformed into the end product (Tiquia, 2002). As a consequence, specific enzymatic activities could provide a way of characterizing the composting process with relation to the rate of transformation of organic residues and the stability of the end product. Mondini et al. (2004) reported that the change in the location of enzymes throughout the composting process, i.e. from extracellular to complexed with humic-like substances, might be useful at the moment of evaluating compost stability, taking into account that not all enzymes will be as equally reliable as a stability index. Despite of the fact that the measurement of enzymatic activities is easy, quick and inexpensive, it is difficult to establish general threshold values to apply enzymatic activities as indicators of compost stability due to the widely different organic substrates involved in the composting processes. Thus, for compost characterization, it is necessary to follow the dynamics of enzymatic activities over time.

Having discussed the previous parameters, determining the change in DOC content with composting time seems to be the most suitable measurement to evaluate the stability of the composting material. It fulfilled the largest number of requirements, it followed a consistent trend and reached a critical value; moreover, it was neither time-consuming nor expensive and was easy to interpret. However, this does not mean that this measurement is equally accurate to evaluate the stability of all source materials and full composting facilities. The creation of databases, showing which protocols are most effective taking into account the source material, the composting conditions and if the experiment was done in a laboratory or at full scale will help us to choose correctly the different parameters for the evaluation of compost quality.

Conclusions

The active phase was accompanied by a significant increase in EC, mineral N, extractable P and basal respiration. However, lower levels of ergosterol and phosphomonoesterase, protease and cellulase activities were found during this phase. The maturation period was characterized by an increase in pH, EC and phosphomonoesterase activity and a decrease in C to N ratio, DOC, mineral N, extractable P, basal respiration and protease activity. The turning of maturation piles and the sporadic addition of leachates from the cattle farm to these piles influenced several parameters such as the pH and NO_3^- content resulting in values that are not typical from the maturation stage. Moreover, a maturation phase of 80 d was enough to obtain a stable compost as outlined by several parameters, but not to obtain a mature compost (i.e. with a low degree of phytotoxicity).

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12

1 Table 1. Variation in the chemical properties of cattle manure with composting time.

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	Composting time (days)				
	Thermophilic		Maturation		
	0	15	80	180	270
EC (mS cm^{-1})	1.30 ± 0.08c	2.10 ± 0.11b	2.80 ± 0.23a	2.90 ± 0.16a	3.00 ± 0.12a
pH	8.26–8.30b	8.07–8.86b	9.20–9.40a	9.50–9.75a	9.48–9.72a
Total C (%)	39.90 ± 0.28a	38.50 ± 0.26a	32.15 ± 0.53b	33 ± 0.53b	25 ± 1.22c
Total N (%)	2.40 ± 0.09b	2.20 ± 0.03b	2.90 ± 0.05a	3.10 ± 0.04a	2.40 ± 0.13b
C to N ratio	17 ± 0.74a	17.50 ± 0.33a	11.40 ± 0.34b	10.75 ± 0.09b	10.60 ± 0.12b
DOC (mg kg^{-1} dw)	7000 ± 700a	7300 ± 280a	3400 ± 610b	3100 ± 250b	2200 ± 10b
NH_4^+ (mg kg^{-1} w)	600 ± 90b	1200 ± 10a	300 ± 40c	120 ± 10c	250 ± 30c
NO_3^- (mg kg^{-1} dw)	20 ± 10b	700 ± 60a	20 ± 10b	50 ± 10b	70 ± 10b
NH_4^+ to NO_3^- ratio	30 ± 14a	1.71 ± 0.58b	15 ± 7.10a	2.4 ± 0.96b	3.57 ± 0.89b
Available P (mg kg^{-1})	170 ± 10b	350 ± 20a	120 ± 30bc	110 ± 10bc	70 ± 10c

Values are means ± standard error ($n = 5$). Values within the same row followed by the same letter are not significantly different according to Tukey's test ($P = 0.05$).

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1 Figure legends

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3 Figure 1. Changes in (a) Cmic, (b) ergosterol content and (c) basal respiration of the
4 initial cattle manure (t =0) and the composting material collected at the end of the active
5 pahse and at different times during the maturation stage (i.e. 80, 180 and 270 d). Values
6 are means ± standard error (n =5). Different letters indicate significant differences at
7 $p<0.05$ (Tukey HSD).

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10 Figure 2. Changes in (a) alkaline phosphomonoesterase, (b) proteas, (c) cellulase and
11 (d) β-glucosidase of the initial cattle manure (t =0) and the composting material
12 collected at the end of the active pahse and at different times during the maturation
13 stage (i.e. 80, 180 and 270 d). Values are means ± standard error (n =5). Different letters
14 indicate significant differences at $p<0.05$ (Tukey HSD).

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17 Figure 3. Principal component analysis of the chemical, microbiological and
18 biochemica parameters of the initial cattle manure (t=0) and the composting material
19 collected at the end of the active pahse and at different times during the maturation
20 stage (i.e. 80, 180 and 270 d).

21

22 Figure 4. Changes in the germination index of the initial cattle manure (t =0) and the
23 composting material collected at the end of the active pahse and at different times
24 during the maturation stage (i.e. 80, 180 and 270 d). Values are means ± standard error
25 (n =5). Different letters indicate significant differences at $p<0.05$ (Tukey HSD).

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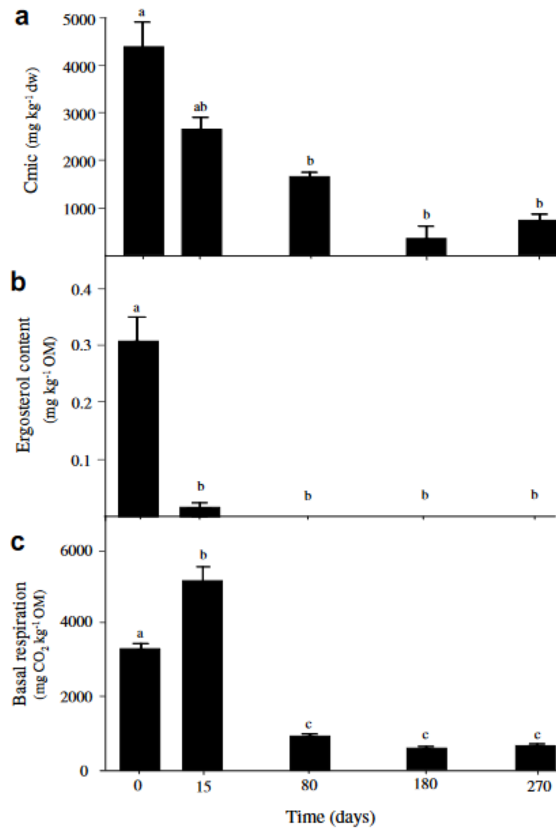

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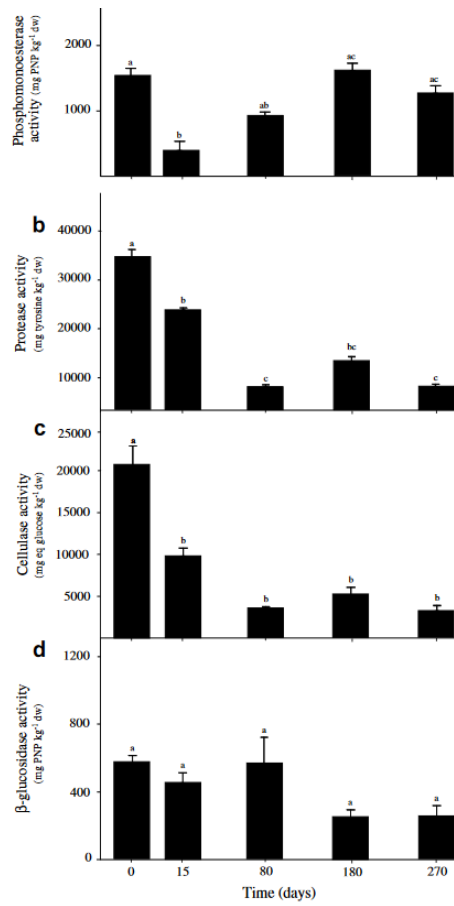
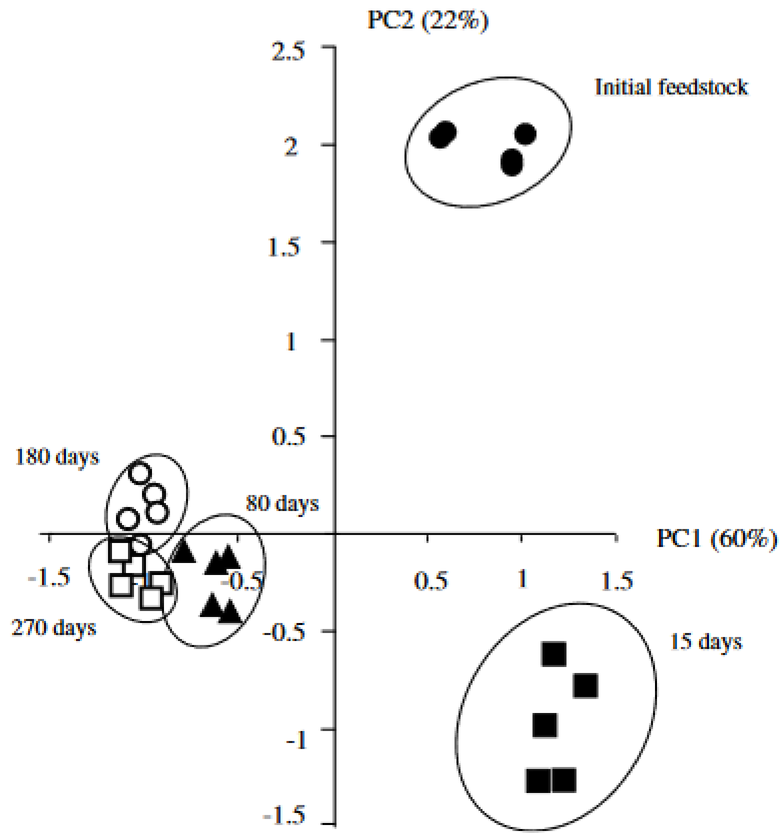


Figure 2



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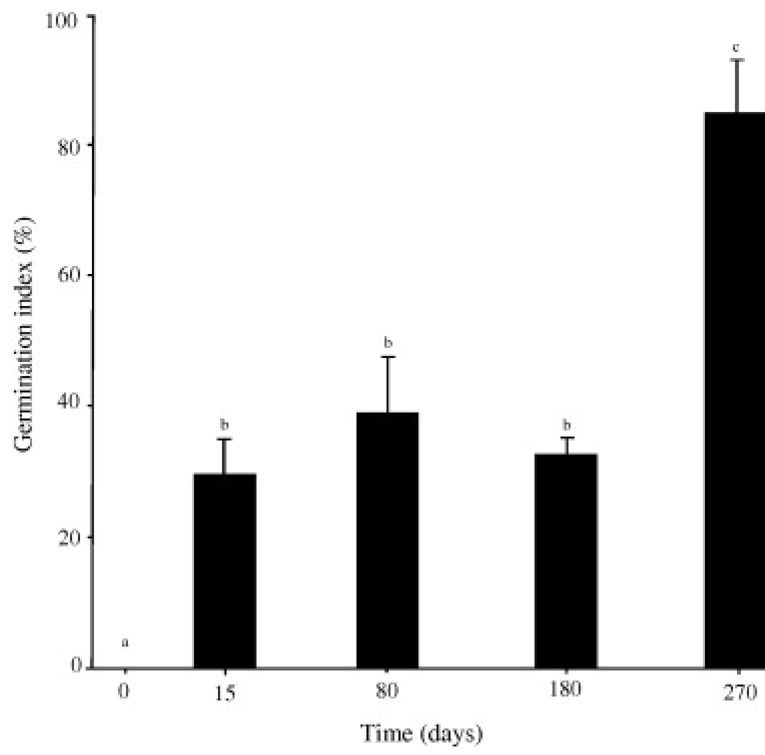

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3 Figure 4