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Changes in the chemical and biological characteristics of grape marc vermicompost during a two-year production period

Natielo Almeida Santana[a,*], Rodrigo Josemar Seminoti Jacques[a], Zaida Inês Antoniolli[a], Hugo Martínez-Cordeiro[b], Jorge Domínguez[b]

[a] Federal University of Santa Maria, Department of Soil Science, 97119-900 Santa Maria, Rio Grande do Sul, Brazil
[b] Grupo de Ecoloxía Animal (GEA), Universidade de Vigo, E-36310 Vigo, Pontevedra, Spain

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ABSTRACT

In this study, the changes in the chemical and biological characteristics of a grape marc vermicompost during a two-year production period were studied to determine the time required for completion of the maturation phase and production of stable vermicompost. Grape marc was added to the surface of a vermireactor monthly for two years. At the end of the experiment, the resulting vermicompost was 100 cm deep and consisted of layers of fresh (top) to processed (bottom) grape marc approximately 15, 30, 180, 360, 540 and 720 days old. Several chemical and biological characteristics were measured in each vermicompost layer at the end of the experiment: the moisture content; pH; electrical conductivity; organic matter content; total and dissolved carbon content; C:N ratio; nutrient content; earthworm density; basal respiration; and the activity of 12 enzymes associated with the carbon, nitrogen, phosphorus and sulfur cycles. The earthworm density increased 30-fold during the two-year period. In addition, the pH and electrical conductivity as well as the organic matter, total carbon, dissolved organic nitrogen, phosphorus, potassium and copper contents all decreased, while the calcium, sulfur, zinc and manganese contents increased. Basal respiration and carboxylesterase, peroxidase and catalase activities were highest during the first 30 days. By contrast, the urease, acid phosphatase, alkaline phosphatase and arylsulfatase activities were greater after 30 days. The major changes in vermicompost occurred during the initial phase of vermicomposting. However, the chemical and biological characteristics continued to vary during the maturation phase because of microbial activity. Thus, the quality of vermicompost greatly depended on the duration of the maturation (conditioning) phase; therefore, it was difficult to determine the time required to yield a stable product.

1. Introduction

Vermicomposting is an inexpensive, efficient and easily applicable biotechnological process in which potentially hazardous organic waste is converted into a high-quality organic fertilizer (Shak et al., 2014; Lim et al., 2016; Busato et al., 2016; Lv et al., 2016). In the vermicomposting process, earthworms and microorganisms act together to alter the chemical, physical and biological characteristics of organic waste, thus generating vermicompost (Domínguez et al., 2010; Lleó et al., 2013; Gómez-Brandón et al., 2019). The final quality of the vermicompost depends on factors such as the chemical and physical characteristics of the substrate used to feed the earthworms, the earthworm species involved, the management practices used and the length of the conditioning period (Bisen et al., 2011; Domínguez and Gómez-Brandón, 2013).

The vermicomposting process can be divided into two phases. The initial phase, also called the active phase, is characterized by intense earthworm activity (Lores et al., 2006; Aira et al., 2007; Gómez-Brandón et al., 2011). During this phase, earthworms process organic waste by ingesting, fragmenting and reducing the volume of the material and by assimilating readily biodegradable compounds (sugars, organic acids, proteins, peptides) (Fornes et al., 2012; Domínguez et al., 2019). Earthworms thus alter the physical and chemical characteristics and the microbial composition of the substrate (Hanc et al., 2019).

The second maturation (or conditioning) phase of vermicomposting begins as the earthworms move towards undigested substrate and is characterized by a predominance of microbial activity in the worm-processed material (Aira et al., 2007). The intensity and duration of the maturation phase varies and depends on the characteristics of the active phase of vermicomposting (Aira and Domínguez, 2008; Domínguez

* Corresponding author.
E-mail address: natielosantana@gmail.com (N.A. Santana).

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et al., 2010). During the maturation phase, microorganisms alter the substrate via the production of extracellular enzymes. Characteristics such as the pH, C:N ratio, total and available contents of nutrients and toxic elements, microbial biomass and vermicompost enzymatic activity vary continuously throughout the vermicomposting process (Aira et al., 2007; Romero et al., 2007; Aguiar et al., 2013; Garcia et al., 2014). These alterations ultimately determine the different characteristics of vermicompost processed for different lengths of time (Cao et al., 2016; Khatua et al., 2018).

Studies monitoring the long-term dynamics of the chemical and biological changes that occur in organic waste during vermicomposting are scarce. Previous studies have strongly emphasized the role of earthworms as agents that modify the characteristics of organic waste, and little attention has been paid to the contribution of microorganisms during the maturation phase. The aim of the present study was to determine the length of time required to yield mature, chemically and biologically stable vermicompost from grape marc. With this aim, the changes in the chemical and biological characteristics of grape marc substrate during a two-year vermicomposting process were determined.

2. Materials and methods

2.1. Substrate and earthworms

White grape marc (*Vitis vinifera* cv. Albariño) was obtained from the Terras Gauda winery in Pontevedra (Galicia, northwestern Spain) and stored in cold rooms at approximately −4 °C until needed. The chemical and physical characteristics of the fresh grape marc used are listed in Table 1. The earthworm species *Eisenia andrei* was used for vermicomposting. The initial population density in the vermireactor was 297 ± 20 individuals m^{-2}, which consisted of 82.25 ± 16 mature earthworms m^{-2} and 215 ± 37 juveniles m^{-2}, and there were 63 ± 18 cocoons m^{-2}; the mean earthworm biomass was 58.4 ± 15 g m^{-2}.

2.2. Vermireactor and experimental design

The experiment was conducted in a vertical deposition vermireactor housed in greenhouse facilities belonging to the University of Vigo (Vigo, northwestern Spain). The vermireactor consisted of a metallic container (length of 1.5 m, width of 4 m, depth of 1 m and volume of 6 m^3) fitted with a dark cover. At the beginning of the two-year experiment, a basal layer of vermicompost (depth of 10 cm) that served as a bed for the earthworms was placed in the empty vermireactor. A plastic mesh (5 mm Ø) was placed on top of the basal layer (to prevent fresh plant material from becoming mixed with the processed vermicompost), and approximately 150 kg of grape marc (depth of 6 cm) was placed on top of the mesh. The grape marc was added monthly during the two-year period as successive layers on the surface of the vermireactor. Thus, at the final sampling time, the vermireactor contained a layer of vermicompost with a depth of 100 cm, comprising layers of fresh (top) to processed (bottom) grape marc approximately 15, 30, 180, 360, 540 and 720 days old (Fig. 1). The vermicompost in the reactor was watered daily with an automatic watering system to prevent drying.

2.3. Sample collection

After 720 days, samples were collected from each of the layers, corresponding to the different stages of vermicomposting. To collect the samples, the vermireactor was divided into 12 sections, each with a surface area of 0.5 m^2. In each section, one sample was obtained from each of the six layers of vermicompost with a cylindrical sampler (7.5 cm diameter × 12 cm height), for a total of 72 samples. The samples were stored in plastic containers at −20 °C until chemical and biological analyses.

2.4. Analytical procedures for chemical and biological analyses

The organic matter was determined by the weight-loss method in a muffle furnace (Schulte and Hopkins, 1996). The total carbon and nitrogen contents were determined by an elemental analyzer (CHNS-O EA-1108, Fisons Instruments, USA), and the NH_4^+ and NO_3^- contents were quantified in a continuous-flow autoanalyzer (Bran + luebbe AA3, Seal Analytical, Norderstedt, Germany) (Sims et al., 1995). Dissolved organic carbon (DOC) was determined colorimetrically after moist digestion ($K_2Cr_2O_7$ and H_2SO_4) of aliquots of 0.5 M K_2SO_4 extracts of the samples (Cabrera and Beare, 1993), and dissolved organic nitrogen (DON) was calculated as the difference between the amount of mineral nitrogen (NH_4^+ and NO_3) and the total extractable nitrogen (TEN) (Cabrera et al., 1993). The TEN was determined in the same way as the NO_3^- was after the addition of potassium persulfate and autoclaving for 30 min at 121 °C (to oxidize the nitrogen to NO_3^-). To determine the DOC, the potassium persulfate extracts were added to 0.16 M potassium dichromate, concentrated H_2SO_4 and 6% barium chloride (m/v), after which the mixture was incubated for 30 min at 160 °C. After 24 h, the supernatant was removed, and the sample was analyzed in a spectrophotometer at a wavelength of 590 nm.

The pH and electrical conductivity (CM35, Crison Instruments, Barcelona, Spain) were determined in a 1:10 vermicompost:water suspension. The moisture content was determined by the gravimetric method (Gardener, 1986). The phosphorus, potassium, boron, calcium, copper, iron, magnesium, manganese, zinc and sulfur contents were determined from extracts of dried samples subjected to nitric-perchloric digestion; the contents were measured by an atomic-absorption spectrometer (Varian SpectrAA-250 Plus, Varian Inc., Walnut Creek, CA, USA) according to the USEPA 3050B method. The available Olsen P content was determined according to the methods of Olsen et al. (1954).

The earthworm population was sampled with the same cylinder (7.5 cm diameter × 12 cm height) at the end of the experiment and in the upper layers of the vermireactor (the deeper layers did not contain any worms or cocoons). The earthworms were classified as adults or juveniles, and the number of cocoons and the biomass of the individuals were quantified.

The microbial activity was determined by measuring the rate of CO_2 evolution from 5 g (fresh weight) subsamples during a 6-hour incubation period. The evolved CO_2 was trapped in 0.02 M NaOH and subsequently measured by titration with HCl to a phenolphthalein endpoint after the addition of excess $BaCl_2$ (Anderson, 1982).

The 12 enzymes considered were associated with the carbon cycle (β-glucosidase, carboxylesterase-NA, carboxylesterase-NPB and laccase), the nitrogen cycle (protease, urease and arginine deaminase), the phosphorus cycle (alkaline and acid phosphatase), the sulfur cycle (arylsulfatase) and antioxidative capability and microbial activity (peroxidase, laccase and catalase). The enzymatic activities were determined according to the methods of Sánchez-Hernandez et al. (2015). As such, 1 g of fresh vermicompost and 50 mL of distilled water were homogenized for 30 min at room temperature (~20 °C) in Falcon tubes in an orbital shaker at 50 rpm (Elmi® Intelli-RM-2 L mixer, Riga, Latvia). Carboxylesterase (EC 3.1.1.1) activity was measured by an interrupted assay with microplates and two substrates (1-naphthyl acetate [1-NA] and 4-nitrophenyl butyrate [4-NPB]) because of the existence of different isoforms of the enzyme (Sánchez-Hernandez et al., 2015). Acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) and β-glucosidase (EC 3.2.1.21) activities were measured according to the methods of Popova and Deng (2010) with modifications, as described by Sanchez-Hernandez et al. (2017). Urease (EC 3.5.1.5) activity was quantified by the nonbuffered method of Schinner et al. (1996), and similarly, protease (EC 3.4.21.92) activity was measured according to the methods of Schinner et al. (1996), although 1.5-mL microtubes were used to reduce reagent consumption. Catalase (EC 1.11.1.6) activity was measured according to the methods of Trasar-

Table 1
Chemical and physical characteristics of fresh grape marc and vermicompost approximately 15, 30, 180, 360, 540 and 720 days old. Shown are the mean values ± standard deviations. The means marked with the same letter are not significantly different (Tukey's test, $p < 0.05$). The statistical analysis was performed considering the data of the worm-processed material.

Attribute	Fresh grape marc	Worm-processed material (days)						Δ (%)	Δ (%)
		15	30	180	360	540	720	(0–30 days)	(30–720 days)
Humidity (%)	74.00 ± 0	76.50 ± 6.10^{ns}	78.41 ± 2.79	78.48 ± 0.71	76.98 ± 0.17	79.34 ± 4.59	79.29 ± 4.32	+5.87	+1.10
pH	4.36 ± 0.04	7.72 ± 0.18^{a}	7.73 ± 0.10^{a}	7.65 ± 0.21^{ab}	7.51 ± 0.20^{ab}	7.46 ± 0.14^{b}	7.49 ± 0.10^{ab}	+77.18	−3.10
Cond (mS cm^{-1})	1.34 ± 0.15	2.87 ± 0.51^{a}	2.83 ± 0.35^{a}	2.79 ± 0.84^{ab}	2.43 ± 0.71^{ab}	2.23 ± 0.54^{ab}	1.96 ± 0.60^{b}	+112.78	−30.74
Total C (g kg^{-1} DW)	484.23 ± 1.60	474.67 ± 13.98^{ab}	481.77 ± 11.04^{a}	464.53 ± 24.36^{ab}	441.17 ± 31.15^{b}	449.05 ± 30.94^{ab}	457.73 ± 19.91^{ab}	−0.48	−4.98
OM (%)	91.21 ± 0.30	89.04 ± 2.49^{ab}	90.56 ± 3.11^{a}	85.08 ± 3.25^{b}	85.34 ± 3.49^{ab}	86.97 ± 3.65^{ab}	85.75 ± 4.89^{ab}	−0.68	−5.31
DOC (g kg^{-1} DW)	4.01 ± 0	3.40 ± 1.54^{ns}	2.63 ± 0.75	3.20 ± 1.18	3.34 ± 1.87	2.38 ± 1.28	1.82 ± 0.52	−37.86	−30.79
Total N (g kg^{-1} DW)	20.19 ± 0.62	39.30 ± 2.42^{bc}	41.70 ± 1.90^{ab}	40.05 ± 3.07^{bc}	37.22 ± 2.42^{c}	38.06 ± 2.81^{bc}	39.42 ± 2.84^{bc}	+100.76	−5.46
NH_4^+ (g kg^{-1} DW)	< 0.001	0.65 ± 0.34^{ns}	0.82 ± 0.16	0.70 ± 0.22	0.58 ± 0.27	0.49 ± 0.11	0.49 ± 0.16	High	−40.24
NO_3^- (g kg^{-1} DW)	< 0.001	0.60 ± 0.14^{ns}	0.69 ± 0.45	0.42 ± 0.25	0.44 ± 0.22	0.48 ± 0.30	0.42 ± 0.21	High	−39.13
DON (g kg^{-1} DW)	< 0.001	3.56 ± 0.84^{ab}	4.17 ± 0.99^{a}	3.03 ± 1.08^{ab}	3.40 ± 1.03^{ab}	3.07 ± 1.03^{ab}	2.60 ± 0.79^{b}	High	−37.64
C:N	24.02 ± 0.72	12.07 ± 0.93^{ns}	11.56 ± 0.35	11.59 ± 1.30	11.80 ± 1.30	11.61 ± 0.81	11.65 ± 0.83	−53.98	+0.77
P (g kg^{-1} DW)	4.03 ± 0.08	5.13 ± 0.45^{ab}	6.27 ± 0.77^{a}	5.44 ± 2.20^{ab}	4.30 ± 1.10^{b}	3.85 ± 0.96^{b}	3.71 ± 0.99^{b}	+49.52	−40.82
Olsen P (g kg^{-1} DW)	1.63 ± 0.27	1.74 ± 0.80^{ns}	1.67 ± 0.60	1.38 ± 0.27	1.28 ± 0.38	0.65 ± 0.38	1.32 ± 0.49	+2.73	−20.95
K (g kg^{-1} DW)	30.46 ± 0.56	24.97 ± 3.47^{a}	24.77 ± 3.48^{a}	20.29 ± 7.36^{b}	17.34 ± 4.57^{b}	23.05 ± 4.64^{ab}	15.62 ± 4.40^{b}	−18.82	−36.93
Ca (g kg^{-1} DW)	3.42 ± 0.77	5.90 ± 0.26^{c}	6.80 ± 1.58^{bc}	9.02 ± 3.73^{ab}	9.92 ± 2.07^{a}	7.48 ± 1.46^{a}	9.86 ± 0.06^{a}	+87.76	+45.00
Mg (g kg^{-1} DW)	0.87 ± 0.23	2.28 ± 0.09^{b}	2.38 ± 0.22^{ab}	3.02 ± 0.81^{ab}	3.14 ± 0.61^{ab}	2.94 ± 0.56^{ab}	3.33 ± 0.70^{a}	+166.46	+39.91
S (g kg^{-1} DW)	0.33 ± 0.08	0.89 ± 0.16^{c}	0.99 ± 0.23^{bc}	1.28 ± 0.52^{ab}	1.41 ± 0.42^{a}	1.20 ± 0.26^{ab}	1.55 ± 0.28^{a}	+180.94	+56.56
B (g kg^{-1} DW)	0.07 ± 0.02	0.18 ± 0.10^{ns}	0.20 ± 0.06	0.12 ± 0.05	0.11 ± 0.05	0.11 ± 0.03	0.13 ± 0.06	+168.25	−35.00
Cu (g kg^{-1} DW)	0.02 ± 0	0.42 ± 0.01^{a}	0.46 ± 0.07^{a}	0.30 ± 0.12^{b}	0.20 ± 0.03^{c}	0.29 ± 0.11^{bc}	0.17 ± 0.03^{c}	High	−63.04
Zn (g kg^{-1} DW)	0.01 ± 0	0.12 ± 0.05^{b}	0.15 ± 0.12^{b}	0.46 ± 0.38^{ab}	0.98 ± 0.70^{a}	0.89 ± 0.06^{a}	0.70 ± 0.47^{a}	+1125.00	+366.66
Fe (g kg^{-1}DW)	0.08 ± 0.02	1.04 ± 0.50^{ns}	0.93 ± 0.49	1.74 ± 1.13	1.76 ± 0.82	1.18 ± 0.28	1.78 ± 0.58	+1189.42	+91.39
Mn (g kg^{-1} DW)	0.02 ± 0.01	0.09 ± 0.01^{b}	0.12 ± 0.07^{b}	0.30 ± 0.19^{a}	0.51 ± 0.28^{a}	0.18 ± 0.07^{ab}	0.43 ± 0.15^{a}	+383.33	+258.33

Δ: percent variation in the value of the attribute within the time interval; OM: organic matter; DOC: dissolved organic carbon; DON: dissolved organic nitrogen.

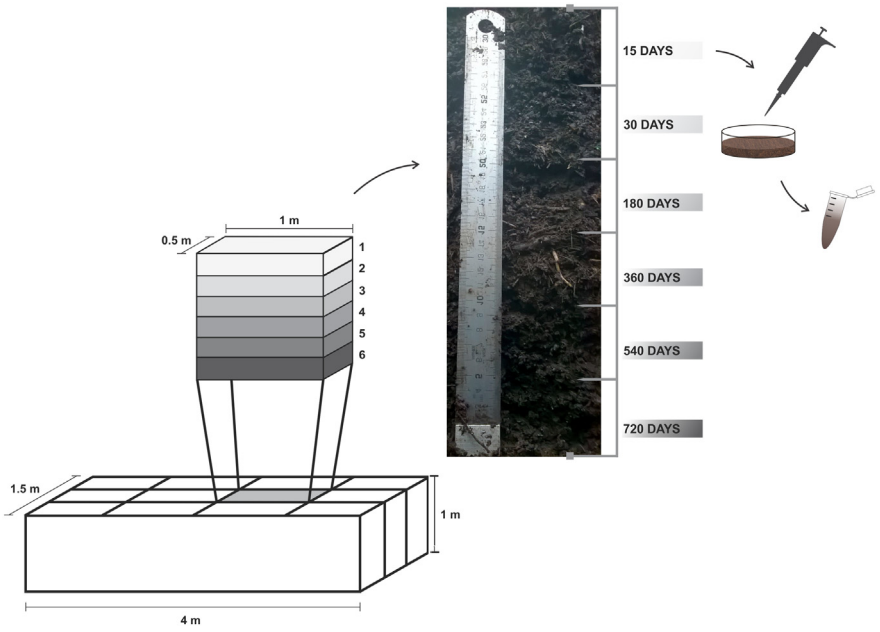

Fig. 1. Diagram of the vertical deposition vermireactor in which the grape marc was added monthly such that at after two years, the resulting vermicompost was 100 cm deep and consisted of layers of fresh (top) to processed (bottom) grape marc approximately 15, 30, 180, 360, 540 and 720 days old, from which samples were collected.

Cepeda et al. (1999), and laccase and peroxidase activities were quantified with pyrogallol (1,2,3-trihydroxybenzene) as the substrate according to the methods of Bach et al. (2013). Arylsulfatase activity (EC 3.1.6.1) was determined with 4-nitrocatechol sulfate as the substrate (Rip and Gordon, 1998).

2.5. Statistical analysis

The normality of the data was examined using the Shapiro-Wilk test ($p < 0.05$). The nutrient and toxic metal contents were square root-transformed to fall within the normal distribution. A one-way ANOVA followed by a Tukey test for pairwise comparisons of the means ($p < 0.05$) was used to test the effect of the vermicomposting production period on chemical and biological characteristics. These characteristics were subjected to a Pearson correlation analysis ($p < 0.05$) in order to verify the relationships between them. To check the quality and to better visualize the correlation, a principal component analysis (PCA) was performed using the STATISTICA 7 program.

3. Results

3.1. Earthworm population

Earthworms (adult and juvenile) were found only in the freshest layers of the vermireactor (< 30 days) and were not observed in deeper layers. The initial (active) phase of vermicomposting was thus considered to last from day 0 to day 30; the maturation phase (involving microorganisms only and not earthworms), from day 30 to day 720.

The density of earthworms at the end of the two-year study period was 10,923 ± 3986 individuals m^{-2} (biomass, 1704.42 ± 561.75 g^{-2}), comprising 289.36 ± 160.35 adults (biomass, 122.97 ± 106.59 g m^{-2}) and 10,634.03 ± 3950.07 juveniles (biomass, 1581.44 ± 663.44 g m^{-2}) in the freshest layers of the vermicompost; in addition, there were 17.02 ± 1.17 cocoons. The earthworm density increased by approximately 30-fold from the start of the experiment, and the earthworm biomass increased by approximately 100-fold.

3.2. Moisture content of the vermicompost

The mean moisture content of the vermicompost (77.57%) did not vary significantly between the different layers (Table 1). Thus, the moisture was effectively retained in all the layers of the vermicompost throughout the process, although slight increases were observed in the lower layers (those subjected to longer vermicomposting times). This finding indicates that excess moisture was not a limiting factor for the earthworms in the different layers and that their preference for the upper layers was determined by other factors.

3.3. Chemical characteristics of the vermicompost

At the end of the two-year study period, most of the chemical characteristics of the vermicompost were altered (Table 1). The main changes in chemical characteristics occurred in the period between 0 and 30 days during the initial vermicomposting phase, especially within the first 15 days (Table 1). Among the 22 characteristics evaluated, 18 varied by > 18.82% (considering the potassium content) during the initial phase, and only four characteristics varied by < 5.87% (considering the moisture content).

The chemical characteristics varied less during the maturation phase than during the initial phase. Nonetheless, the values of some of these characteristics widely varied, including the electrical conductivity (−30.74%), DOC content (−30.79%), NH_4^+ content (−40.24%), NO_3^- content (+39.13%), phosphorus content (−40.82%), and others (Table 1). These changes were mediated mainly by the microorganisms, as little-to-no earthworm activity occurred during the maturation phase.

Relative to the values measured in the initial vermicomposting period, the following characteristics decreased significantly over time: pH ($p < 0.05$), electrical conductivity ($p = 0.001$), total carbon content ($p = 0.017$), organic matter content ($p = 0.020$), DON content ($p = 0.040$), phosphorus content ($p < 0.001$), potassium content ($p < 0.001$) and copper content ($p < 0.001$). The characteristics that did not decrease significantly were the C:N ratio ($p = 0.877$), NH_4^+ content ($p = 0.111$), NO_3^- content ($p = 0.558$), DOC content ($p = 0.184$), Olsen P content ($p = 0.129$) and boron content ($p = 0.129$), and the characteristics that increased significantly over time included the calcium ($p < 0.001$), magnesium ($p = 0.001$), zinc ($p < 0.001$), sulfur ($p < 0.001$) and manganese ($p < 0.001$) contents.

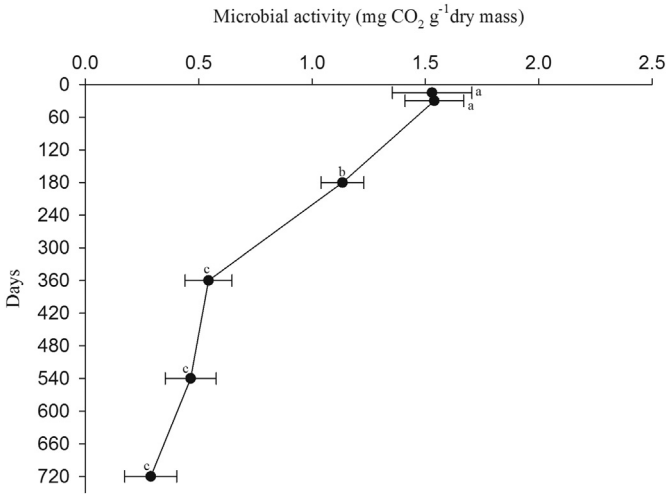

Fig. 2. Microbial activity, measured as basal respiration of the vermicompost during two years of vermicomposting. The bars represent the standard deviations. The means marked with the same letter are not significantly different (Tukey's test, $p < 0.05$).

3.4. 3.4 Biological characteristics of the vermicompost

Microbial activity decreased greatly during the two years of vermicomposting (Fig. 2). CO_2 production was highest ($p < 0.001$, CV = 31.74%) within the first 30 days, followed by a sharp decrease until 360 days, after which it remained low.

The enzymes displaying the highest activity within the first 30 days included the carboxylesterases ($p = 0.005$ for NA and $p = 0.0184$ for NPB), protease ($p = 0.0272$), peroxidase ($p = 0.006$) and catalase ($p = 0.0013$) (Fig. 3). The first three are involved in breaking down organic compounds, and the last two are associated with antioxidative and cellular activity. These results correspond to the microbial activity evaluated by basal respiration, which was greater at the beginning of vermicomposting than later during vermicomposting (Fig. 2).

The urease, acid phosphatase, alkaline phosphatase and arylsulfatase activities increased after 30 days (Fig. 3). These enzymes are involved in breaking down organic compounds, indicating that biochemical changes in organic matter continued to occur during the maturation phase. The β-glucosidase, arginine deaminase and laccase activities did not vary significantly during the vermicomposting process.

3.5. Correlation and principal component analysis

The PCA and the Pearson's correlation showed similar results (Table S1; Fig. 4). The PCA, considered the time of vermicomposting as well as the chemical and biological characteristics, explained 40.23% of the data variance. Most of the variance in the original data set (28.60%) was explained by the first principal component (PC1). The initial phase of vermicomposting was related to the microbial activity, carboxylesterase-NA, carboxylesterase-NPB and glucosidase activities, pH, electrical conductivity, and nutrients (N, NH_4, Cu, Na, K and P). After 180 days of vermicomposting, few variables were related (DON, NO_3, C:N, arginine and urease activities) and were allocated between the groups of variables associated with the initial and final phases of vermicomposting. Acid phosphatase, arylsulfatase, urease and laccase activities; humidity; and nutrients (S, Mg, Ca, Mn, Zn and Fe) were related to the final phase of vermicomposting. Some of the variables presented had weak ($r < 0.40$) to strong ($r > 0.70$) (Liu et al., 2003) Pearson correlations (Table S1). The activity of the microorganisms was correlated with pH, total N, NH_4, B, Cu, K, P, carboxylesterase-NPB, peroxidase and catalase activities. The ions NO_3, P, K and Cu were positively correlated with the electrical conductivity of the vermicompost. All variables related with the initial phase of vermicomposting in the PCA. Arylsulfatase was negatively correlated with OM, total C and DOC (variables related to initial phase of vermicomposting on the PCA) and positively correlated with Fe (related with maturation phase on PCA). Acid phosphatase activity presented a negative correlation with OM, pH, total N, B and Cu (variables related to the initial phase of vermicomposting on the PCA) and a positive correlation with Ca, Fe, Mg, Mn and Mo, variables grouped with the final phase of vermicomposting on the PCA. Humidity was positively correlated with laccase activity and S content, both of which were grouped with the final phase of vermicomposting on the PCA.

4. Discussion

Although high rates of earthworm reproduction have also been observed in other studies (Monroy et al., 2006; Martínez-Cordeiro et al., 2013; Domínguez et al., 2014; Domínguez et al., 2016), the increase observed in the present study was markedly high for this type of substrate. In a previous study by Domínguez et al. (2014) on grape marc substrate, the initial density of earthworms was approximately 300 m^{-2} but increased by slightly more than twofold within 70 days (~700 individuals m^{-2}); however, the density decreased after 112 days (~600 individuals m^{-2}) as the availability of food decreased. The authors demonstrated that when food was readily available, the *E. andrei* population doubled every six months and increased by approximately 50% within the first 15 days. The findings of the present study are consistent with previous findings, as the increase in the earthworm population can be explained by the monthly additions of substrate.

E. andrei requires an environment rich in organic matter to survive and reproduce (Martínez-Cordeiro et al., 2013; García-Sánchez et al., 2017). The conditions in the vermireactor were optimal for survival and reproduction of the earthworms, as the moisture content, temperature and quality of the organic waste were adequate (Yadav and Garg, 2016). The moisture content remained constant throughout the layers of vermicompost (~80% moisture), demonstrating the efficiency of the system in controlling this characteristic both at and below the surface. According to Domínguez et al. (2014), a substrate moisture content close to 85% is optimal for earthworm survival.

During the first 30 days of vermicomposting, the earthworms rapidly degraded the grape marc, with consequent increases in nitrogen, phosphorus, calcium, magnesium, sulfur, boron, copper, zinc, iron and manganese contents. In addition, the physical and chemical changes to the grape marc due to the feeding activity of the earthworms stimulated microbial activity, which also helped to degrade the organic waste. Significant reductions in microbial activity and nutrient contents were observed during the maturation phase (i.e., after 30 days of vermicomposting). The microbial activity probably decreased because of the lower activity of the earthworms, considering that the fresh waste had already been consumed and that more recalcitrant organic material was present in the vermireactor. The reduction in the total or available contents of most nutrients during the maturation phase can be explained by immobilization of the nutrients in the earthworm and microorganism biomass or sorption by the vermicompost. However, the increases in calcium, magnesium, sulfur, zinc and manganese contents were probably due to a shrinkage effect as the mass of the waste decreased (Lv et al., 2016).

Significant changes in most of the evaluated chemical parameters occurred during the maturation phase, although there was no earthworm activity in the older layers. The microbial activity continued, albeit at a low level, and gradually altered the chemical characteristics. In addition, owing to the humification of organic matter, chemical compounds such as functional groups (e.g., carboxylic and phenolic groups) were generated and reacted with the various substances present in the vermicompost, thus further altering the dynamics of availability of many chemical elements (Dores-Silva et al., 2013).

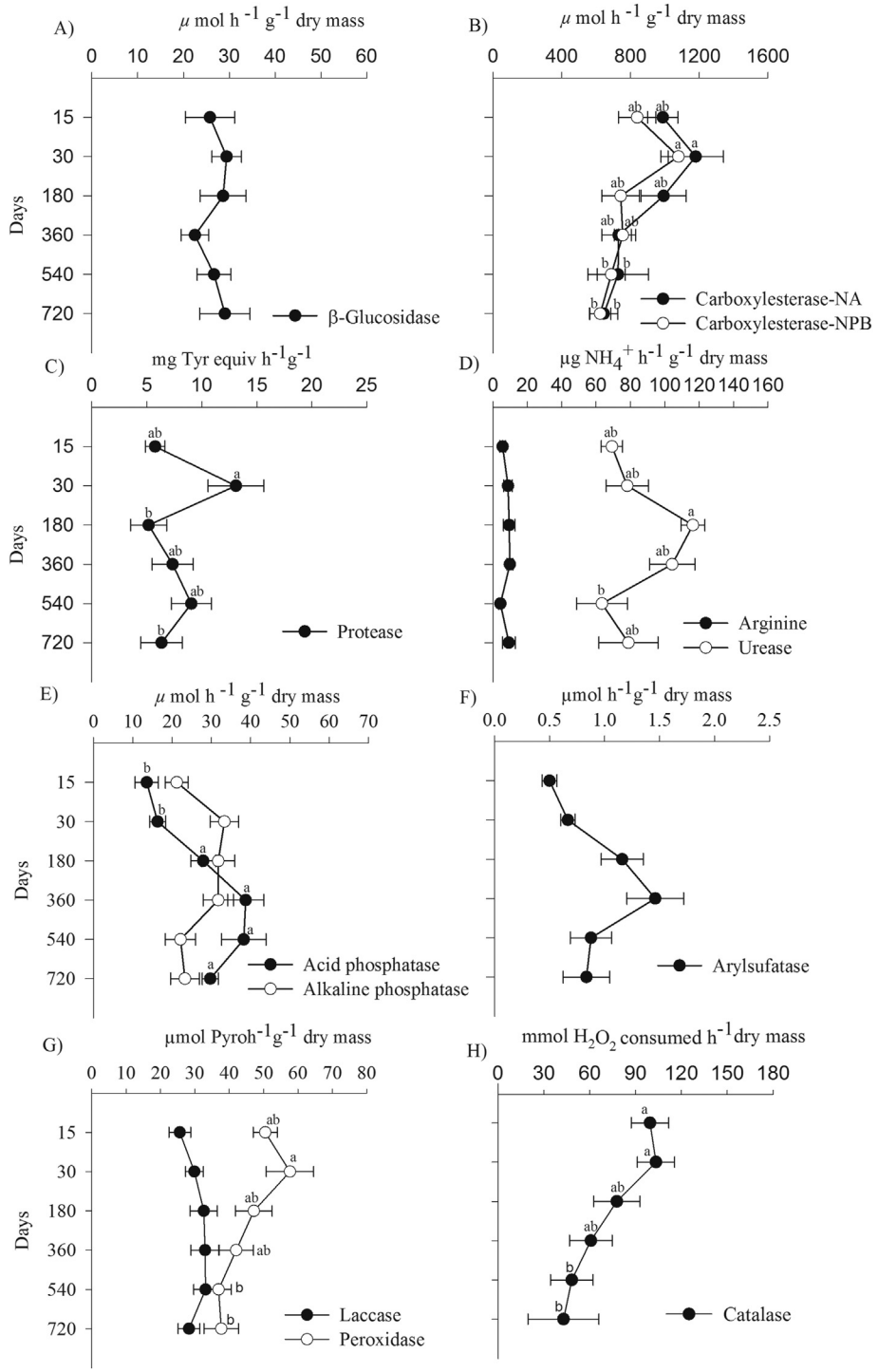

Fig. 3. Activity of enzymes involved in the carbon cycle, including *β*-glucosidase (A), carboxylesterase-NA and carboxylesterase-NPB (B); the nitrogen cycle, including protease (C), arginine deaminase and urease (D); the phosphorus cycle, including acid and alkaline phosphatase (E); and the sulfur cycle, including arylsulfatase (F). Activity of enzymes with antioxidative functions, such as laccase and peroxidase (G), and involved in microbial activity, such as catalase (H). The bars represent the standard deviations. The means marked with the same letter are not significantly different (Tukey's test, $p < 0.05$).

The pH decreased during the maturation phase. Organic matter decomposition and nitrification processes contribute to acidification. Nigussie et al. (2017) and Gogoi et al. (2015) also reported a significant reduction in pH during vermicomposting of plant waste and bovine manure and attributed this phenomenon to the decomposition of organic matter and the release of hydrogen and organic acid into the vermicompost. The authors also stated that the nitrification process contributed to reducing the pH.

The electrical conductivity was optimal (i.e., < 8.0 mS cm^{-1}) for the survival and reproduction of earthworms (Edwards, 1998) but decreased after 180 days of vermicomposting. The electrical conductivity was reported to decrease in other vermicomposting studies (Singh et al., 2010; Shak et al., 2014; Domínguez et al., 2014), which was attributed to significant reductions in NO_3^-, phosphorus, potassium and copper contents because of immobilization of earthworms and microorganism biomass or sorption by vermicompost. The decrease in potassium

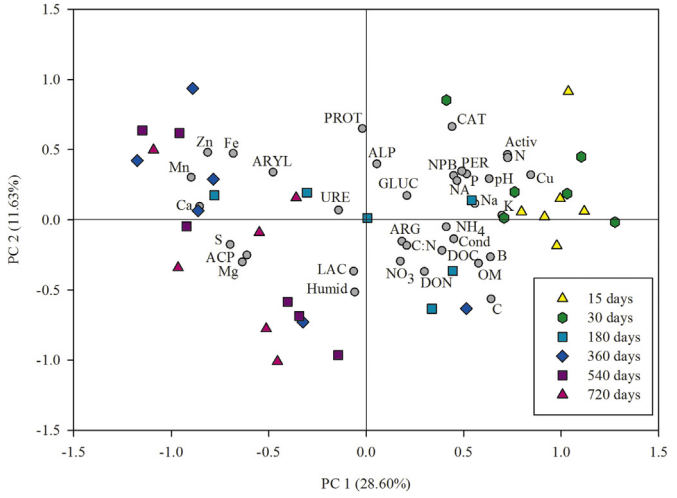

Fig. 4. Principal component analysis (PCA) of chemical and biological characteristics of the grape marc vermicomposted for 15, 30, 180, 360, 540 and 720 days. Acid phosphatase (ACP), alkaline phosphatase (ALP), ammonium (NH_4), arginine (ARG), aryl arylsulfatase (ARYL), boron (B), calcium (Ca), carboxylesterase-NA (NA), carboxylesterase-NPB (NPB), catalase (CAT), copper (Cu), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), electrical conductivity (Cond), glucosidase (GLU), humidity (Humid), iron (Fe), laccase (LAC), magnesium (Mg), manganese (Mn), microbial activity (Activ), nitrate (NO_3), organic matter (OM), peroxidase (PER), phosphorus (P), potassium (K), protease (PROT), ratio C:N (C:N), sodium (Na), sulfur (S), total carbon (C), urease (URE) and zinc (Zn).

content was probably due to immobilization of potassium in the biomass and leaching throughout the vermicompost because of the low ability of potassium to bind to organic matter (Malafaia et al., 2015). The copper content was greater in the vermicompost than in fresh grape marc due to the concentration effect resulting from degradation of the organic compounds by earthworms (Song et al., 2014). However, the copper content decreased during the maturation phase, probably because of strong adsorption of the metal to organic matter promoted by aging of the organic waste (Lv et al., 2016). Gogoi et al. (2015) and Soobhany et al. (2015a) reported a significant reduction in the Cu content of organic waste during the vermicomposting process.

Earthworms accelerate the decomposition of grape marc, which in turn causes high microbial activity (measured as basal respiration) at the beginning of the process of vermicomposting followed by a decrease in the mature phase (Soobhany et al., 2015b). From 30 days onwards, a decrease in microbial activity occurred because of the reduced availability of labile carbon compounds (Huang et al., 2013; Soobhany et al., 2015a). This finding was reflected in the enzymatic activity within the vermicompost. Regarding the enzymes involved in the carbon cycle, the carboxylesterase activity decreased after 30 days of vermicomposting. These enzymes are serine hydrolases and are very important with respect to the environment, as they are involved in the detoxification of anticholinesterase pesticides (organophosphorus and methylcarbamate). The activity of microbial carboxylesterases increases in the presence of earthworms (Sanchez-Hernandez et al., 2014), as observed in the present study, in which the highest activity of these enzymes was observed in the layer with the highest density of earthworms (< 30 days).

Regarding the enzymes involved in the nitrogen cycle, only protease and urease activities changed with vermicomposting time. The protease activity increased because of the greater amount of plant waste available during the first 30 days of the vermicomposting process, which resulted in an increase in NH_4^+ content (Aira et al., 2007). The urease activity increased as the NH_4^+ content decreased (180 days) but then decreased because of the reduction in readily biodegradable fractions of organic matter (Sudkolai and Nourbakhsh, 2017). According to Lakshmi et al. (2014), as vermicomposting progresses, the availability of carbon compounds to microorganisms decreases, reflecting the reduction in microbial biomass and, in turn, urease activity.

Alkaline phosphatase activity was greater in the intermediate period of vermicomposting (30 to 360 days), during which there was relatively high substrate availability and appropriate pH for activity. The high pH and the high available phosphorus content (Olsen P) in the initial layers in the vermireactor led to a decrease in acid phosphatase activity (Fernández-Gómez et al., 2010). The activity of this enzyme was greater after 180 days of vermicomposting, when the vermicompost was more mature, which favors phosphatase activity (García-Sánchez et al., 2017).

Arylsulfatase activity increased after 180 days of vermicomposting when there was greater availability of organic esters containing sulfate (high S_{total}). Arylsulfatase is inhibited by a high concentration of boron (Khafaji and Tabatabai, 1979), which was observed at the beginning of vermicomposting. The boron content decreased after 180 days, which may have allowed greater arylsulfatase activity.

The peroxidase and catalase enzymes behaved similarly, as the activity of both was high in the initial phase but decreased as the vermicompost matured. These enzymes are involved in oxidative stress and are produced in greater amounts as microbial metabolism accelerates. In addition, the generation of reactive oxygen species increases in the presence of excess copper (Cabiscol et al., 2000), as observed in the initial layers of vermicompost. Liu et al. (2012) reported that earthworms cause an increase in catalase activity in vermicompost as a result of their own activity or by favoring microbial activity.

The findings demonstrate that vermicomposting in a vertical deposition vermireactor greatly increased the earthworm density and that the vermicomposting process altered the chemical and biological characteristics of the vermicompost, which was derived from plant waste. The action of the high density of earthworms led to an increase in microbial activity, with increases in the activity of some enzymes at the beginning of the vermicomposting process (in the original worm-processed material), further degrading the grape marc. Consequently, the contents of organic matter, carbon, dissolved and total nitrogen and some nutrients increased. The final quality of the vermicompost depends on the intensity of the active phase. However, significant changes in the chemical and biological characteristics of the vermicompost also occurred during the relatively long maturation phase. Owing to the absence of earthworms and the humification of organic matter during this phase, the microbial activity was low but continuously and gradually altered the chemical characteristics. Thus, the quality of the vermicompost depended on the length of the maturation phase because of alterations in chemical and biological characteristics during the two-year vermicomposting process.

5. Conclusions

The grape marc rapidly degraded during the initial phase of vermicomposting (< 30 days) due to the intense activity of the earthworms and microorganisms. The changes caused by microorganisms during the maturation phase continued to significantly alter the vermicompost, making it impossible to determine the length of time required to produce a chemically mature and biologically stable vermicompost.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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