The Microbiology of Vermicomposting

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I WHAT IS VERMICOMPOSTING?

Although Darwin (1881) 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 more than a century until this was taken seriously as a field of scientific knowledge or even a real technology. Vermicomposting is a mesophilic bio-oxidative process in which detritivorous earthworms interact intensively with microorganisms and soil invertebrates within the decomposer community, strongly affecting decomposition processes, accelerating the stabilization of organic matter,

and greatly modifying its physical and biochemical properties (Edwards and Bohlen 1996; Domínguez 2004; Edwards et al. 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, thus dramatically increasing microbiological activity. Earthworms also modify microbial biomass and activity through stimulation, digestion, and dispersion in the casts (Figure 5.1) and interact closely with other biological components of the vermicomposting system, thereby affecting the structure of the 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.

Vermicompost, the end product of vermicomposting, is a finely divided peat-like material with high porosity and water-holding capacity and a low C:N ratio; it 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 but also phosphorus, potassium, calcium, and magnesium for plants. Vermicompost also contains plant growth hormones produced by microorganisms and plant growth regulators such as humates, in the production of which microorganisms also play a role.
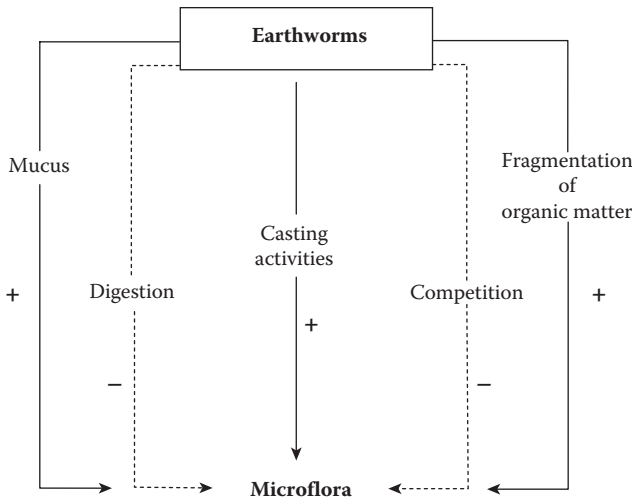

Figure 5.1 Positive (+) and negative (–) effects of earthworms on microbial biomass and activity.

II VERMICOMPOSTING FOOD WEB

Vermicomposting systems sustain a complex microbial and invertebrate food web that results in the recycling of organic matter and release of nutrients. 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 substrate quality are the main properties of these systems (Sampedro and Domínguez 2008). The most numerous and diverse members of this food web are microorganisms, although there are also abundant protozoa and many invertebrates of varying sizes, including nematodes, microarthropods, and large populations of earthworms (Monroy 2006; Sampedro and Domínguez 2008). These invertebrates cover a range of trophic levels—some feed primarily on microbes (bacteriovores and fungivores), on organic waste (detritivores), or on a mixture of organic matter and microbes (microbio-detritivores), whereas others feed on animals (carnivores) or across different trophic levels (omnivores); (Sampedro and Domínguez 2008).

The primary consumers of the vermicomposting food web are the microorganisms (mainly bacteria, fungi, and ciliates) that break down and mineralize organic residues. Microorganisms are the most numerically abundant and diverse members of the vermicomposting food web and include many thousands of different organisms. Secondary and higher-level consumers, that is, the soil invertebrates, including the earthworms, exist together with 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 that microorganisms act on. As decomposers die, more food is added to the food web for other decomposers.

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

III THE PROCESS OF VERMICOMPOSTING

The vermicomposting process includes two different phases involving the activity of earthworms, (a) an active phase during which earthworms process wastes, thereby modifying their physical state and microbial composition (Lores et al. 2006) and (b) a maturation-like phase marked by the displacement of the earthworms toward fresher

layers of undigested waste, during which the microbes take over the decomposition of the earthworm-processed waste (Domínguez 2004; Lazcano et al. 2008). The duration of the active phase is not fixed, and it depends on the species and population densities of earthworms and the rates at which they ingest and process the wastes.

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 transit through the earthworms' intestines. These modifications include the addition of sugars and other substances, modification of the microbial diversity and activity, modification of the microfaunal 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 earthworm gut. These microbes produce extracellular enzymes that can degrade cellulose and phenolic compounds, thereby further enhancing the degradation of ingested material. Other physical modifications of the substrate are caused by the burrowing activities of earthworms, including aeration and homogenization of the substrate, which also favor microbial activity and further decomposition (Domínguez 2004). The proximate activities of earthworms enhance the mineralization of both carbon and nitrogen in the substrate significantly, and such effects are in proportion to the earthworm population densities (Aira et al. 2008).

Upon completion of GAPs, the resultant earthworm casts undergo cast-associated processes (CAPs), which are more closely associated with the aging processes, the action of the microflora and microfauna presents in the substrate, and the physical modification of the egested materials. 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 almost 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 process, vermicompost reaches its optimum in terms of biological properties that promote plant growth and suppress plant diseases. Currently, there is insufficient information regarding when this optimum is achieved, how we can determine it in each case, and whether this optimum has some kind of expiration date. It is important to note that it is possible that the optimal quality may be achieved only in natural ecosystems built from a correct site-specific balance of soil, plants, microorganisms, macroorganisms including earthworms, and climate. However, it is not possible yet to determine easily when a vermicompost is optimal and thus, this can be known only after its application.

IV EFFECTS OF EARTHWORMS ON MICROBIAL COMMUNITIES DURING VERMICOMPOSTING

Microorganisms are the main agents of biochemical decomposition, whereby earthworms are involved in the indirect stimulation of microbial populations

through fragmentation of organic matter, that is, 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 microorganisms, because whether the earthworms stimulate or depress the microbiota, or modify the structure and function of microbial communities, they can have quite different effects on the rates and form of decomposition of organic matter. To address these questions we performed an experiment in our laboratory with mesocosms, filled with cow manure either with 10 mature earthworms belonging to *E. andrei* or without earthworms ($n = 5$ each). We used cow manure as the substrate, which is known to support a dense decomposer food web (Sampedro and Domínguez 2008). The mesocosms consisted of 2 L plastic jars filled with 200 g (fresh weight) of substrate. We used the epigeic earthworm *E. andrei* (Bouché 1972), which is distributed widely and easy to manage under laboratory conditions. We allowed mature earthworms (375 ± 7 mg; mean individual fresh weight $\pm$ 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 and stored them at random in a scientific incubator (20°C and 90% humidity); after 1 month, the earthworms were removed, and vermicompost and control samples were collected and processed immediately 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 by measuring the bacterial and fungal growth rates by the incorporation of radioactively labeled 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.

A Microbial Biomass

Microbial communities were characterized by PFLA profiles (Zelles 1999) and these analyses revealed that earthworm activity impacted 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 (Figure 5.2a); the presence of earthworms reduced total microbial biomass by approximately four to five times relative to the control without earthworms. 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) were chosen to represent the bacterial biomass; and the fungal biomarker 18:2ω6,9 was used to indicate fungal biomass (Frostegård and Bååth 1996; Zelles 1997).
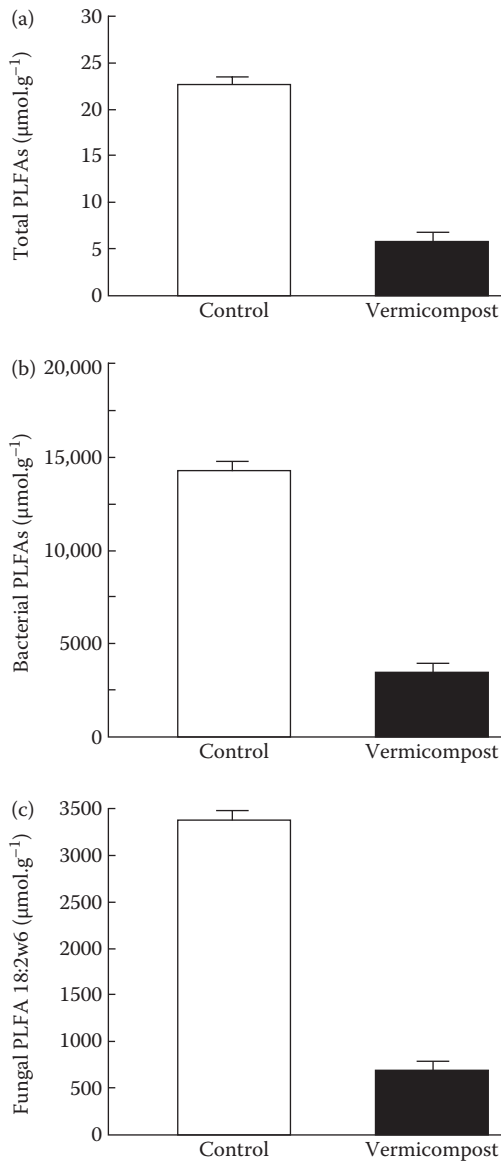
Figure 5.2 Impact of earthworms (*Eisenia andrei*) on microbial communities after vermicomposting in cow manure (for 1 month). (a) Total phospholipid fatty acids (PLFAs), a measure of microbial biomass; (b) bacterial biomass, calculated as the sum of the bacterial PLFA markers: i14:0, i15:0, a15:0, i16:0, 16:1ω5, 16:1ω7, i17:0, a17:0, 10Me18:0, 18:1ω7, cy17:0, and cy19:0; and (c) PLFA 18:2ω6,9, a measure of fungal biomass. Values are means ± SE. Control is the same treatment without earthworms.

The abundance of both bacteria and fungi was drastically reduced by the earthworms after 1 month of vermicomposting (Figure 5.2b and c). Earthworms can reduce microbial biomass directly by selectively feeding on bacteria and fungi (Schönholzer et al. 1999) or indirectly by accelerating the depletion of resources available for the microbes.

B Bacterial and Fungal Growth

In our studies bacterial growth was estimated by the use of the leucine incorporation technique (Bååth et al. 2001), and fungal growth with the acetate-in-ergosterol incorporation technique (Bååth 2001). Earthworm activity greatly decreased bacterial growth rates but did not affect fungal growth rates after 1 month of vermicomposting (Figure 5.3). Animal manures are microbe-rich environments in which bacteria constitute the largest fraction, with fungi mainly present as spores; moreover, the first stages of decomposition in these organic wastes are dominated mainly by bacteria because of the availability of water and easily decomposable substrates. Hence, the activity of earthworms is expected to affect the bacterial growth rates to a greater extent than the fungal growth rate. In addition, carbon availability is a limiting factor for earthworm growth, and it has been reported that earthworms and microorganisms may compete for carbon resources (Tiunov and Scheu 2004); thus, earthworm activity may have reduced the quantity of resources available for microbial communities, and consequently the bacterial growth rates. The fungal growth rate was expected to decrease during the maturation stage, when depletion of more recalcitrant compounds takes place.

C Effects of Earthworms on the Activity of Microbial Communities

There is extensive evidence in the literature suggesting that earthworms and other soil invertebrates grazing on microorganisms enhance microbial activity in the first instance. As a result of this activity, earthworms reduce the later availability of these resources for the microbial communities, and consequently their activity. Thus, in our experiment, the microbial activity, measured as basal respiration, decreased after 1 month of vermicomposting with the earthworm species *E. andrei* (Bouché) (Figure 5.4a).

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. The metabolic quotient

(a)   Bacterial growth rate
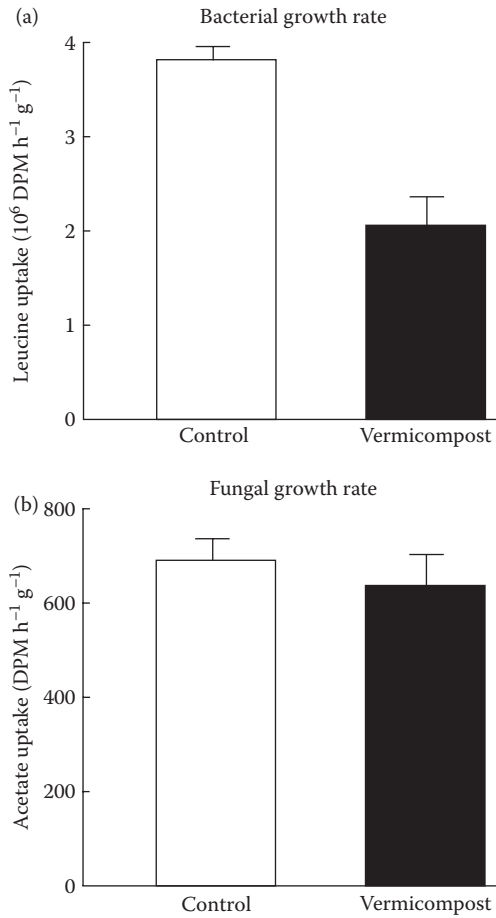

(b)   Fungal growth rate

Figure 5.3 Impact of earthworms (*Eisenia andrei*) on microbial growth after vermicomposting of cow manure (for one month). (a) Bacterial growth rate estimated as incorporation of ac-in-erg. Values are means ± SE. Control is the same treatment without earthworms.

or specific activity of the microbial biomass (qCO_2; microbial respiration per unit biomass) can be used as a measure of microbial efficiency; higher values of qCO_2 indicate that microbial communities are under conditions of higher stress. Thus, less of the energy yielded by substrate metabolism can 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 (Figure 5.4b), 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 increases in the rate of decomposition of organic matter (Figure 5.5a) and in the rates of nitrogen mineralization (Figure 5.5b).
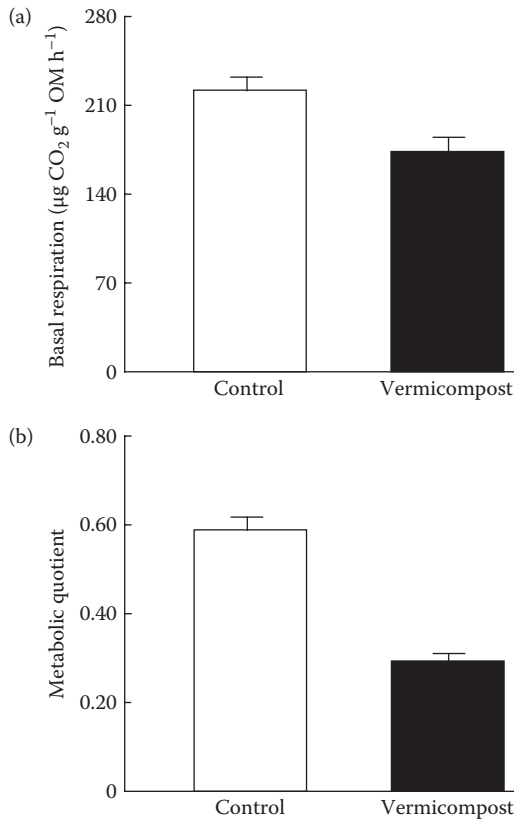
Figure 5.4 Effect of earthworms (*Eisenia andrei*) on microbial community function after vermicomposting of cow manure (for 1 month). (a) Basal respiration, a measure of microbial activity, and (b) the metabolic quotient estimated as the amount of CO_2 released from the sample per unit of biomass. Values are means ± SE. Control is the same treatment without earthworms.

D Effect of Earthworms on Total Coliform Bacteria during Vermicomposting

Earthworms also greatly reduced populations of total coliform bacteria during vermicomposting. Passage through the guts of the earthworm species *Eisenia andrei*, *Eisenia fetida*, and *Eudrilus eugeniae* reduced the populations of total coliforms by 98% relative to that in fresh pig slurry (Figure 5.6a; Monroy et al. 2008, 2009). The same drastic reduction in the populations 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 indicates the effectiveness of vermicomposting in reducing the levels of human pathogens during stabilization of biosolids and other organic wastes. As discussed earlier, digestion
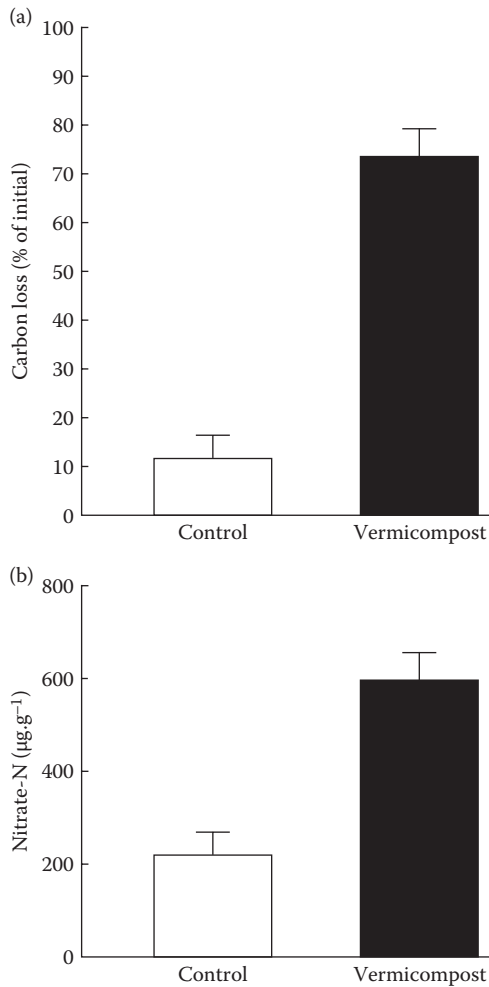
Figure 5.5 (a) Carbon loss (percentage of initial) after 1 month of vermicomposting of cow manure, as affected by the presence of the earthworm *Eisenia andrei.* (b) Effect of earthworms (*Eisenia andrei*) on the amount of Nitrate-N produced after vermicomposting of cow manure (for 1 month). Values are means ± SE. Control is the same treatment without earthworms.

of decaying substrates by earthworms decreases the availability of nutrients for microorganisms, thereby decreasing microbial numbers in the casts and altering the pattern of microbial composition (G. G. 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 (B. A. Brown and Mitchell 1981). Moreover, a negative effect of the passage through the earthworm gut on enterobacteria such as *Serratia marcescens*,
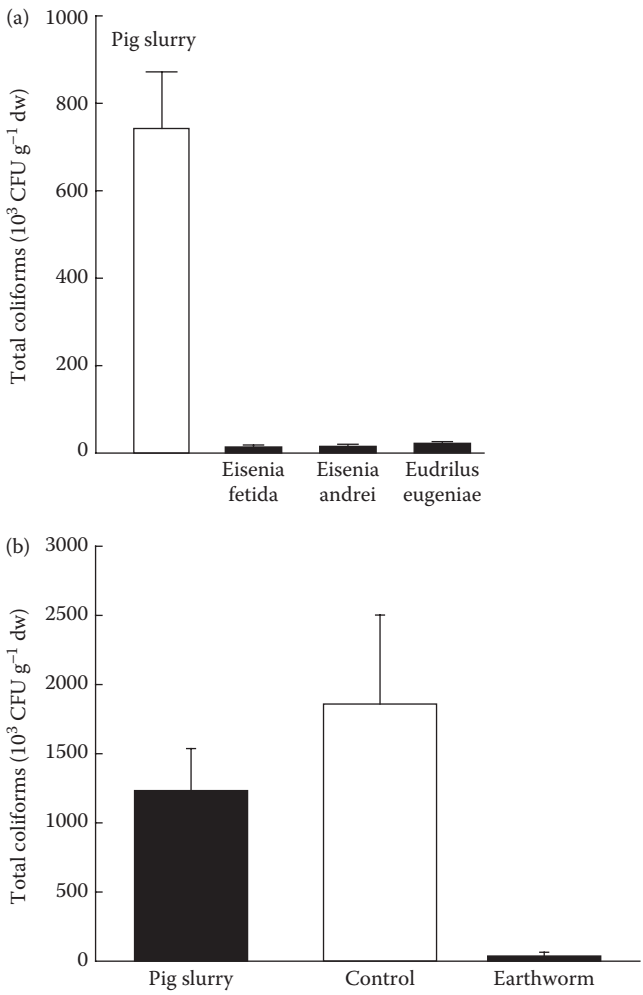
Figure 5.6 Effects of earthworms on total coliforms in pig slurry after (a) the transit through the gut of three species of epigeic earthworms and (b) 2 weeks of vermicomposting with *Eisenia fetida.* Values are means ± SE. Control is the same treatment without earthworms (dw: dry weight).

Escherichia coli, and *Salmonella enteridis* has been observed by several authors (Brown and Mitchell 1981), which suggests the occurrence of selective effects on the ingested microorganisms.

E 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, 16:1ω9, 16:1ω7, 16:1ω5, 16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, 10Me17:0, 18:2ω6,9,

18:1ω9, 18:1ω7, 18:0, 10Me18:0, cy19:0, 20:4ω6, 20:5ω3, 20:3ω6) clearly differentiated between the vermicomposts obtained using three different epigeic earthworm species (*E. andrei*, *E. fetida*, and *Perionyx excavatus*), irrespective of which manure type (cow, horse, or rabbit) was used in vermicomposting. This indicates that different PLFA profiles were 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, 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 esters (FAMEs) 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 be especially important in producing plant container growth media for impoverished and/or intensively fertilized soils.

V CONCLUSIONS

Vermicomposting is a bio-oxidative process in which detritivorous 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 since they are involved in the indirect stimulation of microbial populations through fragmentation and ingestion of fresh organic matter. Earthworms reduce overall 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, possibly because earthworms may use fungi for food selectively. After 1 month of vermicomposting the bacterial growth rates decrease in the substrate whereas the fungal growth rates are not affected. Microbial activity can be measured as decreases in basal respiration after vermicomposting. Earthworm activity helps microbial communities use the available energy more efficiently and plays a key role in shaping the structure of the microbial communities in organic wastes during the vermicomposting process. This evidence indicates that detritivorous earthworms directly modulate the decomposer community composition in the short term, thereby accelerating the decomposition of organic matter.

ACKNOWLEDGMENT

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