# Chapter 6
# Vermicomposting as an Eco-Friendly Approach for Recycling and Valorization Grape Waste

**María Gómez-Brandón, Manuel Aira, and Jorge Domínguez**

**Abstract** Finding strategies to treat, dispose of and reuse 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. This is of particular interest for the winemaking industry given its increasing activity worldwide with the subsequent generation of a wide variety of waste streams. The purpose of this chapter is to evaluate the effectiveness of the vermicomposting process as a low-cost and environmentally safe solution for the treatment and valorization of raw and distilled grape marc, the major solid by-products derived from the winery and distillery industry. We give an overview of the performance of the vermicomposting trial together with an in-depth characterization of the respective vermicomposts by looking at a combination of physicochemical, biochemical and microbiological indicators.

**Keywords** Winery waste · Enzymatic activities · Microbial diversity · Vermicompost · Soil amendment

## 6.1 Introduction

The winemaking industry constitutes one of the most important worldwide, agro-industrial sectors from an economic, social and cultural perspective (Hussain et al. 2008). An estimation carried out by the International Organization of Vine and Wine stated that about 260 million hl were produced globally in 2020 (http://www.oiv.int). The European Union (EU) comprises 44% of world wine-growing areas, with Spain, France and Italy as the three Member States accounting for 76% of EU areas under vines. Other major wine producing countries are the United States, Argentina, Chile, Australia and South Africa.

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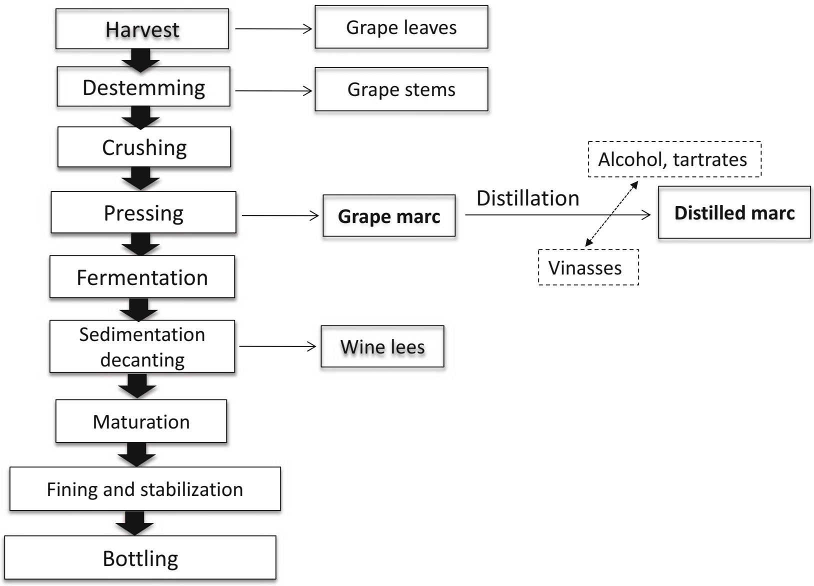


**Fig. 6.1** Processing steps and by-products derived from the winery and distillery industry

*Vitis vinifera* is considered the most commonly cultivated grape variety for wine production (FAO 2015). From grape harvest to bottling, the increasing activity related to the winemaking process generates an ample variety of solid and liquid by-products including vine shoots, grape marc, wine lees, spent filter cakes, vinasses and winery wastewater (Fig. 6.1). Taken together, it raises the necessity to search for profitable and sustainable options to treat, dispose of or to properly reuse these waste streams in order to reduce to a minimum their disposal and avoid negative environmental impacts (Spigno et al. 2017; Muhlack et al. 2018; Gómez-Brandón et al. 2019a). This is of particular interest for grape marc, also known as grape bagasse or grape pomace given that approximately 25% of the grape mass turns into this by-product during wine production (Domínguez et al. 2014).

Grape marc is composed of the seeds, pulp and stalks that remain after pressing the grapes (Domínguez et al. 2016, 2017; Fig. 6.1). Around 1 kg of this by-product results from the production of 5 L of wine, accounting for a worldwide production of 10–13 Mtons per year. A way to economically valorize grape marc is through distillation that permits the recovery of ethanol for its use in the elaboration of alcoholic beverages (Fig. 6.1; Cisneros-Yupanqui et al. 2022). In a simplified way, the sugars present in the raw marc are subjected to alcoholic fermentation under anaerobic conditions, followed by the subsequent distillation that can be carried out in batches or on a continuous basis (Botelho et al. 2020). Nonetheless, the distillation process also involves the generation of distillery effluents including vinasses and distilled grape marc (Zhang et al. 2017; Fig. 6.1) that altogether can represent a challenging waste disposal problem for the winery and distillery industry.

The use of raw and distilled marc as a soil amendment constitutes as an alternative option for their valorization, promoting waste prevention and new recycling goals. Both winery by-products are rich in organic matter and macronutrients that are essential for plant development (Requejo et al. 2016; Domínguez et al. 2016, 2017). However, if not properly treated, their application can cause soil acidification, groundwater pollution and oxygen depletion in soil due to the release of tannins and polyphenols with antimicrobial and phytotoxic effects (Domínguez et al. 2016).

The biological treatment of grape marc, either raw or distilled, through aerobic biodegradation processes offers a promising avenue to handle and process these by-products with the dual purpose of fertilizer production and environment protection (Gómez-Brandón et al. 2019a). The stabilization of raw and distilled grape marc via composting has been explored in this regard (Zhang and Sun 2016; Muhlack et al. 2018; Martínez-Salgado et al. 2019; Paradelo et al. 2019; Pinter et al. 2019; Cortés et al. 2020). Nevertheless, the low pH of grape marc could negatively affect microbial activity and inhibit the transition between mesophilic and thermophilic composting phases (Paradelo et al. 2019). Co-composting with other organic materials has been used as a more effective alternative improving not only the compost quality but also shortening the time required to achieve the stabilization of raw and distilled marc (Zhang and Sun 2016; Costa Barros et al. 2021).

Vermicomposting has also been successfully used to dispose of and treat large quantities of a wide variety of organic wastes (Domínguez and Gómez-Brandón 2012), and can provide a means to overcome the potential limitations of composting with regard to the stabilization of grape marc. The advantage of vermicomposting is twofold: it favours pH neutralization as a result of earthworms' activity (Domínguez et al. 2014; Gómez-Brandón et al. 2020a); and at the same time it offers the possibility to obtain a high-quality fertilizer with beneficial effects on soil and plants (Domínguez et al. 2016, 2017; Gómez-Brandón et al. 2020a, b). The bioconversion of biomass waste into organic fertilizers through the process of vermicomposting is linked to the synergistic actions of earthworms and microbial communities (Domínguez et al. 2010). The underlying biological mechanisms involved largely determine the dynamics of the process and, consequently, the properties of the end product for its further use as a plant growth promoter and in plant disease suppressiveness (Gómez-Brandón and Domínguez 2014).

The purpose of this chapter is to provide an overview of the vermicomposting process as a low-cost and environmentally safe solution for the treatment and valorization of raw and distilled grape marc derived from the winery sector. We will present a case study to illustrate the effectiveness of vermicomposting for the stabilization of these winery by-products together with an in-depth characterization of the final vermicomposts by looking at a combination of physicochemical, biochemical and microbiological indicators.

## 6.2 How Vermicomposting Works: The Experimental Set-up

Raw and distilled marc of the grape variety *Vitis vinifera* L. cv. Mencía, which represents 95% of the annual red grape harvest in northwestern Spain, were processed in pilot-scale vermireactors (6 $m^2$; Fig. 6.2a) using the earthworm *Eisenia andrei* (Oligochaeta, Lumbricidae; Fig. 6.2b). This is one of the most widely used earthworm species in vermicomposting facilities because of its high rate of consumption, digestion and assimilation of organic matter, and its tolerance to a wide range of temperature and moisture conditions (Domínguez and Edwards 2011a). The vermireactors were continuously fed for almost a year with grape marc, either raw or distilled, allowing the population density of earthworms to reach its maximum capacity, with an average value of 11,115 ± 2827 individuals $m^{-2}$ that corresponds with a mean biomass of 1361 ± 415 g $m^{-2}$. This points to the suitability of raw and


**Fig. 6.2** (**a**) Overview of the pilot-scale vermireactors fed with raw and distilled marc; (**b**) Processing of grape marc by the earthworm *Eisenia andrei*; (**c**) Fresh layer of grape marc on top of the plastic mesh for the performance of the vermicomposting trial

distilled marc derived from Mencía grape cultivar as an optimum feedstock for earthworm growth and reproduction providing sufficient energy to sustain large populations in vermicomposting systems.

To evaluate the effectiveness of vermicomposting for the stabilization of grape marc, a new layer of the substrate (raw or distilled marc, 50 kg fresh weight) was placed over a plastic mesh that allowed earthworm migration and prevented the mixing of the processed grape marc and the vermicomposting bedding (Fig. 6.2c). Control reactors containing the same amount of raw or distilled marc in the absence of earthworms were also set up during the vermicomposting trial. All the reactors with and without earthworms were covered with a shade cloth to keep the moisture level of the substrate at approximately 85% during the trial. This is considered an optimum moisture level for the growth and reproduction of epigeic earthworms, as well as for a good performance of the vermicomposting process (Domínguez and Edwards 2011a). After a two-month period, the substrate was completely processed by the earthworms and the raw and distilled grape marc-derived vermicomposts as well as the respective control samples were collected from the surface of the mesh, stored at 4 °C or air-dried depending on the analytical measurement, and further processed for their characterization.

## 6.3 Physicochemical Characterization and Nutrient Content of Grape Marc Vermicompost

Among the physicochemical parameters, pH is one of the most widely used to study the dynamics of the vermicomposting process (Ali et al. 2015), as it affects environmental conditions that are relevant for microbial growth and survival (Jin and Kirk 2018). Changes in pH may influence not only the solubility and bioavailability of nutrients and trace elements over the course of the process (Domínguez et al. 2016, 2017), but also the synthesis and activity of extracellular enzymes (Sánchez-Hernández and Domínguez 2017). Vermicomposting was effective to neutralizing the acidity of raw and distilled grape marc, reaching a pH value close to neutrality in the respective vermicomposts after the two-month period (Table 6.1). An increase in pH towards neutral values as a result of earthworms´ activity is an important asset for the potential usefulness of vermicompost as a soil amendment, considering that the response of crops to organic amendments is more favourable when soil pH ranges from weak-acidic to weak-alkaline levels (Luo et al. 2018; Cataldo et al. 2021).

The electrical conductivity (EC) is also considered an important physicochemical parameter to evaluate during vermicomposting because it reflects the degree of salinity and can largely affect the survival and reproduction of earthworms if it reaches values higher than 8.0 mS $cm^{-1}$ (Domínguez and Edwards 2011b). The EC levels of the studied vermicomposts (circa 0.3 mS $cm^{-1}$; Table 6.1) seem optimal for earthworm survival and were in agreement with those reported in previous vermicomposting trials dealing with grape marc (Cástková and Hanc 2019;

**Table 6.1** Overview of the physicochemical properties and the nutrient content of the vermicomposts obtained from raw and distilled marc after a two-month period. Values are means ± standard error. Nutrient data are on a dry weight (dw) basis

| | Raw marc | | Distilled marc | |
|---|---|---|---|---|
| | Control | Vermicompost | Control | Vermicompost |
| pH | 3.76 ± 0.05 | 6.90 ± 0.03 | 4.30 ± 0.07 | 7.53 ± 0.03 |
| EC (mS $cm^{-2}$) | 0.84 ± 0.02 | 0.31 ± 0.02 | 0.89 ± 0.03 | 0.20 ± 0.01 |
| Ca (mg $kg^{-1}$ dw) | 3680 ± 49 | 4391 ± 196 | 3257 ± 52 | 3669 ± 55 |
| K (mg $kg^{-1}$ dw) | 23,654 ± 568 | 11,956 ± 351 | 17,721 ± 748 | 13,161 ± 415 |
| P (mg $kg^{-1}$ dw) | 3116 ± 61 | 1864 ± 38 | 2582 ± 56 | 1818 ± 96 |
| Mg (mg $kg^{-1}$dw) | 1135 ± 15 | 988 ± 40 | 976 ± 31 | 1047 ± 49 |
| Mn (mg $kg^{-1}$ dw) | 40 ± 0.4 | 37 ± 1 | 39 ± 0.6 | 43 ± 2 |
| Fe (mg $kg^{-1}$ dw) | 92 ± 4 | 129 ± 6 | 98 ± 3 | 134 ± 33 |
| S (mg $kg^{-1}$ dw) | 1528 ± 31 | 1141 ± 45 | 1579 ± 32 | 1650 ± 64 |

Gómez-Brandón et al. 2020a). The grape marc-derived vermicomposts had EC values lower than those in the control treatment likely due to a reduced presence of mineral salts in available forms such as K, P and $NH_4^+$ which are known as main contributors to the electrical conductivity.

Epigeic earthworms are known to accelerate the turnover rate of organic matter during vermicomposting (Domínguez and Gómez-Brandón 2012), by grazing directly on microorganisms and/or by increasing the surface area available for microbial attack through comminution. Supporting this, the initial mass of the raw and distilled marc (50 kg fresh mass; 10.75 kg dry mass) was considerably reduced, by approximately 75%, as a result of the earthworm activity, reaching a final fresh mass of 9.26 kg (2.44 kg dry mass) after 2 months of vermicomposting. The pronounced reduction of the initial mass of grape marc leads to an increase in the density of grape seeds (Domínguez et al. 2014, 2016, 2017). Polyphenolic compounds represent around 7% of seeds' composition and in this regard, Domínguez et al. (2014, 2016) reported that separating the seeds from the vermicompost through sieving may offer a twofold advantage: (i) to obtain a polyphenol-free vermicompost for its safe application into soil considering that some phenolic compounds can negatively affect plant growth and/or disrupt the microbial community balance in the rhizosphere (Jilani et al. 2008) and (ii) to further exploit the nutritional, cosmetic and health-promoting properties of polyphenols recovered from the seeds with respect to their antioxidant, antifungal and scavenging activities (Lores et al. 2013; García-Jares et al. 2015; Álvarez-Casas et al. 2016; Barba et al. 2016).

As a consequence of the enhanced mineralization in the presence of earthworms, macro- and micronutrients such as calcium (Ca) and iron (Fe) appeared in higher concentrations in the grape marc-derived vermicomposts when compared to the control without earthworms (Table 6.1). However, this did not hold for potassium (K) and phosphorus (P) whose contents were much lower in the vermicomposts obtained from raw and distilled marc (Table 6.1). A decrease in these nutrients' concentration throughout the process of vermicomposting might occur via leaching, immobilization by microorganisms or earthworms, or precipitation in the form of non-mineral salts (Domínguez et al. 2018). This decreasing trend in K and P was not observed when cattle manure was used as feedstock in previous vermicomposting trials (Domínguez and Gómez-Brandón 2013). Grape marc is 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. Nonetheless, in vermicomposting applications with cattle manure or other types of manure the feedstock has already passed through the vertebrate gut (i.e. cow, pig, horse).

To sum up, the values reported with regard to the physicochemical and the nutrient properties of raw and distilled grape marc-derived vermicomposts generally matched with those considered to have the quality criteria of a good vermicompost (Domínguez and Edwards 2011b; Domínguez et al. 2017).

## 6.4 Biochemical and Microbiological Characterization of Grape Marc Vermicompost

During vermicomposting, the biochemical decomposition of the organic matter is primarily accomplished through the chemical reactions performed by microorganisms via the production of extracellular enzymes. However, the effects that earthworms have on microbial communities in terms of biomass, activity and/or composition will largely determine the dynamics of the process and the quality of the end product (Gómez-Brandón and Domínguez 2014; Gómez-Brandón et al. 2020b). Bearing this in mind, over the last twenty years our research group has been exploring the relationships between earthworms and microorganisms during vermicomposting of a wide variety of organic wastes by assessing the changes in microbial biomass carbon and nitrogen, as well as in basal respiration as a proxy of microbial activity, and in the activity of extracellular enzymes involved in the major nutrient cycles (Aira et al. 2002, 2006a, b, 2007; Aira and Domínguez 2008, 2011; Domínguez and Gómez-Brandón 2013; Domínguez et al. 2018; Almeida-Santana et al. 2020; Gómez-Brandón et al. 2021). Shifts in community composition over the course of vermicomposting have also been studied by using culture-independent methods such as phospholipid fatty acid analysis (Aira et al. 2011; Gómez-Brandón et al. 2011, 2012, 2013), and more recently by molecular tools based on DNA extraction, followed by amplification (via polymerase chain reaction, PCR) and sequencing of marker genes (Domínguez et al. 2019; Kolbe et al. 2019; Gómez-Brandón et al. 2019b, 2020c; Rosado et al. 2022).

### 6.4.1 Microbial Biomass and Activity

Assessing vermicompost stability is crucial for its potential usefulness as a soil amendment and plant growth promoter. After incorporation to soils, unstable vermicomposts may induce enhanced microbial activity and cause oxygen deficiency and a variety of phytotoxicity problems to plant roots. Stability is related to the degree to which vermicomposts have been decomposed to more stable organic materials and it is typically evaluated by different respirometric measurements and/or by studying the transformations in the chemical characteristics of organic matter (Bernal et al. 2017).

Both the raw and distilled grape marc-derived vermicomposts were characterized by lower levels of microbial biomass carbon and microbial activity assessed as basal respiration when compared to the control without earthworms (Fig. 6.3a, b). Earthworms' activity can reduce microbial biomass directly by selectively feeding on specific taxa during transit through the earthworm gut (Drake and Horn 2007; Aira et al. 2015), or indirectly by accelerating the depletion of resources available for the microbes. In line with this, the resulting vermicomposts were characterized by lower abundances of both bacteria and fungi after the two-month period (Fig. 6.3c,d).

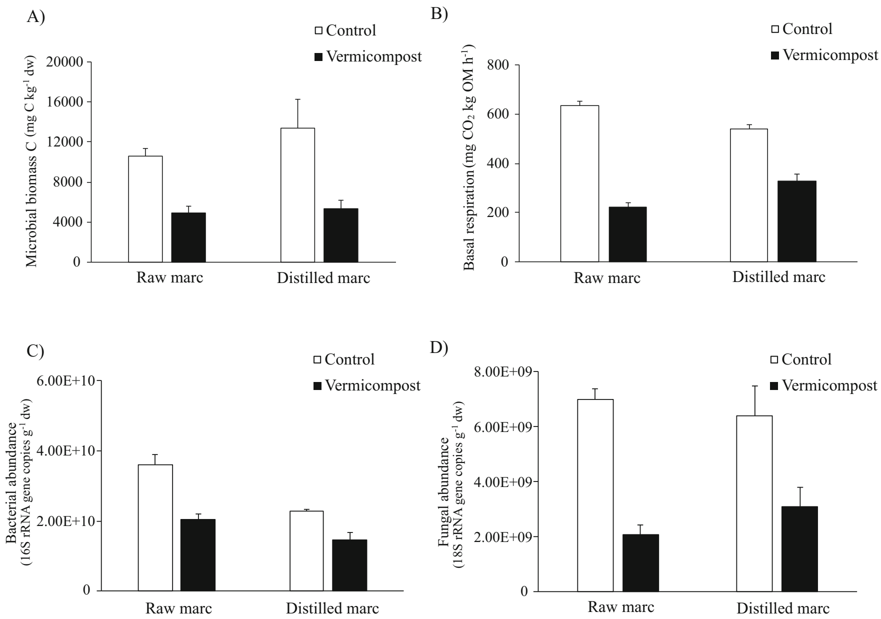


**Fig. 6.3** Impact of earthworms (*Eisenia andrei*) on microbial biomass and activity after vermicomposting of raw and distilled marc for a period of 2 months: (**a**) Microbial biomass carbon; (**b**) Basal respiration, a measure of microbial activity; (**c**, **d**) Bacterial and fungal abundances estimated by real-time PCR. Values are mean ± SE. Control is the same treatment in the absence of earthworms

Altogether, these findings point to the effectiveness of vermicomposting at biologically stabilizing these winery by-products, as reflected by decreases in both microbial biomass and its activity in comparison with the control treatment.

### 6.4.2 Enzymatic Activities

From a functional perspective, the activity of extracellular enzymes has received considerable attention because of their sensitivity to environmental changes and their role in the breakdown or mineralization of major nutrients like carbon (C), nitrogen (N) and P into inorganic forms that can be used by plants (Acosta-Martínez et al. 2018).

Three enzyme activities involved in the C cycle were measured in the grape marc-derived vermicomposts, namely the β-glucosidase, the cellobiohydrolase and the xylosidase (Fig. 6.4a–c). They have a relevant role in organic matter decomposition processes because they catalyse the hydrolysis of cellulose and hemicellulose, two of the main components in the plant debris (Nannipieri et al. 2012). Both β-glucosidase and cellobiohydrolase activities had lower values in the resulting vermicomposts in comparison with the control without earthworms (Fig. 6.4a, b). It is likely that the accelerated depletion of resources due to earthworm activity leads to reduced enzyme activities towards the end of the process (Benítez et al. 2005). The lower levels of these C-associated enzymes were in line with the reduced microbial biomass and activity in the vermicomposts, reinforcing the use of these enzyme activities as indicators of the suitability of vermicomposting for the biological stabilization of raw and distilled marc.

However, the opposite trend was observed for xylosidase whose activity was higher in the vermicompost than in the control samples (Fig. 6.4c). A plausible explanation relies on the increasing concentrations of humic substances that appear as vermicomposting progresses providing chemical support for binding extracellular enzymes, and protecting them against proteases or adverse environmental conditions (Castillo et al. 2013). Likewise, leucine-aminopeptidase activity responsible for the release of amino acids from polypeptides, as well as alkaline phosphomonoesterase reached higher values in the raw and distilled marc-derived vermicomposts (Fig. 6.4d, f). This latter enzyme is involved in the mineralization of organic P into phosphate by hydrolysing phosphoric (mono) ester bonds under alkaline conditions. In contrast, acid phosphomonoesterase showed lower or similar values than those in the control (Fig. 6.4e). Earthworms can alter nutrient cycling and increase N and P uptake by plants through a combination of biochemical and chemical pathways (Medina-Sauza et al. 2019). Taken together, our findings are consistent with these observations providing evidence of the enhancing effect of the earthworm *E. andrei* on certain enzyme activities involved in the breakdown or mineralization of N and P into inorganic forms that can be used by plants.

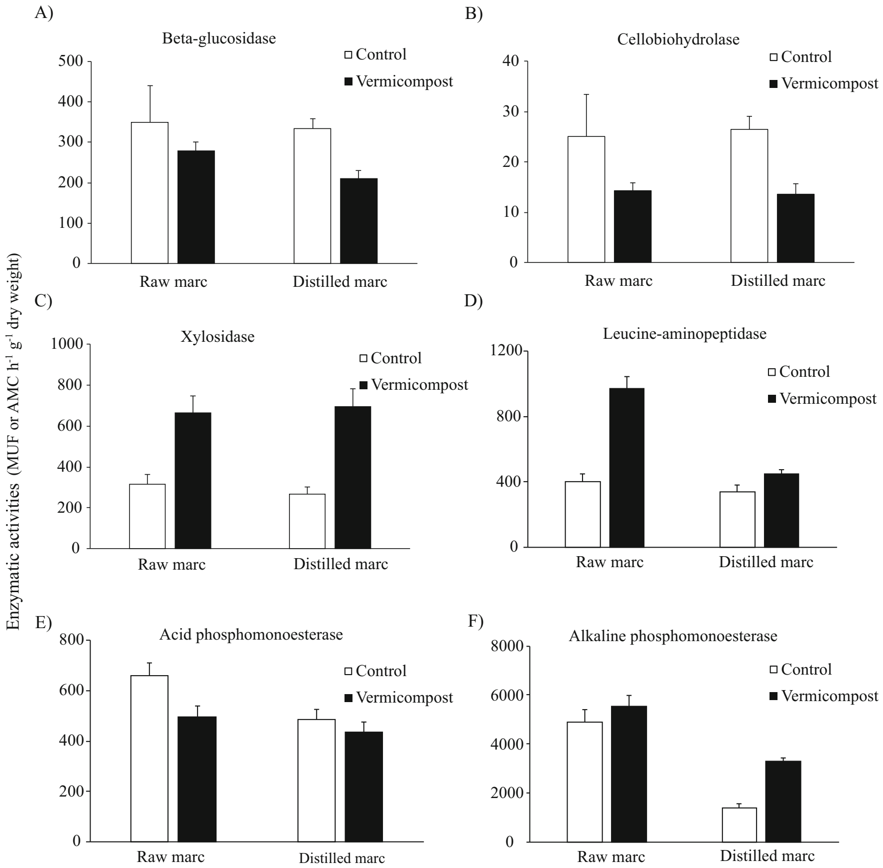


**Fig. 6.4** Impact of earthworms (*Eisenia andrei*) on enzymatic activities related to C-, N- and P-cycles after vermicomposting of raw and distilled marc for a period of 2 months: (**a**) Beta-glucosidase, (**b**) Cellobiohydrolase, (**c**) Xylosidase, (**d**) Leucine-aminopeptidase and (**e**, **f**) Acid and Alkaline phosphomonoesterases. Values are mean ± SE. Control is the same treatment in the absence of earthworms. Units are given as nanomoles of 4-methyl-umbelliferone (MUF) $h^{-1}$ $g^{-1}$ dry weight, except for leucine-aminopeptidase which activity is expressed as nanomoles of 7-amino-4-methyl coumarin (AMC) $h^{-1}$ $g^{-1}$ dry sample

## 6.4.3 *Microbial Richness and Diversity*

Abundant research has shown that the use of vermicomposts has beneficial effects when used as an amendment for soil or plant growth media for a wide variety of agronomic and horticultural crops (Lazcano and Domínguez 2011; Blouin et al. 2019). Various factors such as an improved availability of air and water, the presence of plant growth regulating substances, and the mitigation or suppression of plant diseases have been proposed as plausible, albeit not exclusive, mechanisms by which such improvement is achieved (Gómez-Brandón and Domínguez 2014).

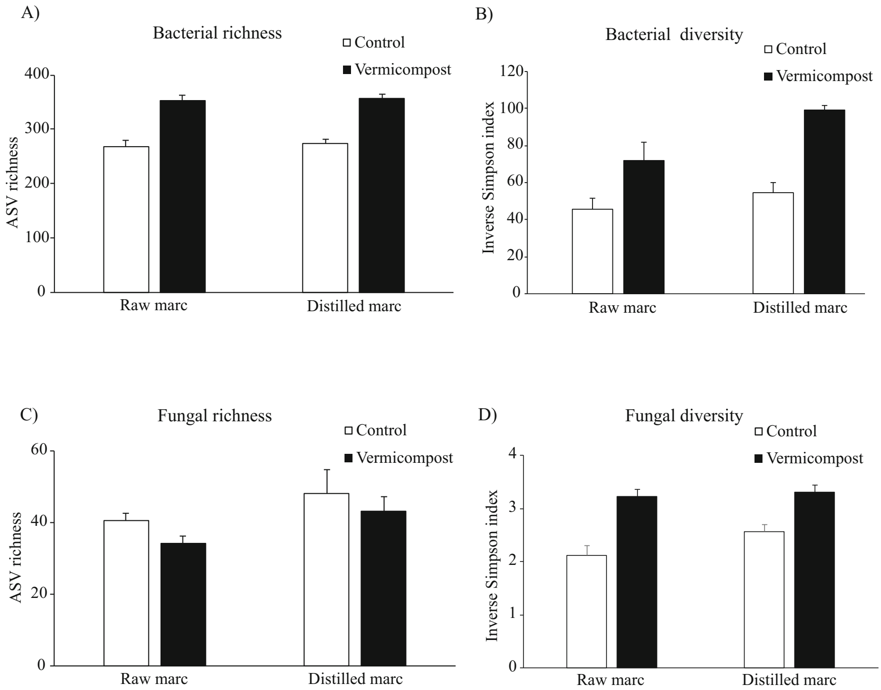


**Fig. 6.5** Impact of earthworms (*Eisenia andrei*) on microbial richness calculated as the number of observed amplicon sequence variant (ASVs), and microbial diversity assessed as the inverse Simpson index after vermicomposting of raw and distilled marc for a period of 2 months: (**a**) Bacterial richness, (**b**) Bacterial diversity, (**c**) Fungal richness and (**d**) Fungal diversity. Values are mean ± SE. Control is the same treatment in the absence of earthworms

Vermicomposts are considered bioactive organic materials and there exist evidence about microbial-based mechanisms that may explain the positive influence of vermicompost on soil and plants (Domínguez et al. 2019; Gómez-Brandón et al. 2020b). We observed that the richness and diversity of bacterial communities were higher in the grape marc-derived vermicomposts compared to the control treatment (Fig. 6.5a, b). The same trend was recorded for fungal diversity (Fig. 6.5d), whilst fungal richness had similar values in the presence and the absence of earthworms (Fig. 6.5c). Microbial diversity may benefit soils in two major ways, by safeguarding a steady nutrient supply through decomposition processes that cope with changing environmental conditions, and secondly by supplying a microbiota that is disease suppressive. In this regard, higher abundances of putative genes involved in the biosynthesis of antibiotics or plant hormone synthesis were found in vermicomposts when compared to the starting material (Domínguez et al. 2019; Kolbe et al. 2019; Gómez-Brandón et al. 2020b).

## 6.5 Conclusions

Vermicomposting has proven to be an effective, environmentally sound management system for processing raw and distilled grape marc derived from the winery industry. Earthworm activity favoured the stabilization of these by-products resulting in a final vermicompost characterized by a higher concentration of macro- and micronutrients essential for plant growth. Moreover, lower values of microbial biomass and microbial activity, indicative of stabilized materials, together with a reduced activity of hydrolytic enzymes involved in cellulose degradation were found after two months of vermicomposting. This was accompanied by an increased microbial richness and diversity in the grape marc-derived vermicomposts reinforcing their potential role as bioactive organic materials for its further use as an amendment for soil or plant growth media.

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