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EARTHWORM AND SOIL POLLUTION

Effectiveness of vermicomposting for bioconversion of grape marc derived from red winemaking into a value-added product

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Abstract

Grape marc, the main solid by-product of the wine industry, can be used as a nutrient-rich organic amendment if treated appropriately before its application into soil. In this study, we evaluated the potential of vermicomposting to process grape marc derived from the red winemaking of Mencía grapes in order to yield a high-quality, polyphenol-free organic vermicompost that could be used as an environmentally friendly fertiliser. We observed that the grape marc from this cultivar appears to be an optimum substrate for feeding earthworms providing optimum conditions for growth and reproduction, and sufficient energy to sustain large populations. Moreover, earthworm activity favoured the stabilisation of the grape marc resulting in a final vermicompost characterised by a higher concentration of macro- and micro-nutrients and a reduced polyphenol content after 112 days of vermicomposting. Lower values of microbial activity, indicative of stabilised materials, were recorded at the end of the process. These findings highlight vermicomposting as an environmentally sound management system for processing grape marc that could easily be scaled up for industrial application.

Keywords Grapes · Wine production · *Eisenia andrei* · Epigeic earthworms · Soil fertiliser · Vermicompost · Polyphenols

Introduction

Grapes (*Vitis vinifera* L.) are one of the most widely grown fruit crops around the world, with a production of around 210 Mtonnes per year, of which 15% are destined to the winemaking process (Dávila et al. 2017). A global production of 281,000,000 hl of wine was reported according to the International Organisation of Vine and Wine (OIV 2014). As a consequence, wineries are considered high-volume generating sectors of organic waste that must be treated, disposed of or reused appropriately in order to avoid any negative environmental impacts (Domínguez et al. 2016, 2017; Fermoso et al. 2018).

The main solid by-product of the wine industry is the grape marc, since it represents approximately 25% of the grape mass during wine production (Devesa-Rey et al. 2011). Grape marc has traditionally been used for ethanol production or as an additive in animal feeding (Fontana et al. 2013). It can also be used as a nutrient-rich organic soil amendment (Bustamante et al. 2008), even though the application of the untreated raw material into soils may result in large-scale contamination of soil, water and air (Christ and Burritt 2013). For instance, its direct use can lead to oxygen depletion in soil, groundwater pollution and greenhouse gases emissions. It can also negatively affect crop growth due to the release of phytotoxic phenolic compounds and tannins into soil (Christ and Burritt 2013).

Over the past years, grape marc has also attracted much interest for researchers, consumers and producers since it represents a low-cost source of an ample range of bioactive compounds (Fontana et al. 2013; Galanakis 2012). Among these, polyphenols have an interesting role in the field of oenology because they are responsible for the organoleptic characteristics of grapes and wines including colour, astringency or bitterness (Ma et al. 2014). In particular, the flavonoids known as

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anthocyanins are responsible, in their monomeric form, for the coloration of the grape skin in red and rose cultivars (Garrido and Borges 2013). They also contribute to the development of polymeric pigments during wine ageing (Puértolas et al. 2010). One of the main features that will determine the polyphenol content of a wine, and consequently of the grape marc, is the winemaking process (Cjevik et al. 2017). During red wine vinification, grape marc will retain a lower proportion of the initial polyphenolic load of the grapes as skins and seeds remain in contact with the fermentation broth for several days. In contrast, in the white winemaking process, the fermentation of the grape juice occurs with a minimal contact, or even without being in contact, with the grape marc that remains as a final residue of the process and retains much of the initial polyphenols of the grapes (Domínguez et al. 2016, 2017).

Specifically, grape marc derived from the red winemaking process provides a raw material rich in several phytochemical compounds including anthocyanins, flavonols, stilbenes and phenolic acids (Amico et al. 2004; Drosou et al. 2015). Nonetheless, it must be properly treated after red wine production in order to further exploit it as a source of these value-added compounds and to avoid any of the above-mentioned negative environmental impacts when used as an organic soil amendment (Muhlack et al. 2018). This gives rise to the necessity of providing profitable and sustainable options by both the scientific community and wine producers for the appropriate management and valorisation of this winemaking by-product (Dávila et al. 2017; Spigno et al. 2017).

In this regard, vermicomposting has been investigated as an alternative to process grape marc at both laboratory (Nogales et al. 2005; Gómez-Brandón et al. 2011) and pilot scales (Domínguez et al. 2014, 2016, 2017) with a dual purpose, i.e. environmental protection and biofertiliser production. The recent studies from Domínguez et al. (2014, 2016, 2017) have shown the potential of vermicomposting to convert white grape marc (*Vitis vinifera* v. Albariño) to high-quality organic fertiliser. Nonetheless, it is still necessary to evaluate the feasibility of grape marc derived from the red winemaking process in large-scale vermicomposting systems in order to yield a high-quality, polyphenol-free organic vermicompost that could be used as an environmentally friendly fertiliser. For this purpose, we monitored the chemical and microbiological changes of grape marc obtained through the red winemaking process of the grape variety *Mencía* in a pilot-scale vermireactor over a period of 112 days. The changes in the content of total polyphenols and, in particular, monomeric anthocyanins were also determined throughout the vermicomposting process. The earthworm population dynamics including the density of cocoons, juveniles and mature individuals as well as the changes in earthworm biomass were monitored under this scenario.

Vitis vinifera L. cv. Mencía is the cultivar that is mostly used for the production of quality red wines in Galicia in the Northwest of the Iberian Peninsula, as it represents nearly 95% of the annual red grape harvest (Soto-Vázquez et al. 2010). Mencía wine is considered as a monovarietal young red wine produced under the Galician designations of origin "Valdeorras DO", "Ribeira Sacra DO" and "Monterrei DO".

Materials and methods

Grape marc and earthworm species

Grape marc samples from the red variety Mencía (*Vitis vinifera sp.*) were kindly provided by the Abadía da Cova winery located in Lugo (Galicia, NW, Spain) immediately after post-processing in October 2016. The grape marc was stored at 4 °C until use and moistened with water during 2 days prior to the experimental setup in order to achieve a suitable level of moisture for the earthworms (~ 85%; Domínguez and Edwards 2011).

Individuals of the earthworm species *Eisenia andrei* were used in the vermicomposting trial and obtained from a stock culture reared in the greenhouse of our research group. This earthworm species is widely used for vermicomposting since it has high rates of consumption, digestion and assimilation of organic matter; tolerance to a wide range of environmental factors; short life cycles; high reproductive rates; and endurance and resistance to handling (Domínguez and Edwards 2011).

Vermireactor setup and sampling strategy

The system used was a rectangular metal pilot-scale vermireactor (4 × 1.5 × 1 m; 6 m^3) housed in a greenhouse with no temperature control as previously described by Domínguez et al. (2014). A 12-cm layer containing mature vermicompost was used as a bed for the earthworms whose initial density was of 203 ± 28 individuals m^{-2}. Afterwards, a 6-cm height layer containing the fresh grape marc (158 kg fresh mass) was placed over a plastic mesh (5-cm mesh size) and covered with a shade cloth to keep the moisture level of the grape marc at approximately 85%. Use of the plastic mesh allows earthworm migration, prevents mixing of the processed grape marc and the vermicompost bedding and facilitates the sampling of grape marc during vermicomposting.

The density and biomass of the earthworm population (adults, juveniles and cocoons) were determined periodically by a random collection of 10 samples, each 6 cm deep (five from above and five from below the plastic mesh) of the material in the vermireactor every 28 days during the trial (112 days) by using a core sampler (7.5-cm diameter and 12-cm height). Five samples comprising 10 g of grape marc and seeds were also collected at random every 28 days for their physico-chemical and microbiological characterisation.

For the determination of the total content of polyphenols and monomeric anthocyanins, the material was sampled as indicated above every 7 and 14 days over the course of vermicomposting. All the samples were stored at − 20 °C in plastic bags until analysis.

Physico-chemical and microbiological analyses

Samples (grape marc plus seeds) 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 organic matter content respectively. Electrical conductivity (EC) and pH were measured in aqueous extracts (1:10 mass/volume) by using a Crison conductivity metre CM35 and a Crison MicropH 2000 pH metre, respectively. Total C and N contents were determined in oven-dried (60 °C) samples, in a Carlo Erba (EA 1108 CHNS-O) 1500 C/N analyser. The total content of macro- and micro-nutrients including P, K, Ca, Mg, S, Fe, Mn, B and Mo was assessed in oven-dried (60 °C) samples, previously subjected to microwave-assisted acid digestion, by optical emission spectrometry with inductively coupled plasma (ICP-OES). Cellulose, hemicellulose and lignin contents were measured by detergent fibre methods (Goering and Van Soest 1970) using the FibreBag System® (Gerhardt, Königswinter, Germany), as described in Aira et al. (2006). Microbial activity was determined by measuring the oxygen consumption using a WTW OxiTop Control System (Weilheim, Germany) according to ISO16072 (2002).

Determination of polyphenols

Total polyphenols were determined by pressurised solvent extraction in a solvent extractor (ASE 150, Dionex, Sunnyvale, CA, USA) as shown in Álvarez-Casas et al. (2014). The total polyphenol content in the grape marc extracts was performed following the Folin–Ciocalteu colorimetric method (Singleton and Rossi Jr 1965) and the absorbance values were measured at 760 nm by spectrophotometry (UV mini-1240, Shimadzu, Tokyo, Japan). The quantification of total phenols was done based on a calibration graph prepared with gallic acid standard solutions and expressed as mg gallic acid equivalents in the liquid extract (mg L^{-1} GAE). The final concentrations of total phenols were expressed as mg gallic acid g^{-1} dry mass (mg GAE g^{-1} dry mass).

Determination of total monomeric anthocyanins

Total monomeric anthocyanins were determined following the pH differential method (Lee et al. 2005). The absorbance values of pH = 1.0 and pH = 4.5 buffer-diluted grape marc extracts were measured at their maximum absorbance wavelength (around 520 nm and 700 nm) by using a Shimadzu UV mini-1240 spectrophotometer (Shimadzu Corporation, Tokyo, Japan). The monomeric anthocyanins were expressed as cyanidin-3-glucoside (cyd-glu, molecular weight of 449.2 g mol^{-1} and molecular absorptivity of 26,900 L cm^{-1} mol^{-1} in aqueous buffer) (Giusti and Wrolstad 2001). Measurements were undertaken in triplicate and the anthocyanin content of each sample solution was calculated using the equation below:

$$A = (A_{\text{vis max}} - A_{700})_{\text{pH}=1.0} - (A_{\text{vis max}} - A_{700})_{\text{pH}=4.5}$$

$$\text{Anthocyanins (mg/L)} = \frac{A \times 449.2 \times \text{DF} \times 1000}{26,900 \times 1}$$

The final concentration of monomeric anthocyanins in the grape marc extracts was expressed as mg of cyanidin-3-glucoside equivalents per g of dry mass (mg CGE g^{-1} dry mass).

Statistical analyses

Data were analysed by repeated measures analysis of variance (ANOVAR) in which the vermireactor represented the subject, and the sampling time was fixed as the within-subject factor. The normality (Shapiro-Wilk's test) and sphericity condition (Mauchly's test) were tested for each variable. Whenever necessary, the sphericity violation was corrected with the Geisser–Greenhouse (G–G) procedure (Potvin et al. 1990). Significant differences in the main effects were analysed by paired comparisons with the Tukey HSD test. All statistical analyses were performed with the SPSS software programme v9.

Results and discussion

Population dynamics of earthworms during vermicomposting of red grape marc

The vermireactor was inoculated with an initial population density of *E. andrei* of 203 ± 28 individuals m^{-2}, including 14 ± 8 mature earthworms m^{-2}, 189 ± 7 juveniles m^{-2} and 70 ± 10 cocoons m^{-2} (Fig. 1), representing a mean biomass of 62 ± 9 g live weight m^{-2}. After the addition of the grape marc, the number of mature earthworms increased significantly until day 56 ($F_{4,16} = 10.34$, $p = 0.002$; Fig. 1); after which and until the end of the trial, no significant changes were recorded (Fig. 1). In the case of juveniles and cocoons, there was also a significant increase in their number from the beginning until day 56 (juveniles: $F_{4,16} = 6.98$, $p = 0.002$; cocoons: $F_{4,16} = 8.63$, $p = 0.0006$), reaching the values of 330 ± 22 juveniles m^{-2} and 108 ± 9 cocoons m^{-2} respectively (Fig. 1). The density of juveniles was then reduced until the end of the process reaching a final value of 205 ± 7 individuals m^{-2} (Fig. 1), while the number of cocoons remained constant until day 112 (Fig. 1). There was a continuous and significant increase of earthworm biomass until day 84 ($F_{4,16} = 36.26$,

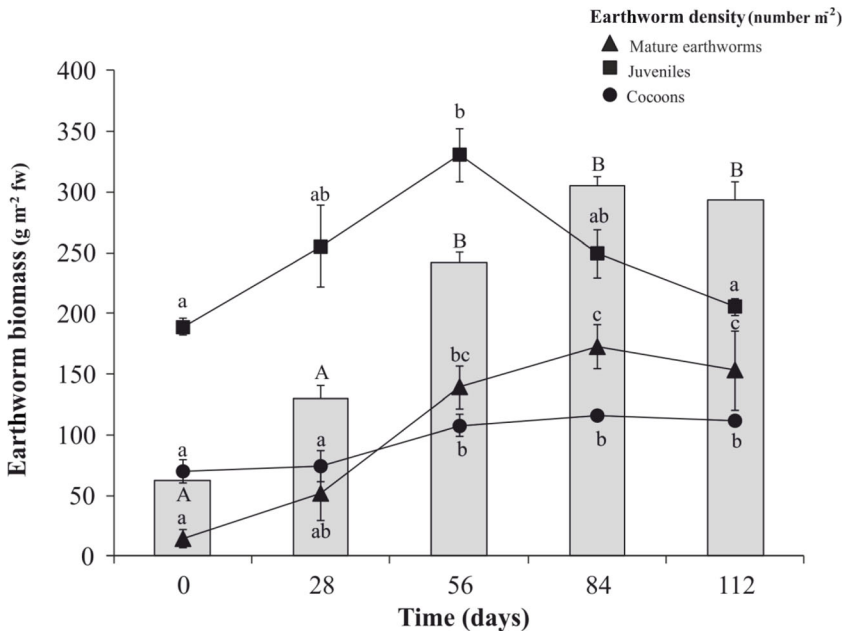

Fig. 1 Earthworm density (number of adults, juveniles and cocoons per m^2) and earthworm biomass (g m^{-2} fresh mass) during vermicomposting of grape marc derived from the red winemaking process of the grape variety Mencía. Grey bars represent earthworm biomass. Different letters (earthworm density, small letters; earthworm biomass, capital letters) indicate significant differences among the sampling times according to Tukey's HSD test. Values are means ± SE ($n = 5$)

$p < 0.0001$), at which it was about 5 times higher (305 g m^{-2} fresh mass) compared with the beginning of the trial (Fig. 1). From day 84 onwards, earthworm biomass remained without change until the end of the trial (Fig. 1).

In the present study, the initial population of earthworms was quite low and despite this, it increased during the first 2 months of vermicomposting as a consequence of the input of organic matter from the grape marc, and was far from its maximum capacity at the end of the process. No more grape marc was added to the vermireactor after the start of the experiment, which clearly influenced the earthworm density was much lower when compared with previous studies dealing with epigeic earthworms (i.e. up to 8000 individuals m^{-2} and 14,600 individuals m^{-2} in cow and pig manures, respectively, Monroy et al. (2006)).

When the vermireactor was later on continuously fed for a couple of years with the same grape marc derived from Mencía red winemaking, we observed that the population density reached maximum values of 12,868 ± 306 individuals m^{-2}, that is 4140 ± 68 mature earthworms m^{-2} and 8728 ± 43 juveniles m^{-2}, which is equivalent to a mean earthworm biomass of 2028 ± 20 g m^{-2} fw. This indicates that the grape marc from this cultivar appears to be an optimum substrate for feeding earthworms providing, on the one end, optimum environmental conditions for earthworm growth and reproduction and, on the other end, sufficient energy to sustain very large populations.

Chemical and microbiological changes during vermicomposting of red grape marc

The moisture content of the grape marc fell within the range of 80–85% (Table 1), which is considered an optimum range for the growth and reproduction of epigeic earthworms, and for a good performance of the vermicomposting process (Domínguez and Edwards 2011). pH is also considered an important driving factor of the dynamics of the process (Ali et al. 2015) since it is often correlated with underlying environmental factors influencing the microbial community such as nutrient availability, and/or the synthesis and activity of enzymes (Luo et al. 2018). In our study, the initial grape marc was characterised by an acidic pH (3.76 ± 0.05; Table 1). Previous work from Paradelo et al. (2013) has shown that the pH of hydrolysed grape marc, which is obtained after pre-treatment for lignocellulosic bioethanol production, was far too acidic for microbial activity and inhibited the transition from mesophilic to thermophilic composting stages. In our case, vermicomposting was found to effectively neutralise the initial acidity associated with the grape marc from the variety Mencía, reaching a pH value of 7.5 ± 0.06 at the end of the trial (Table 1). An increase in pH towards neutral values is of paramount importance to ultimately obtain a high-quality vermicompost. Luo et al. (2018) stated that the response of crops to organic amendments is more favourable when soil pH ranges from weak-acidic to weak-alkaline levels.

Vermicomposting of the grape marc derived from the red winemaking process of Mencía grapes followed the normal pattern of an accelerated decomposition process (Gómez-Brandón and Domínguez 2014). The initial mass of the grape marc (158 kg fresh mass; 24.07 kg dry mass) was considerably reduced, by approximately 78.5%, as a result of the earthworm activity, reaching a final fresh mass of 33.920 kg (6.52 kg dry mass) after 112 days. This mass loss was accompanied by a significant and constant increase in the initial nutrient content of the grape marc, except for P and K, during vermicomposting

Table 1 Changes in the physico-chemical properties of the grape marc (*Vitis vinifera* v. Mencía) throughout the vermicomposting process. Values are means ± standard error ($n = 5$). Different letters within the same row indicate significant differences among the sampling times according to Tukey's HSD test. Data are expressed on a dry weight basis

	Fresh grape marc	Worm-worked material			
Time (days)	0	28	56	84	112
Moisture (%)	84.77 ± 0.60^{a}	83.10 ± 2.52^{ab}	80.27 ± 0.55^{b}	81.58 ± 1.0^{b}	80.77 ± 0.64^{b}
pH	3.76 ± 0.05^{a}	5.49 ± 0.20^{ab}	6.40 ± 0.14^{c}	6.90 ± 0.03^{c}	7.50 ± 0.06^{d}
Electrical conductivity (mS cm^{2})	0.84 ± 0.02^{a}	0.76 ± 0.00^{a}	0.54 ± 0.01^{b}	0.31 ± 0.02^{c}	0.18 ± 0.001^{d}
Organic matter (%)	95.30 ± 0.82^{a}	93.56 ± 0.67^{a}	89.17 ± 0.61^{b}	84.13 ± 1.12^{c}	76.97 ± 0.48^{d}
Total C (g kg^{-1})	512.2 ± 0.81^{a}	510.1 ± 0.81^{ab}	502.6 ± 0.99^{b}	486.0 ± 3.34^{c}	470.98 ± 1.5^{d}
Total N (g kg^{-1})	20.86 ± 0.8^{a}	23.46 ± 0.73^{ab}	29.78 ± 0.41^{b}	35.64 ± 0.87^{c}	40.92 ± 0.32^{d}
C to N ratio	24.64 ± 1.12^{a}	21.82 ± 0.69^{b}	16.87 ± 0.22^{c}	13.66 ± 0.32^{de}	11.64 ± 0.15^{e}
Total P (g kg^{-1})	2.96 ± 0.05^{a}	2.79 ± 0.02^{ab}	2.75 ± 0.01^{ab}	2.57 ± 0.04^{c}	2.45 ± 0.02^{c}
Total K (g kg^{-1})	16.9 ± 0.83^{a}	14.4 ± 0.37^{b}	10.3 ± 0.28^{c}	7.89 ± 0.39^{d}	6.22 ± 0.09^{d}
Total Ca (g kg^{-1})	3.52 ± 0.13^{a}	4.24 ± 0.10^{a}	5.21 ± 0.11^{b}	6.05 ± 0.05^{c}	7.10 ± 0.31^{d}
Total Mg (g kg^{-1})	1.26 ± 0.01^{a}	1.36 ± 0.03^{ab}	1.42 ± 0.03^{bc}	1.52 ± 0.02^{c}	1.72 ± 0.02^{d}
Total S (g kg^{-1})	1.76 ± 0.04^{a}	2.05 ± 0.05^{b}	2.63 ± 0.12^{c}	3.07 ± 0.08^{d}	3.38 ± 0.03^{e}
Total Fe (g kg^{-1})	0.26 ± 0.07^{a}	0.42 ± 0.02^{b}	0.80 ± 0.02^{c}	1.37 ± 0.01^{d}	1.97 ± 0.03^{e}
Total Mn (g kg^{-1})	0.20 ± 0.00^{a}	0.24 ± 0.00^{ab}	0.27 ± 0.00^{b}	0.34 ± 0.00^{c}	0.38 ± 0.00^{d}
Total B (mg kg^{-1})	18.6 ± 2.11^{a}	18.6 ± 0.50^{a}	21.8 ± 0.73^{ab}	24.4 ± 1.32^{bc}	28.0 ± 0.31^{c}
Total Mo (mg kg^{-1})	1.18 ± 0.04^{a}	1.27 ± 0.05^{a}	1.44 ± 0.05^{a}	1.90 ± 0.06^{b}	2.38 ± 0.18^{c}
Lignin (g kg^{-1})	512 ± 14.0^{a}	526 ± 11.2^{a}	587 ± 3.39^{b}	578 ± 13.4^{b}	556 ± 13.4^{ab}
Cellulose (g kg^{-1})	227 ± 10.1^{a}	225 ± 14.3^{a}	215 ± 12.0^{a}	190 ± 4.95^{b}	109 ± 8.66^{c}
Hemicellulose (g kg^{-1})	222 ± 0.61^{a}	192 ± 8.06^{b}	165 ± 0.63^{c}	157 ± 6.70 ^{cd}	109 ± 2.23^{e}

(Ca: $F_{4,16} = 60.31$, $p < 0.0001$; Mg: $F_{4,16} = 28.36$, $p < 0.0001$; Fe: $F_{4,16} = 95.75$, $p < 0.0001$; S: $F_{4,16} = 147.5$, $p < 0.0001$; Mn: $F_{4,16} = 102$, $p < 0.0001$; B: $F_{4,16} = 10.29$, $p = 0.00025$; Mo: $F_{4,16} = 25.91$, $p < 0.0001$), and the highest values for these nutrients were observed at the end of the trial (Table 1).

Furthermore, the concentration of both cellulose and hemicellulose significantly decreased over time (hemicellulose: $F_{4,16} = 60.89$, $p < 0.0001$; cellulose: $F_{4,16} = 22.57$, $p < 0.0001$), registering the lowest values after 112 days (Table 1). Altogether, it indicates that earthworms enhanced the rate of decomposition of the grape marc and promoted the breakdown of polysaccharides, primarily cellulose and hemicellulose and to a lesser extent lignin, during vermicomposting. This can lead to a depletion of resources used by microbes and consequently to a reduction in microbial activity, measured as basal respiration in this study, over the course of vermicomposting ($F_{4,16} = 277.5$, $p < 0.0001$; Fig. 2). In our case, microbial activity significantly decreased until day 56 and then remained without changes until day 112 (Fig. 2). Lower levels of microbial activity are related to a higher degree of stability in the final vermicomposts (Gómez-Brandón and Domínguez 2014). Our findings are in line with those of Gómez-Brandón et al. (2011) in which they showed the potential of *E. andrei* in the stabilisation of grape marc at a lab scale, and over a shorter time period (i.e. after 2 weeks of vermicomposting).

Changes in total phenol and monomeric anthocyanin contents during vermicomposting of red grape marc

The total polyphenol content of the grape marc significantly decreased from 97.5 ± 4.5 mg GAE g^{-1} dry mass (day 0) to 61.2 ± 1.8 mg GAE g^{-1} dry mass in a period of 7 days ($F_{7,16} = 53.96$, $p < 0.0001$; Fig. 3a). From day 7, there was a progressive, albeit slower, reduction in the polyphenol content until day 56 (Fig. 3a). At day 70, polyphenols reached their minimum level (12.8 ± 0.4 mg GAE g^{-1} dry mass) and remained without significant changes until the end of the trial (Fig. 3a). This indicates that a period of 70 days was necessary to reduce most of the polyphenol-associated phytotoxicity from the grape marc derived from the red winemaking process of Mencía grapes. The negative and close correlation between the total polyphenol content and the earthworm biomass ($R = -0.87$, $p = 0.0002$) reinforces the potential and positive

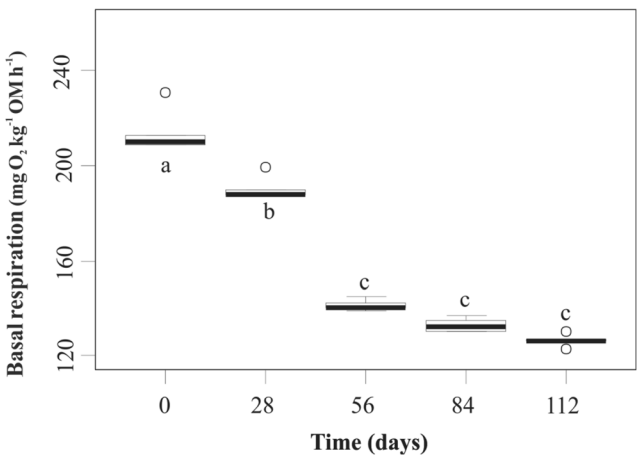

Fig. 2 Changes in microbial activity measured as basal respiration during vermicomposting of grape marc derived from the red winemaking process of the grape variety Mencía. Different letters indicate significant differences among the sampling times according to Tukey's HSD test. Values are means ± SE ($n = 5$)

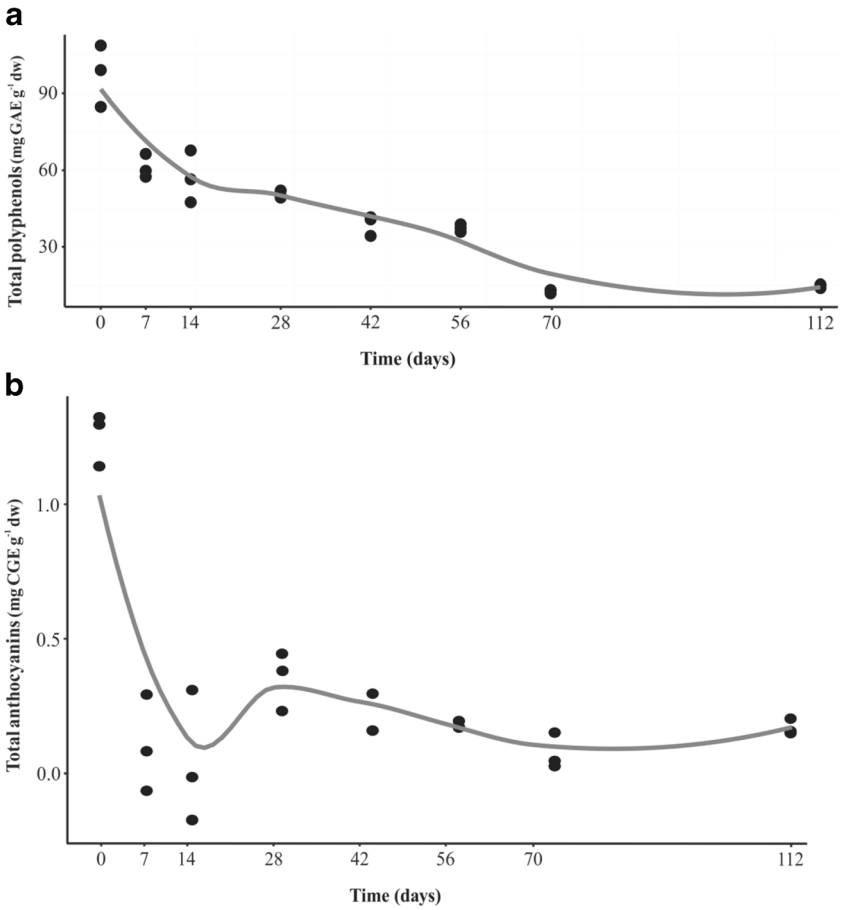

Fig. 3 Changes in the total content of polyphenols (**a**) and monomeric anthocyanins (**b**) during vermicomposting of grape marc derived from the red winemaking process of the grape variety Mencía. Individual values ($n = 3$) are plotted for each time point, and the curve was plotted using the "loess" smoothing method in ggplot2 (Wickham 2016)

role of *E. andrei* in the degradation of polyphenols over the course of vermicomposting.

In contrast to other flavonoids that can be present in both the grape skin and the seeds, the anthocyanins are only found in the skin and in some cases, they also appear in the grape pulp of red cultivars (Garrido and Borges 2013). This makes them more susceptible of degradation, which could explain why for these particular compounds there was a quick and strong reduction of its initial content in only 7 days ($F_{7,16} = 57.93$, $p < 0.0001$, Fig. 3b). This parameter was also closely and negatively related to the earthworm biomass ($R = -0.84$, $p = 0.0006$) over the course of vermicomposting, which provides further evidence of the enhancing effects of the earthworm *E. andrei* on the degradation of polyphenolic compounds.

The lack or low content of polyphenols in the final vermicompost is crucial for its safe application into soil and crop development (Domínguez et al. 2014). This relies on the fact that some phenolic compounds can inhibit plant growth and/or disrupt the microbial community balance by modifying the population and community structure in the rhizosphere (Jilani et al. 2008). Bearing this in mind, grape seeds that usually contain a polyphenolic content, higher than that in the whole grape marc (Negro et al. 2003; García-Jares et al. 2015), were separated from the vermicompost through sieving on day 28 in order to ensure a polyphenol-free vermicompost. As pointed out by Domínguez et al. (2014, 2016, 2017), the recovery of seeds may be suitable for another purpose (Patent no. ES2533501; Domínguez et al. 2015), that is to further use them as a relevant source of polyphenols and other bioactive compounds of interest by exploiting their beneficial properties in the food, pharmaceutical and cosmetic industries.

Our findings are in agreement with those of Domínguez et al. (2014) who observed a rapid and significant reduction in the polyphenolic content of grape marc derived from the white winemaking process of Albariño grapes after only 14 days in a pilot-scale vermireactor in the presence of *E. andrei*. The removal of polyphenols may be related to an increased aeration and organic matter consumption as a result of earthworm activity (Masciandaro et al. 2010), leading to a greater surface area available for microbial colonisation and further decomposition. Nonetheless, the underlying biological mechanisms of how earthworm-microbe interactions affect polyphenols degradation still need to be further investigated (Bi et al. 2016).

In conclusion, our study underscored the potential of the earthworm species *E. andrei* for processing grape marc obtained through the red winemaking process of Mencía grapes

in a large-scale vermicomposting system, turning it into an environmentally friendly organic fertiliser. The final vermicompost was characterised by a higher concentration of macro- and micro-nutrients and, additionally, the total content of polyphenols was significantly reduced during the process which can be indicative of a lower phytotoxicity at the end of the process. Moreover, sieving the worm-worked material separated the vermicompost from the grape seeds which opens a new avenue of exploration for vermicomposting focused on the recovery of these co-products to obtain different bioactive compounds such as polyphenol-rich extracts.

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