CHAPTER 25

New Developments and Insights on Vermicomposting in Spain

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I INTRODUCTION

Nowadays, the technologies of vermiculture and vermicomposting are well established, and there are many commercial enterprises in Spain. However, there are only two vermicompost research groups, the Soil Ecology Laboratory at the University of Vigo and the Department of Environmental Protection, Estación Experimental del Zaidín (EEZ), Consejo Superior de Investigaciones Científícas (CSIC), in Granada, both working on a range of scientific aspects of this discipline. Both of these groups have been developing a comprehensive research program in vermicomposting over the past 20 years, including many different aspects of the process and the effects of the application of vermicomposts on crop protection and pest management. This Chapter summarizes the research on vermicomposting conducted in these two laboratories in Spain. The extent of vermiculture research in Spain makes the country a leader in this new technology.

The research program at the University of Vigo that focuses on the vermicomposting of different types of organic wastes includes the following topics: biology and ecology of earthworms, reproduction and life histories of earthworms, evolutionary ecology of earthworms, ecology of vermicomposting, interactions between earthworms and other microorganisms, interactions between earthworms and soil organisms, microbial ecology of vermicomposting, influence of earthworms on nutrient cycling and nutrient dynamics during vermicomposting, fate of human pathogens during vermicomposting, effects of vermicomposts on the growth of greenhouse and field crops, production of plant growth regulators during vermicomposting, and aging and conservation of vermicomposts.

The overall goal of the research program Biotransformation of Organic Wastes, Protection of Soil and Agricultural Crops at the Department of Environmental Protection, EEZ, CSIC, in Granada, is to contribute sustainable and environmentally friendly practices to soil and crop protection through the use of low-cost bioremediation technologies and the promotion of sustainable development of agricultural systems by means of ecological alternatives. This general objective is approached from a triple perspective: (a) the development of the bioremediation processes, mainly vermicomposting, that favor the biotransformation, recycling, and recovery of polluted organic wastes, as well as the development of low-cost technologies for the protection and bioremediation of soil against organic contaminants; (b) the development of integrated management of pests in agroecosystems using vermicomposts; and (c) the development of methods including the use of vermicomposts to evaluate and maintain biodiversity in sustainable agroecosystems.

II EVALUATION OF ORGANIC WASTES FOR VERMICOMPOSTING

Since vermicomposting is a method of converting solid organic wastes into environmentally friendly and valuable resources for crop production and soil improvement, we evaluated the suitability of different types of organic wastes for the process. We found that vermicomposting works very well for processing sewage sludge and biosolids from wastewater (Elvira et al. 1997; Benítez et al. 1999a, 1999b, 2000; Domínguez et al. 2000, 2003; Plana et al. 2001), paper industry waste (Elvira, Mato, et al. 1995; Elvira, Domínguez, et al. 1995; Elvira, Goicoechea, et al. 1996; Elvira et al. 1997, 1998, 1999), urban residues, food, and animal wastes (Domínguez et al. 1996; Elvira, Domínguez, and Briones 1996; Elvira, Domínguez, and Mato 1996; Domínguez and Edwards 1997; Domínguez, Edwards, et al. 1997, 2001; Atiyeh et al. 2000; Aira et al. 2002, 2006a, 2006b, 2008, 2009; Aira, Monroy, et al. 2007a, 2007b; Aira and Domínguez 2008, 2009; Lazcano et al. 2008; Monroy et al. 2009), and food industry waste (Elvira et al. 1998, 1999; Nogales et al. 1998, 2005, 2008; Nogales, Elvira, et al. 1999; Nogales, Melgar, et al. 1999; Benítez et al. 2002, 2005; Melgar et al. 2002; Saavedra et al. 2006; Romero et al. 2007; Fernández-Bayo et al. 2008; Plaza et al. 2008; Vivas et al. 2009; Gómez-Brandón et al. 2010).

III BIOLOGY OF EARTHWORMS SUITABLE FOR VERMICULTURE

Although more than 3000 species of earthworms have been described, for the great majority of these species, we know only their names, morphologies and location and little about their biology, life cycles, or ecology. Certain epigeic earthworms, with their natural ability to colonize organic wastes and digest and assimilate organic matter, high rates of feedstock consumption, tolerance of a wide range of environmental factors, short life cycles, high reproductive rates, and endurance and tolerance of handling, show good potential for vermicomposting (see Chapters 3 and 4). Few earthworm species display all of these characteristics, and in fact only five have been used extensively in vermicomposting: *Eisenia andrei* Bouché, 1972, *Eisenia fetida* (Savigny, 1826), *Dendrobaena veneta* (Rosa, 1886), *Perionyx excavatus* Perrier, 1872 and *Eudrilus eugeniae* (Kinberg, 1867). We have studied many different aspects of the biology, ecology, and life cycles of four of these species (Elvira, Domínguez, and Briones, 1996; Domínguez and Edwards 1997, 2004; Domínguez, Briones, et al. 1997; Edwards et al. 1998; Domínguez et al. 2000, 2001, 2003, 2005; Aira et al. 2002, 2007; Monroy et al. 2003, 2005, 2006; Domínguez 2004; Pérez-Losada et al. 2005; Tato et al. 2006; Velando et al. 2006, 2008). We have also studied the biology, ecology, and life cycles of other earthworm species, such as *Lumbricus rubellus* and *Dendrodrilus rubidus* (Elvira, Domíguez, and Mato, 1996) and *Octodrilus complanatus* (Monroy et al. 2007). We are studying the ecology of a range of epigeic earthworm species and working in a laboratory screening program that is seeking other species suitable for vermicomposting (see Chapter 4).

A *Eisenia andrei* and *Eisenia fetida* are Two Different Earthworm Species

The closely related species *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 are those used most commonly and globally for the management of organic wastes, and also in studies of ecotoxicology, physiology, and genetics. The problem of their taxonomic status remained unresolved for long time, and in much of the current literature, both species are termed indiscriminately as *E. fetida* or *E. foetida*, and it is often not clear which of the two species is being referred to. We have confirmed that they are two different biological species, reproductively isolated, and that they are also two different phylogenetic species. The reproductive isolation was determined after studying the offspring viability from inter- and intraspecific crosses of both species (Domínguez et al. 2005). Additionally, fully resolved and well-supported phylogenetic trees, based on mitochondrial (COI) and nuclear DNA sequences (28S), confirmed that they are different phylogenetic species (Pérez-Losada et al. 2005). This evidence has important considerations: For vermiculture or vermicomposting *E. andrei* is recommended most often since its growth and reproduction rates are higher than those of *E. fetida*. In ecotoxicological studies it is not possible to assume that contaminants will have the same effect on the two species, since their responses to stress factors could be different. The existence of postcopula but not precopula isolation in sympatric populations clearly affects population dynamics by reducing the fitness of the individuals. For this reason, for applied aspects of vermiculture, it is important keep the two species separated where possible, although the mixed populations often used may still function well in vermicomposting.

IV INFLUENCE OF ENVIRONMENTAL FACTORS ON THE SURVIVAL, GROWTH, AND REPRODUCTION OF VERMICOMPOSTING EARTHWORMS

The survival, reproduction, and growth of earthworms can be affected critically by environmental conditions. We have studied the influence of temperature, moisture content, ammonium content, population densities, type of food, and intra- and interspecific competition on the life histories of the four earthworm species most extensively used in vermicomposting: *E. andrei*, *E. fetida*, *P. excavatus*, and *E. eugeniae* (Elvira, Domínguez, and Briones, 1996; Elvira, Domínguez, and Mato, 1996; Domínguez and Edwards 1997, 2004; Domínguez, Briones, et al. 1997; Edwards et al. 1998; Domínguez et al. 2000, 2001; Domínguez 2004).

V ECOLOGY OF VERMICOMPOSTING

A Earthworms and Microorganisms: Disentangling the Black Box of Vermicomposting

Vermicomposting systems sustain a complex food web in organic wastes, which results in the recycling of the organic matter and release of the nutrients it contains. Biotic interactions between decomposer microorganisms (i.e., bacteria and fungi) and soil invertebrates include competition, mutualism, predation, and facilitation. The rapid changes that occur in both functional diversity and in substrate qualities 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 and life-history patterns, including nematodes and microarthropods, as well as large populations of earthworms (Monroy et al. 2006; Sampedro and Domínguez 2008; Domínguez et al. 2010).

Microorganisms are largely responsible for organic-matter decomposition, but earthworms also affect rates of decomposition directly by feeding on and fragmenting the organic matter. This also affects the rates of decomposition indirectly through interactions with microorganisms, basically involving stimulation or depression of microbial biomass and activity and enzymatic activity (Domínguez 2004; Domínguez et al. 2010). We found that these processes mainly depend on earthworm population densities, with significant decreases in microbial biomass and activity related to increasing numbers of earthworms per unit area or volume of waste and time (Aira et al. 2002, 2008).

We found that the vermicomposting of animal manures with *E. fetida* comprises two separate stages, mainly associated with earthworm activities. Thus, when earthworms are present, not only is microbial biomass and activity enhanced but also rates of mineralization are increased (Aira, Monroy, et al. 2007b, 2007c); moreover, we also found significant increases in fungal populations in this stage that were associated with cellulose degradation (Aira et al. 2006b); this priming of fungal populations was observed in short-term experiments (72 hours; Aira et al. 2008). In animal waste experiments, once the earthworms moved from processed material to new batches of raw manure, the second stage begins. This part is characterized by the stabilization of the manure, with continuous decreases in microbial biomass and activity (Aira, Monroy, et al. 2007a, 2007b, 2007c; Domínguez et al. 2010). Thus, we can expect that microbial communities in the process from manure to vermicompost should change markedly, as we reported in a study on different animal manures and earthworm species (*E. andrei*, *E. eugeniae*, and *Lumbricus rubellus*). Fungal biomass increased significantly in horse manure vermicomposted by *L. rubellus* and in cow manure vermicomposted by all three earthworm species, whereas it decreased significantly in pig manure vermicomposted by *L. rubellus* and *E. eugeniae*. Furthermore, protozoa biomass, undetectable in the animal manures, increased significantly in all vermicomposts produced by the three earthworm species (Lores et al. 2006; Gómez-Brandón et al. 2010). Surprisingly, we found an effect of earthworm species, since the microbial communities in vermicomposts produced by each earthworm species were very similar, independently of the type of parent animal waste (horse, cow and pig manure) clustering together in related groups, mainly due to the above-mentioned changes together with a marked drop in bacterial biomass (Lores et al. 2006; Domínguez et al. 2010; Gómez-Brandón et al. 2010).

Results from analyses of fresh earthworm casts and their parent raw manures demonstrated increases in microbial biomass and decreases in microbial activity (Aira et al. 2006a; Aira and Domínguez 2009); these indicate that the direct effects of *E. fetida* produce changes in microbial populations that can influence the overall dynamics of organic-matter degradation. These decreases in microbial activity can be attributed to reductions in organic C and N in the wastes (Aira and Domínguez 2008). However, analyses of the gut contents of several epigeic earthworm species revealed no changes in bacterial numbers or microbial activity between species (Aira et al. 2009). We inoculated fresh manure with vermicompost to study the indirect effects of earthworms on organic-matter decomposition. We found that the inoculation of vermicomposts into animal manures modified the microbial community functions, separating clearly microbial communities depending on the type of vermicompost, size of inoculum, and time of incubation. These changes all occurred in the same directions, first an increase and then a decrease. These changes in microbial communities, and those found in our vermicomposting experiment, suggest that the indirect effects of earthworms are to alter the dynamics of animal manure decomposition (Aira and Domínguez 2010). However, the extent of these effects was not as great as those we found during vermicomposting; these observations, together with the results of our earthworm casting experiment, suggest the existence of other factors governing relationships between earthworms and microorganisms that become established during vermicomposting.

B Stimulation and Acceleration of Microbial Decomposition by Earthworms during Vermicomposting

Nutrient mineralization is governed directly by the activities of bacteria and fungi, and these activities are strongly affected by soil invertebrates that interact with the microorganisms, and also by food web interactions that determine the transfer of nutrients through the system. Although epigeic earthworms have few direct impacts on mineralization, their indirect effects on microbial biomass and microbial activity are very important in mineralization. These indirect effects include the digestion and release of readily assimilable substances, such as mucus for the microbiota, as well as the transport and dispersal of microorganisms through earthworm casting (Domínguez et al. 2010).

In studies at the University of Vigo we found that earthworms accelerated the rates of organic-matter decomposition during vermicomposting significantly (Atiyeh et al. 2000; Domínguez et al. 2003; Domínguez 2004; Aira et al. 2006b, 2008; Aira, Monroy, et al. 2007a, 2007b; Aira and Domínguez 2008, 2009). Although earthworms can assimilate C from the more labile fractions of organic wastes, their contribution to the total heterotrophic respiration is relatively low due to their relatively poor capacity for assimilation.

Nitrogen mineralization is regulated basically by the availability of dissolved organic nitrogen and ammonium, the activity of the microorganisms, and their relative requirements for C and N. In our studies we found that earthworms also have a great impact on N transformations during vermicomposting, through modifications of the environmental conditions and their interactions with microorganisms; they enhance N mineralization, thereby producing conditions in the organic wastes that favor nitrification, resulting in the rapid conversion of NH_4-N into NO_3-N (Atiyeh et al. 2000; Domínguez 2004; Aira et al. 2008; Aira and Domínguez 2008; Lazcano et al. 2008).

VI VERMICOMPOSTING AND HUMAN PATHOGEN DESTRUCTION

We found that earthworms decreased populations of total coliforms during vermicomposting greatly (see Chapter 16). Thus, passage through the gut of the earthworm species *E. andrei*, *E. fetida*, and *E. eugeniae* reduced population densities of total coliform bacteria by 98%, relative to those in fresh pig slurry (Monroy et al. 2008). We also found similar drastic reductions in the population densities of total coliforms in another experiment after 2 weeks of vermicomposting using *E. fetida* (Monroy et al. 2009).

VII EFFECTS OF VERMICOMPOSTS ON PLANT GROWTH

Earthworms have beneficial physical, biological, and chemical effects on soils, and these effects increase plant growth and crop yields in both natural and agroecosystems (Edwards and Bohlen 1996; Edwards et al. 1998;) (see Chapters 9, 10, and 15). Over the past few years, the Soil Ecology Laboratory at the University of Vigo has been developing a comprehensive research program in vermicomposting, which has included studies on the effects of vermicomposts on plant growth. The effects of vermicomposts on the growth of a variety of crops, including cereals, legumes, vegetables, ornamental and flowering plants, and trees, have been assessed in the greenhouse, and to a lesser degree in field crops (Lazcano, Arnold, et al. 2009; Lazcano, Sampedro, et al. 2009; Lazcano et al. 2010). These investigations have consistently demonstrated that vermicomposts have beneficial effects on plant growth independent of nutrient transformations and availability. Whether vermicomposts are used as soil additives, or as components of horticultural soilless bedding-plant container media, they have improved seed germination consistently, enhanced seedling growth and development, and increased plant productivity and yields, much more than would be possible from the mere conversion of mineral nutrients into more plant-available forms.

VIII EFFECTS OF VERMICOMPOSTS ON PESTICIDE DEGRADATION IN SOILS

Vermicomposting is an efficient way of utilizing organic wastes; in addition, vermicomposts can be added to soil to improve plant productivity, via nutrient additions, suppress plant pathogens, or promote of plant hormone activity, as mentioned previously (see Chapter 9). Otherwise, the research program at the Department of Environmental Protection, EEZ, CSIC, in Granada found that the application of vermicomposts to soils restored much biochemical activity and increased the bacterial diversity in a soil contaminated with trichloroethylene (Moreno et al. 2009). They also found that application of vermicomposts from the winery industries increased the retention of the insecticide imidacloprid in 10 different soils (Fernández-Bayo et al. 2007) and that the sorption capacity of vermicomposts was lower for anionic herbicides than for hydrophobic pesticides (Romero et al. 2006). In these ways, they found that the amendment of soils with vermicomposts from wastes from the winery and alcohol industries reduced the persistence of two pesticides (diuron and imidacloprid) in soils, although this effect depended on the type of pesticide and soil (Fernández-Bayo et al. 2009). Their results suggested that the application of vermicomposts reduced the availability of diuron in soils (Fernández-Bayo et al. 2008). They did not find any correlation between effects of vermicomposts and enzyme activity, which suggests that these effects were mediated more by physicochemical, and not biological, properties of vermicomposts, as repeated by Delgado-Moreno and Peña (2007). These authors studied the degradation of three sulfonylurea herbicides (chlorsulfuron, prosulfuron, bensulfuron) in soils amended with raw olive cake and vermicomposts produced from it. Their results point to chemical rather than biological degradation, probably due to interactions between the three pesticides. The same trend was found for four triazine herbicides since only the addition of vermicomposts boosted the biological degradation rate of triazines (Delgado-Moreno and Peña 2008).

IX SIXTH INTERNATIONAL SYMPOSIUM ON EARTHWORM ECOLOGY

The Sixth International Symposium on Earthworm Ecology (ISEE) was held at the University of Vigo, Spain, on August 31–September 4, 1998. The history of these major symposia on earthworm ecology began in 1981, with the first symposium held in Grange-over-Sands, England (ISEE1). The next gathering of earthworm scientists was in 1985 in Bologna, Italy (ISEE2), then 2 years later in 1987 in Hamburg, Germany (ISEE3), followed by ISEE4 in 1990 in Avignon, France. In 1994, the ISEE shifted out of Europe for the first time, to Columbus, Ohio, in the United States (ISEE5). The following conferences were held again in Europe: In 1998 ISEE6 was held in Vigo, Spain, ISEE7 in 2002 in Cardiff, United Kingdom, and ISEE8 in 2006 in Kraków, Poland. In 2010, ISEE9 will be held in the city of Xalapa in the state of Veracruz (Mexico) from September 5 to September 10, 2010.

In 1998, 200 earthworm scientists from 39 countries met in the city of Vigo, Spain, for the first time. The symposium attracted scientists working on various aspects of earthworm biology and ecology. It gave an opportunity for people researching different disciplines to get together, giving a very stimulating boost to further international research and cooperation in earthworm ecology. During the symposium, 55 oral contributions were presented, accompanied by 165 posters. The symposium was divided into the following sessions:

Session 1. Earthworm biodiversity and biogeography: 5 oral presentations and 19 posters
Session 2. Influence of earthworms on soil organic matter and nutrient dynamics: 5 oral presentations and 19 posters
Session 3. Earthworm ecotoxicology: 6 oral presentations and 25 posters

Session 4. Earthworms in agroecosystems and land use: 9 oral presentations and 22 posters

Session 5. Earthworms and waste management: 7 oral presentations and 31 posters

Session 6. Earthworms and soil physical properties and function: 7 oral presentations and 7 posters

Session 7. Earthworm biology, ecology, and physiology: 9 oral presentations and 27 posters

Session 8. Interactions of earthworms with other organisms: 6 oral presentations and 16 posters

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