Chapter 5
Vermicomposting: Earthworms Enhance the Work of Microbes

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Abstract Vermicomposting, a very efficient method of converting solid organic waste into an environmentally-friendly, useful and valuable resource, is an accelerated process that involves bio-oxidation and stabilization of the waste as a result of the interactions between some species of earthworms and microorganisms. Although microorganisms are the main agents for biochemical decomposition of organic matter, earthworms are critical in the process of vermicomposting. Complex interactions among the organic matter, microorganisms, earthworms and other soil invertebrates result in the fragmentation, bio-oxidation and stabilization of the organic matter.

Contents

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5.1 What Is Vermicomposting?

Although it was Darwin (1881) who first drew attention to the great importance of earthworms in the decomposition of dead plants and the release of nutrients from them, it was necessary to wait for more than a century until this was taken seriously as a field of scientific knowledge or even a real technology.

The cultivation of earthworms in organic wastes has been termed vermiculture, and vermicomposting, the managed processing of organic wastes by earthworms to produce vermicompost, has progressed considerably in recent years. Vermicomposting has been shown to be successful in processing sewage sludge and solids from wastewater (Domínguez et al. 2003; Clark et al. 2007; Pramanik et al. 2007; Suthar 2007), food industry waste (Nogales et al. 1999a, b, 2005), urban residues, food and animal waste (Domínguez and Edwards 1997; Atiyeh et al. 2000; Triphati and Bhardwaj 2004; Aira et al. 2006a, b; Garg et al. 2006; Suthar 2007; Lazcano et al. 2008), and in the paper industry waste (Elvira et al. 1996, 1998; Kaushik and Garg 2003; Gajalakshmi and Abbasi 2004), as well as treating horticultural residues from cultivars (Gajalakshmi et al. 2005; Pramanik et al. 2007; Gupta et al. 2007; Suthar 2007).

Vermicomposting is a bio-oxidative process in which detritivore earthworms interact intensively with microorganisms and other soil fauna within the decomposer community, strongly affecting decomposition processes, accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties (Domínguez 2004). Microorganisms produce the enzymes that cause the biochemical decomposition of organic matter, but earthworms are the crucial drivers of the process as they are involved in the indirect stimulation of microbial populations through fragmentation and ingestion of fresh organic matter, which results in a greater surface area available for microbial colonization, drastically altering biological activity. Earthworms also modify microbial biomass and activity through stimulation, digestion and dispersion in casts (Fig. 5.1) and closely interact with other biological components of the vermicomposting system, thereby affecting the structure of microflora and microfauna communities (Domínguez et al. 2003; Lores et al. 2006). Thus, 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 (see Moore et al. 2004).

Vermicompost, the end product of vermicomposting, is a finely divided peat-like material of high porosity and water holding capacity and contains many nutrients in forms that are readily taken up by plants. High rates of mineralization occur in the organic matter-rich earthworm casts, which greatly enhances the availability of inorganic nutrients, particularly ammonium and nitrates, for plants.

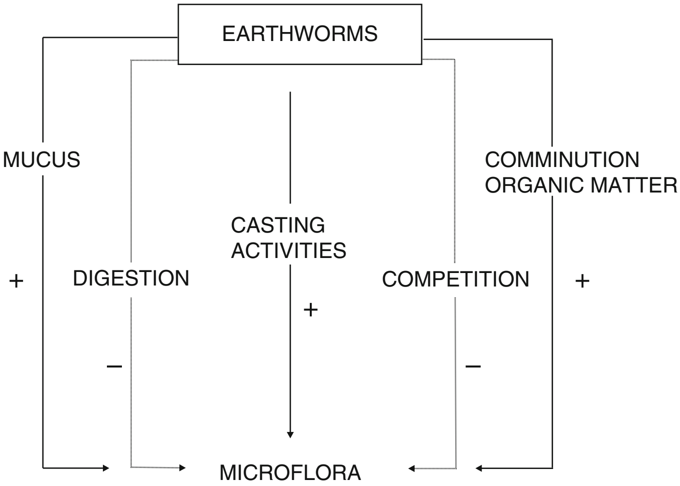


Fig. 5.1 Positive (+) and negative (–) effects of earthworms on microbial biomass and activity. Microbes are mainly dispersed through earthworm casts

5.2 Earthworms

Earthworms are macroscopic clitellate oligochaete annelids that live in soil. They are hermaphroditic animals and display indeterminate growth. Earthworms represent the major animal biomass in most terrestrial temperate ecosystems; they significantly affect soil's physical, chemical and biological properties, and play a key role in modifying soil structure and in accelerating the decomposition of organic matter and nutrient turnover (Edwards and Bohlen 1996; Lavelle and Spain 2001). More than 4,000 species of earthworms have been described, although for the great majority of these species, only the names and morphologies are known, and nothing is 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 (soil feeders) and anecic species (burrowers) live in the soil profile and consume a mixture of soil and organic matter, and thus excrete organo-mineral feces. Epigeic earthworms are litter dwellers and litter transformers; they live in organic horizons, in or near the surface litter and feed primarily on coarse particulate organic matter, ingest large amounts of non-decomposed litter and excrete holorganic fecal pellets. These pellets provide a higher surface to the volume ratio than the original leaf litter, which enhances the rate of decomposition (Lavelle et al. 1997; Lavelle and Spain 2001).

Epigeic earthworms, 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. Few earthworm species display all these characteristics, and in fact only four have been extensively used in vermicomposting facilities: *Eisenia andrei*, *E. fetida*, *Perionyx excavatus* and *Eudrilus eugeniae* (see Domínguez (2004) for details of the life cycles of these species).

5.3 Vermicomposting Food Web

Vermicomposting systems sustain a complex food web that results in the recycling of organic matter. Biotic interactions between decomposers (i.e., bacteria and fungi) and the soil fauna include competition, mutualism, predation and facilitation, and the rapid changes that occur in both functional diversity and in substrate quality are the main properties of these systems (Sampedro and Domínguez 2008). The most numerous and diverse members of this food web are microbes, although there are also abundant protozoa and many animals of varying sizes, including nematodes, microarthropods and large populations of earthworms (Monroy 2006; Sampedro and Domínguez 2008). These fauna cover a range of trophic levels – some feed primarily on microbes (microbial-feeders), organic waste (detritivores), a mixture of organic matter and microbes (microbial-detritivores), whereas others feed on animals (carnivores) or across different trophic levels (Fig. 5.2; Sampedro

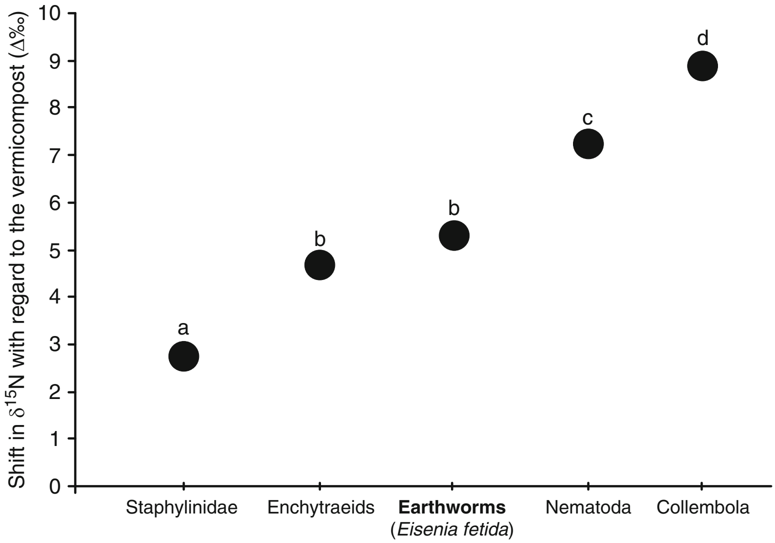


Fig. 5.2 Shift in the $\delta^{15}N$ (expressed as Δ ‰) of some components of the micro- and mesofauna of a pig slurry vermicomposting bin with respect to the fresh vermicompost in which they live. Different letters denote significant differences at $\alpha = 0.01$, Tukey HSD test. (Modified after Sampedro and Domínguez 2008)

and Domínguez 2008). A continuous range of feeding strategies from pure detritivore to pure microbivore has been proposed in detritus-based food webs (Scheu 2002), although the trophic structure and specific resource utilization are poorly understood.

The primary consumers of the vermicomposting food web are the microbes (mainly bacteria, fungi and ciliates) that break down and mineralize organic residues. Microbes are the most numerically abundant and diverse members of the vermicomposting food web, and include thousands of organisms. Secondary and higher-level consumers, i.e., the soil fauna including the earthworms, exist alongside microbes, feeding on and dispersing them throughout the organic matter. As organic matter passes through the gizzard of the earthworms, it becomes finely ground prior to digestion. Endosymbiotic microbes produce extracellular enzymes that degrade cellulose and phenolic compounds, enhancing the degradation of ingested material; and the degraded organic matter passes out of the earthworm's body in the form of casts. As earthworms feed on decaying organic wastes, their burrowing and tunneling activities aerate the substrate and enable water, nutrients, oxygen and microbes to move through it; their feeding activities increase the surface area of organic matter for microorganisms to act upon. As decomposers die, more food is added to the food web for other decomposers (Fig. 5.3; direct

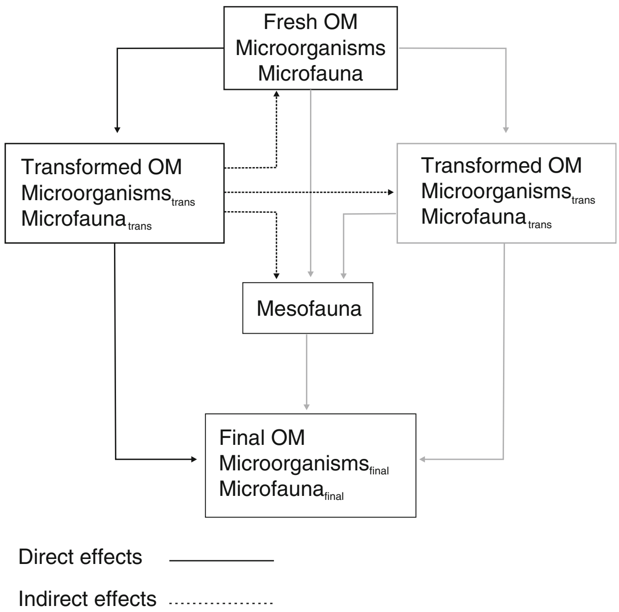


Fig. 5.3 Direct and indirect effects of earthworms on the decomposition of organic matter (OM) during vermicomposting. Here we show the two interacting pathways functioning in the process: the earthworm-mediated pathway (*black lines*) and the microbial pathway (*gray lines*). Both pathways involve intermediated stages of decomposition (here denominated transformed) which result in the final organic matter

effects are those related to direct earthworm activity such as digging, digestion and casting, which initially modify the organic matter, microorganisms and microfauna. Indirect effects are those derived from the direct ones, and include aging and mixing of casts with fresh organic matter or transport of microorganisms, i.e., the interaction of transformed substrates with fresh and transformed substrates by microorganisms).

Earthworms accelerate decomposition processes during vermicomposting (Aira et al. 2006a, 2007a), but it is not clear from where they obtain their energy inputs (decaying organic matter, microorganisms, microfauna or a combination of them). They may utilize different strategies ranging from non-selective substrate feeding to grazing strategies, and have the ability to shift between living and nonliving carbon sources (Domínguez et al. 2003; Sampedro et al. 2006).

5.4 How Vermicomposting Works

The vermicomposting process includes two different phases regarding the activity of earthworms, (i) an active phase during which earthworms process waste, thereby modifying its physical state and microbial composition (Lores et al. 2006), and (ii) a maturation-like phase marked by the displacement of the earthworms towards fresher layers of undigested waste, during which the microbes take over the decomposition of the earthworm's processed waste (Domínguez 2004; Fig. 5.4). As in composting, the duration of the active phase is not fixed, and depends on the species and density of earthworms (the main drivers of the process), and the rates at which they ingest and process the waste (Aira and Domínguez 2008a).

The effect of earthworms on the decomposition of organic waste during the vermicomposting process is, in the first instance, due to gut associated processes (GAPs). These processes include all the modifications that the decaying organic matter and the microorganisms undergo during the intestinal transit. These modifications include the addition of sugars and other substances, modification of the microbial diversity and activity, modification of the microfauna populations, homogenization, and the intrinsic processes of digestion, assimilation and production of mucus and excretory substances such as urea and ammonia, which constitute a readily assimilable pool of nutrients for microorganisms. Decomposition is also enhanced through the action of endosymbiotic microbes that reside in the gut of earthworms. These microbes produce extracellular enzymes that degrade cellulose and phenolic compounds, thereby further enhancing the degradation of ingested material. Other physical modifications of the substrate caused by the digging activities of earthworms, such as aeration and homogenization of the substrate also favour microbial activity and further enhance decomposition (Domínguez 2004). The proximate activity of earthworms significantly enhances the mineralization of both carbon and nitrogen in the substrate, and such effects are in proportion to the earthworm density (Aira et al. 2008). Several authors have reported

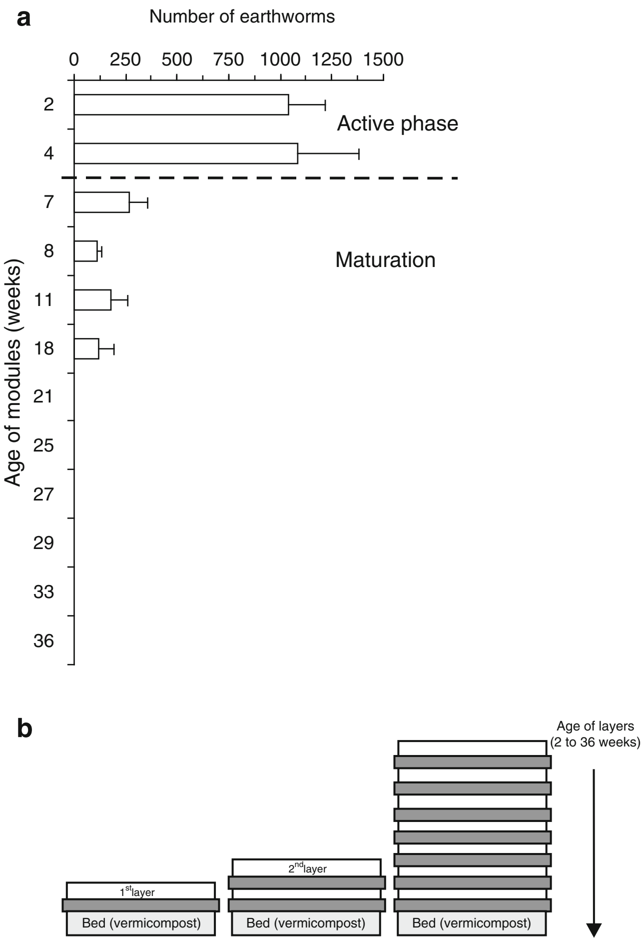


Fig. 5.4 The two phases of vermicomposting depending on earthworm presence in the substrate (active and maturation-like phase). (**a**) Earthworm population in vermireactors at different sampling times. Number of earthworms (means ± SE., n = 3) in each layer, from 2 to 36 weeks of age are shown. (**b**) Scheme of the set up and the procedure for adding new modules during the vermicomposting process. The earthworms moved upwards towards the new modules to which fresh waste had been added. (Modified from Aira et al. 2007a)

similar responses in detritivorous organisms involved in organic matter decomposition (Aira et al. 2002; Vetter et al. 2004).

Upon completion of GAPs, the resultant earthworm casts undergo cast associated processes (CAPs), which are more closely associated with aging processes, the action of the microflora and microfauna present in the substrate and with the physical modification of the egested materials (days to weeks; Parthasarathi and Ranganathan 2000; Aira et al. 2005). During these processes the effects of earthworms are mainly indirect and derived from the GAPs. It is important to note that in vermicomposting systems, earthworm casts are always mixed with material not ingested by the earthworms, and the final vermicompost consists of a mixture of the two different fractions. During this aging, vermicompost will reach its optimum in terms of biological properties promoting plant growth and suppressing plant diseases (see Chap. 8, de Bertoldi 2010; Chap. 11, Fuchs 2010). Currently, there is insufficient information regarding when this “optimum” is achieved, how we can determine it in each case and if this “optimum” has some kind of expiration date. It is important to note that the optimal quality may only be achieved in natural ecosystems built from the correct site-specific balance of soil, plants, microorganisms, macroorganisms including earthworms and climate. However, it is not possible to easily determine when a vermicompost sample is “optimal” and thus, only after application, can this be known.

5.5 Stimulation and Acceleration of Microbial Decomposition by Earthworms during Vermicomposting

Nutrient mineralization is directly governed by the activities of bacteria and fungi and these activities are strongly affected by the soil fauna that lives alongside the microbes, and also by food web interactions that determine the transfer of nutrients through the system. Although epigeic earthworms have little direct impact on mineralization, their indirect effects on microbial biomass and activity are very important. These indirect effects include digestion and release of readily assimilable substances, such as mucus for microbiota (Brown and Doube 2004), as well as the transport and dispersal of microorganisms through casting. Earthworms ingest a mixture of organic wastes and microorganisms during vermicomposting and some of this material will be digested, but they also excrete large amounts of rather fragile fecal material in which further microbial growth is enhanced by favorable conditions of moisture and the intense mixing that has occurred in the gut. Other earthworms or members of the mesofauna may subsequently ingest those pellets and assimilate a further set of substrates made available by the most recent burst of microbial activity (Lavelle et al. 1997; Fig. 5.3). Earthworm casts play an important role in decomposition because they contain nutrients and microbiota different from those contained in the material prior to ingestion (Aira et al. 2006b; Aira and Domínguez 2008b). This enables better exploitation of resources either because of the appearance of microbial species in fresh substrate or the pool of readily assimilable compounds in the casts.

It is well known that earthworms accelerate the rate of organic matter decomposition during vermicomposting (Atiyeh et al. 2000; Domínguez et al. 2003; Domínguez 2004; Triphati and Bhardwaj 2004; Aira and Domínguez 2008a,b; Aira et al. 2006b, 2007a,b, 2008; Fig. 5.5). Although earthworms can assimilate carbon from the more labile fractions of organic wastes, their contribution to the total heterotrophic respiration is very low due to their poor capacity for assimilation.

Fig. 5.5 Carbon loss (% of initial) after 1 month of vermicomposting of cow manure, as affected by the presence of the earthworm *Eisenia andrei*. Values are means ± SE. Control is the same treatment without earthworms

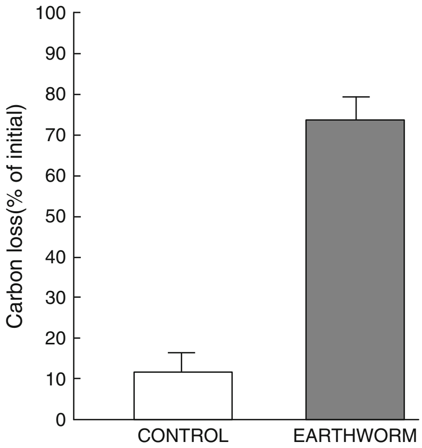
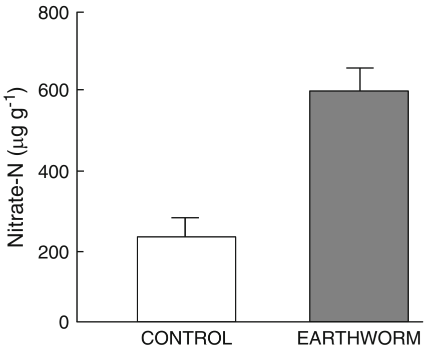


Fig. 5.6 Effect of earthworms (*Eisenia andrei*) on the amount of Nitrate-N produced after vermicomposting of cow manure (for 1 month). Values are means ± SE. Control is the same treatment without earthworms



Nitrogen mineralization is regulated by the availability of dissolved organic nitrogen and ammonium, the activity of the microorganisms and their relative requirements for carbon and nitrogen. Earthworms also have a great impact on nitrogen transformations during vermicomposting through modifications of the environmental conditions and their interactions with microbes; they enhance nitrogen mineralization, thereby producing conditions in the organic wastes that favour nitrification, resulting in the rapid conversion of ammonium-nitrogen into nitrates (Atiyeh et al. 2000; Domínguez 2004; Lazcano et al. 2008; Aira et al. 2008; Aira and Domínguez 2008b; Fig. 5.6).

The effects of microbial-feeding fauna on microbial activity and nutrient mineralization are generally positive. Enhanced C mineralization results from increased turnover rate, activity and respiration of grazed microbial populations, whereas enhanced N mineralization is mainly due to the direct excretion of excess N. In general, grazers have lower assimilation efficiencies than the microbes upon which they graze, and therefore they excrete the excess nutrients in biologically available forms (e.g., protozoa preying on bacterial populations are assumed to release about one-third of the N consumed; Bardgett 2005). This release of nutrients in fact constitutes remobilization of the nutrients that were bound up in the microbial biomass, and has been termed the "microbial loop" (Clarholm 1994).

5.6 Effects of Earthworms on Microbial Communities during Vermicomposting

Microorganisms are the main agents of biochemical decomposition, whereas earthworms are involved in the indirect stimulation of microbial populations through comminution of organic matter, i.e., by increasing the surface area available for microbes. Earthworms also modify the microbial populations through digestion, stimulation and dispersion in casts. Therefore, it is necessary to establish the effects of earthworms on the microorganisms, because whether the earthworms stimulate or depress microbiota, or modify the structure and function of microbial communities, they would have different effects on the decomposition of organic matter. To address these questions we performed an experiment in our laboratory with mesocosms filled with cow manure with ten mature earthworms and without earthworms ($n = 5$ each). We used cow manure as the substrate, which is known to support a dense decomposer foodweb (Sampedro and Domínguez 2008). The mesocosms consisted of 2 L plastic jars filled with 200 g (fresh weight, fw) of substrate. We used the epigeic earthworm *E. andrei* Bouché, 1972, broadly distributed and easy to manage under lab conditions. We allowed mature individuals (375 ± 7 mg; mean individual fw ± standard error of the mean) to shed their gut contents on moistened tissue paper for 24 h at room temperature before the experiment. We covered the jars (containing the substrate and the earthworms) with perforated lids, stored them at random in a scientific incubator (20°C and 90% humidity) and after 1 month, earthworms were removed and vermicompost and control samples were collected and immediately processed for microbial analyses. Viable microbial biomass was determined as the sum of all identified phospholipid fatty acids (PLFAs). The structure of the microbial community was assessed by PLFA analysis; and some specific PLFAs were used as biomarkers to determine the presence and abundance of specific microbial groups. Microbial community function was determined measuring the bacterial and fungal growth rates by the incorporation of radioactively labelled leucine into proteins and radioactively labeled acetate into the fungal-specific lipid ergosterol, respectively. The metabolic quotient, a parameter that evaluates the efficiency of microorganisms in utilizing organic C compounds, was also determined. The data were analyzed by one-way ANOVA. Post hoc comparisons of means were performed by a Tukey HSD test at $\alpha = 0.05$ test.

5.6.1 Effects of Earthworms on the Structure of Microbial Communities

5.6.1.1 Microbial Biomass

Assessment of microbial communities by PLFA (Zelles 1999) revealed that earthworms impact greatly on microbial community structure and function. We found that the activity of earthworms reduced the viable microbial biomass measured as the total content of PLFAs after 1 month of vermicomposting (Fig. 5.7a); the presence of earthworms reduced total microbial biomass by approximately four to five times relative to the control without earthworms. Earthworm activity also reduced the ratio of fungal to bacterial PLFA (Fig. 5.7b) indicating, that the decrease in fungal PLFA was proportionally higher than that of the bacterial PLFA.

Certain specific PLFAs can be used as biomarkers to determine the effect of earthworms on the presence and abundance of specific microbial groups. The sum of PLFAs characteristic of Gram-positive bacteria (iso/anteiso branched-chain PLFAs), Gram-negative bacteria (monounsaturated and cyclopropyl PLFAs) and actinomycetes (10Me branched PLFAs) was chosen to represent the bacterial biomass; and the fungal biomarker 18:26,9 was used to indicate fungal biomass (Frostegård and Bååth 1996; Zelles 1997). The abundance of both bacteria and fungi was drastically reduced by the earthworms after 1 month of vermicomposting

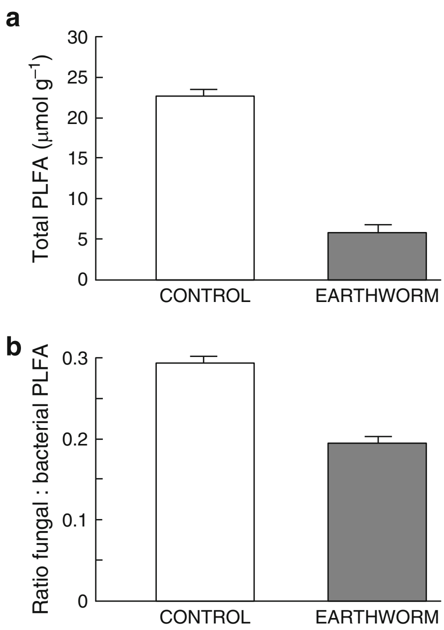


Fig. 5.7 Impact of earthworms (*Eisenia andrei*) on microbial communities after vermicomposting of cow manure (for 1 month): (**a**) Total PLFA, a measure of microbial biomass, and (**b**) the ratio of fungal:bacterial PLFA, a measure of shifts in the relative abundance of fungi and bacteria within the whole microbial community. Values are means ± SE. Control is the same treatment without earthworms

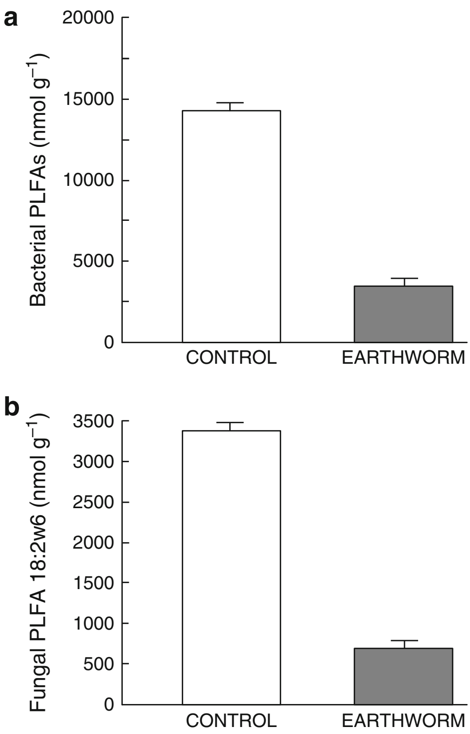


Fig. 5.8 Effect of earthworms (*Eisenia andrei*) on microbial communities after vermicomposting of cow manure (for 1 month): (**a**) Bacterial biomass, calculated as the sum of the bacterial PLFA markers: i14:0, i15:0, a15:0, i16:0, 16:1w5, 16:1w7, i17:0, a17:0, 10Me18:0, 18:1w7, cy17:0 and cy19:0 and (**b**) PLFA 18:2ω6,9, a measure of fungal biomass. Values are means ± SE. Control is the same treatment without earthworms

(Fig. 5.8). Earthworms can reduce microbial biomass directly by selective feeding on bacteria and fungi (Schönholzer et al. 1999) or indirectly by accelerating the depletion of resources for the microbes.

5.6.1.2 Bacterial and Fungal Growth

In our studies bacterial growth was estimated by the use of the leucine incorporation technique (Bååth 1994), as modified by Bååth et al. (2001), and fungal growth with the acetate-in-ergosterol incorporation technique (Newell and Fallon 1991) as modified by Bååth (2001). Earthworm activity greatly decreased the bacterial growth rate and did not affect the fungal growth rate after 1 month of vermicomposting (Fig. 5.9). Animal manures are microbiologically-rich environments in which bacteria constitute the largest fraction, with fungi mainly present as spores (Garrett 1981); moreover, the first stages of decomposition in these organic wastes are mainly dominated by bacteria because of the availability of water and easily decomposable substrates. Hence, the activity of earthworms is expected to affect the bacterial growth rate to a greater extent than the fungal growth rate. In addition, carbon availability is a limiting factor for earthworm growth and it has been

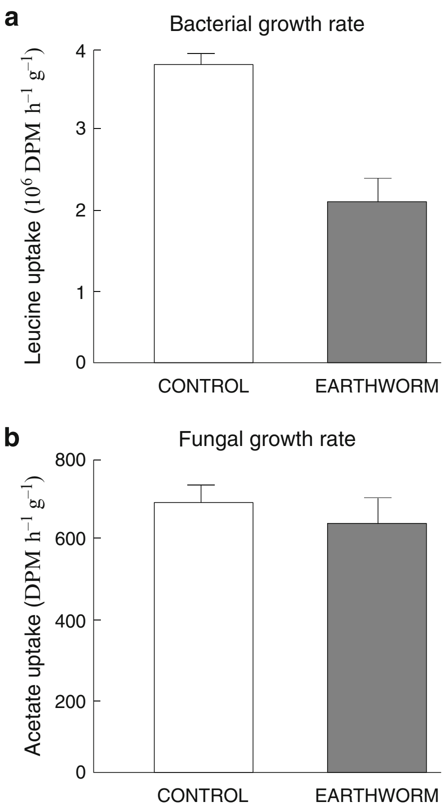


Fig. 5.9 Impact of earthworms (*Eisenia andrei*) on microbial growth after vermicomposting of cow manure (for 1 month): (**a**) Bacterial growth rate estimated as incorporation of leucine, and (**b**) Fungal growth rate estimated as incorporation of ac-in-erg. Values are means ± SE. Control is the same treatment without earthworms

reported that earthworms and microorganisms may compete for carbon resources (Tiunov and Scheu 2004); thus, earthworm activity may have reduced the quantity of resource available for microbial communities, and consequently the bacterial growth rate. The fungal growth rate is expected to decrease during the maturation stage, when depletion of more recalcitrant compounds takes place.

5.6.2 *Effects of Earthworms on the Activity of Microbial Communities*

As discussed earlier in this chapter, there is extensive evidence in the literature suggesting that earthworms and other soil animals grazing on microbes enhance microbial activity at the first instance. As a result of this, earthworm activity reduces later the availability of the resources for the microbial communities, and consequently their activity. Thus, in our experiment, the microbial activity

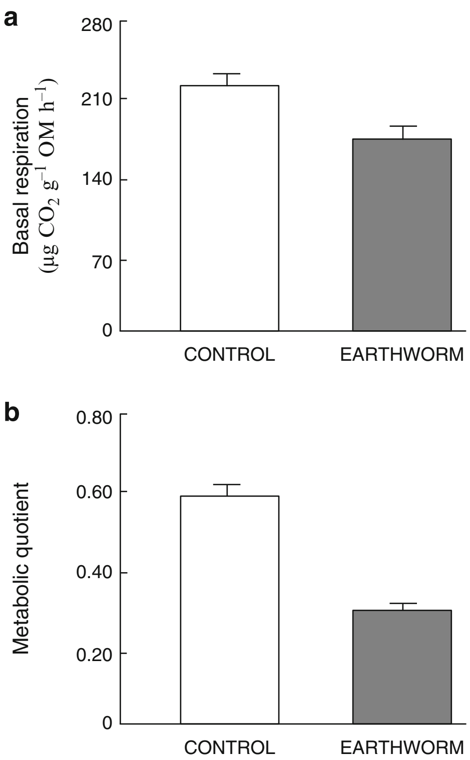


Fig. 5.10 Effect of earthworms (*Eisenia andrei*) on microbial community function after vermicomposting of cow manure (for 1 month): (**a**) Basal respiration, a measure of microbial activity, and (**b**) the metabolic quotient estimated as the amount of CO_2 released from the sample per unit of biomass. Values are means ± SE. Control is the same treatment without earthworms

measured as basal respiration decreased after 1 month of vermicomposting with the earthworm species *E. andrei* (Fig. 5.10a).

Organic carbon taken up by the heterotrophic microbial communities is partitioned between microbial cell biomass production, metabolite excretion and respiration. The proportion of substrate carbon retained as microbial biomass relative to carbon respired as CO_2 depends on the efficiency of microbial growth (i.e., the efficiency with which substrates are incorporated into biomass and by-products), as well as on the degree of protection of microbial biomass in the organic matrix and on the rate of decomposition of bacterial and fungal by-products by other microorganisms. Thus, the lower the microbial growth efficiency or the less protected the biomass, the greater the amount of carbon lost as CO_2 (Six et al. 2006). The metabolic quotient or specific activity of the microbial biomass (qCO_2; microbial respiration per unit biomass) can be used as a measure of microbial efficiency (Anderson and Domsch 1993; Wardle and Ghani 1995); higher values of qCO_2 indicate that microbial communities are under conditions of higher stress. Thus, less of the energy yielded by substrate metabolism will be used for biosynthetic purposes. An important portion of this energy will be expended on cell maintenance and lost as CO_2. Earthworm activity reduced the metabolic quotient after 1 month of vermicomposting (Fig. 5.10b), indicating, that microbial communities used the available energy more efficiently in the presence of earthworms. As a consequence, the system functioned much better, as shown by the large increase in the rate of decomposition of the organic matter (Fig. 5.5.) and in the rate of nitrogen mineralization (Fig. 5.6). The effect of earthworms on C and N mineralization rates is density-dependent (Aira et al. 2008).

5.6.3 Effect of Earthworms on Total Coliforms during Vermicomposting

Earthworms also greatly reduced the presence of total coliforms during vermicomposting. The passage through the gut of the earthworm species *E. andrei*, *E. fetida* and *Eu. eugeniae* reduced the density of total coliforms by 98%, relative to fresh pig slurry (Fig. 5.11a) (Monroy et al. 2008). The same drastic reduction in the density of total coliforms was also found in another experiment after 2 weeks of vermicomposting with *E. fetida* (Monroy 2006). The reductions in total coliforms were similar to those reported by Eastman et al. (2001) for these and other human pathogens, which indicate the effectiveness of vermicomposting at reducing the levels of human pathogens during stabilization of biosolids and other organic wastes. As discussed earlier, digestion of decaying substrate by earthworms decreases the availability of nutrients for microorganisms, thereby decreasing microbial numbers in casts and altering the microbial composition (Brown 1995). There is increasing evidence that earthworms have a specific gut microflora (Karsten and Drake 1995; Horn et al. 2005), and the decrease in total coliforms also may be related to competitive interactions between coliforms and microorganisms that are specific to the earthworm gut (Brown and Mitchell 1981). Moreover, the negative effect of the passage through the earthworm gut observed in enterobacteria such as *Serratia marcescens*, *Escherichia coli* and *Salmonella enteridis* (Day 1950; Brüsewitz 1959; Brown and Mitchell 1981) suggests the occurrence of selective effects on the ingested microorganisms.

5.6.4 Effect of Earthworms on the Composition of Microbial Communities

The discriminant analysis of 25 PLFAs (i14:0, 14:0, i15:0, a15:0, 15:0, i16:0, 16:19, 16:17, 16:15, 16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, 10Me17:0, 18:26,9, 18:19, 18:17, 18:0, 10Me18:0, cy19:0, 20:46, 20:53, 20:36) clearly differentiated the vermicomposts obtained with three different epigeic earthworm species (*E. andrei*, *E. fetida* and *P. excavatus*), irrespective of what manure type (cow, horse or rabbit) was used in vermicomposting (Fig. 5.12). This indicates that there were

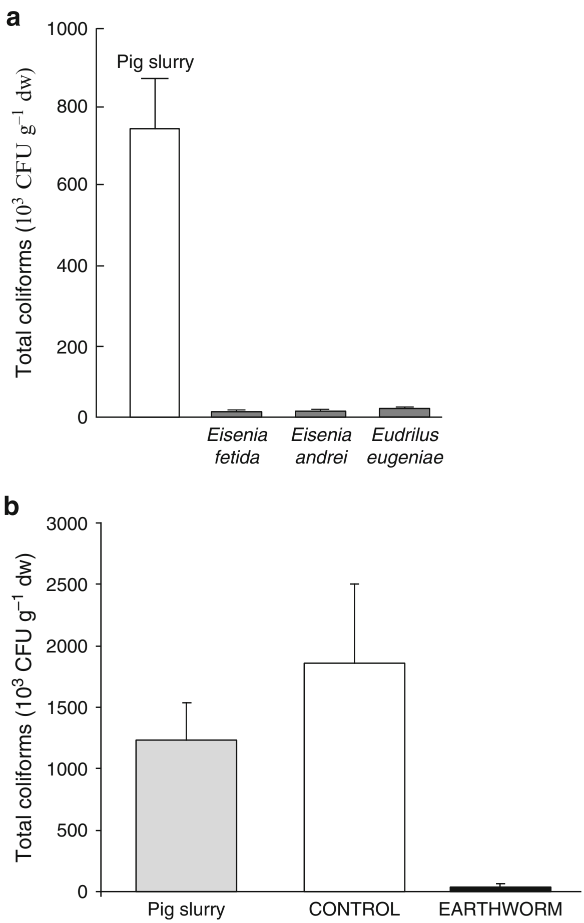


Fig. 5.11 Effect of earthworms on total coliforms in pig slurry after: (**a**) the transit through the gut of three species of epigeic earthworms, and (**b**) 2 weeks of vermicomposting with *Eisenia fetida*. Values are means ± SE. Control is the same treatment without earthworms

different PLFA profiles associated with the vermicomposts, not related to the type of animal manure used, but rather to the earthworm species and/or their endosymbiotic gut microflora. Moreover, the separation between vermicomposts and control substrates (manures processed without earthworms) was also very clear (Fig. 5.12), indicating that earthworms play a key role in shaping the structure of the microbial community in organic wastes during the vermicomposting process. Similar results were also found with fatty acid methyl ester (FAME) profiles (Lores et al. 2006). From this perspective and since different vermicomposts produced by different earthworm species and from different types of organic wastes contain an enormous and specific variety of microorganisms, it is possible to obtain specific vermicomposts for different practical applications. This may especially be important in

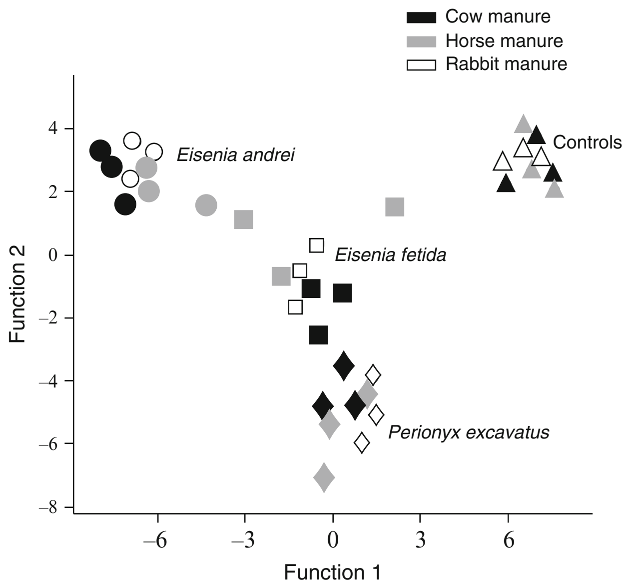


Fig. 5.12 Earthworm species-specific modification of the microbial community composition after vermicomposting of three animal manures (cow, horse and rabbit) for 1 month. Discriminant analysis of the PLFAs (Wilks´λ = 0.00099, $p < 0.0001$) from the vermicompost obtained with three epigeic earthworm species (*Eisenia andrei*, *E. fetida* and *Perionyx excavatus*). Controls are the same treatment without earthworms. Functions 1 and 2 represent 65 and 29% of the variance respectively

producing plant container media and for impoverished and/or intensively fertilized soils.

5.6.5 Molecular Tools Applied to Vermicomposting Studies

Molecular tools are commonly used for investigating microbial communities in ecological studies (see Hultman et al. 2010, Insam et al. 2010, Knapp et al. 2010, Minz et al. 2010). Such tools include clone libraries, fluorescent in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) analysis. Each of these methods measures different aspects of the community such as diversity, in situ detection, and community dynamics, and all of them are based on 16S rRNA gene sequences (Deutschbauer et al. 2006). Although these techniques nowadays are frequently used in composting research (Danon et al. 2008; Franke-Whittle et al. 2009), their application in vermicomposting is very scarce (Fracchia et al. 2006;

Vivas et al. 2009; Sen and Chandra 2009). Application of these techniques has shown that compost and vermicompost differ greatly in their microbial communities, and that a higher microbial diversity exists in vermicompost relative to the initial substrate than in compost (Fracchia et al. 2006; Vivas et al. 2009; Sen and Chandra 2009).

5.7 Conclusions

Vermicomposting is a bio-oxidative process in which detritivore earthworms interact intensively with microorganisms in decomposition processes, accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties. Earthworms are crucial drivers of the process as they are involved in the indirect stimulation of microbial populations through fragmentation and ingestion of fresh organic matter. Earthworms reduce microbial biomass and activity during the vermicomposting process. The activity of epigeic earthworms drastically reduces the viable microbial biomass during the vermicomposting process and this reduction is proportionally higher for fungi than for bacteria. After 1 month of vermicomposting the bacterial growth rate decreases in the substrate whereas the fungal growth rate is not affected. Microbial activity measured as basal respiration decreases after vermicomposting. Earthworm activity helps microbial communities to use the available energy more efficiently and plays a key role in shaping the structure of the microbial community in organic wastes during the vermicomposting process. These evidences indicate that detritivorous earthworms directly modulate the decomposer community composition in the short term accelerating the decomposition of organic matter.

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