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Twenty years of the earthworm biotechnology research program at the University of Vigo, Spain

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ABSTRACT

The technologies of vermiculture and vermicomposting are well established and now a days there are several commercial enterprises in Spain. The Soil Ecology Laboratory at the University of Vigo works on a wide range of scientific aspects of this discipline and has been developing a comprehensive research program in earthworm biotechnology over the past 20 years, including many different aspects of earthworm biology and ecology, of the vermicomposting process and of the effects of the application of the vermicompost on crop protection. This paper summarizes the research on vermicomposting conducted in our laboratory.

Keywords: *Earthworm biotechnology, vermicompost, organic waste, greenhouse, nutrient cycling*

1.0 INTRODUCTION

The research program at the University of Vigo based on the vermicomposting of different types of organic waste 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 vermicompost on the growth of greenhouse and field crops, production of plant growth regulators during vermicomposting and aging and conservation of vermicompost.

2.0 EVALUATION OF ORGANIC WASTES FOR VERMICOMPOSTING

Since vermicomposting is a method of converting solid organic waste into an environmentally-friendly and valuable resource for crop production and soil improvement, we have evaluated the suitability of many different types of organic wastes for the process. We found that vermicomposting works very successfully for processing sewage sludge and biosolids from wastewater (Domínguez *et al*. 2000, 2003; Elvira *et al*. 1997; Plana *et al*., 2001), paper industry waste (Elvira *et al*. 1995a, 1995b, 1996b, 1997, 1998, 1999); urban residues, food and animal wastes (Aira *et al*. 2002, 2006a, 2006b, 2007a, 2007b, 2008, 2009a; Aira and Domínguez 2008, 2009, 2011; Atiyeh et al. 2000; Domínguez *et al*. 1996, 1997b,

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2001; Domínguez and Edwards 1997; Elvira *et al*. 1996a, 1996c; Gómez-Brandón *et al*. 2011a, 2011c, 2012; Lazcano *et al*. 2008; Monroy *et al*. 2009;) and food industry waste (Elvira *et al*. 1998, 1999; Gómez-Brandón *et al*. 2010, 2011b).

3.0 BIOLOGY OF EARTHWORMS SUITABLE FOR VERMICULTURE AND VERMI-COMPOSTING

Although almost 5,000 species of earthworms have been described, for the great majority of these species, we know only their names and morphologies, and little is known about their biology, life cycles or ecology. Certain epigeic earthworms, with their natural ability to colonize organic wastes; high rates of feedstock consumption, digest and assimilate organic matter; show tolerance to a wide range of environmental factors; short life cycles, high reproductive rates, and endurance and tolerance of handling, show good potential for vermicomposting. Few earthworm species display all these characteristics, and in fact only five have been extensively used in vermicomposting facilities: *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 these four earthworm species (Aira *et al*. 2002, 2007; Domínguez and Edwards 1997; Domínguez *et al*. 1997a, 2000, 2001, 2003, 2005; Domínguez 2004; Domínguez and Edwards 2004; Elvira *et al*. 1996a; Edwards *et al*. 1998; Monroy *et al*. 2003, 2005, 2006; Pérez-Losada *et al*. 2005; Porto *et al*. 2012; Tato *et al*. 2006; Velando *et al*. 2006, 2008). We also have studied the biology, ecology and life cycles of other earthworm species as *Lumbricus rubellus* and *Dendrodrilus rubidus* (Elvira *et al*. 1996c) and *Octodrilus complanatus* (Monroy *et al*. 2007). We are currently studying the lyfe cycles and ecology of a range of epigeic earthworm species and working in a laboratory screening programme looking for other species suitable for vermicomposting and vermiculture.

3.1 *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 most commonly used globally for the management of organic wastes, and also in ecotoxicology, physiology and genetics studies. 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 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 intra-specific 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; 2012). This evidence implies important considerations; in vermiculture or vermicomposting *E. andrei* is recommended more since its growth and reproduction rates are higher than *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 the 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.

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

Survival, reproduction and growth of earthworms can be critically affected by environmental conditions. We have studied the influence of: temperature, moisture content, ammonium content, population density, type of food, intra- and inter-specific competition on the life histories of four earthworm species most extensively used in vermicomposting (*Eisenia andrei*, *Eisenia fetida*, *Perionyx excavatus* and *Eudrilus eugeniae*) (Domínguez 2004; Domínguez and Edwards 1997, 2004; Domínguez *et al*. 1997, 2000, 2001; Elvira *et al*. 1996a,1997c; Edwards *et al*. 1998).

5.0 ECOLOGY OF VERMICOMPOSTING

5.1 Earthworms and microorganisms: disentangling the black box of vermicomposting

Vermicomposting systems sustain a complex food web in organic wastes that results in the recycling of the organic matter and release of nutrients it contains. Biotic interactions between decomposers (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 (Aira *et al*., 2002; Sampedro and Domínguez 2008; Gómez-Brandón *et al*., 2011b). 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, microarthropods as well as the large populations of earthworms (Monroy *et al*. 2006, 2008, 2011; Sampedro and Domínguez 2008; Domínguez *et al*. 2010).

Microorganisms are largely responsible for organic matter decomposition, but earthworms may also affect rates of decomposition directly by feeding on and fragmenting the organic matter which indirectly affect decomposition through interactions with microorganisms, basically involving stimulation or depression of microbial biomass and activity and enzymatic activity (Aira *et al*. 2007; Domínguez 2004; Domínguez *et al*. 2010; Gómez-Brandón *et al*. 2011a, 2011b, 2011c, 2012). We found that these processes are mainly dependent on earthworm population density, with significant decreases in microbial biomass and activity related to increasing numbers of earthworms and time (Aira *et al*. 2002, 2008; Gómez-Brandón *et al*. 2011a).

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 microbial biomass and activity enhanced but also mineralization rates are increased (Aira *et al*. 2007b, 2007c); moreover, we also found significant increases in fungal populations in this stage that was 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 to 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 *et al*. 2007a, 2007b, 2007c; Domínguez *et al*. 2010).

Thus, we can expect that microbial communities from manure to vermicompost should change markedly as we reported in a study on different animal manures and earthworm species (*Eisenia andrei, Eudrilus eugeniae and Lumbricus rubellus*); in this way, fungal biomass increased significantly in horse manure vermicomposted by *L. rubellus* and in cow manure vermicomposted by the 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 obtained with the three earthworm species (Lores *et al*. 2006; Gómez-Brandón *et al*. 2011c, 2012). Surprisingly, we found a strong general effect of earthworm species, since the microbial communities of vermicomposts produced by each earthworm species were very similar, independently of the 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 (Domínguez *et al*. 2010; Gómez-Brandón *et al*. 2011c,2012; Lores *et al*. 2006).

Results from analyzing fresh earthworm casts and their parent raw manure, showed increases in microbial biomass and decreases in microbial activity (Aira *et al*. 2006a; Aira and Domínguez 2009; Gómez-Brandón *et al*. 2011c); these indicate that the direct effects of *E. fetida,* produce changes in microbial populations that can influence the overall dynamics of organic matter degradation. The decreases in microbial activity can be attributed to reductions in organic C and N in the wastes (Aira and Dominguez 2008). However, the analysis of the gut contents of several epigeic earthworm species revealed no changes in bacterial numbers or microbial activity (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 vermicompost into animal manures modified the microbial community functions, separating clearly microbial communities depending on the type of vermicompost, inoculation dose and time of incubation. These changes were all in the same direction, first an increase and then a decrease. The changes in microbial communities and those found in the vermicomposting experiment suggested that the indirect effects of earthworms are able to alter the dynamics of animal manures decomposition (Aira and Domínguez 2011). However, the extent of these effects was not as great as those we found during vermicomposting; this, together with the results of the earthworm casting experiment suggest the existence of other factors governing the relationships between earthworms and microorganisms that established during vermicomposting.

5.2 Stimulation and acceleration of microbial decomposition by earthworms during vermin-composting

Nutrient mineralization is governed directly by the activities of bacteria and fungi and these activities are strongly affected by soil inverteb rates 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. These indirect effects include 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 accelerate the rates of organic matter decomposition during vermicomposting significantly (Aira and Domínguez 2008a,b, Aira *et al*. 2006b, 2007a, 2007b, 2008;Atiyeh *et al*. 2000, Domínguez 2004; Domínguez *et al*. 2003; Gómez-Brandón *et al*. 2010, 2011a, 2011b, 2011c, 2012). Although earthworms can assimilate carbon from the more labile fractions of organic wastes, their contribution to the total heterotrophic respiration is relatively low due to their 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 favour nitrification, resulting in the rapid conversion of NH_4-N into $N0_3$-N (Aira *et al*. 2008; Aira and Domínguez 2008b; Atiyeh *et al*. 2000; Domínguez 2004; Lazcano *et al*. 2008).

6.0 VERMICOMPOSTING AND HUMAN PATHOGEN DESTRUCTION

We found that earthworms greatly decreased the presence of total coliforms during vermicomposting. Thus, the passage through the gut of the earthworm species *Eisenia andrei*, *Eisenia fetida* and *Eudrilus eugeniae* reduced the population densities of total coliforms by 98%, relative to those in fresh pig slurry (Monroy *et al*. 2008). We also found the same drastic reductions in the population density of total coliforms in another experiment after two weeks of vermicomposting with *E. fetida* (Monroy *et al*. 2009). Finally, in an industrial-scale experiment we also found a selective reduction of the pathogenic load of cow manure. Thus there was not any modification for *Clostridium,* total coliforms and *Enterobacteria*, whereas levels of faecal enterococci, faecal coliforms and *Escherichia coli* were reduced to acceptable levels (Aira *et al*. 2011).

7.0 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 1998). 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 into 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 *et al*. 2009a, 2009b, 2010a, 2010b, 2011a, 2011b). These investigations have demonstrated consistently 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, vermicomposts 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.

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