Waste Management 135 (2021) 1–10

Contents lists available at ScienceDirect

Waste Management

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

Changes in the nutrient dynamics and microbiological properties of grape marc in a continuous-feeding vermicomposting system

María Gómez-Brandón [*], Hugo Martínez-Cordeiro, Jorge Domínguez

Grupo de Ecoloxía Animal (GEA), Universidad de Vigo, Vigo 36310, Spain

A R T I C L E I N F O

Keywords:
Winery wastes
Eisenia andrei
Vermicompost
Organic amendment

A B S T R A C T

Finding strategies to reuse and treat organic wastes is of utmost need. Biological processes offer the possibility to transform them into safer end products with benefits for both agriculture and the environment. Moreover, it represents an ecologically-sound and economically attractive alternative to landfill disposal and incineration. In this work, we evaluated the feasibility of vermicomposting to treat and process grape marc, the main solid by-product of the wine industry. The long-term changes in grape marc derived from both white and red winemaking processes were assessed throughout the process of vermicomposting from a physico-chemical and microbiological perspective. New layers of fresh marc were added sequentially in the presence and absence of earthworms (*Eisenia andrei*) forming an age gradient during a 42-week period. An optimal moisture level of 70% was maintained over the course of the process. The pH fell within weak-alkaline levels through the layerś profile and the electrical conductivity was between 200 and 300 μS cm^{-1}, providing optimum conditions for earthworm growth. The mass loss caused by earthworm activity led to an increased content of macro- and micronutrients at the end of the trial. An overall decrease in microbial biomass and its activity, indicative of a stabilised material, was also recorded with depth of layer. Altogether, this points to vermicomposting as a suitable management system for processing grape marc with a dual purpose, that is fertilizer production and environment protection. This is especially relevant in the current attempts to reach a fully circular economy.

1. Introduction

Given the economic importance of the winemaking industry worldwide (http://www.oiv.int in Mateo and Maicas, 2015), increasing attention has been given to the management and valorization of grape marc and other winery by-products as reviewed by Cortés et al. (2020), Domínguez et al. (2016, 2017) and Gómez-Brandón et al. (2019a). In this regard, vermicomposting has been shown to effectively transform this winery by-product and its distillery effluent into a nutrient-rich, biologically active and polyphenol-free organic vermicompost (Almeida-Santana et al., 2020; Částková and Hanč, 2019; Domínguez et al., 2014, 2016, 2017; García-Sánchez et al., 2017; Gómez-Brandón et al., 2019a,b; 2020a,b,c; Kolbe et al., 2019).

The properties of the final vermicompost and its potential usefulness as an organic amendment are inextricably linked to the earthworms ‘activity over the course of the vermicomposting process (Domínguez et al., 2016, 2017; Gómez-Brandón et al., 2019a). In the short-term, epigeic earthworms are known to accelerate the rate of organic matter decomposition (Domínguez et al., 2010), and alter the nutrient pools and microbial communities by ingesting, fragmenting and reducing the volume of the initial material during the transit through the earthworm gut (Domínguez and Gómez-Brandón, 2012). Indeed, rapid shifts in the microbial biomass and activity as well as in the microbial community composition of the grape marc have been reported within the first fifteen days of vermicomposting (Gómez-Brandón et al., 2011a, 2019b; Kolbe et al., 2019). These compositional changes were further accompanied by a rapid increase in the metabolic capacity of the grape marćs bacterial communities as reflected by a higher abundance of genes related to cellulose metabolism (Kolbe et al., 2019).

The changes occurred during the initial stages of vermicomposting will largely affect the performance of the process (Gómez-Brandón and Domínguez, 2014), as the modified microbial communities will be released into the vermicomposting system as part of the earthworm casts (Aira et al., 2015). The inoculation of these egested materials into the raw substrate was found to alter the rate of organic matter decomposition, similar to that when earthworms are present (Aira et al., 2011). In addition, significative changes in the nutrient dynamics and microbiome composition have been reported during the cast ageing period (Aira

---

[*] Corresponding author.
E-mail addresses: mariagomez@uvigo.es, mariagomezbrandon@gmail.com (M. Gómez-Brandón).

https://doi.org/10.1016/j.wasman.2021.08.004
Received 24 May 2021; Received in revised form 15 July 2021; Accepted 3 August 2021

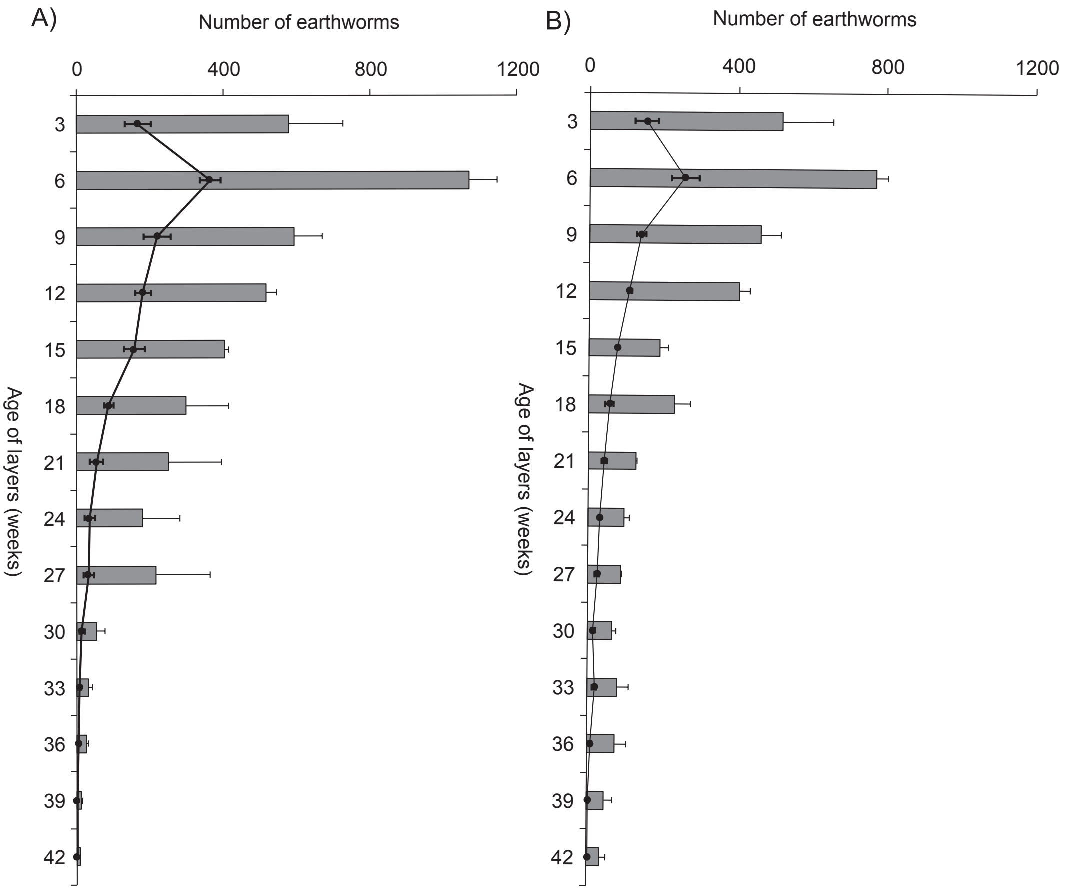

Fig. 1. Number of earthworms in each layer, from 3 to 42 weeks of age, of the reactors fed with grape marc from the cultivars Albariño (A) and Mencía (B). The biomass of earthworms (black circles) in each layer is also shown. The corresponding units are given in grams. Values are means ± SE.

et al., 2010, 2019). This latter period is characterized by a gradual decrease of labile carbon and nitrogen pools (Aira et al., 2010) that can lead to a successional variation in the bacterial communities according to their trophic characteristics (from copiotrophs to oligotrophs; Aira et al., 2019). These long-term changes mainly rely on the activity of microorganisms via the production of extracellular enzymes. In fact, higher levels of urease, acid phosphatase and arylsulfatase were found after one year of vermicomposting of grape marc when compared to the first 30 days of the process (Almeida-Santana et al., 2020). All in all, it underlines that a period of ageing is necessary for a proper use of the grape marc derived vermicompost as plant growth promoter and in plant disease suppressiveness.

To date, most studies on vermicomposting have primarily focused on marc derived either from white (Almeida-Santana et al., 2020; Domínguez et al., 2014; Kolbe et al., 2019) or red grape varieties (Gómez-Brandón et al., 2019b, 2020b). These previous studies were carried out at a pilot-scale and did not include a control treatment without earthworms. Nonetheless, comparative studies integrating both types of marc and assessing their long-term changes over the course of vermicomposting are largely missing. In contrast to red winemaking, the fermentation of the grape juice takes place with minimal or no contact with the grape marc during white wine vinification. These distinct procedures involved in the winemaking processes of red and white varieties are expected to distinctly shape the chemical and microbial composition of the resulting marc and consequently, the performance of the vermicomposting process (Domínguez et al., 2016, 2017). Therefore, the objective of the present study was to evaluate and compare the long-term effects of the epigeic earthworm *Eisenia andrei* on the nutrient content of grape marc derived from both white and red winemaking processes, as well as on their physico-chemical and microbiological properties over a period of 42 weeks. In this regard, a control treatment was also included so as to evaluate the changes as a result of earthworm activity during the process of vermicomposting. At the end of the trial each experimental unit was composed by a profile of layers of increasing age that permitted us to study the different phases of interaction between earthworms and microorganisms throughout the course of the process.

2. Material and methods

2.1. Substrate

White grape marc (*Vitis vinifera* L. cv. Albariño) and red grape marc (*Vitis vinifera* L. cv. Mencía) were kindly provided by Terras Gauda and Abadía da Cova wineries located in Galicia (northwestern Spain). The main physicochemical properties and nutrient content of the raw grape marc derived from white and red grape varieties can be found in Almeida-Santana et al. (2020) and Gómez-Brandón et al. (2020b), respectively. Both types of marc were stored at 4 °C until needed, turned and moistened with water for two days prior to the vermicomposting trial.

2.2. Experimental set up and sampling procedure

The mesocosms comprised PVC modules of an external diameter of 30 cm and a height of 70 cm. Each mesocosm was initially comprised of one module containing a base layer of vermicompost from grape marc that acted as a bed for the earthworms, and another module containing a layer of fresh marc (2 kg, fresh weight, fw). The bottom of each module consisted of a mesh (5 mm pore size) that permitted earthworm mobility

Table 1

Changes in moisture, organic matter content, pH and electrical conductivity through the layers of reactors with and without earthworms (Ew) fed with grape marc from the varieties Albariño and Mencía. Values are means with standard error in brackets.

	Moisture (%)				Organic matter (%)				pH				Electrical conductivity (μS cm^{-1})			
	Albariño		Mencía		Albariño		Mencía		Albariño		Mencía		Albariño		Mencía	
Age of layers (weeks)	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew
3	65.4 (1.83)	63.7 (3.64)	59.9 (3.78)	63.4 (1.30)	92.9 (1.01)	91.2 (3.33)	95.1 (0.83)	93.6 (1.01)	9.20 (0.61)	8.23 (0.27)	7.46 (0.34)	7.93 (0.23)	726 (130.6)	378 (85.5)	336 (52.9)	277 (3.9)
6	69.8 (2.43)	66.1 (0.45)	67.5 (0.64)	70.0 (0.49)	88.8 (0.83)	91.4 (1.02)	93.7 (0.38)	95.2 (1.54)	8.63 (0.11)	8.30 (0.24)	7.64 (0.15)	7.42 (0.23)	566 (87.6)	345 (67.3)	321 (41.9)	203 (64.8)
9	68.5 (1.25)	67.2 (2.02)	70.5 (2.53)	67.6 (1.40)	95.2 (1.54)	93.1 (2.05)	89.5 (0.68)	94.5 (1.12)	7.75 (0.17)	7.29 (0.18)	6.99 (0.09)	6.86 (0.10)	334 (32.9)	183 (9.50)	208 (21.5)	152 (16.0)
12	67.6 (0.30)	66.9 (0.86)	70.1 (0.79)	67.4 (0.92)	90.9 (1.58)	90.6 (0.77)	95.7 (0.45)	94.5 (0.53)	8.13 (0.03)	7.96 (0.10)	7.51 (0.06)	7.47 (0.11)	378 (20.3)	273 (79.5)	191 (16.8)	156 (12.3)
15	69.8 (0.83)	67.5 (0.58)	70.3 (1.26)	72.9 (1.86)	90.3 (0.67)	92.0 (2.09)	90.9 (4.25)	92.8 (1.15)	8.72 (0.09)	8.97 (0.16)	7.68 (0.05)	7.69 (0.10)	354 (19.8)	280 (52.3)	191 (9.10)	183 (7.90)
18	73.6 (0.45)	69.3 (1.09)	70.8 (3.17)	72.0 (2.90)	92.1 (0.58)	92.2 (2.65)	94.1 (1.62)	96.1 (1.13)	7.69 (0.09)	7.66 (0.09)	7.24 (0.07)	7.27 (0.05)	313 (53.0)	347 (72.8)	168 (10.4)	188 (24.0)
21	71.8 (1.35)	73.6 (2.41)	72.1 (0.69)	72.3 (0.78)	92.0 (2.33)	90.5 (0.64)	93.1 (0.10)	94.3 (1.03)	8.69 (0.13)	8.82 (0.09)	7.68 (0.07)	7.84 (0.19)	380 (12.0)	379 (16.7)	243 (28.4)	197 (43.8)
24	73.0 (0.45)	70.9 (1.40)	71.5 (0.62)	70.4 (1.80)	92.4 (0.75)	92.3 (1.11)	97.2 (1.07)	92.9 (0.97)	8.54 (0.05)	8.61 (0.11)	7.62 (0.03)	7.72 (0.13)	298 (14.2)	352 (21.0)	219 (25.2)	201 (29.4)
27	73.6 (0.66)	72.3 (1.88)	72.7 (3.01)	71.9 (3.07)	88.4 (1.14)	90.2 (0.92)	96.5 (0.75)	95.7 (1.57)	7.98 (0.06)	7.67 (0.19)	7.10 (0.15)	6.99 (0.06)	316 (44.1)	263 (18.0)	191 (6.20)	206 (15.0)
30	74.0 (2.37)	73.5 (0.06)	72.3 (0.44)	73.0 (1.19)	91.4 (0.93)	91.5 (1.13)	94.6 (1.14)	94.6 (0.85)	8.29 (0.08)	7.98 (0.15)	7.50 (0.06)	7.74 (0.10)	320 (33.2)	300 (20.9)	175 (11.8)	172 (17.3)
33	71.8 (1.96)	76.7 (2.69)	72.3 (1.65)	70.2 (0.64)	96.1 (2.22)	93.5 (1.00)	96.5 (1.00)	97.3 (0.57)	8.46 (0.01)	8.55 (0.13)	7.36 (0.13)	7.54 (0.04)	316 (53.8)	304 (44.2)	224 (1.80)	190 (11.3)
36	72.4 (2.85)	70.7 (2.34)	73 (0.94)	71.5 (0.93)	92.5 (3.01)	94.8 (1.89)	95.8 (0.56)	93.8 (1.10)	7.66 (0.14)	7.76 (0.07)	7.08 (0.10)	7.22 (0.02)	359 (52.6)	281 (20.6)	168 (17.9)	168 (15.4)
39	70.8 (1.30)	69.3 (0.68)	72.0 (1.61)	71.5 (2.18)	92.9 (0.23)	92.4 (0.39)	97.0 (1.34)	94.3 (0.93)	8.69 (0.07)	8.70 (0.18)	7.22 (0.22)	7.68 (0.01)	273 (35.3)	314 (26.3)	178 (5.30)	227 (53.9)
42	69.2 (0.41)	69.9 (2.49)	71.3 (2.20)	72.9 (1.65)	94.8 (1.03)	92.1 (1.10)	96.0 (0.77)	89.6 (5.46)	8.70 (0.12)	8.50 (0.13)	7.46 (0.07)	7.55 (0.10)	296 (17.7)	356 (52.1)	189 (2.80)	224 (57.5)

Table 2
Changes in the content of total C and N, and the C to N ratio through the layers of reactors with and without earthworms (Ew) fed with grape marc from the varieties Albariño and Mencía. Values are means with standard error in brackets.

	Total C (g)				Total N (g)				C to N ratio			
	Albariño		Mencía		Albariño		Mencía		Albariño		Mencía	
Age of layers (weeks)	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew
3	283	112	374	159	12.4	4.93	14.7	6.34	23.4	23.3	25.5	25.2
	(13.4)	(6.40)	(33.5)	(6.15)	(1.70)	(0.66)	(0.69)	(0.13)	(2.50)	(1.99)	(1.78)	(1.40)
6	220	121	283	131	15.3	4.15	16.1	5.89	15.2	29.5	17.9	23.0
	(14.4)	(4.59)	(7.36)	(2.91)	(3.03)	(0.38)	(1.40)	(0.66)	(2.29)	(2.25)	(1.98)	(3.21)
12	246	115	259	178	11.4	3.80	11.0	10.8	25.2	32.7	24.6	16.7
	(7.55)	(4.73)	(6.46)	(9.87)	(2.84)	(0.88)	(1.71)	(0.69)	(7.44)	(5.50)	(3.56)	(1.55)
18	197	115	247	187	10.4	5.57	14.9	10.3	20.3	21.6	19.6	18.5
	(10.2)	(4.78)	(25.6)	(16.8)	(1.86)	(0.71)	(4.6)	(1.40)	(3.97)	(3.66)	(4.92)	(1.37)
24	227	122	251	209	7.63	4.81	9.34	9.96	29.7	25.4	27.2	21.8
	(8.45)	(11.0)	(10.0)	(5.91)	(0.33)	(0.40)	(0.92)	(1.37)	(0.54)	(0.79)	(1.74)	(3.24)
33	235	114	260	254	10.6	4.79	9.73	9.00	22.5	24.5	26.8	28.6
	(20.0)	(14.7)	(15.8)	(9.54)	(1.43)	(0.94)	(0.24)	(0.92)	(1.39)	(1.94)	(2.22)	(2.08)
42	269	154	263	233	10.5	5.50	10.9	8.93	26.4	28.5	24.2	26.4
	(6.23)	(22.2)	(24.5)	(14.5)	(1.10)	(0.97)	(1.04)	(0.93)	(3.21)	(2.03)	(1.49)	(1.58)

between modules and prevented fresh material to be mixed with earthworm-processed material. New modules with the same amount of fresh substrate were added sequentially according to the feeding activity of the earthworm population. This procedure allowed the addition of each module to be dated within the mesocosms (Gómez-Brandón et al., 2011b). We set up twelve of the abovementioned mesocosms; six each for Albariño and Mencía grape marc respectively. For each type of marc, three mesocosms were inoculated with 400 individuals of the earthworm species *Eisenia andrei* and three were left without earthworms (control). All of the mesocosms were kept in a conditioned room with temperature set to 20 °C throughout the duration of the trial. After 42 weeks, the experimental mesocosms resembled a time profile and consisted of fourteen modules of increasing age that were dismantled after 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39 and 42 weeks. For each module the earthworms were removed manually from the substrate, counted and weighed. The amount of substrate remaining in each module was also weighed for each time point. In each mesocosm three samples of substrate per module were collected at random and mixed gently prior to analysis.

2.3. Physico-chemical analyses

Samples were dried 24 h at 105 °C and combusted 5 h at 550 °C in a muffle furnace (Carbolite, CWF 1000) for the determination of the moisture and the organic matter contents, respectively. The pH and electrical conductivity (CM35, Crison Instruments, Barcelona, Spain) were determined in a 1:10 vermicompost:distilled water suspension. Total C and N contents were assessed in oven-dried (60 °C) samples, in a CarloErba (EA 1108 CHNS-O) 1500 C/N analyzer. The total content of macro- and micronutrients including P, K, Ca, Mg, S, Fe, Mn, and B was determined from extracts of dried samples previously subjected to nitric-perchloric digestion by optical emission spectrometry with inductively coupled plasma (ICP-OES) according to the USEPA 3050B method (1996).

2.4. Microbial biomass and microbial activity

Microbial biomass C was determined by the chloroform fumigation–extraction method using a $K_{EC} = 2.64$ (Vance et al., 1987). Microbial activity was assessed as basal respiration by measuring the rate of evolution of CO_2 after 6 h of incubation. The evolved CO_2 was trapped in NaOH and then measured by titration with HCl to a phenolphthalein endpoint after adding excess of $BaCl_2$ (Anderson, 1982).

2.5. DNA extraction and real-time PCR

DNA was extracted from 0.25 g (fw) of sample using DNeasy PowerSoil Kit (Qiagen) according to manufacturer's protocols. DNA quality was evaluated using BioTek's Take3™ Multi-Volume Plate (Synergy™ Multi-Mode Microplate Reader, Bio-Tek Instruments, Inc.). The amount of DNA in each sample was determined by Qubit fluorometric quantitation prior to real-time PCR.

Quantitative real-time PCR (qPCR) analysis was performed to determine the 16S rRNA gene copy number of bacteria, and the 18S rRNA gene copy number of fungi by using the primer pairs 1055f/1392r (bacteria, Bardelli et al., 2017) and FF390/FR1 (fungi, Prévost-Bouré et al., 2011) respectively. The samples were run against 10-fold dilution curves of standards consisting of genomic DNA extracted from cultures of *Nitrosomonas europaea* (DSMZ 21879) for bacteria, and *Fusarium solani* (DSMZ 10696) for fungi. DNA concentrations of the standards were measured using Qubit fluorometric quantitation and freshly prepared for each run. Each reaction had a total volume of 20 μL and contained 1 ng of DNA template, 0.4 μM of each forward and reverse bacterial or fungal primer, 0.6 mg mL^{-1} BSA, distilled water (RNase/ DNase free), and 10 μL of 1X Luminaris HiGreen qPCR Master Mix, High ROX (Thermo Scientific). Samples and standards were run in triplicate on a StepOnePlus (Applied Biosystems). The cycling conditions were as follows: after an initial denaturation at 94 °C (bacteria) and 95 °C (fungi) for 10 min, thermal cycling comprised 40 cycles of 20 s at 95 °C, 15 s at 58 °C and 30 s at 72 °C for bacteria; and 15 s at 95 °C, 30 s at 50 °C and 30 s at 72 °C for fungi. To check for product specificity and potential primer dimer formation, runs were completed with a melting analysis starting from 60 °C to 95 °C with temperature increments of 0.25 °C and a transition rate of 5 s.

2.6. Statistical analyses

Data were analysed by repeated measures analysis of variance (ANOVAR). Single reactors were considered as subjects. Earthworm treatment (presence and absence) and the type of marc (white and red grape marc) were fixed as between-subject factors, and the age of layers was fixed as a within-subject factor. The normality and the variance homogeneity of the dataset were tested prior to ANOVAR. The sphericity violation was corrected (if necessary) with the Geisser-Greenhouse procedure.

3. Results and discussion

In line with Domínguez et al. (2016, 2017) and Gómez-Brandón et al.

Table 3

Changes in the content of macro- and micronutrients through the layers of reactors with and without earthworms (Ew) fed with grape marc from the varieties Albariño and Mencía. Values are means with standard error in brackets.

	Ca (mg)				K (mg)				P (mg)				Mg (mg)			
	Albariño		Mencía		Albariño		Mencía		Albariño		Mencía		Albariño		Mencía	
Age of layers (weeks)	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew
3	2300	1000	3288	1465	14,034	5646	13,843	6843	2219	773	2314	1070	913	386	1299	591
	(138)	(151)	(433)	(146)	(3076)	(1461)	(3901)	(1671)	(158)	(235)	(588)	(162)	(105)	(60)	(295)	(90)
6	2419	1187	2523	1234	15,325	5252	8250	3445	2379	667	2277	677	1095	437	793	332
	(89)	(50)	(96)	(83)	(986)	(496)	(288)	(77)	(137)	(44)	(154)	(23)	(36)	(26)	(34)	(22)
12	2651	1209	2408	1796	12,960	5446	6938	5354	1870	605	1574	1079	1210	523	736	595
	(159)	(100)	(193)	(45)	(642)	(545)	(364)	(351)	(224)	(69)	(134)	(149)	(81)	(47)	(43)	(42)
18	2676	1377	1913	1460	11,030	5969	8822	6390	1641	749	1113	1022	1189	581	804	663
	(153)	(108)	(186)	(178)	(368)	(483)	(782)	(891)	(74)	(64)	(117)	(233)	(59)	(47)	(72)	(101)
24	1849	1086	2357	2258	7611	4545	8373	8581	1167	690	1450	1919	643	395	808	1002
	(165)	(156)	(62)	(165)	(939)	(979)	(1173)	(1494)	(97)	(66)	(165)	(194)	(140)	(52)	(72)	(141)
33	1768	879	2140	2015	9785	3773	7895	6590	1787	733	1254	1208	652	315	773	681
	(89)	(96)	(184)	(128)	(933)	(515)	(2636)	(1480)	(326)	(150)	(254)	(251)	(69)	(37)	(160)	(128)
42	2009	1236	2285	2155	7937	4364	7974	6466	1548	751	1294	1418	755	396	806	673
	(104)	(163)	(154)	(244)	(824)	(443)	(1953)	(1708)	(114)	(94)	(99)	(253)	(46)	(72)	(128)	(158)

	S (mg)				Fe (mg)				Mn (mg)				B (mg)			
	Albariño		Mencía		Albariño		Mencía		Albariño		Mencía		Albariño		Mencía	
Age of layers (weeks)	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew	Control	Ew
3	1152	572	1602	709	83.1	39.9	111	55.3	31.0	25.1	45.8	22.3	20.9	8.27	29.9	12.4
	(94)	(121)	(159)	(6.97)	(12.1)	(7.42)	(12.2)	(8.29)	(3.75)	(12.4)	(10.7)	(2.53)	(5.31)	(1.94)	(9.24)	(3.06)
6	1128	477	1251	536	75.2	40.3	85.6	40.9	35.3	20.9	20.5	10.3	25.3	11.8	17.9	8.93
	(48)	(22)	(71)	(36)	(5.38)	(9.87)	(9.25)	(11.4)	(1.83)	(6.98)	(0.66)	(0.39)	(1.26)	(0.56)	(1.98)	(0.40)
12	1140	495	1229	873	61.7	43.4	87.3	66.7	33.8	17.7	20.6	14.6	29.5	12.5	18.2	11.5
	(95)	(56)	(100)	(88)	(7.24)	(4.91)	(11.8)	(12.7)	(1.90)	(1.96)	(2.31)	(1.18)	(2.75)	(0.82)	(1.23)	(1.11)
18	1232	593	1159	990	94.0	41.2	87.5	101	35.9	18.3	28.4	22.4	26.7	14.0	18.5	12.4
	(28)	(58)	(148)	(171)	(8.94)	(5.96)	(14.6)	(28.2)	(0.61)	(1.67)	(3.00)	(3.38)	(1.71)	(1.32)	(3.41)	(1.97)
24	726	561	1098	1694	47.5	54.5	138	184	22.5	25.4	40.2	63.7	14.4	8.50	17.5	17.8
	(111)	(62)	(97)	(569)	(9.83)	(8.29)	(36.5)	(27.4)	(4.13)	(10.7)	(7.75)	(10.0)	(4.73)	(2.10)	(2.74)	(1.76)
33	1027	511	1088	979	67.5	35.7	124	64.1	24.1	11.3	28.0	26.2	12.8	6.23	15.6	14.1
	(76)	(53)	(57)	(84)	(6.19)	(5.11)	(20.0)	(5.04)	(1.94)	(1.11)	(6.60)	(5.24)	(3.53)	(1.50)	(5.93)	(4.65)
42	1106	586	1254	1113	97.6	44.4	140	214	28.6	16.1	38.7	49.2	13.0	7.45	15.3	13.6
	(172)	(168)	(99)	(120)	(6.61)	(9.67)	(24.6)	(61.3)	(1.91)	(3.87)	(4.17)	(14.4)	(1.97)	(1.19)	(4.17)	(4.29)

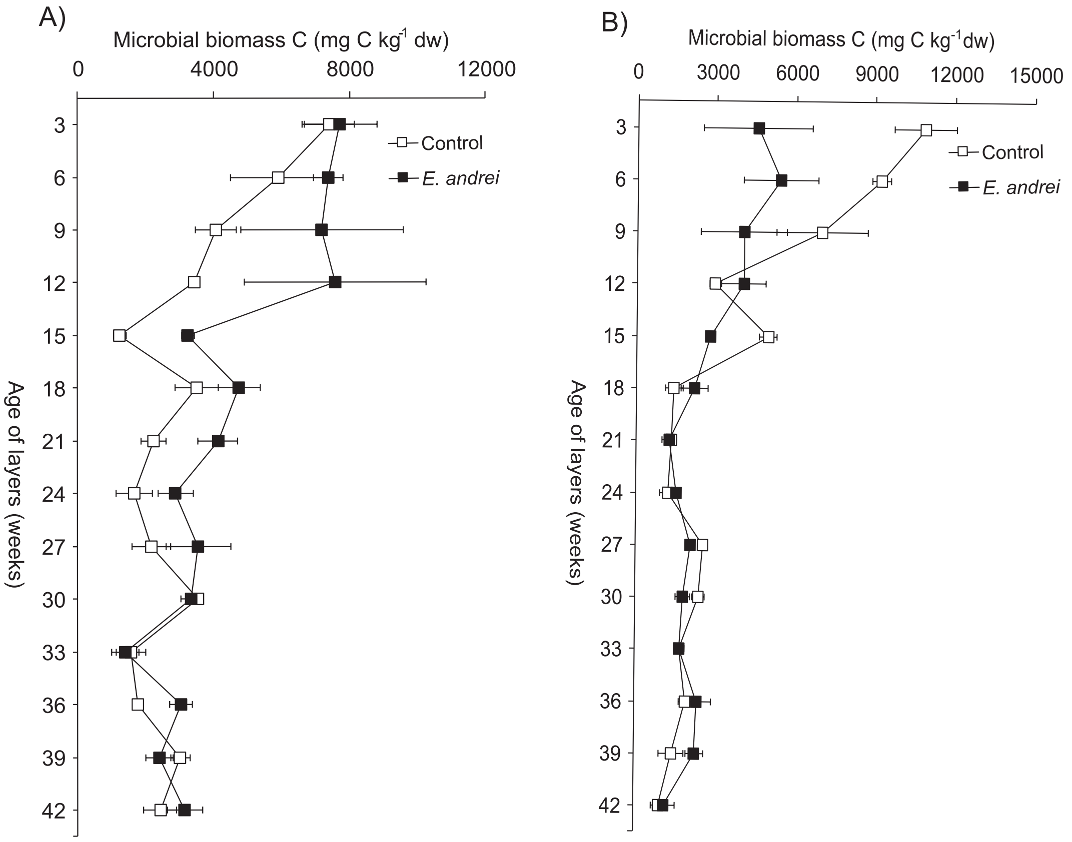

Fig. 2. Microbial biomass carbon in layers of reactors fed with grape marc from the cultivars Albariño (A) and Mencía (B) with *E. andrei* (filled symbols) and without *E. andrei* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of grape marc are shown on the y axis.

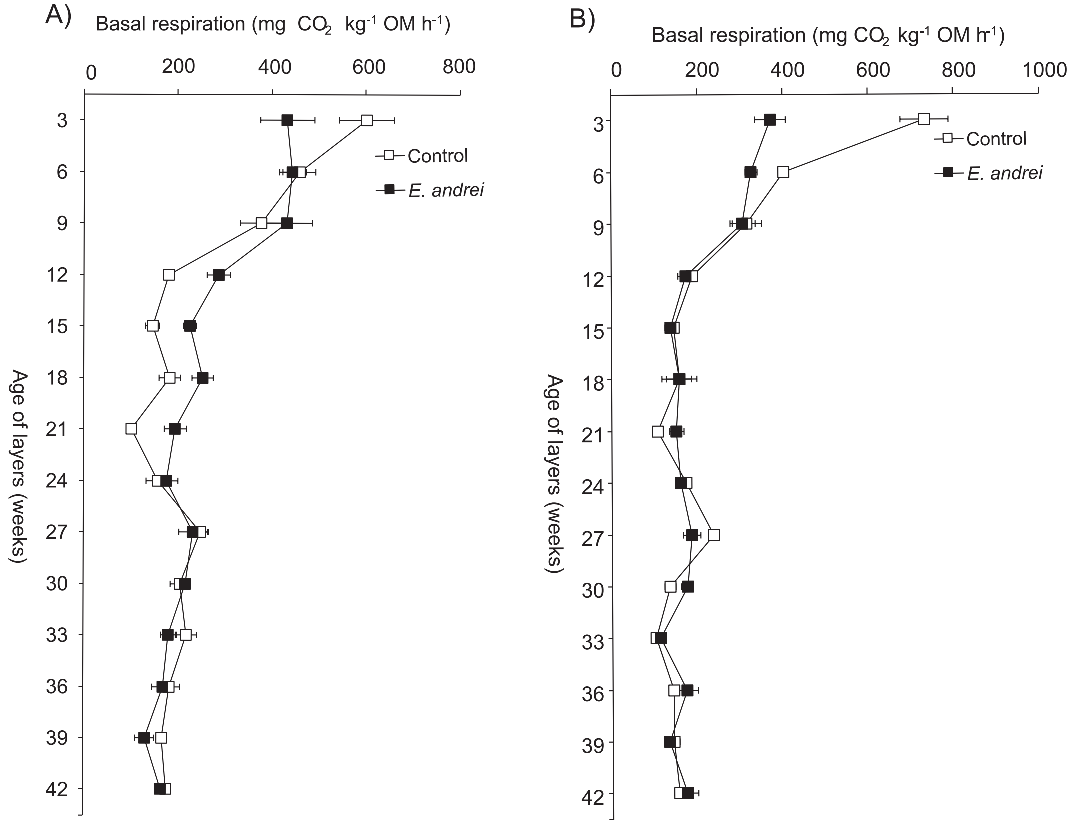

Fig. 3. Microbial activity, assessed as basal respiration, in layers of reactors fed with grape marc from the cultivars Albariño (A) and Mencía (B) with *E. andrei* (filled symbols) and without *E. andrei* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of grape marc are shown on the y axis.

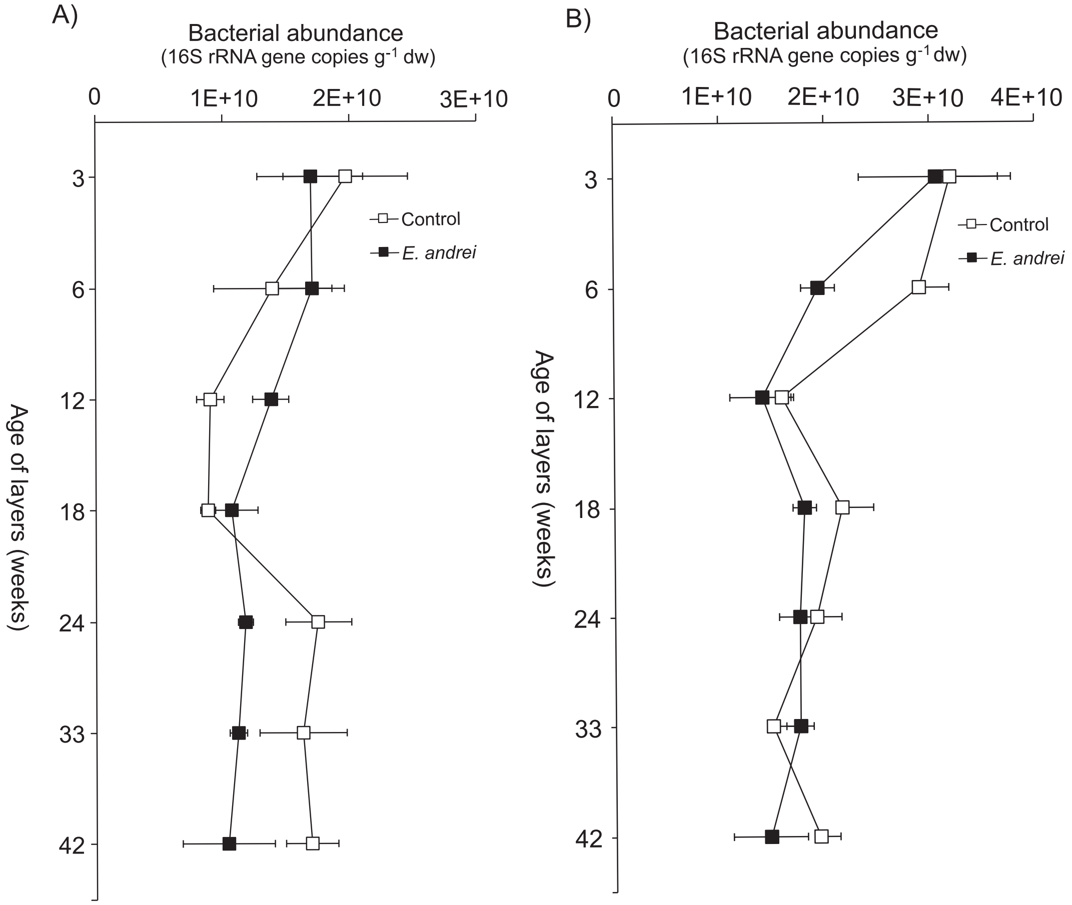

Fig. 4. Bacterial abundance, assessed by real-time PCR, in layers of reactors fed with grape marc from the cultivars Albariño (A) and Mencía (B) with *E. andrei* (filled symbols) and without *E. andrei* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of grape marc are shown on the y axis.

(2020b), our findings provide further evidence that using grape marc as a feedstock results in suitable environmental conditions for earthworm growth and reproduction, offering sufficient energy to sustain large populations in vermicomposting systems. We observed that both the number and the biomass of earthworms were closely and positively related in the reactors fed with the two types of marc (Albariño: $R^2 = 0.9023$; Mencía: $R^2 = 0.9408$). After the addition of the grape marc, we found that the mean number of earthworms increased from 400 to 579 ± 147 and 518 ± 135 individuals in the 3-week-old layers of Albariño and Mencía mesocosms, representing a mean biomass of 169 ± 34 g and 153 ± 32 g respectively (Fig. 1A, B). A higher number was then recorded in the 6-week-old layers for both types of marc, with 1071 ± 77 and 770 ± 30 earthworms per mesocosm and a mean biomass of 365 ± 28 and 258 ± 36 g for Albariño and Mencía grape varieties (Fig. 1A, B). Such values were similar to those reported by Aira et al. (2007) in a continuous-feeding vermicomposting system with pig slurry. These authors found higher earthworm densities in the 2- and 4-week-old layers with mean values of 1040 ± 180 and 1080 ± 300 individuals in each layer and an average biomass of 240 ± 61 and 280 ± 64 g, respectively. As expected, and likely due to the migration of earthworms towards the newly added layers of undigested substrate, the number of earthworms (Albariño: $F_{13,28} = 30.15$, $p < 0.0001$; Mencía: $F_{13,28} = 26.72$, $p < 0.0001$) and their biomass (Albariño: $F_{13,28} = 12.80$, $p < 0.0001$; Mencía: $F_{13,28} = 24.93$, $p < 0.0001$) steadily decreased through the grape marc layers of increasing age (Fig. 1A, B). More specifically, average values of 9 ± 1 and 34 ± 8 individuals with mean weights of 2 ± 0.09 and 4 ± 0.08 g were recorded in the 42-week-old layer of the Albariño and Mencía mesocosms, respectively (Fig. 1A, B). An average moisture content of 70% was maintained in the mesocosms fed with the two types of marc for the duration of the vermicomposting trial (Table 1). This is considered an optimum level of moisture for the growth and reproduction of the earthworm species *E. andrei*, as well as for a good performance of the vermicomposting process (Domínguez and Edwards, 2011). The pH also constitutes an important factor affecting earthworm survival; epigeic earthworms can tolerate a pH range from 5 to 9 (Domínguez and Edwards, 2011). In this regard, processing grape marc via vermicomposting has been reported as an effective means of neutralizing the initial acidity associated with this winery by-product (Domínguez et al., 2014; Gómez-Brandón et al., 2020b,c).

In our study, the pH of the grape marc fell within weak-alkaline levels through the layers‘ profile in the presence and the absence of earthworms (Table 1), with higher mean values in those mesocosms fed with Albariño (mean pH = 8.4 and 7.5 for Albariño and Mencía, respectively; $F_{1,104} = 143.3$, $p < 0.0001$). These pH levels reinforce the potential use of the grape marc-derived vermicompost as an amendment for soil and other plant growth media, since crops respond to organic amendments more favorably when soil pH ranges from weak-acidic to weak-alkaline levels (Luo et al., 2018). Likewise, EC may affect the suitability of vermicomposts as organic amendments (Gómez-Brandón and Domínguez, 2014), since plants may face phytotoxic and osmotic problems owing to high salt concentrations. In the presence of earthworms, mean EC values of 311 and 196 µS cm^{-1} were recorded in the Albariño and Mencía mesocosms (Table 1). These EC levels seem optimal (i.e., < 8.0 mS cm^{-1}; Domínguez and Edwards, 2011) for the survival and reproduction of earthworms, and were consistent with those reported in previous vermicomposting trials dealing with grape marc (Částková and Hanč, 2019; Gómez-Brandón et al., 2020b). The differences in EC relative to the control without earthworms were more

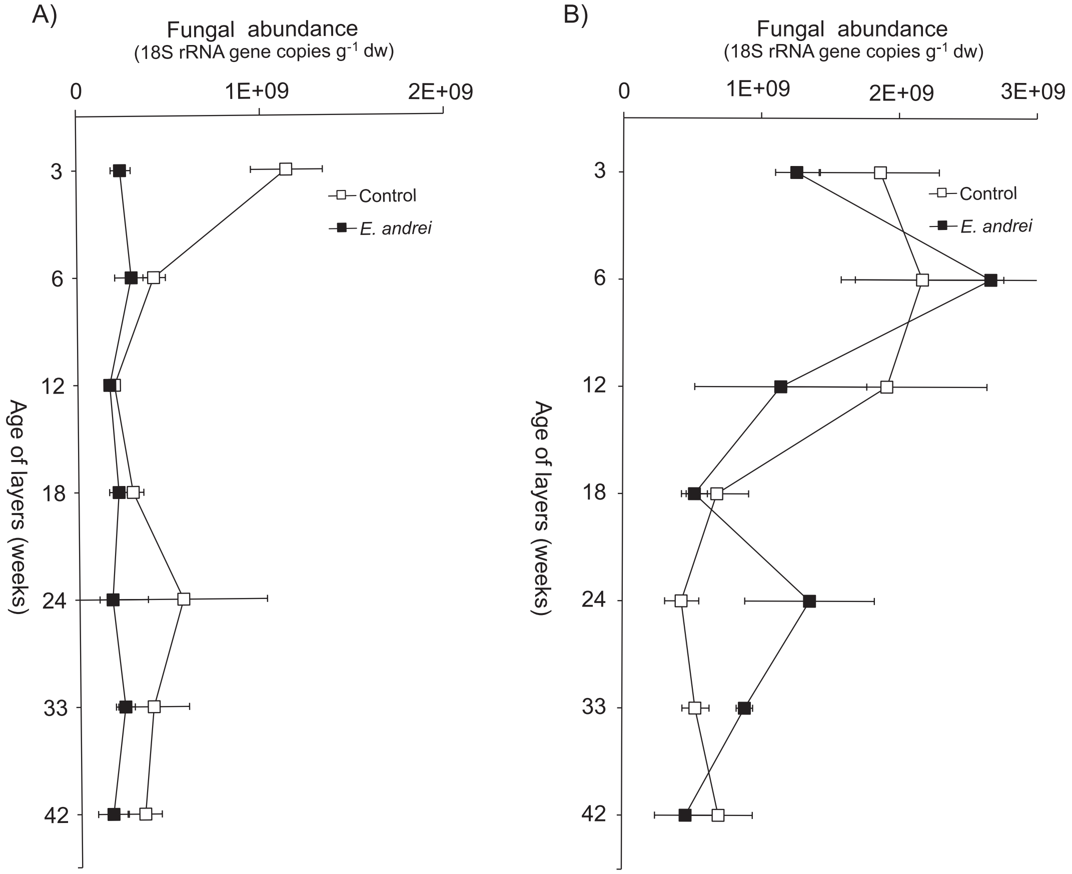

Fig. 5. Fungal abundance, assessed by real-time PCR, in layers of reactors fed with grape marc from the cultivars Albariño (A) and Mencía (B) with *E. andrei* (filled symbols) and without *E. andrei* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of grape marc are shown on the y axis.

evident, about 1.5-fold greater compared to the treatment with earthworms, in the grape marc layers of between 3- and 9-weeks old (earthworm presence × age of layers, $F_{13,104} = 3.48$, $p < 0.0001$). A decreasing trend in EC was observed through these first three layers (Table 1), likely due to a reduction in soluble ion concentrations via leaching or precipitation in the form of non-soluble salts (Domínguez et al., 2018). No major changes with the age of the layers were observed later on in in this parameter until the end of the trial (Table 1).

Earthworm activity significantly reduced the total C and N contents of the grape marc when compared to the control (around 1.5–2 times lower; Table 2), particularly for the grape variety Albariño (earthworm presence × type of marc, *total C*: $F_{1,48} = 9.6$, $p = 0.015$; *total N*: $F_{1,48} = 8.4$, $p = 0.018$). Such a decrease was more accused in the layers ranging from 3 to 12 weeks old (earthworm presence × age of layers, *total C*: $F_{6,48} = 11.0$, $p < 0.0001$; *total N*: $F_{6,48} = 5.84$, $p < 0.0001$; Table 2), which are characterised by higher earthworm densities. This is in agreement with the findings from Aira et al. (2008) who found a linear density-dependent response of the C and N mineralization to the earthworm density. Enhanced C mineralization in the presence of earthworms results from increased turnover rate, activity and respiration of grazed microbial populations, whereas enhanced N mineralization is mainly due to the direct excretion of excess N (Domínguez et al., 2010). Earthworms are involved in the indirect stimulation of microbial populations through fragmentation and ingestion of fresh organic matter, which results in a greater surface area available for microbial colonization, altering the biological activity and accelerating the rate of organic matter decomposition during vermicomposting (Domínguez et al., 2010; Gómez-Brandón and Domínguez, 2014).

As occurred for total C and N, earthworms promoted a significant decrease in the content of the other macronutrients (Table 3), particularly in the 3- to- 12-week-old layers, being around 2–3 times lower relative to the control without earthworms for both types of marc (earthworm presence × age of layers, *Ca*: $F_{6,48} = 7.60$, $p < 0.0001$; *K*: $F_{6,48} = 2.93$, $p = 0.016$; *P*: $F_{6,48} = 10.07$, $p < 0.0001$; *Mg*: $F_{6,48} = 4.76$, $p = 0.001$; *S*: $F_{6,48} = 4.72$, $p = 0.01$). As shown in Table 3, a similar trend as a result of earthworm activity was observed for the content of micronutrients including Fe ($F_{6,48} = 2.90$, $p = 0.017$), Mn ($F_{6,48} = 3.40$, $p = 0.007$), and B ($F_{6,48} = 2.24$, $p = 0.04$). The mass loss caused by the activity of earthworms resulted in an increased content of C and N as well as for most of the other macro- and micronutrients in the Albariño and Mencía mesocosms at the end of the vermicomposting trial (42 weeks) when compared to the youngest marc layers (Tables 2 and 3).

In our study, the C to N ratio reached higher values in comparison with those reported in earlier studies (Domínguez et al., 2014; Gómez-Brandón et al., 2020). In the presence of earthworms, a mean C to N ratio of 28.5 ± 2.03 and 26.4 ± 1.58 was recorded after 42 weeks in Albariño and Mencía mesocosms (Table 2); while values of 12 were found by Domínguez et al. (2014) and Gómez-Brandón et al. (2020b) after 112 days of vermicomposting of marc derived from white and red grape varieties. These latter studies used larger reactors with only an initial input of the feedstock, while in the present study fresh grape marc was added periodically through the 42 week-trial.

As previously shown by Aira et al. (2007), a reduction in Cmic used as a proxy for microbial biomass and in microbial activity assessed as basal respiration was observed with the increasing age of layers, particularly until the week 12th, in both with and without earthworm treatments for Albariño and Mencía mesocosms (Cmic: $F_{13,104} = 16.96$, $p < 0.0001$, Fig. 2A,B; basal respiration: $F_{13,104} = 27.40$, $p < 0.0001$, Fig. 3A, B). Similarly, Almeida-Santana et al. (2020) reported a decreasing trend in basal respiration during a two-year vermicomposting period of white grape marc. Decreases in microbial activity were also detected with depth of layer by Gómez-Brandón et al. (2013) in a continuous-feeding vermicomposting system with rabbit manure. The overall effect of earthworms on microbial biomass (Cmic; Fig. 2), as well as on bacterial and fungal abundances (qPCR-based gene copies; Figs. 4-5) and microbial activity (Fig. 3) was not significant, irrespective of the type of marc and the age of layers.

The lack of differences in Cmic relative to the control without earthworms was also recorded by Aira et al. (2007); while Gómez-Brandón et al. (2011b) reported a decreased Cmic with earthworm activity during vermicomposting of pig slurry. In this latter study, the phospholipid fatty acid content instead of Cmic was used as a proxy for microbial biomass. The starting material is an important distinction between the current work and those from Aira et al. (2007) and Gómez-Brandón et al. (2011b). Our study started with a plant material of lignocellulosic nature and the resulting vermicompost can be thought to represent the process of a single gut – that is, the starting material passed only through the earthworm gut (Domínguez et al., 2019). On the contrary, in the case of vermicomposting applications with pig slurry or other types of manure the feedstock has already passed through the vertebrate gut (i.e., pig, cow, horse).

4. Conclusions

Our findings provide further evidence about the suitability of vermicomposting for processing grape marc based on a continuous feeding system, turning it into an environmentally friendly organic fertilizer. The final vermicompost derived from both Albariño and Mencía grape varieties had optimum levels of moisture, pH and EC for its application into soil. The mass loss caused by earthworm activity led to an increased content in macro- and micronutrients at the end of the trial. Lower values of microbial biomass and its activity, indicative of a stabilised material, were also recorded after the 42-week period in both with and without earthworm treatments. The treatment of grape marc, the main solid by-product of the wine industry, through vermicomposting served a dual purpose, that is fertilizer production and environment protection.

CRediT authorship contribution statement

María Gómez-Brandón: Investigation, Methodology, Formal analysis, Visualization, Writing – original draft. **Hugo Martínez-Cordeiro:** Conceptualization, Methodology. **Jorge Domínguez:** Conceptualization, Investigation, Methodology, Formal analysis, Visualization, Funding acquisition, 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

This study was supported by the Spanish Ministerio de Economía y Competitividad [AGL2017-86813-R] and the Xunta de Galicia [grant numbers ED431F 2018/07 and ED431B 2019/038]. MGB acknowledges support by the Programa Ramón y Cajal [RYC-2016-21231; Ministerio de Economía y Competitividad]. The authors would like to thank Alberto Da Silva and Nariane de Andrade for their valuable help with the laboratory analyses. Paul Fraiz is also acknowledged for his valuable help with English wording and editing.

References

Aira, M., Monroy, F., Domínguez, J., 2007. *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. Microb. Ecol. 54 (4), 662–671.

Aira, M., Sampedro, L., Monroy, F., Domínguez, J., 2008. Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. Soil Biol. Biochem. 40 (10), 2511–2516.

Aira, M., Lazcano, C., Gómez-Brandón, M., Domínguez, J., 2010. Ageing effects of casts of *Aporrectodea caliginosa* on soil microbial community structure and activity. Appl. Soil Ecol. 46 (1), 143–146.

Aira, M., Domínguez, J., Wright, J., 2011. Earthworm effects without earthworms: inoculation of raw organic matter with worm-worked substrates alters microbial community functioning. PLoS ONE 6 (1), e16354.

Aira, M., Bybee, S., Pérez-Losada, M., Domínguez, J., Marchesi, J., 2015. Feeding on microbiomes: effects of detritivory on the taxonomic and phylogenetic bacterial composition of animal manures. FEMS Microbiol. Ecol. 91 (11), fiv117. https://doi.org/10.1093/femsec/fiv117.

Aira, M., Pérez-Losada, M., Domínguez, J., 2019. Microbiome dynamics during cast ageing in the earthworm *Aporrectodea caliginosa*. Appl. Soil Ecol. 139, 56–63.

Almeida-Santana, N., Seminoti Jacques, R.J., Antoniolli, Z.I., Martínez-Cordero, H., Domínguez, J., 2020. Changes in chemical and biological characteristics of grape marc vermicompost during a two-year production period. Appl. Soil Ecol. 154, 103587.

Anderson, J.P.E., 1982. Soil Respiration. In: Page, A.L. (Ed.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. Agronomy monograph No. 9. ASA-SSSA, Madison, pp. 831–871.

Bardelli, T., Gómez-Brandón, M., Ascher-Jenull, J., Fornasier, F., Arfaioli, P., Francioli, D., Egli, M., Sartori, G., Insam, H., Pietramellara, G., 2017. Effects of slope exposure on soil physico-chemical and microbiological properties along an altitudinal climosequence in the Italian Alps. Sci. Tot. Environ. 575, 1041–1055.

Částková, T., Hanč, A., 2019. Change of the parameters of layers in a large-scale grape marc vermicomposting system with continuous feeding. Waste Manage. Res. 37 (8), 826–832.

Cortés, A., Moreira, M.T., Domínguez, J., Lores, M., Feijoo, G., 2020. Unraveling the environmental impacts of bioactive compounds and organic amendment from grape marc. J. Environ. Manage. 272, 111066. https://doi.org/10.1016/j.jenvman.2020.111066.

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: from Wastes to Resources. Springer-Verlag, Berlin Heildelberg, pp. 93–114.

Domínguez, J., Edwards, C.A., 2011. Biology and ecology of earthworm species used for vermicomposting. In: Edwards, C.A., Arancon, N.Q., Sherman, R.L. (Eds.), Vermiculture Technology: Earthworms, Organic Waste and Environmental Management. CRC Press, Boca Raton, FL, pp. 25–37.

Domínguez, J., Gómez-Brandón, M., 2012. Management of Organic Waste. In: Sunil Kumar, Ajay Bharti (Eds.). InTech Open Access Publisher, pp. 29–48.

Domínguez, J., Martínez-Cordeiro, H., Álvarez-Casas, M., Lores, M., 2014. Vermicomposting grape marc yields high quality organic biofertiliser and bioactive polyphenols. Waste Manage. Res. 32 (12), 1235–1240.

Domínguez, J., Martínez-Cordeiro, H., Lores, M., 2016. Earthworms and grape marc: simultaneous production of a high-quality biofertilizer and bioactive-rich seeds. In: Morata, A., Loira, I. (Eds.), Grape and Wine Biotechnology. Intech Open Science, Rijeka, Croatia, pp. 167–183.

Domínguez, J., Sánchez-Hernández, J.C., Lores, M., 2017. Vermicomposting of winemaking products. In: Galanakis, C.M. (Ed.), Handbook of Grape Processing By-Products: Sustainable Solutions. Academic Press Elsevier, London, pp. 55–78.

Domínguez, J., Gómez-Brandón, M., Martínez-Cordeiro, H., Lores, M., 2018. Bioconversion of Scotch broom into a high-quality organic fertiliser: vermicomposting as a sustainable option. Waste Manage. Res. 36 (11), 1092–1099.

Domínguez, J., Aira, M., Kolbe, A.R., Gómez-Brandón, M., Pérez-Losada, M., 2019. Changes in the composition and function of bacterial communities during vermicomposting may explain beneficial properties of vermicompost. Sci. Rep. 9, 9657.

García-Sánchez, M., Taušnerová, H., Hanč, A., Tlustoš, P., 2017. Stabilization of different starting materials through vermicomposting in a continuous-feeding system: changes in chemical and biological parameters. Waste Manage. 62, 33–42.

Gómez-Brandón, M., Lazcano, C., Lores, M., Domínguez, J., 2011a. Short-term stabilization of grape marc through earthworms. J. Hazard. Mat. 187 (1-3), 291–295.

Gómez-Brandón, M., Aira, M., Lores, M., Domínguez, J., 2011b. Changes in microbial community structure and function during vermicomposting of pig slurry. Bioresour. Technol. 102 (5), 4171–4178.

Gómez-Brandón, M., Lores, M., Domínguez, J., 2013. Changes in chemical and microbiological properties of rabbit manure in a continuous-feeding vermicomposting system. Bioresour. Technol. 128, 310–316.

Gómez-Brandón, M., Domínguez, J., 2014. Recycling of solid organic wastes through vermicomposting: microbial community changes throughout the process and use of vermicompost as a soil amendment. Crit. Rev. Environ. Sci. Technol. 44 (12), 1289–1312.

Gómez-Brandón, M., Lores, M., Insam, H., Domínguez, J., 2019a. Strategies for recycling and valorization of grape marc. Crit. Rev. Biotechnol. 39 (4), 437–450.

Gómez Brandón, M., Aira, M., Kolbe, A.R., de Andrade, N., Pérez-Losada, M., Domínguez, J., 2019b. Rapid bacterial community changes during vermicomposting of grape marc derived from red winemaking. Microorganisms 7 (10), 473. https://doi.org/10.3390/microorganisms7100473.

Gómez-Brandón, M., Aira, M., Domínguez, J., 2020a. In: Earthworm Assisted Remediation of Effluents and Wastes. Springer Singapore, Singapore, pp. 125–140. https://doi.org/10.1007/978-981-15-4522-1_8.

Gómez-Brandón, M., Lores, M., Martínez-Cordeiro, H., Domínguez, J., 2020b. Effectiveness of vermicomposting for bioconversion of grape marc derived from red winemaking into a value-added product. Environ. Sci. Poll. Res. 27 (27), 33438–33445.

Gómez-Brandón, M., Aira, M., Santana, N., Pérez-Losada, M., Domínguez, J., 2020c. Temporal dynamics of bacterial communities in a pilot-scale vermireactor fed with distilled grape marc. Microorganisms 8 (5), 642. https://doi.org/10.3390/microorganisms8050642.

Kolbe, A.R., Aira, M., Gómez-Brandón, M., Pérez-Losada, M., Domínguez, J., 2019. Bacterial sucession and functional diversity during vermicomposting of the white grape marc Vitis vinifera v. Albariño. Sci. Rep. 9, 7472.

Luo, G., Li, L., Friman, V.-P., Guo, J., Guo, S., Shen, Q., Ling, N., 2018. Organic amendments increase crop yields by improving microbe-mediated soil functioning of agroecosystems: a meta-analysis. Soil Biol. Biochem. 124, 105–115.

Mateo, J.J., Maicas, S., 2015. Valorization of winery and oil mill wastes by microbial technologies. Food Res. Int. 73, 13–25.

Prévost-Bouré, N.C., Christen, R., Dequiedt, S., Mougel, C., Lelièvre, M., Jolivet, C., Shahbazkia, H.R., Guillou, L., Arrouays, D., Ranjard, L., 2011. Validation and application of a PCR primer set to quantify fungal communities in the soil environment by real- time PCR. PLoS ONE 6, e24166.

USEPA, 1996. Method 3050B: Acid digestion of sediments, sludges, soils. Revision 2, USEPA.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19 (6), 703–707.