

Vermicomposting: Composting with Earthworms to Recycle Organic Wastes

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1. Introduction

The overproduction of organic wastes has led to the use of inappropriate disposal practices such as their indiscriminate and inappropriately-timed application to agricultural fields. These practices can cause several environmental problems, including an excessive input of potentially harmful trace metals, inorganic salts and pathogens; increased nutrient loss, mainly nitrogen and phosphorus, from soils through leaching, erosion and runoff; and the emission of hydrogen sulphide, ammonia and other toxic gases (Hutchison et al., 2005). However, if handled properly, organic wastes can be used as valuable resources for renewable energy production, as well as sources of nutrients for agriculture, as they provide high contents of macro- and micronutrients for crop growth and represent a low-cost alternative to mineral fertilizers (Moral et al., 2009).

The health and environmental risks associated with the management of such wastes could be significantly reduced by stabilizing them before their disposal or use. Composting and vermicomposting are two of the best known-processes for the biological stabilization of a great variety of organic wastes (Domínguez & Edwards, 2010a). However, more than a century had to pass until vermicomposting, i.e. the processing of organic wastes by earthworms was truly considered as a field of scientific knowledge or even a real technology, despite Darwin (1881) having already highlighted the important role of earthworms in the decomposition of dead plants and the release of nutrients from them.

In recent years, vermicomposting has progressed considerably, primarily due to its low cost and the large amounts of organic wastes that can be processed. Indeed, it has been shown that sewage sludge, paper industry waste, urban residues, food and animal waste, as well as horticultural residues from cultivars may be successfully managed by vermicomposting to produce vermicomposts for different practical applications (reviewed in Domínguez, 2004). Vermicompost, the end product of vermicomposting, is a finely divided peat-like material of high porosity and water holding capacity that contains many nutrients in forms that are readily taken up by plants.

Vermicomposting is defined as a bio-oxidative process in which detritivore earthworms interact intensively with microorganisms and other fauna within the decomposer community, accelerating the stabilization of organic matter and greatly modifying its

physical and biochemical properties (Domínguez, 2004). The biochemical decomposition of organic matter is primarily accomplished by microorganisms, but earthworms are crucial drivers of the process as they may affect microbial decomposer activity by grazing directly on microorganisms (Aira et al., 2009; Monroy et al., 2009; Gómez-Brandón et al., 2011a), and by increasing the surface area available for microbial attack after comminution of organic matter (Domínguez et al., 2010) (Figure 1). These activities may enhance the turnover rate and productivity of microbial communities, thereby increasing the rate of decomposition. Earthworms may also affect other fauna directly, mainly through the ingestion of microfaunal groups (protozoa and nematodes) that are present within the organic detritus consumed (Monroy et al., 2008); or indirectly, modifying the availability of resources for these groups (Monroy et al., 2011) (Figure 1).

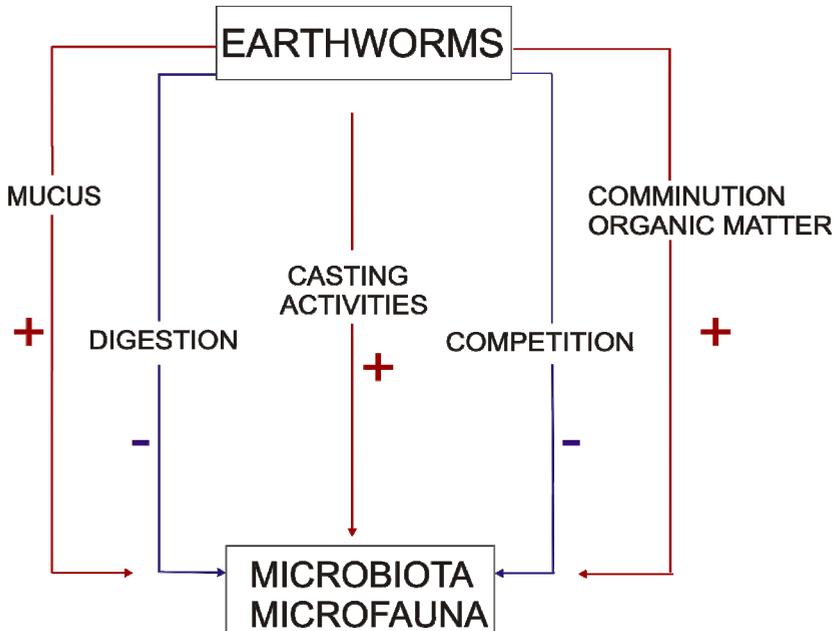


Fig. 1. Positive (+) and negative (-) effects of earthworms on microbiota and microfauna (modified from Domínguez et al., 2010).

Furthermore, earthworms are known to excrete large amounts of casts (Figure 1), which are difficult to separate from the ingested substrate (Domínguez et al., 2010). The contact between worm-worked and unworked material may thus affect the decomposition rates (Aira & Domínguez, 2011), due to the presence of microbial populations in earthworm casts different from those contained in the material prior to ingestion (Gómez-Brandón et al., 2011a). In addition, the nutrient content of the egested materials differs from that in the ingested material (Aira et al., 2008), which may enable better exploitation of resources, because of the presence of a pool of readily assimilable compounds in the earthworm casts. Therefore, the decaying organic matter in vermicomposting systems is a spatially and temporally heterogeneous matrix of organic resources with contrasting qualities that result from the different rates of degradation that occur during decomposition (Moore et al., 2004).

2. Earthworm species suitable for vermicomposting

Earthworms represent the major animal biomass in most terrestrial temperate ecosystems (Edwards & Bohlen, 1996). Indeed, more than 8,300 species of earthworms have been described (Reynolds & Wetzel, 2010), although for the great majority of these species only the names and morphologies are known, and little is yet known about their biology, life cycles and ecology. Different species of earthworms have different life histories, occupy different ecological niches, and have been classified, on the basis of their feeding and burrowing strategies, into three ecological categories: epigeic, anecic and endogeic (Bouché 1977). Endogeic species (soil feeders) forage below the surface soil, ingest high amounts of mineral soil and form horizontal burrows. Anecic species (burrowers) live in deeper zones of mineral soils, ingest moderate amounts of soil, and feed on litter that they drag into their vertical burrows. And, epigeic earthworms (litter dwellers and litter transformers) live in the soil organic horizon, in or near the surface litter, and mainly feed on fresh organic matter contained in forest litter, litter mounds and herbivore dung, as well as in man-made environments such as manure heaps. These latter species, with their natural ability to colonize organic wastes; 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 show good potential for vermicomposting (Domínguez & Edwards, 2010b). In fact, few epigeic earthworm species display all these characteristics, and only four have been extensively used in vermicomposting facilities: *Eisenia andrei*, *Eisenia fetida*, *Perionyx excavatus* and *Eudrilus eugeniae* (Figure 2).



Fig. 2. Earthworm species *Eisenia andrei* (top left), *Eisenia fetida* (top right), *Eudrilus eugeniae* (bottom left) and *Perionyx excavatus* (bottom right).

3. How does vermicomposting work?

The vermicomposting process includes two different phases regarding earthworm activity: (i) an active phase during which earthworms process the organic substrate, thereby modifying its physical state and microbial composition (Lores et al., 2006), and (ii) a maturation phase marked by the displacement of the earthworms towards fresher layers of undigested substrate, during which the microorganisms take over the decomposition of the earthworm-processed substrate (Aira et al., 2007; Gómez-Brandón et al., 2011b). The length of the maturation phase is not fixed, and depends on the efficiency with which the active phase of the process takes place, which in turn is determined by the species and density of earthworms (Domínguez et al., 2010), and the rate at which the residue is applied (Aira & Domínguez, 2008).

More specifically, the impact of earthworms on the decomposition of organic waste during the vermicomposting process is initially due to *gut associated processes* (GAPs) (Figure 3), i.e., via the effects of ingestion, digestion and assimilation of the organic matter and microorganisms in the gut, and then casting (Gómez-Brandón et al., 2011a). Specific microbial groups respond differently to the gut environment (Schönholzer et al., 1999) and selective effects on the presence and abundance of microorganisms during the passage of organic material through the gut of these earthworm species have been observed. For instance, some bacteria are activated during passage through the gut, whereas others remain

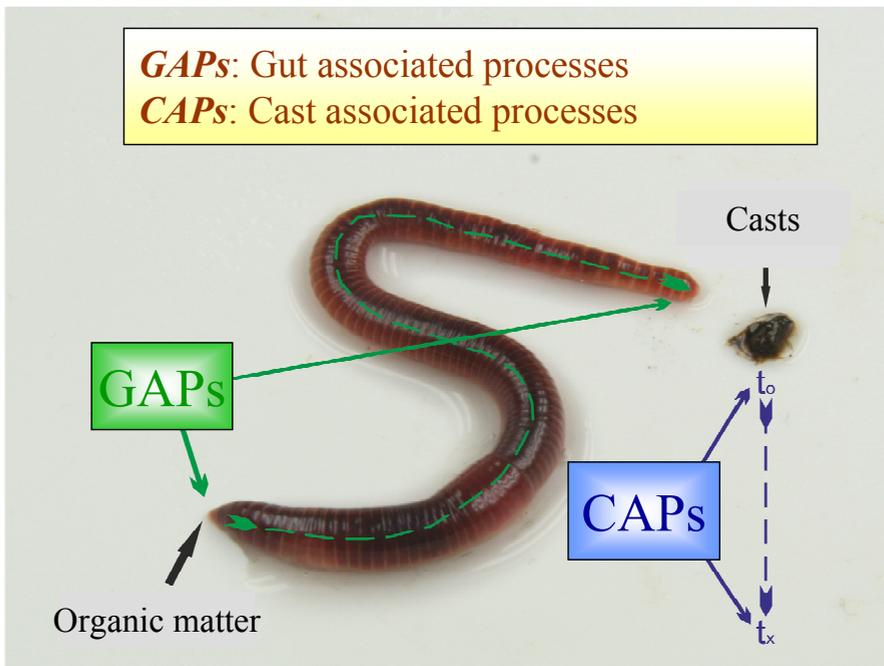


Fig. 3. Earthworms affect the decomposition of organic matter during vermicomposting through ingestion, digestion and assimilation in the gut and then casting (*gut associated processes*); and *cast associated processes*, which are more closely related with ageing processes.

unaffected and others are digested in the intestinal tract and thus decrease in number (Drake & Horn, 2007; Monroy et al., 2009). Such selective effects on microbial communities as a result of gut transit may alter the decomposition pathways during vermicomposting, probably by modifying the composition of the microbial communities involved in decomposition, as microbes from the gut are then released in faecal material where they continue to decompose egested organic matter. Indeed, as mentioned above, earthworm casts contain different microbial populations to those in the parent material (Domínguez et al., 2010), and in turn it is expected that the inoculum of those communities in fresh organic matter promotes modifications similar to those found when earthworms are present, altering microbial community levels of activity and modifying the functional diversity of microbial populations in vermicomposting systems (Aira & Domínguez, 2011).

Upon completion of GAPs, the resultant earthworm casts undergo *cast associated processes* (CAPs; Figure 3), which are more closely related to ageing processes, the presence of unworked material and to physical modification of the egested material (weeks to months). During these processes the effects of earthworms are mainly indirect and derived from the GAPs (Aira et al., 2007). In addition, during this aging, vermicompost is expected to reach an optimum in terms of its biological properties, thereby promoting plant growth and suppressing plant diseases (Domínguez et al., 2010). However, little is yet known about when this “optimum” is achieved, how we can determine it in each case and if this “optimum” has some kind of expiration date.

4. Effects of earthworms on the structure and activity of microbial communities during vermicomposting

Since vermicomposting is a biological process, microorganisms play a key role in the evolution of the organic materials and in the transformations they suffer from wastes to safe organic amendments or fertilizers (vermicompost). Therefore, the effects that earthworms have on the microorganisms must be established because if the earthworms were to stimulate or depress microbiota or modify the structure and activity of microbial communities, they would have different effects on the decomposition of organic matter, and in turn on the quality of the final product. To address these questions we performed three laboratory experiments, with the following objectives:

- i. To investigate whether and to what extent the earthworm *E. andrei* is capable of altering the structure and activity of microbial communities through the gut associated processes.
- ii. To investigate how the earthworm species affect the structure and activity of microbial communities during the active phase of vermicomposting.
- iii. To investigate the effectiveness of the active phase of vermicomposting for the short-term stabilization of a plant residue.

4.1 How do earthworms affect microbial communities through the gut associated processes?

To provide further light into the effect of gut transit on microbial communities, we carried out an experiment with microcosms filled with cow manure and inoculated with 25 mature individuals of the earthworm species *E. andrei*. The microcosms consisted of 250 mL plastic

containers filled to three quarters of their capacity with sieved, moistened vermiculite. A plastic mesh was placed over the surface of the vermiculite and 100 g (fresh weight, fw) of the substrate was placed on top of the mesh, to avoid mixing the substrate with the vermiculite bedding (Figure 4a). The microcosms were covered with perforated lids and stored in random positions in an incubation chamber, at 20 °C and 90% relative humidity, for three days (Figure 4b). Control microcosms consisted of each type of manure incubated without earthworms. Each treatment was replicated five times. In order to obtain cast samples, earthworms were removed from the microcosms, washed three times with distilled water and placed in Petri dishes on moistened filter paper (Figure 4b). Casts from the same Petri dish were then collected with a sterile spatula and pooled for analysis in 1.5 mL Eppendorf tubes (Figure 4b); the same amount of manure samples were also collected from the control microcosms. Viable microbial biomass was determined as the sum of all identified phospholipid fatty acids (PLFAs) (Zelles, 1999). The structure of microbial communities was assessed by PLFA analysis; some specific PLFAs were used as biomarkers to determine the presence and abundance of specific microbial groups (Zelles, 1997). The sum of PLFAs characteristic of Gram-positive (iso/anteiso branched-chain PLFAs), and Gram-negative bacteria (monounsaturated and cyclopropyl PLFAs) were chosen to represent bacterial PLFAs, and the PLFA 18:2 ω 6c was used as a fungal biomarker. Total

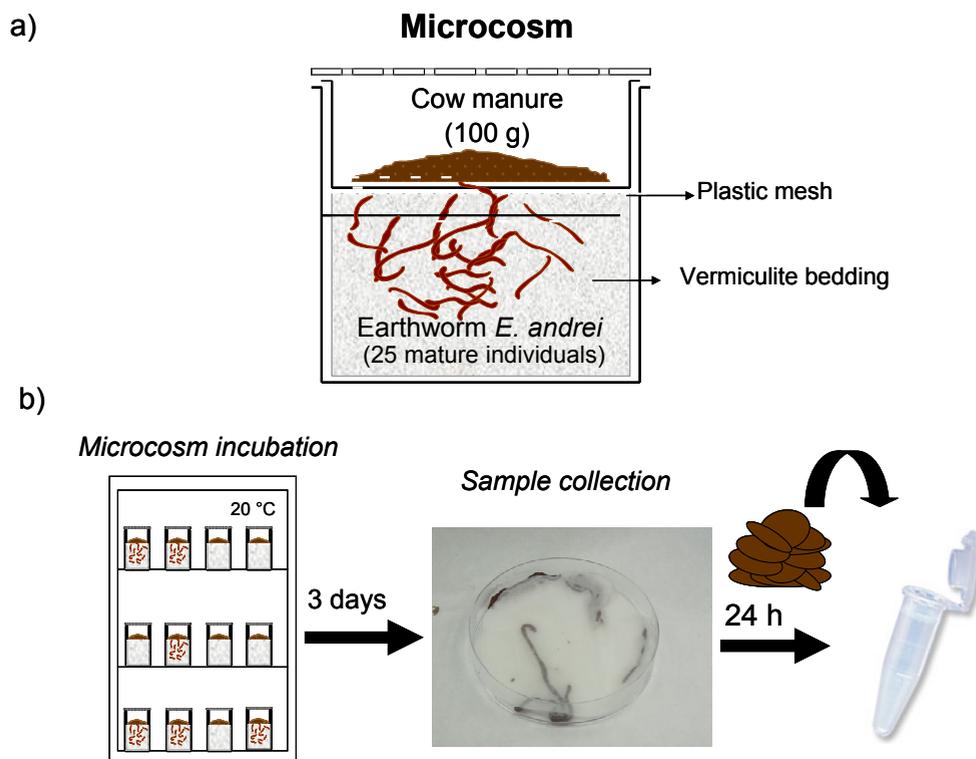


Fig. 4. Scheme of the (a) microcosm and (b) procedure for incubation of microcosms and collection of cast samples.

microbial activity was determined by hydrolysis of fluorescein diacetate (FDA), a colourless compound that is hydrolysed by both free and membrane bound enzymes, to release a coloured end product (fluorescein) that can be measured by spectrophotometry (Adam & Duncan, 2001). The data were analysed by a one-way ANOVA test, at $\alpha = 0.05$.

4.1.1 Microbial biomass

Recent reports suggest that the digestion of organic material by epigeic earthworms has negative effects on microbial biomass (Aira et al., 2006, 2009; Monroy et al., 2009). The present data are consistent with these findings, since we found a reduction in the viable microbial biomass as a result of the passage of the fresh substrate through the gut of the earthworm species *E. andrei* (Figure 5). More specifically, the total content of PLFAs was 1.5 times higher in the control treatment ($1868.11 \pm 129.02 \mu\text{g g}^{-1} \text{dw}$) than that in earthworm casts ($1249.87 \pm 158.43 \mu\text{g g}^{-1} \text{dw}$).

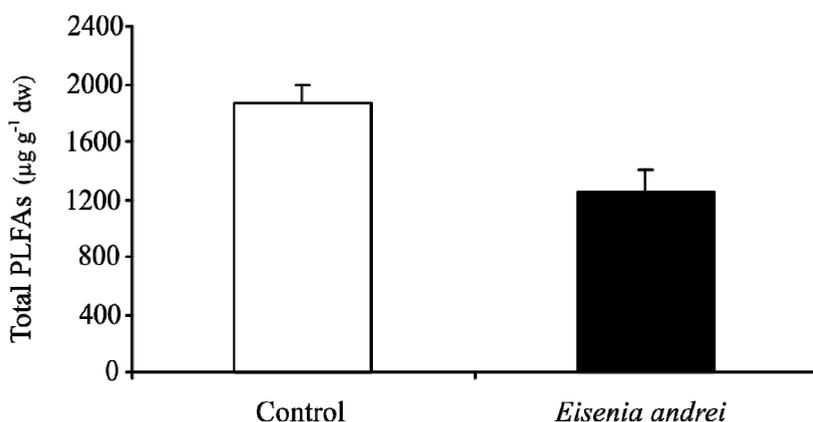


Fig. 5. Changes in the viable microbial biomass, measured as total PLFAs, after the passage of cow manure through the gut of the earthworm species *Eisenia andrei*. Values are means \pm SE. Control is the manure incubated without earthworms.

Epigeic earthworms possess a diverse pool of digestive enzymes which enables them to digest bacteria, protozoa, fungi and partly decomposed plant debris (Zhang et al., 2000). Indeed, bacterial populations decreased in cow manure after transit through the earthworm gut (Figure 6a). As occurred with microbial biomass, bacterial PLFAs were 1.5 times lower in cast samples relative to the control (Figure 6a). However, the passage of cow manure through the earthworm gut affected fungal populations to a lesser extent than bacteria (Figure 6b).

Animal manures are microbial-rich environments in which bacteria constitute the largest fraction (around 70% of the total microbial biomass as assessed by PLFA analysis), with fungi mainly present as spores (Domínguez et al., 2010). Thus, earthworm activity is expected to have a greater effect on bacteria than on fungi in these organic substrates. These contrasting short-term effects on bacterial and fungal populations with earthworm activity are thus expected to have important implications on decomposition pathways during vermicomposting, because there exist important differences between both microbial

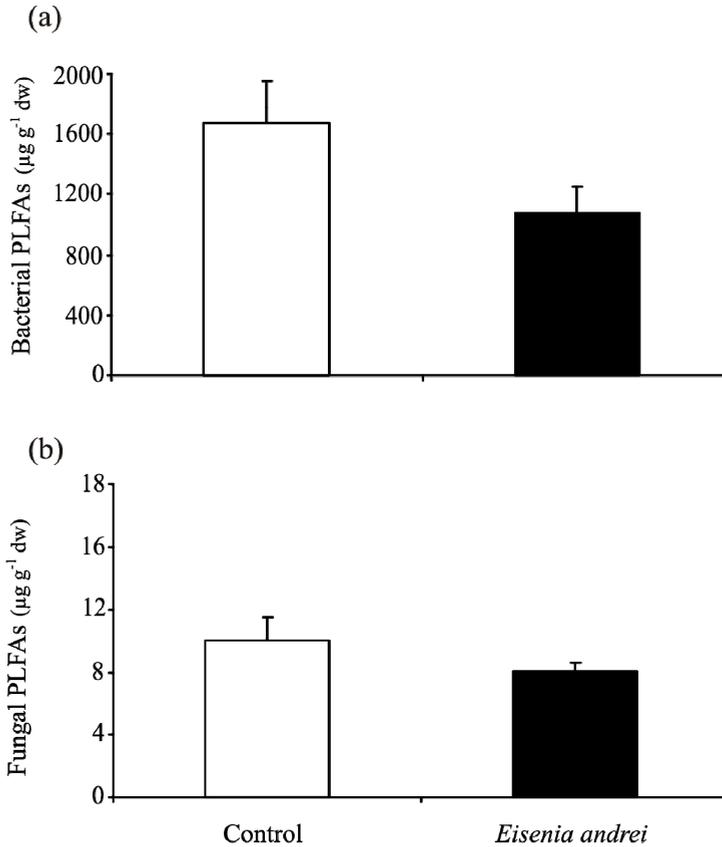


Fig. 6. Changes in (a) bacterial biomass calculated as the sum of the bacterial PLFA markers: i14:0, i15:0, a15:0, i16:0, a17:0, 16:1 ω 7, 17:1 ω 7, 18:1 ω 7, cy17:0 and cy19:0, and (b) PLFA 18:2 ω 6c, a measure of fungal biomass, after the passage of cow manure through the gut of the earthworm species *Eisenia andrei*. Values are means \pm SE. Control is the manure incubated without earthworms.

decomposers related to resource requirements and exploitation. This is based on the fact that bacteria are more competitive in the use of readily decomposable compounds and have a more exploitative nutrient use strategy by rapidly using newly produced labile substrates (Bardgett & Wardle, 2010); whereas fungi are more competitive with regard to the degradation of more slowly decomposable compounds such as cellulose, hemicellulose and lignin (de Boer et al., 2005).

4.1.2 Microbial activity

The transit of the organic material through the gut of the earthworm *E. andrei* reduced the microbial activity, measured as FDA hydrolysis, relative to the control (Figure 7). We found up to a 30% reduction in the microbial activity from the control treatment ($524.8 \pm 60.1 \mu\text{g fluorescein g}^{-1} \text{ dw h}^{-1}$) to earthworm casts ($208.0 \pm 21.7 \mu\text{g fluorescein g}^{-1} \text{ dw h}^{-1}$). Similar

decreases in microbial activity were reported in casts of *Eu. eugeniae* and *E. fetida* fed on pig and cow manures respectively (Aira et al., 2006; Aira & Domínguez, 2009).

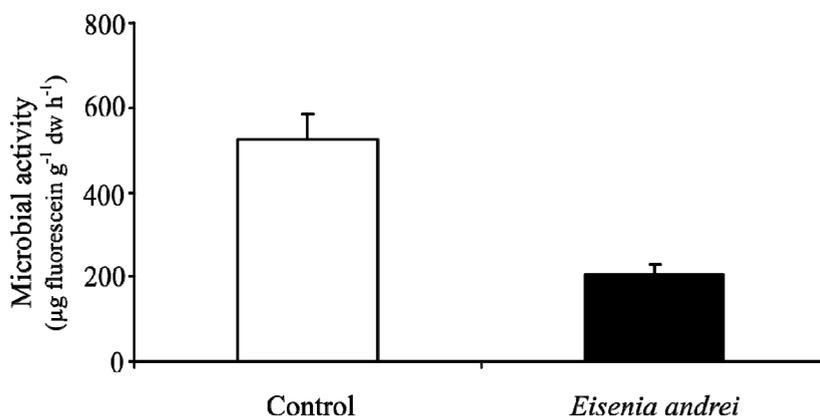


Fig. 7. Changes in microbial activity assessed by fluorescein diacetate hydrolysis, after the passage of cow manure through the gut of the earthworm species *Eisenia andrei*. Values are means \pm SE. Control is the manure incubated without earthworms.

4.2 How does the earthworm species affect microbial communities?

Earthworms of different functional groups, or even different species within the same functional group, have a particular mode of food selection, ingestion, digestion, assimilation and movement, thus their importance in mixing, decomposition or nutrient release, as well as in the structure and activity of microbial communities will vary both qualitatively and quantitatively (Curry & Schmidt, 2007). To determine how the earthworm species shape the relationships between earthworms and microorganisms during the active phase of vermicomposting, we performed an experiment with mesocosms filled with cow manure and inoculated with 10 mature individuals of the earthworm species *Eisenia andrei*, *Eisenia fetida* and *Perionyx excavatus*. The mesocosms consisted of 2 L plastic containers filled to three quarters of their capacity with sieved, moistened vermiculite. A plastic mesh was placed over the surface of the vermiculite and 200 g (fresh weight, fw) of the substrate was placed on top of the mesh, to avoid mixing the substrate with the vermiculite bedding. The mesocosms were covered with perforated lids and stored in random positions in an incubation chamber, at 20 °C and 90% relative humidity. Control mesocosms consisted of each type of manure incubated without earthworms. Each treatment was replicated three times. The length of the active phase depends greatly on the rates at which the earthworms ingest and process the substrate (Domínguez et al., 2010). The high rate of consumption, digestion and assimilation of organic matter by these earthworm species resulted in the substrates being completely processed by the earthworms in one month, as previously shown by Lores et al. (2006). After this time (i.e., active phase), the earthworms were removed from the mesocosms and the processed material was collected from the surface of the vermiculite. The same amount of sample was also collected from the control mesocosms.

The viable microbial biomass was assessed as the sum of all identified PLFAs and certain PLFAs were used as biomarkers to determine the presence and abundance of specific microbial groups. Microbial community function was determined by measuring the bacterial and fungal growth rates. Bacterial growth was estimated by the incorporation of radioactively labelled leucine into proteins (Bååth, 1994), as modified by Bååth et al. (2001); fungal growth was estimated by the incorporation of radioactively labelled acetate into the fungal-specific lipid ergosterol Newell & Fallon (1991), with modifications by Bååth (2001). Total microbial activity was also assessed by measuring the rate of evolution of CO₂. The data were analyzed by a one-way ANOVA test. Post hoc comparisons of means were performed by a Tukey HSD test, at $\alpha = 0.05$.

4.2.1 Microbial biomass

The viable microbial biomass was about 3.8 times lower in the presence of *E. andrei* than that in the control (Figure 8), while no such pronounced decrease was detected in relation to the activity of *E. fetida* and *P. excavatus* (Figure 8). Similarly, the activity of *E. andrei* drastically reduced the bacterial and fungal biomass in cow manure, relative to the control (3.7 and 5.3 times, respectively), after the active phase of vermicomposting (Figure 9).

In the present study, the earthworm species *E. andrei* could have reduced the abundance of these microbial groups directly through ingestion, digestion and assimilation in the gut, and/or indirectly by accelerating the depletion of resources for the microbes, since greater losses of carbon were found as a result of earthworm activity after the active phase of vermicomposting (data not shown). However, the second explanation seems more likely to justify the reduction in fungal populations, since no significant changes were found in this microbial group after the passage through the gut of *E. andrei* (see experiment 1).

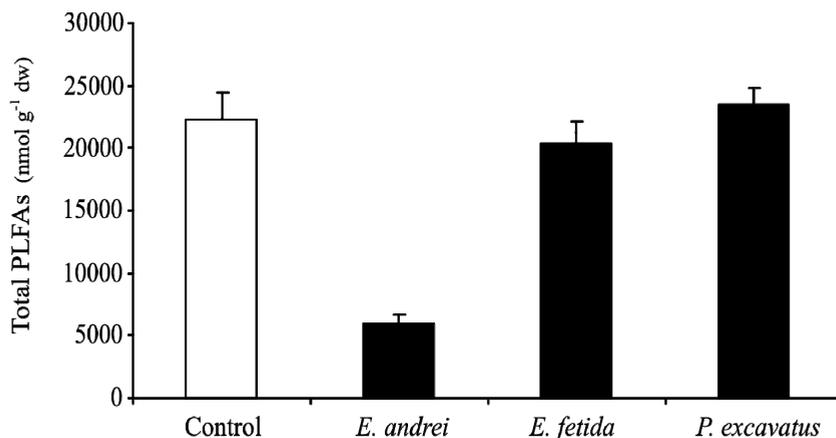


Fig. 8. Changes in the viable microbial biomass, measured as total PLFAs, of cow manure after being processed by the epigeic earthworm species *Eisenia andrei*, *Eisenia fetida* and *Perionyx excavatus* during the active phase of vermicomposting. Values are means \pm SE. Control is the manure incubated without earthworms.

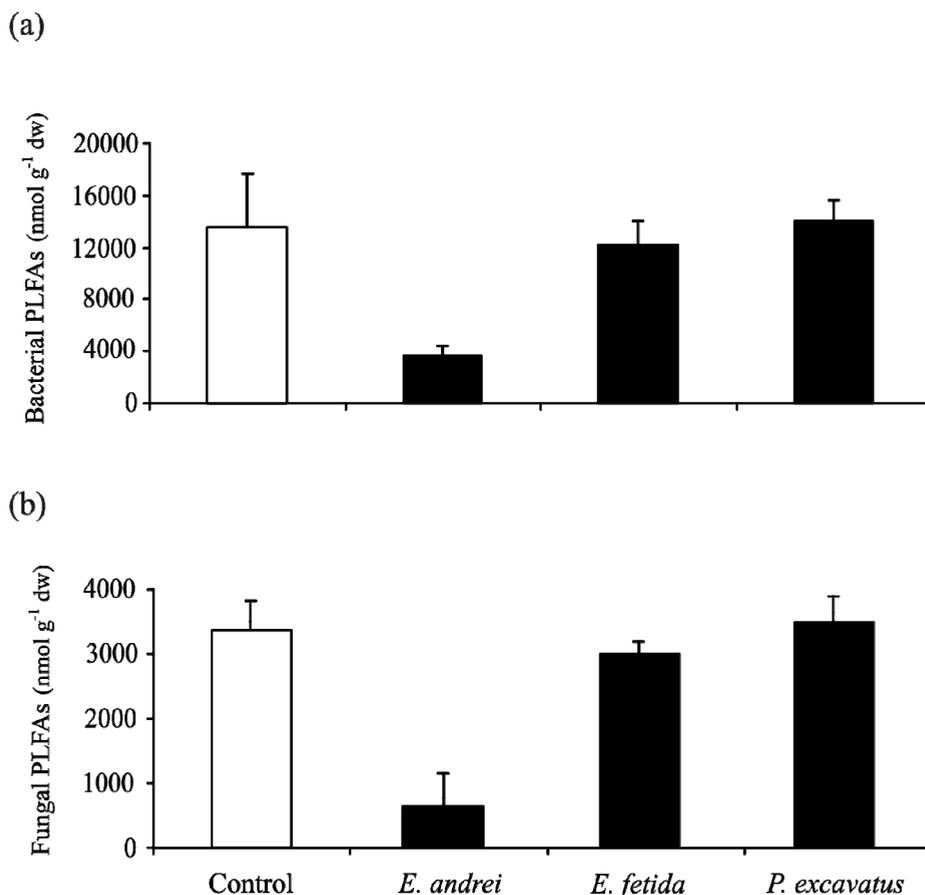


Fig. 9. Changes in (a) bacterial biomass calculated as the sum of the bacterial PLFA markers: i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 10Me16:0, 10Me17:0, 10Me18:0, 16:1 ω 7, 18:1 ω 7, cy17:0 and cy19:0, and (b) PLFA 18:2 ω 6c, a measure of fungal biomass, of cow manure after being processed by the epigeic earthworm species *Eisenia andrei*, *Eisenia fetida* and *Perionyx excavatus* during the active phase of vermicomposting. Values are means \pm SE. Control is the manure incubated without earthworms.

4.2.2 Microbial activity

E. andrei reduced the bacterial growth rate by approximately 1.5 times relative to the control without earthworms after the active phase of vermicomposting (Figure 10a); no significant differences were detected with *E. fetida* and *P. excavatus* (Figure 10a). Despite the consistent effects on bacterial growth, earthworm activity did not affect the fungal growth rate (data not shown). Microbial activity in cow manure followed the same pattern as the bacterial

growth rate (Figure 10b). As mentioned before, bacteria constitute the largest fraction of the microbiota in animal manures, and they are therefore expected to contribute greatly to the respiration rate.

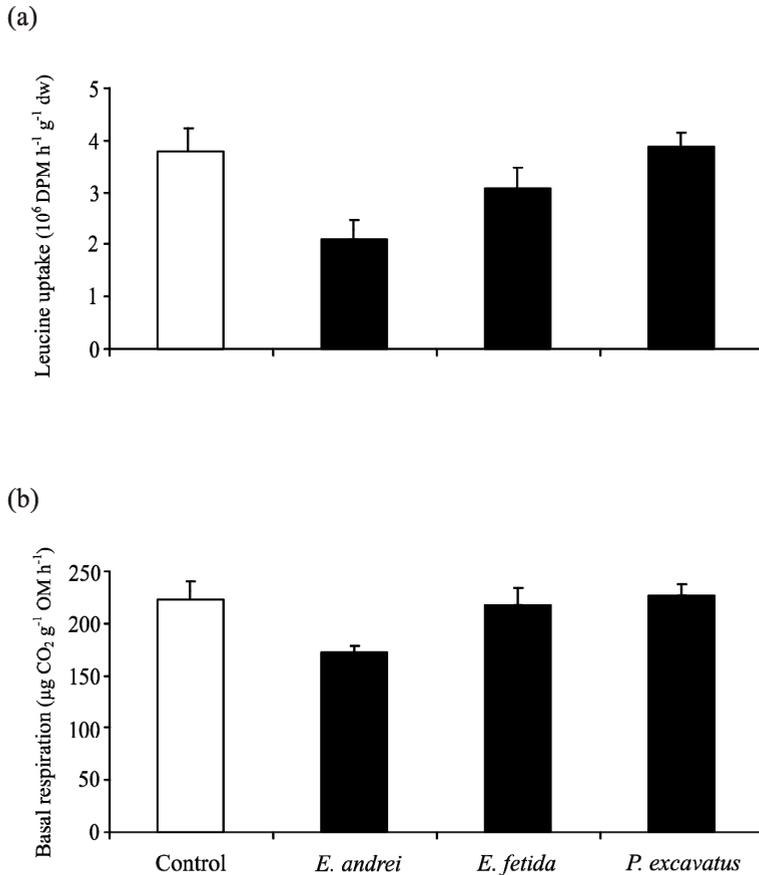


Fig. 10. Changes in (a) bacterial growth rate, estimated as leucine uptake and (b) microbial activity, measured as basal respiration, of cow manure after being processed by the epigeic earthworm species *Eisenia andrei*, *Eisenia fetida* and *Perionyx excavatus* during the active phase of vermicomposting. Values are means \pm SE. Control is the manure incubated without earthworms.

The above-mentioned results highlight the potential of *E. andrei* for biodegrading organic substrates. The species *E. andrei* and *E. fetida* are closely related, although *E. andrei* predominates in mixed cultures, especially when there is no substrate limitation, as

occurred in this experiment, indicating that it is a more extreme r strategist than *E. fetida*, as shown by more rapid growth and reproduction (Domínguez et al., 2005).

4.3 How do earthworms affect microbial communities of a plant residue in the short-term?

In this study we evaluated the effectiveness of the active phase of vermicomposting for the short-term stabilization of grape marc, a lignocellulosic enriched residue that consists of the stalks, skin, pulp and seeds remaining after the grape crushing and pressing stages in wine production (Flavel et al., 2005). This by-product is a valuable resource as a soil fertilizer with high contents of macro- and micronutrients for crop growth (Bertran et al., 2004). However, the overproduction of grape marc – more than 750,000 ton per year in Spain (Fernández-Bayo et al., 2007) – has become a problem that requires strategies for its disposal and/or management. Whilst composting has been widely used for the treatment of winery wastes (Bertran et al., 2004; Marhuenda-Egea et al., 2007; Fernández et al., 2008; Bustamante et al., 2009; Paradelo et al., 2010), there are still very few studies on the application of vermicomposting as a methodological alternative to recycling such wastes (Nogales et al., 2005; Romero et al., 2007, 2010).

The vermicomposting of grape marc was performed in mesocosms that consisted of plastic containers (2 L), which were filled to three quarters of the capacity with moistened (80% moisture content) and mature vermicompost in order to ensure the survival of the earthworms. Five hundred juvenile and adult specimens of the epigeic earthworm species *Eisenia andrei* were placed on the surface of the vermicompost. One kilogram (fresh weight) of grape marc was placed on a mesh on the surface of the vermicompost and was rewetted by spraying it with 20mL of tap water. The mesocosms were covered with perforated lids and stored in random positions in an incubation chamber, at 20 °C and 90% relative humidity. Control mesocosms consisted of the grape marc incubated without earthworms. Each treatment was replicated five times. The high density of earthworms used and the relatively rapid gut transit time of the epigeic earthworm species *E. andrei*, around 2.5–7 h, resulted in the grape marc being completely processed by the earthworms in 15 days. After this time (i.e., active phase), the earthworms were removed from the mesocosms and the processed material was collected from the surface of the vermicompost bedding. The same amount of sample was also collected from the control mesocosms. The viable microbial biomass was assessed as the sum of all identified PLFAs and certain PLFAs were used as biomarkers to determine the presence and abundance of specific microbial groups. Microbial community function was determined by measuring the total microbial activity assessed by basal respiration, and by determining the activity of enzymes involved in C and N cycles, i.e. protease and cellulase activities.

4.3.1 Microbial biomass

Earthworm activity reduced the viable microbial biomass measured as total PLFAs relative to the control without earthworms ($96.90 \pm 1.04 \mu\text{g mL}^{-1}$ and $113.60 \pm 1.04 \mu\text{g mL}^{-1}$ for treatments with and without earthworms). Similarly, the presence of earthworms also reduced the abundance of both bacteria and fungi after the active phase of vermicomposting of grape marc (Figure 11).

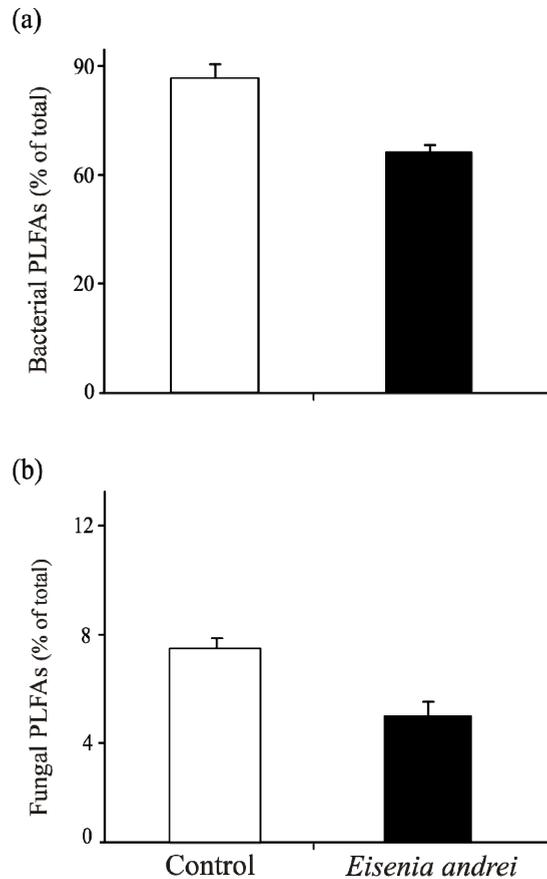


Fig. 11. Changes in (a) bacterial biomass calculated as the sum of the bacterial PLFA markers: i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 16:1 ω 7, 17:1 ω 7, cy17:0 and cy19:0, and (b) PLFAs 18:1 ω 9c and 18:2 ω 6c, a measure of fungal biomass, of grape marc after being processed by the epigeic earthworm species *Eisenia andrei* during the active phase of vermicomposting. Values are means \pm SE. Control is the grape marc incubated without earthworms.

4.3.2 Microbial activity

As occurred in the two previous experiments, the total microbial activity measured as basal respiration was about 1.7 times lower in the presence of *E. andrei* than that in the control without earthworms (Figure 12). This suggests that the presence of earthworms favoured the stabilization of the residue, as shown by Lazcano et al. (2008). These authors found that

both vermicomposting treatments (vermicomposting and a combination of composting and vermicomposting) produced more stabilized substrates than the active phase of composting in terms of microbial activity.

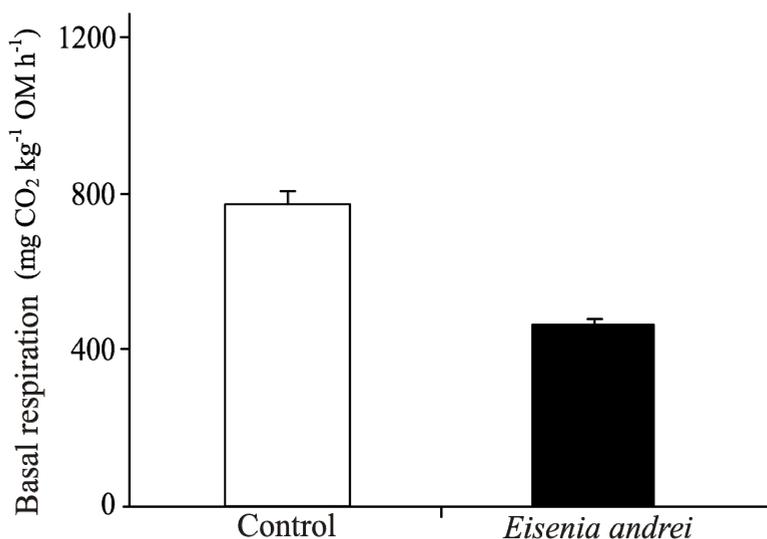


Fig. 12. Changes in microbial activity assessed by basal respiration of grape marc after being processed by the epigeic earthworm species *Eisenia andrei* during the active phase of vermicomposting. Values are means \pm SE. Control is the grape marc incubated without earthworms.

The study of enzyme activities has been shown to be a reliable tool for characterizing the state and evolution of the organic matter during vermicomposting (Benítez et al., 2005), as they are implicated in the biological and biochemical processes that transform organic wastes into stabilized products. In the present study, earthworm activity greatly reduced the activities of the protease (Figure 13a) and cellulase enzymes (Figure 13b) in comparison with the control. These findings are in agreement with microbial activity data, which reinforces that a higher degree of stability was reached after the active phase of vermicomposting. Similarly, Lazcano et al. (2008) reported lower values of protease activity, relative to the control, after vermicomposting and composting with subsequent vermicomposting (3 and 4.4 times lower, respectively). However, they did not find any differences in relation to this enzyme activity after the active phase of composting, indicating that the vermicomposted materials were significantly more stabilized than the compost.

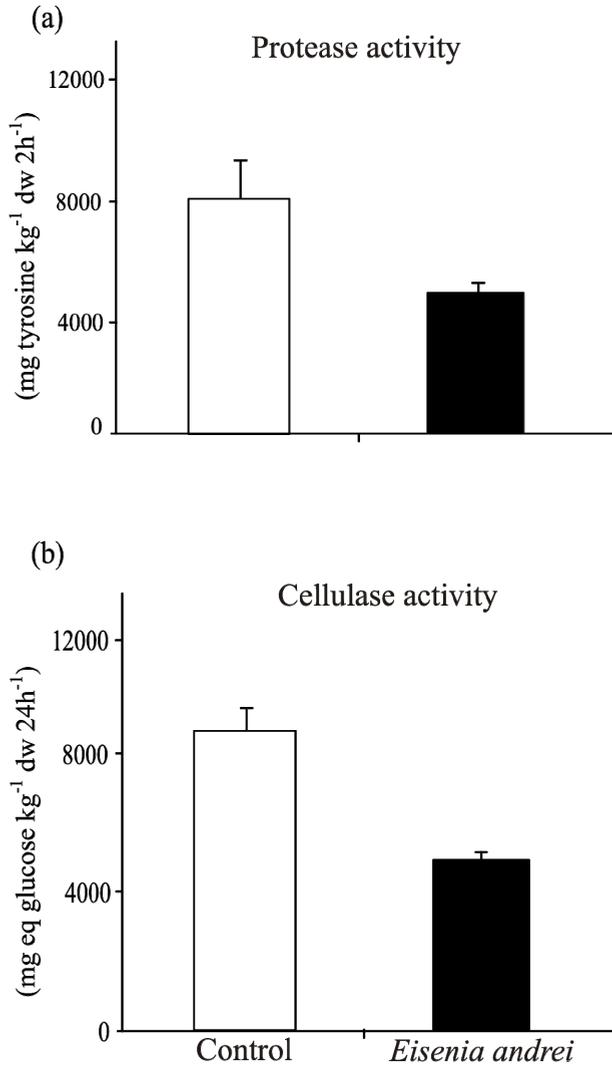


Fig. 13. Changes in (a) protease activity, and (b) cellulase activity of grape marc after being processed by the epigeic earthworm species *Eisenia andrei* during the active phase of vermicomposting. Values are means \pm SE. Control is the grape marc incubated without earthworms.

5. Conclusions

Detritivorous earthworms interact intensively with microorganisms during vermicomposting, thus accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties. Digestion of the ingested material is the first step in earthworm-microorganism interactions. Passage of organic material through the

gut of epigeic earthworms reduced the viable microbial biomass and affected the abundance of bacteria to a greater extent than fungi. Microbial activity also decreased after transit of the microorganisms through the earthworm gut. Accordingly, the presence of earthworms reduced microbial biomass and activity after the active phase of vermicomposting, although this effect depended on the earthworm species involved. The bacterial growth rate also decreased in the substrate, whereas the fungal growth rate was not affected after one month. The speed at which these transformations occurred made the active phase of vermicomposting a suitable stage for studying the relationships between earthworms and microorganisms and permitted us to understand the chemical and biological consequences of earthworm activities. Ultimately, these findings provide valuable information for the understanding of the transformations that organic matter undergoes during vermicomposting and, in addition constitute a powerful tool for the development of strategies leading to a more efficient process for the disposal and/or management of organic wastes.

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7. References

- Adam, G. & Duncan, H. (2001). Development of a Sensitive and Rapid Method for the Measurement of Total Microbial Activity Using Fluorescein Diacetate (FDA) in a Range of Soils. *Soil Biology and Biochemistry*, vol. 33, No. 7-8, pp. 943-951, ISSN 0038-0717.
- Aira, M., Monroy, F. & Domínguez, J. (2006). Changes in Microbial Biomass and Microbial Activity of Pig Slurry After the Transit Through the Gut of the Earthworm *Eudrilus eugeniae* (Kinberg, 1867). *Biology and Fertility of Soils*, vol. 42, No. 4, pp. 371-376, ISSN 0178-2762.
- 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. *Microbial Ecology*, vol. 54, No. 4, pp. 662-671, ISSN 0095-3628.
- Aira, M. & Domínguez, J. (2008). Optimizing Vermicomposting of Animal Wastes: Effects of Dose of Manure Application on Carbon Loss and Microbial Stabilization. *Journal of Environmental Management*, vol. 88, No. 4, pp. 1525-1529, ISSN 0301-4797.
- 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 Biology and Biochemistry*, vol. 40, No. 10, pp. 2511-2516, ISSN 0038-0717.
- Aira, M. & Domínguez, J. (2009). Microbial and Nutrient Stabilization of Two Animal Manures After the Transit Through the Gut of the Earthworm *Eisenia fetida* (Savigny, 1826). *Journal of Hazardous Materials*, vol. 161, No. 2-3, pp. 1234-1238, ISSN 0304-3894.

- Aira, M.; Monroy, F. & Domínguez, J. (2009). Changes in Bacterial Numbers and Microbial Activity of Pig Slurry during Gut Transit of Epigeic and Anecic Earthworms. *Journal of Hazardous Materials*, vol. 162, No. 2-3, pp. 1404-1407, ISSN 0304-3894.
- Aira, M. & Domínguez, J. (2011). Earthworm Effects without Earthworms: Inoculation of Raw Organic Matter with Worm-Worked Substrates Alters Microbial Community Functioning. *Plos One*, vol. 6, No. 1, pp. 1-8, ISSN 1932-6203.
- Bååth, E. (1994). Measurement of Protein Synthesis by Soil Bacterial Assemblages with the Leucine Incorporation Technique. *Biology and Fertility of Soils*, vol. 17, No. 2, pp. 147-153, ISSN 0178-2762.
- Bååth, E. (2001). Estimation of Fungal Growth Rates in Soil Using ¹⁴C-Acetate Incorporation into Ergosterol. *Soil Biology and Biochemistry*, vol. 33, No. 14, pp. 2011-2018, ISSN 0038-0717.
- Bååth, E.; Petterson, M. & Söderberg, K.H. (2001). Adaptation of a Rapid and Economical Microcentrifugation Method to Measure Thymidine and Leucine Incorporation by Soil Bacteria. *Soil Biology and Biochemistry*, vol. 33, No. 11, pp. 1571-1574, ISSN 0038-0717.
- Bardgett, R.A. & Wardle, D.A. (2010). *Aboveground-Belowground Linkages*, Oxford University Press, ISBN 978-0-19-954687-9, New York.
- Benítez, E.; Sanz, H. & Nogales, R. (2005). Hydrolytic Enzyme Activities of Extracted Humic Substances During the Vermicomposting of a Lignocellulosic Olive Waste, *Bioresource Technology* vol. 96, No. 7, pp. 785-90, ISSN 0960-8524.
- Bertran, E.; Sort, X.; Soliva, M. & Trillas, I. (2004). Composting of Winery Waste: Sledges and Grape Stalks. *Bioresource Technology*, vol. 95, No. 2, pp. 203-208, ISSN 0960-8524.
- Bustamante, M.A.; Paredes, C.; Morales, J.; Mayoral, A.M. & Moral, R. (2009). Study of the Composting Process of Winery and Distillery Wastes Using Multivariate Techniques. *Bioresource Technology*, vol. 100, No. 20, pp. 4766-4772, ISSN 0960-8524.
- Curry, J.P. & Schmidt, O. (2007). The Feeding Ecology of Earthworms - a Review. *Pedobiologia*, vol. 50, No. 6, pp. 463-477, ISSN 0031-4056.
- Darwin, C. (1881) *The Formation of Vegetable Mould through the Action of Worms with Observations on their Habits*, Murray, London.
- De Boer, W.; Folman, L.B.; Summerbell, R.C. & Boddy, L. (2005). Living in a Fungal World: Impact of Fungi on Soil Bacterial Niche Development. *FEMS Microbiology Reviews*, vol. 29, No. 4, pp. 795-811, ISSN 1574-6976.
- Domínguez, J. (2004). State of the Art and New Perspectives on Vermicomposting Research, In: *Earthworm Ecology*, C.A. Edwards, (Ed.), 401-424, CRC Press, ISBN 1-884015-74-3, Boca Raton, Florida.
- Domínguez, J.; Ferreiro, A. & Velando, A. (2005). Are *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 (Oligochaeta, Lumbricidae) Different Biological Species? *Pedobiologia*, vol. 49, pp. 81-87, ISSN 0031-4056.
- Domínguez, J. & Edwards, C.A. (2010a). Relationships between Composting and Vermicomposting: Relative Values of the Products, In: *Vermiculture Technology: Earthworms, Organic Waste and Environmental Management*, C.A. Edwards; N.Q. Arancon; R.L. Sherman, (Eds.), 1-14, CRC Press, ISBN 9781439809877, Boca Raton, Florida.
- Domínguez, J. & Edwards, C.A. (2010b). Biology and Ecology of Earthworm Species Used for Vermicomposting, In: *Vermiculture Technology: Earthworms, Organic Waste and*

- Environmental Management*, C.A. Edwards; N.Q. Arancon; R.L. Sherman, (Eds.), 25-37, CRC Press, ISBN 9781439809877, Boca Raton, Florida.
- Domínguez, J.; Aira, M. & Gómez-Brandón, M. (2010). Vermicomposting: Earthworms Enhance the Work of Microbes, In: *Microbes At Work: From Wastes to Resources*, H. Insam; I. Franke-Whittle; M. Goberna, (Eds.), 93-114, Springer, ISBN 978-3-642-04042-9, Heidelberg, Germany.
- Drake, H.L. & Horn, M.A. (2007). As the Worm Turns: the Earthworm Gut as a Transient Habitat for Soil Microbial Biomes. *Annual Review of Microbiology*, vol. 61, pp. 169-189, ISSN 0066-4227.
- Edwards, C.A. & Bohlen, P.J. (1996). *Biology and Ecology of Earthworms*, Chapman and Hall, London.
- Flavel, T.C., Murphy, D.V., Lalor, B.M., & Fillery, I.R.P. (2005). Gross N Mineralization Rates after Application of Composted Grape Marc of Soil. *Soil Biology and Biochemistry*, vol. 37, No. 7, pp. 1397-1400, ISSN 0038-0717.
- Fernández-Bayo, J.D.; Nogales, R. & Romero, E. (2007). Improved Retention of Imidacloprid (Confidor®) in Soils by Adding Vermicompost from Spent Grape Marc. *Science of Total Environment*, vol. 378, No. 1-2, pp. 95-100, ISSN 0048-9697.
- Fernández, F.J.; Sánchez-Arias, V.; Villaseñor, J. & Rodríguez, L. (2008). Evaluation of Carbon Degradation during Co-composting of Exhausted Grape Marc with Different Biowastes. *Chemosphere*, vol. 73, No. 5, pp. 670-677, ISSN 0045-6535.
- Gómez-Brandón, M.; Aira, M.; Lores, M. & Domínguez, J. (2011a). Epigeic Earthworms Exert a Bottleneck Effect on Microbial Communities through Gut Associated Processes. *Plos One*, vol. 6, No. 9, pp.1-9, ISSN 1932-6203.
- Gómez-Brandón, M.; Aira, M.; Lores, M. & Domínguez, J. (2011b). Changes in Microbial Community Structure and Function During Vermicomposting of Pig Slurry. *Bioresource Technology*, vol. 102, No. 5, pp. 4171-4178, ISSN 0960-8524.
- Hutchinson, M.L.; Walters, L.D.; Avery, S.M.; Munro, F. & Moore, A. (2005). Analyses of Livestock Production, Waste Storage, and Pathogen Levels and Prevalences in Farm Manures. *Applied and Environmental Microbiology*, vol. 71, No. 3, pp. 1231-1236, ISSN 1098-5336.
- Lazcano, C.; Gómez-Brandón, M. & Domínguez, J. (2008). Comparison of the Effectiveness of Composting and Vermicomposting for the Biological Stabilization of Cattle Manure. *Chemosphere*, vol. 72, No. 7, pp. 1013-1019, ISSN 0045-6535.
- Lores, M.; Gómez-Brandón, M.; Pérez-Díaz, D. & Domínguez, J. (2006). Using FAME profiles for the Characterization of Animal Wastes and Vermicomposts. *Soil Biology and Biochemistry*, vol. 38, No. 9, pp. 2993-2996, ISSN 0038-0717.
- Marhuenda-Egea, F.C.; Martínez-Sabater, E.; Jordá, J.; Moral, R.; Bustamante, M.A.; Paredes, C. & Pérez-Murcia, M.D. (2007). Dissolved Organic Matter Fractions Formed during Composting of Winery and Distillery Residues: Evaluation of the Process by Fluorescence Excitation-Emission Matrix. *Chemosphere*, vol. 68, No. 2 pp. 301-309, ISSN 0045-6535.
- Monroy, F.; Aira, M. & Domínguez, J. (2008). Changes in Density of Nematodes, Protozoa and Total Coliforms after Transit through the Gut of Four Epigeic Earthworms (Oligochaeta). *Applied Soil Ecology*, vol. 39, No. 2, pp. 127-132, ISSN 0929-1393.
- Monroy, F.; Aira, M. & Domínguez, J. (2009). Reduction of Total Coliform Numbers during Vermicomposting is Caused by Short-term Direct Effects of Earthworms on

- Microorganisms and Depends on the Dose of Application of Pig Slurry. *Science of the Total Environment*, vol. 407, No. 20, pp. 5411-5416, ISSN 0048-9697.
- Monroy, F.; Aira, M. & Domínguez, J. (2011). Epigeic Earthworms Increase Soil Arthropod Populations during First Steps of Decomposition of Organic Matter. *Pedobiologia*, vol. 54, No. 2, pp. 93-99, ISSN 0031-4056.
- Moore, J.C.; Berlow, E.L.; Coleman, D.C.; de Ruiter, P.C.; Dong, Q.; Johnson, N.C.; McCann, K.S.; Melville, K.; Morin, P.J.; Nadelhoffer, K.; Rosemond, A.D.; Post, D.M.; Sabo, J.L.; Scow, K.M.; Vanni, M.J. & Wall, D.H. (2004). Detritus, Trophic Dynamics and Biodiversity. *Ecology Letters*, vol. 7, pp. 584-600.
- Moral, R.; Paredes, C.; Bustamante, M.A.; Marhuenda-Egea, R. & Bernal, M.P. (2009). Utilisation of Manure Composts by High-Value Crops: Safety and Environmental Challenges. *Bioresource Technology*, vol. 100, No. 22, pp. 5454-5460, ISSN 0960-8524.
- Newell, S.Y. & Fallon, R.D. (1991). Toward a Method for Measuring Instantaneous Fungal Growth Rates in Field Samples. *Ecology*, vol. 72, No. 5, pp. 1547-1559, ISSN 0012-9658.
- Nogales, R.; Cifuentes, C. & Benítez E. (2005). Vermicomposting of Winery Wastes: a Laboratory Study. *Journal of Environmental Science and Health Part B* vol. 40, No. 4, pp. 659-673, ISSN 0360-1234.
- Paradelo, R.; Moldes, A. & Barral, M. (2010). Utilization of a Factorial Design to Study the Composting of Hydrolyzed Grape Marc and Vinification Lees. *Journal of Agriculture and Food Chemistry*, vol. 58, No. 5, pp. 3085-3092.
- Reynolds, J. W. & Wetzel, M. J. (2010). *Nomenclatura Oligochaetologica. Supplementum Quartum. A catalogue of names, descriptions and type specimens of the Oligochaeta*, Illinois Natural History Survey Special Publication, Chicago.
- Romero, E.; Plaza, C.; Senesi, N.; Nogales, R. & Polo, A. (2007). Humic Acid-Like Fractions in Raw and Vermicomposted Winery and Distillery Wastes. *Geoderma*, vol. 139, No. 3-4, pp. 397-406, ISSN 0016-7061.
- Romero, E.; Fernández-Bayo, J; Díaz, J.M.C. & Nogales, R. (2010). Enzyme Activities and Diuron Persistence in Soil Amended with Vermicompost Derived from Spent Grape Marc and Treated with Urea. *Applied Soil Ecology*, vol. 44, No. 3, pp.198-204, ISSN 0929-1393.
- Schönholzer, F.; Dittmar, H. & Zeyer, J. (1999). Origins and Fate of Fungi and Bacteria in the Gut of *Lumbricus terrestris* L. Studied by Image Analysis. *FEMS Microbiology Ecology*, vol. 28, No. 3, pp. 235-248, ISSN 1574-6941.
- Zelles, L. (1997). Phospholipid Fatty Acid Profiles in Selected Members for Soil Microbial Communities. *Chemosphere*, No. 1-2, vol. 35, pp. 275-294, ISSN 0045-6535.
- Zelles, L. (1999). Fatty Acid Patterns of Phospholipids and Lipopolysaccharides in the Characterization of Microbial Communities in Soil: a Review. *Biology and Fertility of Soils*, vol. 29, No. 2, pp. 111-129, ISSN 0960-8524.
- Zhang, B.; Li, G.; Shen, T.; Wang, J. & Sun, Z. (2000). Changes in Microbial Biomass C, N, and P and Enzyme Activities in Soil Incubated with the Earthworms *Metaphire guillelmi* or *Eisenia fetida*. *Soil Biology and Biochemistry*, vol. 32, No. 2, pp. 2055-2062, ISSN 0038-0717.