Bioresource Technology 345 (2022) 126572

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Vermicomposting of cow manure: Effect of time on earthworm biomass and chemical, physical, and biological properties of vermicompost

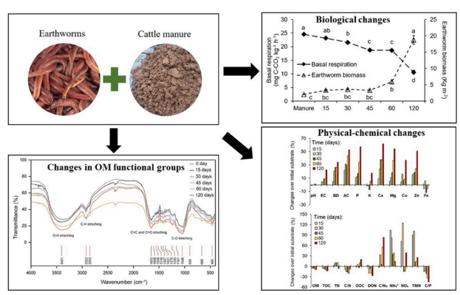
Rodrigo Ferraz Ramos [a], Natielo Almeida Santana [b], Nariane de Andrade [a], Izabelle Scheffer Romagna [a], Bárbara Tirloni [c], Andressa de Oliveira Silveira [b], Jorge Domínguez [d], Rodrigo Josemar Seminoti Jacques [a,*]

[a] *Department of Soil, Federal University of Santa Maria, Roraima Ave., 1000, Camobi, Santa Maria, RS, Brazil*
[b] *Department of Sanitary and Environmental Engineering, Federal University of Santa Maria, Roraima Ave., 1000, Camobi, Santa Maria, RS, Brazil*
[c] *Department of Chemistry, Federal University of Santa Maria, Roraima Ave., 1000, Camobi, Santa Maria, RS, Brazil*
[d] *Grupo de Ecoloxía Animal (GEA), Universidade de Vigo, E-36310 Vigo, Pontevedra, Spain*

HIGHLIGHTS

- Forty-four variables were evaluated during 120 days of vermicomposting.
- The first 45 days characterized the initial vermicomposting phase.
- The largest physical–chemical changes occurred in the final phase.
- At least 120 days are required to produce earthworm matrices.
- The dissolved organic matter aromaticity was modified by vermicomposting.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Cattle manure
Eisenia andrei
Nutrient
Organic matter aromaticity
Infrared spectroscopy

ABSTRACT

Vermicomposting is a biological process for efficient cattle manure treatment, but the vermicomposting time determines the quality of the vermicompost. The objective of this study was to evaluate the effect of cattle manure vermicomposting time on earthworm biomass and the changes in physical, chemical, and biological in properties of the vermicompost. The cattle manure was inoculated with *Eisenia andrei* earthworms and conducted vermicomposting for 0, 15, 30, 45, 60, and 120 days. The analysis of 44 chemical, physical, and biological properties allowed the vermicomposting process to be divided into initial (<45 days) and final (45–120 days) phases. The initial phase was characterized by high microbial activity and the final by high physical–chemical transformation of the vermicompost and an increase in earthworm density. The organic matter aromaticity increased until the 45th day, subsequently decreasing. Although 30 d of vermicompost are sufficient to obtain a high-quality organic fertilizer, 120 d are necessary for producing matrices.

* Corresponding Author.
E-mail address: rodrigo@ufsm.br (R. Josemar Seminoti Jacques).

https://doi.org/10.1016/j.biortech.2021.126572
Received 22 October 2021; Received in revised form 6 December 2021; Accepted 10 December 2021
Available online 15 December 2021

1. Introduction

The increasing global demand for animal protein has resulted in the intensification of confined livestock farming (Gilbert et al., 2021). Consequently, large amounts of cattle manure are produced in small areas, and appropriate environmental residue management is a problem in many countries (Udo et al. 2011). Well-treated and managed cattle manure is rich in plant nutrients (Powers et al., 2019; Rini et al., 2020). Furthermore, organic fertilization under technical criteria has other benefits, including nutrient cycling and less use of chemical fertilizers, which are expensive and made from non-renewable, polluting sources (Li et al., 2017). However, insufficient treatment of organic waste may cause nitrogen losses through volatilization, eutrophication, and environmental contamination with pathogens, antibiotics, heavy metals, among other consequences (Lazcano et al., 2008; Ali et al., 2021).

Vermicomposting is an economical and environmentally friendly method to treat this residue (Cao et al., 2021). Ingestion and fragmentation of the organic substrate occur along with material volume reduction at the beginning of the vermicomposting process, characterized by intense earthworm activity (Domínguez et al., 2017). In this phase, earthworms and the microbial community preferably use easily assimilable molecules (simple carbohydrates, peptides, proteins, vitamins, etc.), while structurally complex (recalcitrant) molecules tend to accumulate in the substrate (Gómez-Brandón et al., 2019). Microorganisms perform a predominant role during the vermicomposting maturation phase, continuing the transformation of organic compounds digested by earthworms (Gómez-Brandón et al., 2020). As a result, the vermicompost produced has high levels of organic matter, nutrients, and plant growth-promoting substances produced by earthworm enteric bacteria, making it an excellent fertilizer (Banerjee et al., 2019).

One of the less-studied variables of vermicomposting is the degree of organic matter aromaticity owing to the need for complex spectral methods including specific ultraviolet absorbance (SUVA) and Fourier-transform infrared spectroscopy (FT-IR) (Zhu et al., 2016; Bhat et al., 2017; Che et al. 2020). However, these spectral methods are essential for understanding transformations promoted by earthworms and microorganisms in the substrate through the different vermicomposting phases. For example, Zhu et al. (2016) observed an increase in organic matter aromaticity throughout the vermicomposting process using FT-IR analysis.

Although vermicomposting is a traditional process, few studies have analyzed chemical, physical, and biological changes, including the aromaticity of organic matter, that occur in the substrate during a long period of vermicomposting. Vermicompost is normally produced and marketed after a production period of only 40–60 days (Che et al., 2020; Rini et al., 2020), and most previous studies evaluated changes in nutrient availability occurring during a short vermicomposting process (<60 days). However, the knowledge of changes in several properties of substrate and in earthworm biomass during a longer period of vermicomposting helps determine when this process should take place to meet the interest of the producer. Ultimately, the variation in vermicomposting time will determine the quality of the vermicompost and amount of earthworm biomass produced.

This is particularly important as there is a growing demand for earthworm biomass in manufacturing feed (e.g., fish farming and poultry activities) and matrices for urban vermicomposting. Only a few studies have evaluated the best time for vermicomposting to obtain high earthworm biomass (Tedesco et al., 2020). Therefore, the aim of the study was to evaluate the effect of cattle manure vermicomposting time on earthworm biomass and the changes in the physical, chemical, and biological properties of the vermicompost.

2. Material and methods

2.1. Substrate and earthworms

Vermicomposting was conducted using confined cattle manure without anti-parasitic treatment. The manure was stored in the dark for 15 days in polypropylene bags; then, 4.0 kg samples were placed into a 5 L vermireactor (20 × 20 × 28 cm) and inoculated with 20 adult *Eisenia andrei* individuals (equivalent to 5,000 earthworms per m^{-2}).

2.2. Experimental design and vermicomposting

The experiment was a randomized design with six treatments and six repetitions. Treatments were defined by vermicomposting time: 0 (substrate), 15, 30, 45, 60, and 120 days. Thus, 36 vermireactors were maintained at 28 °C (±2.0), and their humidity was maintained at ~ 75% of the field capacity by adding water, as necessary. Earthworms were inoculated onto the surface of cattle manure and covered by nonwoven fabric to prevent light exposure. Six experimental units were removed on each of the abovementioned dates to analyze earthworm density, and 500 g substrate samples were homogenized and stored at −20 °C to evaluate microbiological variables. Another 500 g sample was dried in an oven at 65 °C until a constant mass was achieved, sieved, and then used to determine the chemical and physical variables.

2.3. Biological analyses

The density of adults, juveniles, and cocoons was determined for each vermireactor by manual counting. Fresh biomass was determined before earthworms were killed by gradual temperature reduction and freezing. Subsequently, earthworms were dried in a forced-air oven at 68 °C until reaching constant mass to determine the dry mass of juveniles and adults and total dry mass. The reproduction rate was calculated as the relationship between the number of cocoons and adult earthworms in each vermicomposting time. The relative growth rate in each vermicomposting time was calculated as the individual biomass of adult earthworms in the initial substrate (~410 mg ind^{-1}), given per Eq. 1:

$$Growthrate = \frac{\overline{X}tx - \overline{X}ti}{Ntx}$$

Where: $\overline{X}tx$ is the average biomass (mg) in × time, $\overline{X}ti$ is the average initial biomass (mg), and *Ntx* is the time (days) from the beginning of the vermicomposting process.

The vermicompost microbial activity was determined as the CO_2 production in 20 g of wet-mass samples incubated in hermetically sealed flasks for 6 h at 28 °C. The produced CO_2 was captured using 0.1 M NaOH, titrated with standardized HCl (0.02 M), after adding phenolphthalein and excess $BaCl_2$ (Anderson, 1982).

2.4. Physicochemical analysis of the vermicompost

The vermicompost wet mass was determined by weighing the content of each vermireactor and was dried in an oven at 65 °C until a constant mass was achieved (Embrapa, 2017). The pH (water, 1:1) and electrical conductivity (EC) (water, 1:10) of dry substrate samples were determined. The Ca, Mg, Cu, Zn, Fe, Ni, Cr and Pb were determined in an atomic absorption spectrophotometer (AAS, VarianSpectrAA-600, Australia), K was determined using a flame photometer (DM62, Digimed, Brazil), and P was determined using a spectrophotometer (SF325NM, Bel Engineering, Italy). The N and C contents were determined in an elemental analyzer (Flash1112, Thermo Finnigan, Italy) The NH_4^+ and NO_3^- + NO_2^- content was determined using the micro-Kjeldahl method. The bulk density (BD) of the substrate was determined using the beaker method (Jain et al., 2018).

The Zhu et al. (2016) method was modified to determine the dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) content in the vermicompost. Fresh samples were diluted with distilled water (1:10 solid/water ratio) and mixed for 16 h using a horizontal agitator (200 rpm) at 27 °C. After decanting samples for 2 h, the supernatant was extracted and filtered through 0.45 μm membranes to remove solid particles. The filtrate DOC and DON were determined using a total organic carbon (TOC) analyzer (Shimadzu TOC-LCPN, Japan). The C/N and C/P ratios were calculated as per Biruntha et al. (2020), and the dissolved C/N ratio was calculated as the ratio between DOC and DON contents.

2.5. Infrared and ultraviolet visible absorbance spectroscopy

Ultraviolet visible absorbance (UV–VIS) spectroscopy was performed with a spectrophotometer (Shimadzu UV–Vis Spectrophotometer UV-2600, Kyoto, Japan) in the wavelength range of 200–800 nm. All solutions were diluted to DOC concentrations of 1 mg mL^{-1}. The specific UV absorption was determined by normalizing the UV absorbance at 254 nm ($SUVA_{254}$) to the corresponding concentration of DOC and UV cell path length. E4/E6 was the ratio of absorbance measured at 465 and 665 nm. FT-IR spectra were recorded on a Bruker VERTEX 70 spectrophotometer in the 4000–400 cm^{-1} region (4 cm^{-1} of resolution; 64 scans were performed on each sample). The pallets were obtained by pressing a finely ground mixture of 3 mg of vermicompost and 100 mg of dried KBr (spectroscopic grade) under reduced pressure.

2.6. Data analysis

Statistical analyses were performed under the R statistical environment (R Core Team, 2019). Analysis of variance followed by the Tukey test were used to determine the significant difference at a 95% confidence level using the ExpDes.pt package (Ferreira et al., 2018). Spearman correlation coefficient ($P < 0.01$) of quantitative variables was conducted, and the correlation matrix was generated using the corrplot package of R (Wei and Simko, 2017). Finally, principal component analysis (PCA) was conducted using the Stats package.

3. Results and discussion

This study provides additional evidence on the use of cattle manure in vermicomposting processes to reduce environmental impact and obtain a high-quality fertilizer and high earthworm biomass. Changes in vermicompost quality, that is 44 chemical, physical, and biological properties of the organic fertilizer, and earthworm population during 120 days of vermicompost were analyzed. Among which stand out complex spectral methods to assess changes in the aromaticity of organic matter. Unlike most studies already performed, analyses occurred over short time intervals of up to 60 days of vermicomposting, and a final analysis was performed at 120 days. This is a longer period of vermicomposting compared with that reported by most studies (<60 days).

3.1. Microbial activity and earthworm population dynamic

The vermicomposting process can be divided into an initial and a final phase based on earthworm density and microbial respiration (Lazcano et al., 2008; Garcia-Sanchez et al., 2017). The initial phase persisted for 45 days and was characterized by high microbial activity

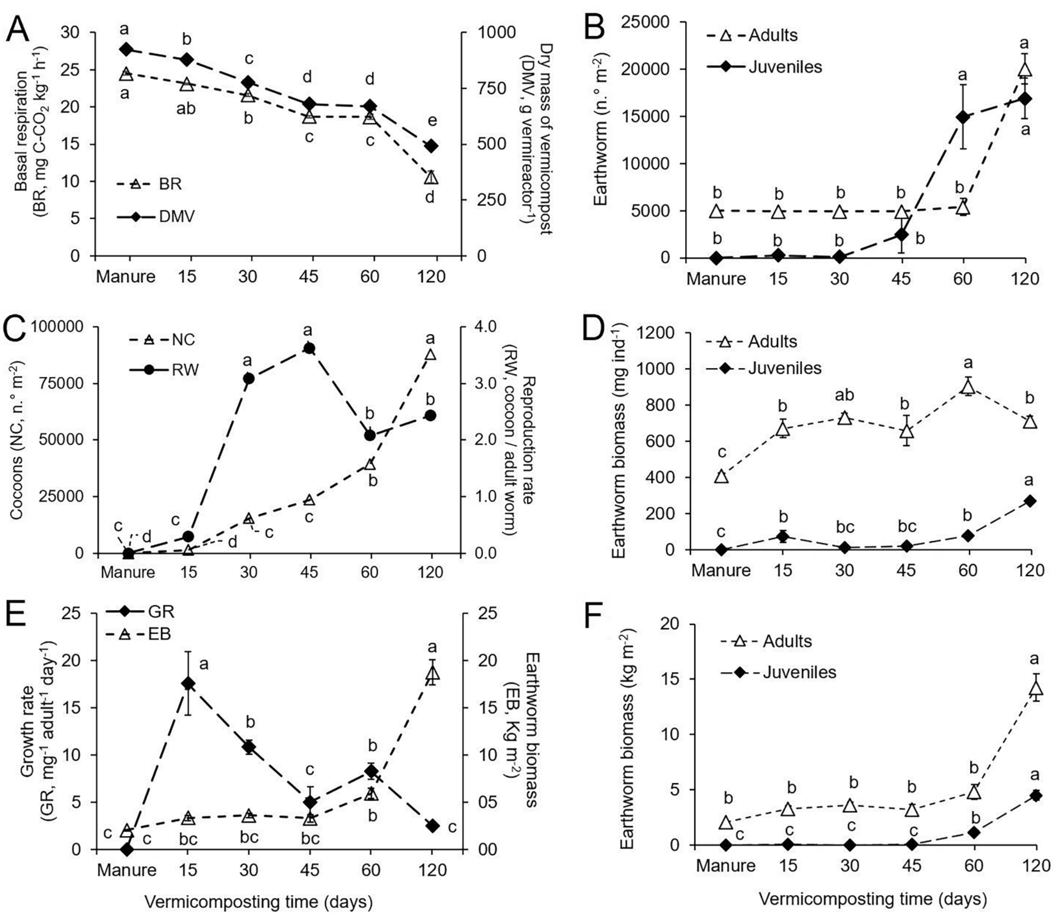


Fig. 1. (A) Basal respiration and dry mass of the vermicompost, (B) adult and juvenile earthworm density, (C) number of cocoons and reproduction rate, (D) adult and juvenile earthworm biomass, (E) growth rate and total earthworm biomass, and (F) adult and juvenile biomass of *Eisenia andrei* during 120 d of cattle manure vermicomposting. Dots correspond to mean values ± standard error. The means followed by the same letter are not significantly different (Tukey's test, $P < 0.05$).

(average of 23 mg C-CO_2 $kg^{-1}h^{-1}$, P < 0.0001) (Fig. 1A) and showed no significant changes in adult and juvenile earthworm populations (Fig. 1B). The final phase was from the 45th to the 120th day and was characterized by an ~ 45% reduction in microbial activity (average of 15 mg C-CO_2 $kg^{-1}h^{-1}$, P < 0.0001) and dry mass of vermicompost (Fig. 1A) and a steep increase in earthworm cocoon population in the vermicompost (Fig. 1C).

A previous study showed a high earthworm activity in the initial phase due to ingestion, mechanical fragmentation, and substrate degradation, increasing the particle surface area and stimulating microbial activity (Domínguez et al., 2010). The final phase of vermicomposting is characterized by reduced earthworm and microorganism activity because of the low availability of fresh residues and increases in recalcitrant organic matter (Gómez-Brandón et al., 2020; Santana et al., 2020).

Nevertheless, the results showed that the highest adult, juvenile, and earthworm cocoon populations (P < 0.0001) occurred in the final phase. Data from Fig. 1D-E show that earthworms with high body mass increase during the first 15 days (Fig. 1D, mg per adult d^{-1}) because of intense substrate degradation. After reaching ~ 600 mg ind^{-1} (Fig. 1D, the initial body mass was ~ 410 mg ind^{-1}), the earthworms directed resources toward reproduction, causing an increase in cocoon production (Fig. 1C, cocoon per adult) between day 15 and 45. Hence, an increase in the number of juvenile earthworms after 45 days and adults from 60 days was observed (Fig. 1B). The earthworm population increase observed at the end of the vermicomposting process occurred because of high substrate degradation by earthworms in addition to increases in biomass (Fig. 1E-F) and reproduction during the initial phase.

Population density is a significant variable to determine the adequacy of an organic substrate as earthworm food (Tedesco et al., 2020). A steep increase in the number of adults, juveniles, and cocoons was observed between the initial population and that after 120 days. The adult population increased 3.8 times, reaching 19,000 ind m^{-2} (Fig. 1B). The juvenile population reached 15,000 ind m^{-2} (Fig. 1B), and the cocoon production increased to ~ 87,000 cocoons m^{-2} (Fig. 1C). Lalander et al. (2015) observed maximum densities of 3,086 adults m^{-2} and 12,344 juveniles m^{-2} after 109 days, 83% and 18% lower than those observed in this study, respectively. In addition, it was possible to produce ~ 19 kg of fresh earthworm biomass (Fig. 1E), being ~ 14 kg of adult earthworm biomass (Fig. 1F) in 120 days of vermicomposting. Thus, our results demonstrated the adequacy of cattle manure as earthworm substrate and that vermicomposting must last at least 120 days to promote earthworm production. Garcia-Sanchez et al. (2017) also observed that the maximum density of *E. andrei* was achieved on the 120th day of vermicomposting in different organic residues.

Earthworms can be used for diverse objectives; live matrices can be sold for bio-transforming organic residues and feeding animals, such as poultry, birds, and frogs. It is also possible to transform earthworms into high-protein animal feed (Chachina et al., 2015; Bhat et al., 2018; Santana et al., 2019; Tedesco et al., 2019). Furthermore, studies have shown the potential for producing mealworms for human consumption (Sun and Jiang 2017; Tedesco et al., 2019, 2020).

3.2. Physical-chemical characteristics of vermicomposting and correlation analysis

After 120 days, earthworm activity changed 19 out of 27 physical–chemical variables (Table 1). Only moisture (P = 0.8492), pH (P = 0.9842), dissolved organic carbon (P = 0.2067), nitrogen (P = 0.2486), iron (P = 0.8478), nickel (P = 0.9999), chrome (P = 0.8121), and lead (P = 0.9822) content did not differ significantly during 120 days of vermicomposting. Moisture remained stable during vermicomposting, with an average of 78% (w/w). These values help explain the success of this vermicomposting process. Santana et al. (2020) affirmed that a 78% moisture content (w/w) during grape pomace vermicomposting was adequate for developing *E. andrei*. The substrate dry mass decreased by 35% (m/m) during the 120 days, while the bulk density increased by 34% (m/v). The increase in bulk density relates to the increase in organic compound decomposition and a disproportionate reduction between the substrate mass and volume (Jain et al., 2018). The treatment of organic residues aims to decrease the substrate mass and volume because it increases nutrient concentration and commercial value and

Table 1
Chemical and physical variables of fresh cattle manure and samples vermicomposted for 15, 30, 45, 60, and 120 d. Mean values ± standard error are presented.

Attribute	Manure	Worm-processed material (days)					Δ (%)		
		15	30	45	60	120	0–45	45–120	Limits[1]
Humidity (%)	77.6 ± 0.4 ns	77.8 ± 0.4	78.4 ± 0.6	77.3 ± 0.6	77.5 ± 0.9	77.7 ± 0.4	– 0.4	0.5	–
VM (g / vermireactor dw)	924 ± 0.0 a	878 ± 5.3b	777 ± 5.6c	679 ± 5.4 d	669 ± 7.1 d	490 ± 10.4 e	−26.9	−27.2	–
Bulk density (g cm^{-3})	0.38 ± 0.0c	0.40 ± 0.0 bc	0.46 ± 0.0 ab	0.48 ± 0.0 a	0.48 ± 0.0 a	0.51 ± 0.0 a	+ 26.4	+ 6.8	–
pH	8.2 ± 0.2 ns	8.2 ± 0.0	8.1 ± 0.0	8.2 ± 0.0	8.2 ± 0.0	8.3 ± 0.0	– 0.07	+ 1.29	> 6
EC (mS cm^{-1})	2.2 ± 0.0c	2.3 ± 0.1 bc	2.3 ± 0.1 bc	2.4 ± 0.0 bc	2.4 ± 0.0b	2.7 ± 0.1 a	+ 8.9	+ 14.9	–
OM (%)	84.7 ± 0.3 a	81.3 ± 1.7 ab	79.9 ± 1.1 bc	80.2 ± 0.5 bc	78 ± 1.1 bc	76.6 ± 0.3c	– 5.3	– 4.4	–
TOC (g kg^{-1} dw)	394.2 ± 5.7 a	372.7 ± 4.7 ab	370.5 ± 2.7 ab	361.2 ± 2.5 bc	351.4 ± 10.1 bc	341.3 ± 5.6c	– 8.4	– 5.5	>100
Total N (g kg^{-1} dw)	26.4 ± 0.5 bc	27.5 ± 0.6 ab	29.1 ± 0.3 a	28.9 ± 0.2 a	29.4 ± 0.4 a	24.8 ± 0.9c	+ 9.2	– 14.2	>5
C / N ratio (total)	15 ± 0.4 a	13.6 ± 0.2 bc	12.7 ± 0.1 bcd	12.5 ± 0.1 cd	11.9 ± 0.2 d	13.8 ± 0.3 bc	– 16.3	+ 10.5	<20
C/P ratio	69.0 ± 2.3 a	58.7 ± 2.2 abc	60.6 ± 3.4 ab	54.4 ± 3.6 bc	46.2 ± 3.2 cd	37.8 ± 1.2 d	−21.2	−30.5	–
DOC (g kg^{-1})	2.39 ± 0.2 ns	2.37 ± 0.1	2.52 ± 0.1	2.30 ± 0.2	2.63 ± 0.2	2.87 ± 0.2	– 3.8	+ 24.9	–
DON (g kg^{-1})	0.67 ± 0.1 ns	0.69 ± 0.0	0.54 ± 0.0	0.63 ± 0.1	0.54 ± 0.1	0.49 ± 0.1	– 6.5	– 21.8	–
Dissolved C / N ratio	3.6 ± 0.2 ab	3.5 ± 0.2b	4.8 ± 0.6 ab	3.8 ± 0.4 ab	5.6 ± 2.0 ab	6.6 ± 1.1 a	+ 6.1	+75.1	–
Total mineral N (mg kg^{-1} dw)	154.7 ± 10.3b	293.1 ± 8.1 a	311.3 ± 27.4 a	173.6 ± 3.1b	177.4 ± 5.3b	194.5 ± 10.8b	+ 12.2	+ 12	–
NH_4^+ (mg kg^{-1} dw)	84.8 ± 2.2c	173.1 ± 4.3 a	154.1 ± 10.2 a	114.1 ± 2.8b	117 ± 4.5b	97 ± 8.4 bc	+ 34.6	– 15	–
NO_3^- + NO_2^- (mg kg^{-1} dw)	70 ± 9.0c	120 ± 3.9 ab	157.3 ± 20.1 a	59.5 ± 5.6c	60.4 ± 6.0c	97.5 ± 8.7 bc	– 14.9	+ 63.9	–
Ash content (g kg^{-1})	153.1 ± 2.4c	186.6 ± 17.3 bc	201.2 ± 10.8 ab	198.3 ± 4.7 ab	220.4 ± 11.5 ab	233.9 ± 3.4 a	+ 29.5	+ 17.9	
P (g kg^{-1} dw)	5.8 ± 0.3c	6.4 ± 0.2 bc	6.5 ± 0.2 bc	6.7 ± 0.4 bc	7.7 ± 0.4 ab	9.1 ± 0.3 a	+ 17.3	+ 34.3	–
K (g kg^{-1} dw)	19.2 ± 0.6 ab	19 ± 0.8 ab	18.9 ± 0.6 ab	17.8 ± 1.8b	20.2 ± 0.6 ab	22.6 ± 0.7 a	– 7.3	+ 26.6	–
Ca (g kg^{-1} dw)	2.1 ± 0.1b	2.3 ± 0.4 bc	2.6 ± 0.1 bc	2.9 ± 0.2 ab	2.9 ± 0.1 ab	3.4 ± 0.2 a	+ 34.8	+ 17.8	–
Mg (g kg^{-1} dw)	9.2 ± 0.2c	9.7 ± 0.4c	10.2 ± 0.4c	10.9 ± 0.9 bc	12.6 ± 0.5 ab	14.2 ± 0.4 a	+ 18.4	+ 29.8	–
Cu (mg kg^{-1} dw)	17.4 ± 0.6b	18.2 ± 0.5b	20.2 ± 1.5 ab	18.6 ± 1.6b	18.9 ± 1.0 ab	23.9 ± 0.9 a	+ 6.9	+ 28.3	<70
Zn (g kg^{-1} dw)	0.53 ± 0.1b	0.61 ± 0.1b	0.62 ± 0.0b	0.63 ± 0.0b	0.66 ± 0.0 ab	0.80 ± 0.1 a	+17.8	+ 27.2	<200
Fe (g kg^{-1} dw)	1.7 ± 0.3 ns	1.6 ± 0.2	1.8 ± 0.2	1.5 ± 0.1	1.6 ± 0.0	1.7 ± 0.1	– 7.6	+ 7.7	–
Ni (mg kg^{-1} dw)	0.57 ± 0.4 ns	0.58 ± 1.3	0.53 ± 0.6	0.71 ± 2.0	0.36 ± 1.5	0.74 ± 0.4	+ 24.6	+ 4.7	<175
Cr (mg kg^{-1} dw)	4.6 ± 4.5 ns	4.1 ± 1.0	8.9 ± 5.3	4.4 ± 2.9	3.7 ± 1.5	9.9 ± 6.4	– 4.6	+125.2	<70
Pb (mg kg^{-1} dw)	1.2 ± 0.9 ns	1.0 ± 1.5	1.4 ± 0.3	1.7 ± 0.5	1.9 ± 0.3	2.0 ± 0.2	+ 46.3	+ 15.0	<150

Means followed by the same letter are not significantly different (Tukey's test, P < 0.05).[1] (MAPA, 2006; MAPA, 2011; MAPA, 2020). (–) Attribute not mentioned in the regulations.

reduces cost of organic fertilizer transportation and application, among other benefits (Udo et al., 2011; Powers et al., 2019).

Biological transformations throughout the cattle vermicomposting process caused decreases in OM ($P = 0.0002$) and TOC ($P < 0.0001$), possibly indicating an increase in organic matter humification. In general, the loss of C through respiration tends to be faster than decreases in N, causing decreases in the vermicompost C/N relationship (Li et al., 2020), as observed in this study. The NH_4^+ content expectedly increased during the initial phase (35%) and decreased in the final phase (15%) due to the precedence of ammonification over nitrification. Despite large fluctuations during the 120 days, the $NO_3^- + NO_2^-$ content increased in the final phase. In general, epigean earthworms significantly affect N transformations during vermicomposting due to their biomass increases, microbial activity stimulation, and modifications in the vermicompost environmental conditions (Domínguez and Gómez-Brandón, 2013).

Despite DOC and DON content remaining unmodified throughout the vermicomposting process, there was a significant increase in the dissolved C/N ratio ($P = 0.0201$), indicating a decrease in N content of the vermicompost soluble fraction. Lazcano et al. (2008) suggested that the reduction in DOC and DON contents is desirable for vermicomposting because high contents may be harmful to plants because of their facilitated degradation by the soil microbiota and consequent reduction of oxygen concentration near roots. Organic fertilizers containing < 4.0 g DOC kg^{-1} are considered safe for plant growth (Gómez-Brandón et al., 2008), as obtained in this study.

The sharpest percentage increases in P, K, Mg, Cu, Zn, Fe, and Cr contents occurred during the final phase, with the sharpest increase in Ca, Ni, and Pb content in the initial phase (Table 1). The increase in nutrient availability at the end of vermicomposting compared with the initial substrate relates to organic matter degradation, nutrient mineralization, and a decrease in substrate volume (due to concentration) (Jain et al., 2018; Cao et al., 2021). Decreases in K content during the initial 45 days coincided with the sharpest increase of earthworm growth and reproduction, indicating possible immobilization of K by those organisms and the microbial community (Malafaia et al., 2015).

Table 1 shows a comparison of all the physical–chemical variables, and 63% of variables experienced their greatest change (Δ%) in the final phase (45–120 days) of vermicomposting. However, there was intense earthworm and microorganism activity in the initial phase. One reason

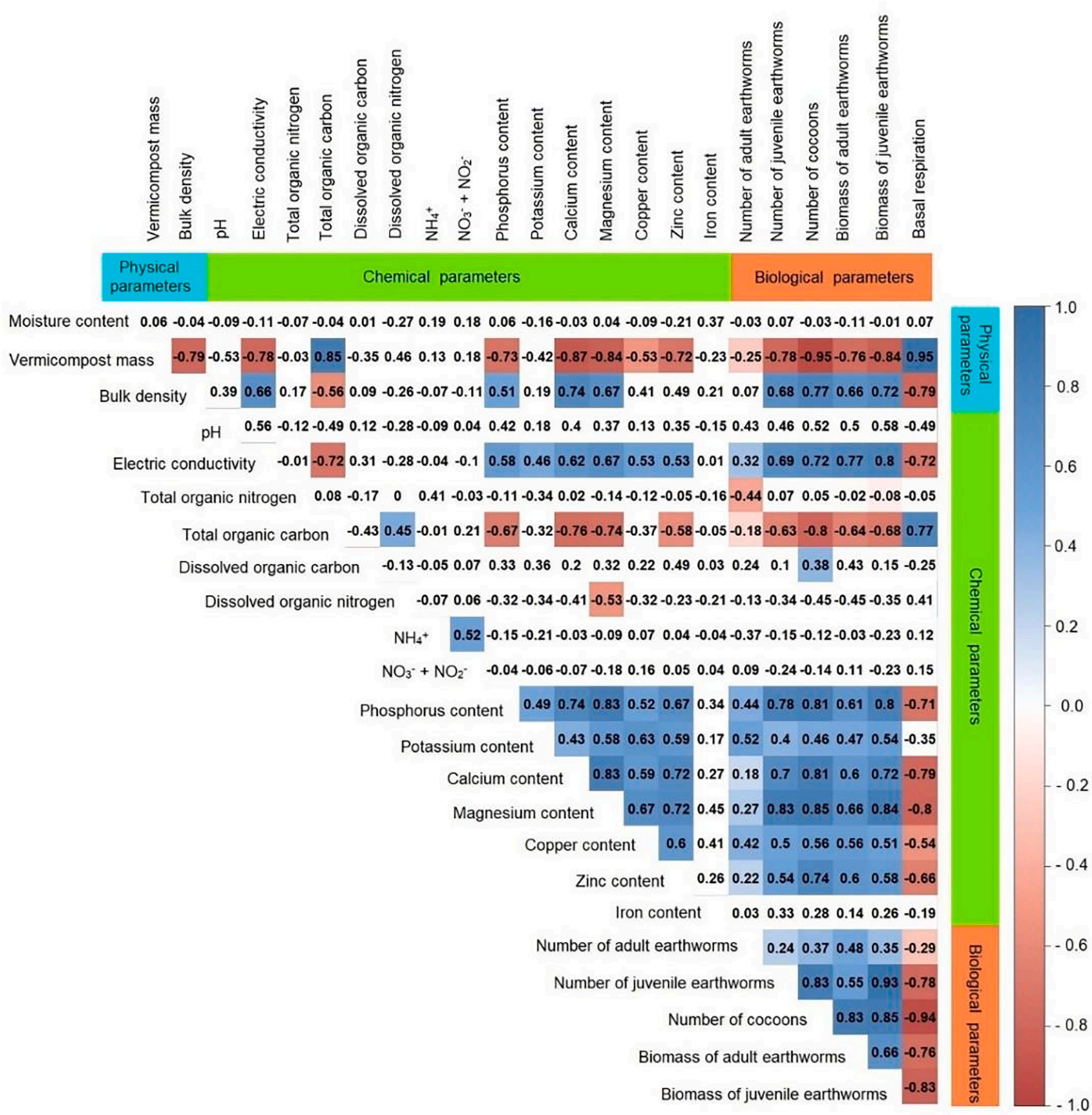


Fig. 2. Spearman correlation between physical, chemical, and biological variables in cattle manure vermicomposted using *Eisenia andrei*. Cells with insignificant correlations ($P < 0.01$) are shown in white.

for this behavior is the longer final phase (~75 days) when compared with the initial phase (~45 days). Furthermore, data from Fig. 1 help to understand this behavior. The earthworm growth rate (mg per adult d^{-1}) and microorganism activity were lower in the final phase; however, increases in the number and biomass of adult and juvenile earthworms indicate significant feeding activity, with consequential physical–chemical modification in the substrate until the end of vermicomposting. Finally, although microbial activity was low, it continued and contributed to the change in the substrate in this phase, indicating that the stabilization process of vermicomposting for 120 days was incomplete.

Good quality vermicompost for agricultural usage, as per Brazilian and international laws, were sufficiently obtained after 30 days (Brinton 2000, MAPA 2020). All parameters regulated in the laws (P, K, Ca, Mg, Cu, Zn, Fe, TOC, TN, and C/N ratio) were within the permissible limits (Table 1). Furthermore, the highest mineral nitrogen ($NH_4^+ + NO_3^- + NO_2^-$) content was observed after 30 days (Table 1), an important nutrient for plant growth which is normally used to determine the required dose of vermicompost. Although there was an increase in heavy metal contents (Cu and Zn), they were substantially lower than the permissible limits for organic agricultural substrates of international laws (Cu = 100 mg kg^{-1} and Zn = 4.0 g kg^{-1}) (Balachandar et al., 2021).

The strong positive correlation between microbial activity and substrate mass ($\rho = 0.95$) and TOC ($\rho = 0.77$) (Fig. 2) indicates that higher carbon content, easily degradable during the initial vermicomposting phase, determines microbial action. Increases in earthworm population result in substrate mass reduction and degradation, causing increases in nutrient content (Rini et al., 2020; Cao et al., 2021), indicated by the significantly positive correlation between the number of adult earthworms and nutrient content (P = 0.44, K = 0.52, Ca = 0.18, Mg = 0.27, Cu = 0.42, Zn = 0.22, and EC = 0.32) (Fig. 2). The significantly negative correlation between microbial respiration and the remaining biological variables resulted from the distinction among the vermicomposting phases: the initial phase characterized by high microbial activity and low earthworm density and biomass and the final phase characterized by decreases in microbial activity and higher earthworm density and biomass. The positive correlation between inorganic nitrogen forms ($\rho = 0.52$) is caused by nitrification promoted by the microbial community with the N-NH_4^+ mineralization in vermicomposts, resulting in rapid transformation into $NO_3^- + NO_2^-$ (Lv et al., 2019).

3.3. Principal components analysis

PCA showed that 50.1% and 9.1% of the data remained in PC1 and PC2, respectively (Fig. 3). Most of the variables that contributed to total covariance in PC1 were biological, while chemical variables played the same role in PC2 (see supplementary materials). The PCA allowed variables to be grouped according to each vermicomposting phase. The variables, $NO_3^- + NO_2^-$, NH_4^+, basal respiration, and mass of vermicompost, were closely related and correlated to the initial phase (smaller ellipse of Fig. 3), while TOC, DON, and TN formed an intermediate group between both phases (ellipses overlap). The initial phase of vermicomposting was characterized by intense microbial activity that promoted the reduction in cattle manure volume by converting organic matter into CO_2 and consequently promoted nitrogen mineralization (Santana et al., 2020). However, all other variables (EC, BD, number of adults and juveniles, biomass of adults and juveniles, number of cocoons, and P, K, Mg, Cu, and Zn content) were grouped in the final phase (larger ellipse of Fig. 3). The production of high biomass of earthworms only occurs in the final stage of vermicomposting when there is also a

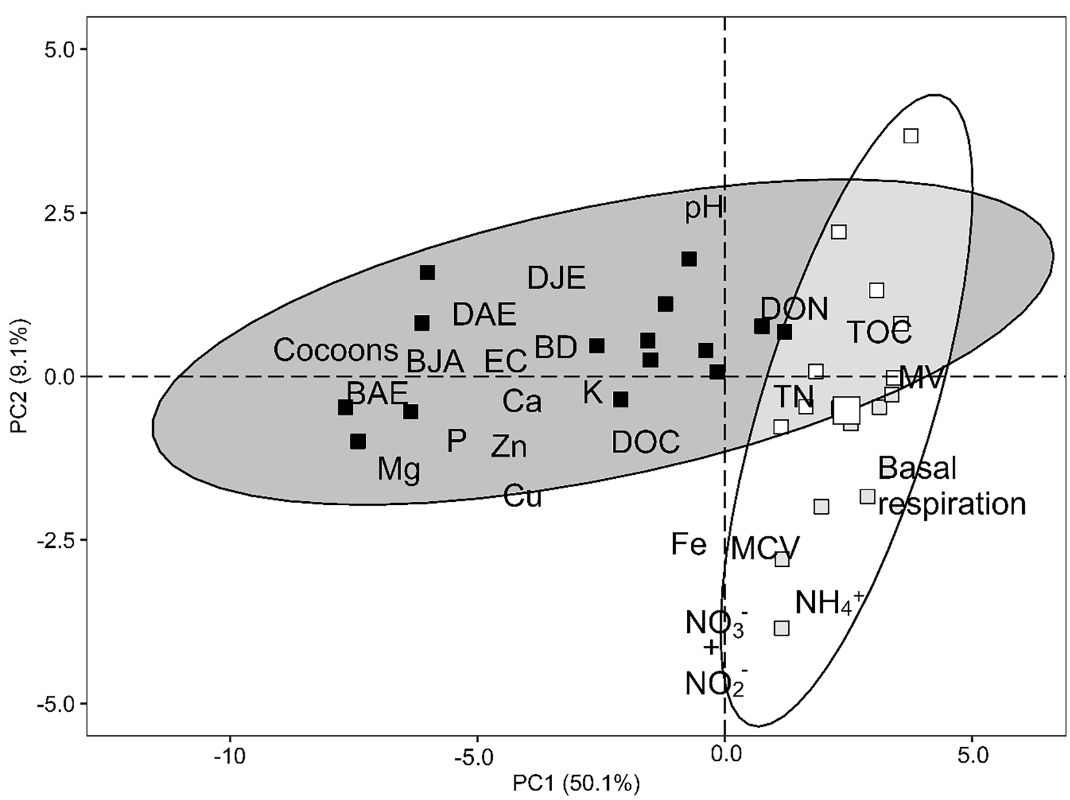


Fig. 3. Principal components analysis among physical, chemical, and biological variables in cattle manure vermicomposted using *Eisenia andrei*. White and black squares are individual values grouped in the initial and final phases, respectively. The ellipse areas in white and black represent variables grouped in the initial and final vermicomposting phases, respectively. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TOC, total organic carbon; TN, total organic nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; Zn, zinc; Cu, copper; Fe, iron; BD, bulk density; MV, mass of vermicompost; $NO_3^- + NO_2^-$, nitrite + nitrate; NH_4^+, ammonium; EC, electrical conductivity; BAE, biomass of adult earthworms; BJE, biomass of juvenile earthworms; DAE, number of adult earthworms; DJE, number of juvenile earthworms; and MCV, moisture of the vermicompost.

higher concentration of nutrients because of the mineralization of more easily degradable natural compounds and consequent reduction in the volume of the substrate (ZZiwa et al., 2021).

3.4. $SUVA_{254}$, E4/E6 and FT-IR spectroscopy

$SUVA_{254}$ (specific UV absorption at 254 nm) has been used as an index for aromatic compounds in the water extractable organic matter (WEOM) (Weishaar et al., 2003). $SUVA_{254}$ values of the DOM corroborate the division into initial and final vermicomposting stages (Table 2). These values increased until the 45th day, a period characterized by intense substrate degradation. Earthworms and microorganisms likely acted selectively towards aliphatic molecules, targeting those which are easily degradable and contain a higher nutritional value (Che et al., 2020). According to Caricasole et al. (2010), the increase in $SUVA_{254}$ could be associated with the fast transformation of non-aromatic compounds, which led to a relative enrichment in aromatics. Additionally, an increase of $SUVA_{254}$ value proposes a higher degree of aromaticity and molecular weight (He et al., 2011). After 45 days, $SUVA_{254}$ values decreased from 4.09 to 3.53 L/(mg m), likely due to the increase in earthworm density. The coelomic fluid, constantly expressed by earthworms, and coprolites have high gluconate proteins and glycosidic molecule contents, enriching the substrate with new aliphatic molecules with low molecular mass (Gómez-Brandón et al., 2011; Yadav et al. 2015). Although this variable was not measured, it was possible to observe higher substrate stickiness in reactors as earthworm density increased, especially after 60 days. The E4/E6 ratio is related to the degree of aromatic polycondensation and the molecular weight of the humic substances (Zhu et al. 2016). In the present study, the E4/E6 ratio was not a clear indicator of changes in organic matter (Table 2). However, Saab and Martin-Neto (2007) demonstrated that the E4/E6 ratio is more associated with condensed aromatic groups and not with the total aromaticity of the samples.

FT-IR spectroscopy ensures easy identification of chemical functional groups, where the change in the intensity of the absorption band can be used to assess vermicompost stability (Lim and Wu, 2015). FT-IR spectra obtained from different treatments show differences in the relative intensity of absorption bands (see supplementary materials). The final vermicompost showed a significant decrease in the peak intensity at 3401 cm^{-1} (O – H stretch), indicating phenol and carbohydrate decomposition (Srivastava et al., 2020). The reduction in peak intensity at 2922–2853 cm^{-1} (C – H stretching of aliphatic methylene groups) during the treatment suggests degradation of lipid and carbohydrates due to a decrease in aliphatic structures. A decreasing relative intensity was observed at approximately 1653 cm^{-1} (C = O stretching of amide groups, quinonic C = O and/or C = O of H-bonded conjugated ketones) (Zhu et al., 2016). The peak intensities increased at 1606–1421 cm^{-1} (C = C stretching of aromatic groups) because of elevated levels of aromatic groups during vermicomposting. The peak at 1046 cm^{-1} is related to C – O stretching of polysaccharides. These results corroborate the $SUVA_{254}$ observations and indicate a preferential degradation of easily degradable compounds by earthworms and microorganisms, increasing the DOM aromaticity.

Earthworm population and microbial activity divide vermicompost into initial (45 days) and final (45–120 days) phases. The vermicompost produced for 30 days has optimal moisture, pH, conductivity, and nutrient content. The largest physical–chemical transformations and major earthworm density occurred in the final phase. In the initial period of vermicomposting, the interative effect between the earthworms and the high microbial activity promoted an increase in the degree of aromaticity of the organic matter. However, the large increase in earthworm density resulted in the formation of less aromatic organic matter. Vermicomposting time promotes changes in the functional groups of organic matter because of the degradation of phenols, carbohydrates, and lipids, resulting in the reduction of aliphatic structures and increasing the degree of aromaticity of organic matter.

Table 2
$SUVA_{254}$ and E4/E6 values of DOC from vermicomposting.

Attribute	Manure	Worm-processed material (days)				
		15	30	45	60	120
$SUVA_{254}$ (L/(mg m))	3.3 ± 0.3 ab	3.1 ± 0.1b	2.7 ± 0.2b	4.1 ± 0.4 a	3.1 ± 0.2 ab	3.5 ± 0.2 ab
E4/E6	4.8 ± 0.0c	4.9 ± 0.0b	4.7 ± 0.0 d	5.0 ± 0.0 a	4.7 ± 0.0 d	5.0 ± 0.0 a

4. Conclusions

Vermicomposting time has a role in modifying the chemical, physical, and biological properties of the vermicompost, including the aromaticity of organic matter. Because of the high activity of earthworms and microorganisms at the beginning of vermicomposting, in 30 days it is already possible to use this organic fertilizer in agriculture. However, the largest physical–chemical transformations of the vermicompost and the increase in the earthworm population occur from 45 to 120 days. Therefore, vermicomposting must last at least 120 days to produce *Eisenia andrei* earthworm matrices.

CRediT authorship contribution statement

Rodrigo Ferraz Ramos: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Natielo Almeida Santana:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Nariane de Andrade:** Investigation, Methodology. **Izabelle Scheffer Romagna:** Investigation, Methodology. **Bárbara Tirloni:** Investigation, Methodology. **Andressa de Oliveira Silveira:** Conceptualization, Investigation, Methodology. **Jorge Domínguez:** Conceptualization, Methodology. **Rodrigo Josemar Seminoti Jacques:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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.

Acknowledgements

The authors are grateful to the National Council of Scientific and Technological Development (*Conselho Nacional de Desenvolvimento Científico e Tecnológico* - CNPq) and Coordination for the Improvement of Higher Education Personnel (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - CAPES) (Finance Code 001) for providing scholarships and financial support for this research. We are also grateful to Eduarda Calegari for supporting the collection from reactors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2021.126572.

References

Ali, M., Khanom, A., Nahar, K., Ali, Y., Azad, A.K., Rahman, M., 2021. Effect of manure application on net nitrification rates, heavy metal concentrations and nitrifying archaea/bacteria in soils. Bull. Environ. Contam. Toxicol. 106, 707–713.

Anderson, J.P.E., 1982. Soil respiration. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Chemical and Microbiological Properties, second ed. ASA-SSSA, Madison, pp. 831–871.

Balachandar, R., Biruntha, M., Yuvaraj, A., Thangaraj, R., Subbaiya, R., Govarthanan, M., Kumar, P., Karmegam, N., 2021. Earthworm intervened nutrient recovery and greener production of vermicompost from *Ipomoea staphylina* - An invasive weed with emerging environmental challenges. Chemosphere 263, 128080.

Banerjee, A., Biswas, J.K., Pant, D., Sarkar, B., Chaudhuri, P., Rai, M., Meers, E., 2019. Enteric bacteria from the earthworm (*Metaphire posthuma*) promote plant growth and remediate toxic trace elements. J. Environ. Manage. 250, 109530.
Biruntha, M., Karmegam, N., Archana, J., Selvi, B.K., Paul, J.A.J., Balamuralikrishnan, B., Chang, S.W., Ravindran, B., 2020. Vermiconversion of biowastes with low-to-high C/N ratio into value added vermicompost. Bioresour. Technol. 297, 122398.
Bhat, S.A., Singh, S., Singh, J., Kumar, S., Bhawana, V.A.P., 2018. Bioremediation and detoxification of industrial wastes by earthworms: Vermicompost as powerful crop nutrient in sustainable agriculture. Bioresour. Technol. 252, 172–179.
Bhat, S.A., Singh, J., Vig, A.P., 2017. Instrumental characterization of organic wastes for evaluation of vermicompost maturity. Journal of Analytical Science and Technology 8, 2.
Brinton, W.F., 2000. Compost quality standards & guidelines, first ed. Woods End Research Laboratory, New York.
Cao, Y., Tian, Y., Wu, Q., Jianshe, L.i., Zhu, H., 2021. Vermicomposting of livestock manure as affected by carbon-rich additives (straw, biochar and nanocarbon): A comprehensive evaluation of earthworm performance, microbial activities, metabolic functions and vermicompost quality. Bioresour. Technol. 320, 124404.
Caricasole, P., Provenzano, M.R., Hatcher, P.G., Senesi, N., 2010. Chemical characteristics of dissolved organic matter during composting of different organic wastes assessed by 13C CPMAS NMR spectroscopy. Bioresour. Technol. 101 (21), 8232–8236.
Chachina, S.B., Voronkova, N.A., Baklanova, O.N., 2015. Biological remediation of the engine lubricant oil-contaminated soil with three kinds of earthworms, *Eisenia fetida*, *Eisenia andrei*, *Dendrobena veneta*, and a mixture of microorganisms. Procedia Engineering 113, 113–123.
Che, J., Lin, W., Ye, J., Liao, H., Yu, Z., Lin, H., Zhou, S., 2020. Insights into compositional changes of dissolved organic matter during a full-scale vermicomposting of cow dung by combined spectroscopic and electrochemical techniques. Bioresour. Technol. 301, 121757.
Domínguez, J., Gómez-Brandón, M., 2013. The influence of earthworms on nutrient dynamics during the process of vermicomposting. Waste Manag. Res. 31 (8), 859–868.
Domínguez, J., Aira, M., Gómez-Brandón, M., 2010. Vermicomposting: earthworms enhance the work of microbes. In: Insam, H., Franke-Whittle, I., Goberna, M. (Eds.), Microbes at Work. Springer, Berlin, pp. 93–114.
Domínguez, J., Sanchez-Hernandez, J.C., Lores, M., 2017. Vermicomposting of winemaking by-products. In: Galanakis, C.M. (Ed.), Handbook of grape processing by-products. Academic Press, London, pp. 55–78.
Embrapa, 2017. Soil Analysis Methods Manual, third ed. Brazilian Agricultural Research Corporation, Brasília.
Ferreira, E.B., Cavalcanti, P.P., Nogueira, D.A., 2018. ExpDes.pt: Pacote Experimental Designs (Portuguese). R package version 1.2.0, 2018.
Garcia-Sanchez, M., Taušnerová, H., Hanc, A., Tlustoš, P., 2017. Stabilization of different starting materials through vermicomposting in a continuous-feeding system: changes in chemical and biological parameters. Waste Management 62, 33–42.
Gilbert, W., Thomas, L.F., Coyne, L., Rushton, J., 2021. Review: Mitigating the risks posed by intensification in livestock production: the examples of antimicrobial resistance and zoonoses. Animal: The international journal of animal biosciences 15, 100123.
Gómez-Brandón, M., Lazcano, C., Domínguez, G., 2008. The evaluation of stability and maturity during the composting of cattle manure. Chemosphere 70 (3), 436–444.
Gómez-Brandón, M., Aira, M., Lores, M., Domínguez, J., 2011. Changes in microbial community structure and function during vermicomposting of pig slurry. Bioresour. Technol. 102, 4171–4178.
Gómez-Brandón, M., Aira, M., Kolbe, A.R., Andrade, N., Perez-Losada, M., Domínguez, J., 2019. Rapid bacterial community changes during vermicomposting of grape marc derived from red winemaking. Microorganisms 7, 473.
Gómez-Brandón, M., Aira, M., Santana, N.A., Perez-Losada, M., Domínguez, J., 2020. Temporal dynamics of bacterial communities in an industrial-scale vermireactor fed with distilled grape marc. Microorganisms 8, 1–19.
He, X.S., Xi, B.D., Wei, Z.M., Guo, X.J., Li, M.X., An, D., Liu, H.L., 2011. Spectroscopic characterization of water extractable organic matter during composting of municipal solid waste. Chemosphere 82 (4), 541–548.
Jain, M.S., Jambhulkar, R., Kalamdhad, A.S., 2018. Biochar amendment for batch composting of nitrogen rich organic waste: Effect on degradation kinetics, composting physics and nutritional properties. Bioresour. Technol. 253, 204–213.
Lalander, C.H., Komakech, A.J., Vinnerås, B., 2015. Vermicomposting as manure management strategy for urban small-holder animal farms - Kampala case study. Waste Management 39, 96–103.
Lazcano, C., Gómez-Brandón, M., Domínguez, J., 2008. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. Chemosphere 72 (7), 1013–1019.
Li, H., Feng, W.-T., He, X.-H., Zhu, P., Gao, H.-J., Sun, N., Xu, M.-G., 2017. Chemical fertilizers could be completely replaced by manure to maintain high maize yield and soil organic carbon (SOC) when SOC reaches a threshold in the Northeast China Plain. Journal of Integrative Agriculture 16 (4), 937–946.
Li, Y., Yang, X., Gao, W., Qiu, J., Li, Y., 2020. Comparative study of vermicomposting of garden waste and cow dung using *Eisenia fetida*. Environ. Sci. & Pollut. 27, 9646–9657.
Lim, S.L., Wu, T.Y., 2015. Determination of maturity in the vermicompost produced from palm oil mill effluent using spectroscopy, structural characterization and thermogravimetric analysis. Ecol. Eng. 84, 515–519.
Lv, B., Zhang, D., Chen, Q., Cui, Y., 2019. Effects of earthworms on nitrogen transformation and the correspond genes (amoA and nirS) in vermicomposting of sewage sludge and rice straw. Bioresour. Technol. 287, 121428.
Malafaia, G., Costa Estrela, D., Guimarães, A.T.B., Araújo, F.G., Leandro, W.M., Lima Rodrigues, A.S., 2015. Vermicomposting of different types of tanning sludge (liming and primary) mixed with cattle dung. Ecol. Eng. 85, 301–306.
MAPA, 2006. SDA normative instructions n. 27, seventh ed. Official Journal of the Union, Brasília.
MAPA, 2011. Normative instructions n. 46 (plant and animal production), first ed. Official Journal of the Union, Brasília.
MAPA, 2020. Normative instructions n. 61, 134 ed. Official Journal of the Union, Brasília.
R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Powers, S.M., Chowdhury, R.B., MacDonald, G.K., Metson, G.S., Beusen, A.H.W., Bouwman, A.F., Hampton, S.E., Mayer, B.K., McCrackin, M.L., Vaccari, D.A., 2019. Global opportunities to increase agricultural independence through phosphorus recycling. Earth's Future 7 (4), 370–383.
Rini, J., Deepthi, M.P., Kulandaivel, S., Ramasamy, T.N., Natchimuthu, K., Palanisamy, K., 2020. Nutrient recovery and vermicompost production from livestock solid wastes with epigeic earthworms. Bioresour. Technol. 313, 123690.
Santana, N.A., Ferreira, P.A.A., Tarouco, C.P., Schardong, I.S., Antoniolli, Z.I., Nicoloso, F.T., Jacques, R.J.S., 2019. Earthworms and mycorrhization increase copper phytoextraction by *Canavalia ensiformis* in sandy soil. Ecotoxicol. Environ. Saf. 182, 109383.
Santana, N.A., Jacques, R.J.S., Antoniolli, Z.I., Martínez-Cordeiro, H., Domínguez, J., 2020. Changes in the chemical and biological characteristics of grape marc vermicompost during a two-year production period. Applied Soil Ecology 154, 103587.
Sun, Z., Jiang, H., 2017. Nutritive evaluation of earthworms as human food. In: Mikkola, H. (Ed.), Future Foods. IntechOpen, London.
Srivastava, V., Goel, G., Thakur, V.K., Singh, R.P., Araujo, A.S.F., Singh, P., 2020. Analysis and advanced characterization of municipal solid waste vermicompost maturity for a green environment. J. Environ. Manage. 255, 109914.
Tedesco, D.E.A., Castrica, M., Tava, A., Panseri, S., Balzaretti, C.M., 2020. From a food safety prospective: the role of earthworms as food and feed in assuring food security and in valuing food waste. Insects 11, 293.
Tedesco, D.E.A., Conti, C., Lovarelli, D., Biazzi, E., Bacenetti, J., 2019. Bioconversion of fruit and vegetable waste into earthworms as a newprotein source: The environmental impact of earthwormmeal production. Sci. Total Environ. 683, 690–698.
Udo, H.M.J., Aklilu, H.A., Phong, L.T., Bosma, R.H., Budisatria, I.G.S., Patil, B.R., Samdup, T., Bebe, B.O., 2011. Impact of intensification of different types of livestock production in smallholder crop-livestock systems. Livestock Science 139, 22–29.
Wei, T., Simko, V., 2017. R package "corrplot": Visualization of a Correlation Matrix. Version 84.
Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ. Sci. Technol. 37 (20), 4702–4708.
Yadav, A., Suthar, S., Garg, V.K., 2015. Dynamics of microbiological parameters, enzymatic activities and worm biomass production during vermicomposting of effluent treatment plant sludge of bakery industry. Environ. Sci. Pollut. Res. 22, 14702–14709.
Zhu, W., Yao, W., Du, W., 2016. Heavy metal variation and characterization change of dissolved organic matter (DOM) obtained from composting or vermicomposting pig manure amended with maize straw. Environ. Sci. Pollut. Res. 23, 12128–12139.
Zziwa, A., Jjagwe, J., Kizito, S., Kabenge, I., Komakech, A.J., Kayondo, H., 2021. Nutrient recovery from pineapple waste through controlled batch and continuous vermicomposting systems. J. Environ. Manage. 279, 111784.