Chapter 2
State-of-the-Art and New Perspectives on Vermicomposting Research: 18 Years of Progress

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Abstract Vermiculture and vermicomposting are well-established technologies and nowadays constitute a thriving industry that is becoming increasingly important throughout the world. Members of the Soil Ecology Laboratory at the University of Vigo have been studying a wide range of scientific aspects of this discipline and have developed a comprehensive vermicomposting research programme over the past 30 years. This research has included many different aspects of earthworm biology and ecology, the vermicomposting process, and the use of vermicompost for improving plant growth and health. This chapter summarizes the research on vermicomposting conducted in my laboratory, and it represents an up-date of the original text entitled "State-of-the-Art and New Perspectives on Vermicomposting Research", written in 2004 and included in the book Earthworm Ecology. Here, I synopsize the main advances and current state of the art after 18 years of continuous progress in scientific, technical, and industrial-commercial vermicomposting endeavours, illustrating the coming of age of this discipline.

Keywords Enzyme activity · Bioremediation · Earthworm biology · Gut-associated processes · Industrial waste

2.1 Introduction

At the end of the twentieth century, vermicomposting—the transformation of organic waste into a humus-like material called vermicompost mediated by the synergistic actions of earthworms and microorganisms—began to be considered an attractive alternative to thermophilic composting for recycling and conversion of organic matter into organic fertilizers and soil-improving amendments that can be used in horticulture and agriculture. Vermicomposting is now a well-established

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H. A. Mupambwa et al. (eds.), *Vermicomposting for Sustainable Food Systems in Africa*, Sustainable Agriculture and Food Security,
https://doi.org/10.1007/978-981-19-8080-0_2

technology and constitutes a thriving industry that is becoming increasingly important throughout the world.

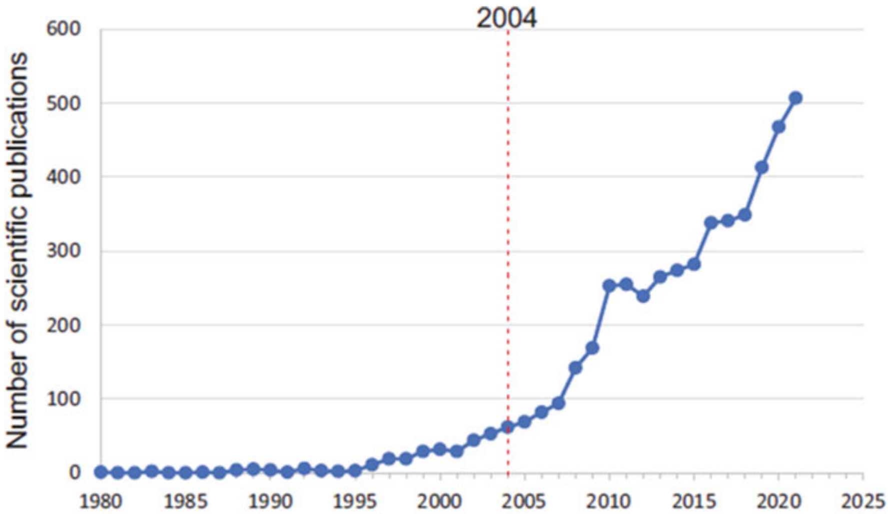
The Soil Ecology Laboratory (Group of Animal Ecology, GEA) at the University of Vigo (Spain) has conducted comprehensive research on vermicomposting over the past 30 years, studying a wide range of scientific aspects of this discipline, including earthworm biology and ecology, the characterization, functioning and ecology of the vermicomposting process, and the effects of vermicompost on soil and plants. This chapter aims to summarize the research on vermicomposting conducted in my laboratory, and it represents an up-date of the original chapter entitled "State-of-the-Art and New Perspectives on Vermicomposting Research", written in 2004 and included in the book Earthworm Ecology. In this new chapter, I aim to synopsize the main advances and current state of the art after 18 years of continuous progress in scientific, technical, and industrial-commercial aspects of vermicomposting, illustrating the coming of age of this discipline.

Results of an internet survey conducted using three bibliographic search engines (Scopus®, Web of Knowledge®, Google Scholar) and one general search engine (Google Search) revealed that major developments in vermicomposting occurred during the first quarter of the twenty-first century (Fig. 2.1). The main figure shows the number of scientific reports published every year on vermicomposting, with the first document found in the bibliographic search of papers published in 1980. The table shows the output of Google Scholar searches (also academic results but with more wide-reaching information, with 1490 results before 2004, and 24,300 results in total) and the total output of Google Search (1580 results before 2004, and 1,060,000 results in total).

In short, vermicomposting has expanded enormously at various different levels: home application for the management of family or individual household waste, application on farms and in different industrial facilities associated with the agri-food sector, and industrial implementation with the development of large, important companies worldwide.

2.2 Vermicomposting Technologies

Vermicomposting technologies vary depending on the scale of application, ranging from very simple, low technology methods, such as containers, waste heaps and windrows, to medium-scale, moderately complex systems and to automated continuous flow vermireactors. Today, vermicomposting is basically applied at three scales: household or educational; farms and primary sector companies; and industrial level.



	Google Scholar	Google
Before 2004	1490	1580
Total	24300	1060000

Fig. 2.1 Development of vermicomposting during the last quarter of the twentieth century and the first quarter of the twenty-first century. The search was conducted (March 2022) using three bibliographic engines (Scopus®, Web of Knowledge®, Google Scholar) and one general search engine (Google Search). The dotted line indicates the year (2004) in which the original chapter "State-of-the-Art and New Perspectives on Vermicomposting Research" was published in the book Earthworm Ecology

2.3 Vermicomposting: A Brief Definition

Vermicomposting is the transformation of solid organic waste into vermicompost, which is an excellent biofertilizer. The transformation involves an enhanced bio-oxidation process in which the interactions between epigeic earthworms and microorganisms accelerate the decomposition and stabilization of organic matter and substantially modify the physical, chemical and biological properties of the organic waste. Although earthworms are crucial in the process and drastically alter the biological activity, microorganisms produce the enzymes responsible for the biochemical decomposition of organic matter. The continuous grazing of earthworms on fungi and bacteria reduces microbial biomass and greatly modifies the structure of microbial communities. Decomposition is accelerated during vermicomposting by the huge increase in microbial activity induced by earthworm movements.

2.4 Vermicomposting Earthworms

Earthworms are hermaphroditic, iteroparous soil-living animals with indeterminate growth. Different earthworm species have different life histories and ecological strategies, occupying different soil niches. Among the more than 7000 species described to date, very few (~6) are suitable for vermicomposting. In vermicomposting systems, pure organic matter acts as both the habitat and food, and as soil is not involved, only epigeic earthworms can be used in the process. The earthworm species used in vermicomposting are generally those mentioned in the original chapter (from 2004), and there have been few changes in this respect since then. The earthworms used in vermicomposting are the temperate species *Eisenia andrei* (Bouché 1972) and *Eisenia fetida* (Savigny 1826) and the tropical species *Eudrilus eugeniae* (Kinberg 1867) and *Perionyx excavatus* (Perrier 1872). The biology, life cycle and growth and reproduction rates of these earthworms have been widely reported. Among these species, *E. andrei* and *E. fetida* are the most frequently used, with *E. andrei* being the most widespread and common in vermicomposting companies and farms worldwide. Although it has not yet been generally accepted that *E. fetida* and *E. andrei* are different species, this has been demonstrated and is now recognized by most specialists (Fig. 2.2).

In 2004, it was emphasized that *Eisenia* spp. are temperate organisms, and that the use of tropical species, in particular *P. excavatus* and the African nightcrawler *Eudrilus eugeniae*, would potentially be useful in tropical regions. Although experimental data indicate their aptitude and good performance in vermicomposting, neither of these species has yet replaced *Eisenia* as the queen of vermicomposting worldwide, in all climates, ranging from continental, cold, mid-latitude and temperate climates to equatorial and tropical zones. Some interesting initiatives have been made to find other alternatives, with local species that would at least closely match the quality and aptitude of *Eisenia*. *Dichogaster annae* has been found in some vermicomposting facilities in Brazil, but little is known regarding its life cycle and distribution. *Eudrilus eugeniae* is an African species that, because of its size and life cycle, is more suitable for vermiculture than for vermicomposting. Other epigeic earthworm belonging to the family Eudrilidae, *Hyperiodrilus africanus*, has been also found in compost heaps and litter layers of high organic soils in western and central Africa. However, to date no earthworm species that is more suitable for vermicomposting than *Eisenia* has been found.

2.5 Materials Used in Vermicomposting

In 2004, vermicomposting was basically considered an alternative method of waste treatment. In fact, the first scientific studies focused on the treatment of sewage sludge and animal manures. Many types of waste required pretreatment or conditioning, sometimes by composting, before being acceptable to earthworms. In the

Fig. 2.2 *Eisenia andrei* (above) and *Eisenia fetida* (below) are different earthworm species

intervening years, we have learned that vermicomposting is effective and can be applied to any degrading organic material in a solid or semi-solid state (up to 90% moisture), and that acclimatization or pretreatment of the materials is not necessary.

Of course, thousands of different situations can exist depending on the type of farm and scope, clearly differentiating at least the three levels mentioned above (home vermicomposting for processing domestic waste, medium-scale vermicomposting for management of waste from agro-livestock or agri-food farms, and large-scale, industrial vermicomposting, mainly conducted by waste treatment companies). In medium and large scale operations, the materials processed are usually homogeneous, and mixtures of different materials are generally only processed in domestic vermicomposting. This is important, as we have learned that earthworms become adapted to certain diets, or in other words process homogeneous physical, chemical and microbiological materials, and they do not grow or reproduce as well when these characteristics vary widely and unpredictably.

2.6 Environmental Conditions of Vermicomposting

For maximum efficiency in vermicomposting, very dense earthworm populations must be maintained. This can only be achieved by maintaining ideal conditions for earthworm growth and reproduction. In general, the moisture content of the substrate is a critical factor. The metabolic rates of both earthworms and microorganisms are determined by water availability, so that if the moisture content is too low, both earthworm and microbial activity will be greatly reduced. By contrast, excessively high moisture levels (above 90%) can lead to anaerobic decomposition, and in the case of materials that are dense or have small particle size, the vermicompost will be sludgy and remain wet, forming rocky material that will be difficult to handle, preserve, transport and apply. The optimal moisture content for vermicomposting ranges between 75 and 85%.

Temperature is also important and clearly influences the vermicomposting process. *Eisenia* spp., which as mentioned are the most widely used species in vermicomposting, are temperate species from mid latitudes and develop well at between 15 and 25 °C. At higher temperatures, growth of these species is greatly affected by loss of water, which slows down the process or even leads to death of the earthworms. Temperature must therefore be carefully monitored and controlled. The worms tolerate temperatures below 10 °C, although their metabolism, growth, and reproduction decrease, and vermicomposting thus slows down considerably.

Other environmental factors or physical and chemical conditions will also influence the life cycle of the worms, i.e. their growth and reproduction, as in the case of animals that breathe through their skin and are therefore very sensitive to gas exposure. In 2004, information was already available about the optimum ranges and tolerance to these important variables, including pH, ammonia nitrogen and ammonium gas, and the salt content of the substrate. More data have since been published in relation to the ranges of tolerance of earthworms to other environmental parameters, mainly physical-chemical but also biological. However, I do not think it relevant to report this information here and I believe that the integral management of the vermicomposting system and the care and maintenance of the matrix structure are more important in the present context. As already mentioned, vermicomposting earthworms live in the same substrate that serves as food. The ideal conditions for vermicomposting systems include a healthy matrix and appropriate physical and chemical conditions. The fresh material, i.e. the waste or by-products, to be processed is added to this matrix (or beside it in lateral systems), so that earthworms will process it on demand. When the conditions are suitable and the food is supplied ad libitum or in excess, the population begins to increase and will do so almost exponentially until the carrying capacity or maximum level (more than 20,000 worms per square metre in our pilot studies) is reached. The environmental conditions of the system should then be maintained and the food material should be added continuously for processing by the worms. Maximal rates of waste processing, which are very high, can thus be reached.

The key steps in effective vermicomposting are as follows: (1) addition of the parent material to the surface in thin layers, at frequent intervals, allowing earthworms to move up and concentrate in the upper 15–20 cm of the vermicomposting matrix and to continue to move upwards with the addition of new layers. Vermicomposting operations can be mechanized with a suitable balance between mechanization costs and labour savings; (2) maintenance of aerobic conditions and optimal moisture and temperature ranges in the substrate, avoiding addition of materials with excessive amounts of salts. The addition of fresh materials in thin layers prevents overheating through thermophilic composting, although the heat generated is usually sufficient to maintain suitable temperatures for earthworm growth during cold winter periods. Thus, to maintain a reasonable temperature, vermicomposting should be done under cover; additional heating is not necessary if the waste addition is managed well with application of thicker layers during cold periods to provide some heat derived from thermophilic composting. The temperature can be reduced in summer by using fans or water misting cooling systems.

2.7 How Vermicomposting Works: Stages of the Process

In the chapter written in 2004, a move from the classical approach of considering vermicomposting as the mere addition of earthworm to organic waste, to an approach differentiating two different processes, gut-associated processes (GAP) and cast-associated processes (CAP), was proposed. Vermicomposting involves an active phase, in which earthworms are critical, and a maturation phase, which takes place once the worms leave the substrate, and where microorganisms are the key players. The active phase comprises all of the processes associated with the passage of the waste through the earthworm intestines (GAPs: gut-associated processes). In the maturation phase, earthworm casts begin ageing, while the associated microbial communities experience further turnover (i.e. cast-associated processes, CAPs) (Fig. 2.3). In a prospective study, we recently found that (1) most of the bacterial (96%) and fungal (91%) taxa were eliminated during vermicomposting of sewage sludge, mainly through gut-associated processes (GAP), and (2) that the modified microbial communities in the earthworm casts (faeces) later undergo a process leading to more diverse microbiota than those found in the original sewage sludge (Fig. 2.3).

2.8 Fate of Human Pathogens During Vermicomposting

Animal manures and slurries, sewage sludge and biosolids are generally recycled and applied to agricultural land as the most economical and environmentally sustainable means of treatment and reuse. These materials are valuable as fertilizers and can help maintain soil quality and fertility. However, as they often contain

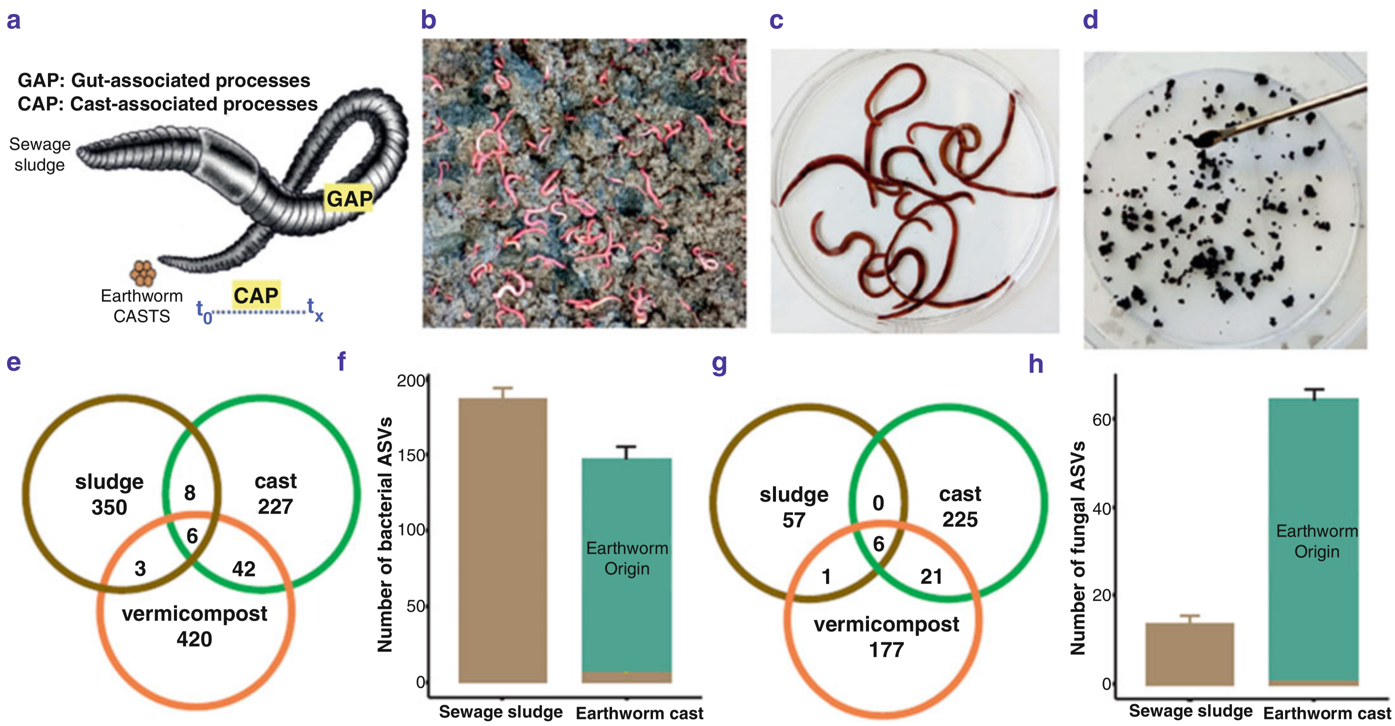


Fig. 2.3 (**a**) GAP and CAP processes during vermicomposting of sewage sludge, (**b**) Earthworms (*Eisenia andrei*) living and feeding in fresh sewage sludge in the vermireactor, (**c**) Sampling dishes used to collect fresh earthworm cast samples. (**d**) Fresh earthworm casts collected from sampling dishes. Changes in richness and diversity of bacteria and fungi during vermicomposting of sewage sludge. Venn diagrams showing the absolute number of (**e**) bacterial and (**g**) fungal ASVs found in sewage sludge, fresh earthworms casts and vermicompost (3 months old). Effect of GAP (gut-associated processes) on the richness and diversity of (**f**) bacteria and (**g**) fungi. Modified from Domínguez et al. (2021)

pathogenic microorganisms, spreading them on land can lead to pathogens entering the food chain. Therefore, controlling and reducing the levels of human pathogens in animal waste before this is applied to agricultural land will reduce contamination of soil and the surrounding water by pathogens.

Earthworms can reduce the levels of different human pathogens in animal waste via gut-associated mechanisms. The degree of reduction largely depends on the earthworm species and/or the pathogen considered. However, the mechanism and magnitude of this selective elimination during the processing of large amounts of sewage sludge in short vermicomposting processes are currently not very well known.

Elimination of human pathogens during the vermicomposting process is a key part of the process and crucial for its industrial application. Studies in our laboratory have revealed drastic reductions in coliform bacteria (including *E. coli*) during the vermicomposting process. There is also some evidence, which must be demonstrated at larger scales, that the vermicomposting process eliminates or drastically reduces the presence of enteric viruses and eggs of intestinal parasites. However, the biological mechanisms linking the presence of worms to the elimination of pathogens are not fully known. In addition to confirming this elimination, it is essential to determine how long it takes for pathogens to be eliminated from sewage sludge and animal wastes and to determine the underlying mechanisms, the triggering factors and when and how and under what circumstances of the process these occur.

Several studies have reported important reductions in microbial human pathogens during vermicomposting of sewage sludge and animal manures. We have found that earthworm activity, mainly during the gut-associated processes, is a critical factor leading to the rapid reduction of pathogens during vermicomposting (see previous section). The mechanisms involved in reducing or eliminating microbial pathogens may include direct effects of physical disruption during grinding in the earthworm gizzard, microbial inhibition by antimicrobial substances or microbial antagonists produced by the earthworms themselves, and destruction of microorganisms by enzymatic digestion and assimilation.

2.9 Vermicompost Properties

Vermicompost, the final product of vermicomposting, is known commercially as earthworm humus, earthworm castings or vermicasts. It is very fine, porous, biologically stable material with a high water retention capacity, low C/N ratio, high content of nutrients in forms that are easily assimilated by plants. It also includes a rich, complex microbial community with a wide range of beneficial effects to soil-plant systems.

Vermicompost improves soil health and fertility, increases the nutrient content and microbial content of soils, improves water retention and reduces the need for fertilizers and pesticides.

Vermicompost and liquid derivatives have been shown to increase the growth and productivity of many crops. These effects seem to be independent of the chemical nutrients provided and could be the outcome of biological mechanisms derived from microbial activity. The effects of vermicompost on plant growth vary depending on the plant species (and even the variety) considered, as well as the starting material, production process, storage time, and type of soil and potting medium. In studies by our group using next-generation sequencing (NGS) approaches, we have found that different microbial communities emerge during vermicomposting depending on the earthworm species and the starting material, which may imply different biological properties and functional capacities for stimulation of plant growth. The research carried out to date highlights the enormous complexity of vermicompost-plant interactions, and more detailed studies will make important contributions to organic and ecological agriculture and to soil ecology, allowing us to further unravel the complex web of relationships between plants and soil microbial communities.

2.10 Vermicomposting and Enzymatic Activity

The breakdown of macromolecules (e.g. cellulose, hemicellulose, lignin and tannins) during vermicomposting requires the action of various extracellular enzymes, most of which are produced by microorganisms. Changes in enzyme activities during vermicomposting have been extensively studied with the aim of elucidating the biochemical interactions between earthworms and microorganisms during the decomposition of organic matter. Vermicompost contains high amounts of extracellular enzymes involved in nutrient cycling (e.g. phosphatases, glucosidases, cellulase, protease and ureases) and in the degradation of organic pollutants (e.g. laccases, peroxidases and carboxylesterases). We have found that microbial communities are greatly altered by the vermicomposting process, and that the final vermicompost contains a high diversity of bacteria and fungi. Thus, vermicompost has a high load of extracellular enzymes, which are stabilized by organic matter. Recent studies in our laboratory have revealed very high enzymatic activities, including carboxylesterase activity, in vermicompost derived from different types of organic waste. In addition, the gradual increase in the concentrations of humic substances involved in vermicomposting provides some chemical support for the binding of extracellular enzymes, making them more stable and protecting them from proteases or adverse conditions such as temperature changes and desiccation. Therefore, unsurprisingly, the activity of extracellular enzymes remains very high in the final vermicompost. It is important to determine the biological properties of the final vermicompost after drying or ageing, to improve its conservation, management and application.

2.11 Vermicomposting and Bioremediation

The capacity of organisms to accumulate, adsorb, and/or degrade pollutants has led to their potential use for remediation or treatment of contaminated environments. Soil bioremediation involves any process in which a biological system is used to remove environmental pollutants from soil. Natural bioremediation is mediated by native organisms and is often a slow process. With the aim of speeding up this process and accelerating the biodegradation of environmental contaminants, there has been a tendency to focus on biostimulation, which involves the inoculation of non-native microorganisms and the addition of nutrients and other chemicals to increase microbial growth.

The use of vermicomposting and vermicompost for bioremediation purposes is not widespread, although vermicompost is basically organic matter that is rich in chemical and biological nutrients and microorganisms, and that therefore promotes and enhances microbial growth (biostimulation). The synergistic actions of earthworms and microorganisms during vermicomposting enhances detoxification processes and accelerates the removal of pollutants from all types of organic waste. Vermicompost is a microbiologically active substrate, and thus its addition to soil is a way of inoculating the soil with non-native microorganisms that can degrade organic pollutants.

Bioremediation can be done in situ, to treat contaminated sites directly, or ex situ, to treat substrates that have been removed from contaminated sites. In the case of vermicomposting, bioremediation can be achieved in both ways, by applying vermicompost in situ and by processing contaminated materials ex situ to eliminate or reduce the pollutant loads. The vermicomposting process can be considered an ex situ bioremediation process in the sense that it eliminates or reduces the possible pollutant potential of the treated materials. Although less common, in situ bioremediation can be carried out by applying vermicompost as a source of microorganisms, with the aim of decontaminating soil.

The concentration of heavy metals in the substrate increases during vermicomposting because of the loss of mass. However, the bioavailability of the metals decreases since they are sequestered in organo-mineral complexes with humic acids and other polymerized organic fractions. Vermicompost is therefore a good sorbent and can reduce the bioavailability and toxicity of metals when applied to the soil or used in biofilters to detoxify wastewater.

Characteristics such as the high organic matter content and high microbial abundance and diversity, together with the presence of pollutant-detoxifying exoenzymes, make vermicompost an ideal substrate for the bioremediation of contaminated soils. Vermicompost is an organic carbon-rich substrate, and although the total amount of organic matter in the vermicompost is lower than in the original feedstock, the content of humic substances is higher. These humic substances facilitate the formation of metal-humic complexes and the adsorption of organic contaminants. Vermicompost contains a high diversity of microorganisms and extracellular enzymes, and the rate of biodegradation of pollutants is therefore expected to increase in soils treated with vermicompost. These bioaugmentation properties have been addressed in several studies.

Some studies performed in our laboratory have reported the existence of a high level of carboxylesterase activity in vermicompost derived from different types of organic waste. The enzymatic activity is variable, and the rate largely depends on the substrate used and the earthworm activity. Vermicompost can bind organic pollutants, indicating its strong bioremediation power. The incorporation of vermicompost in topsoil could provide a molecular and biochemical barrier reducing the movement of the contaminants and enhancing their biodegradation. Although significant advances have been made in different research laboratories, the use of vermicompost for the bioremediation of contaminated soils and to prevent the effects of pesticides and other organic contaminants on soil function require further research.

In the case of organophosphorus pesticides, carboxylesterase-mediated detoxification involves the formation of a stable enzyme-pesticide complex by the direct interaction between the organophosphorus molecule and the active site of the enzyme. The findings of experiments conducted in our laboratory to study carboxylesterase activity in vermicompost suggest that this enzyme irreversibly binds the organophosphorus chlorpyrifos-oxon, acting as a molecular scavenger of this type of pesticide.

To assess the enzymatic bioremediation potential of vermicompost, we have explored the stability of the carboxylesterase activity in the vermicompost in response to desiccation and ageing. We have found that the esterase activity is very stable, probably due to strong interactions with the humic substances in the vermicompost. This chemical binding protects and stabilizes the enzymes against physicochemical and biological degradation.

Another promising application is the vermicomposting of sludge derived from wastewater treatment (WWTP sludge) as a tertiary treatment to accelerate the degradation of contaminants, including emerging pollutants, antibiotic resistance genes and microplastics.

The amount of sewage sludge generated in wastewater treatment plants is increasing steadily, with hundreds of million tons produced every year all over the world. Microplastics are small (<5 mm diameter) synthetic polymer wastes, derived from a wide range of sources, including clothing, personal care products, and polymer manufacturing and processing industries. The widespread use of plastics, coupled with their resistance to degradation, leads to their accumulation and adverse impacts on the environment. Plastics can adsorb pollutants, such as polycyclic aromatic hydrocarbons, heavy metals, polybrominated diphenyl ethers, and pharmaceutical substances, and may cause chronic toxicity by bioaccumulation in organisms.

Wastewater treatment plants are main sinks of microplastics derived from daily human activities. These plants are quite effective in removing microplastics from the water preventing them from entering natural aquatic systems at this stage. However, during the treatment, the microplastics are retained in the sewage sludge. Land-applied sludge is thus a main source of terrestrial contamination with microplastics, which subsequently enter natural aquatic systems. Therefore, it is important to establish ways of removing plastics from sewage sludge and of assessing the environmental impact of land-applied sludge in terrestrial and aquatic ecosystems. Regulations on the use of sewage sludge and biosolids in agriculture stipulate limits for the contents of human pathogens, and maximum rates of application of metals and nutrients to the soil. Concentration limits vary for all contaminants, and contamination by microplastics has not yet been addressed.

Rates of degradation of plastics and biopolymers are limited by the hydrolysis of ester bonds, and extracellular microbial enzymes (including carboxylesterases) catalyse the hydrolysis of plastic polymers. During vermicomposting the drastic changes that take place in the structure and function of the microbial communities are concomitant with significant increases in extracellular detoxifying enzymatic activity. This activity should trigger and accelerate the enzymatic degradation of plastics. The same approach could potentially be applied to other families of contaminants, such as antibiotic resistance genes, human pathogens and emerging pollutants, which are not usually monitored but can enter terrestrial ecosystems and ultimately aquatic ecosystems causing environmental and health-related problems.

2.12 Conservation of Vermicompost and Processing Strategies

Vermicompost is very rich in microorganisms, and the main qualities that make it a superior fertilizer are its excellent biological properties. These properties have been related to nutrient cycling in the soil or plant growth substrate, and also to the provision of bioactive substances that act as plant growth enhancers (including precursors of auxins, gibberellins and cytokinins, enzymes, humic acids, and beneficial microorganisms and their metabolic products).

The moisture content of vermicomposting substrates should be between 75 and 85% during the process; however, the final moisture content varies greatly, depending on the starting material from which the vermicompost is made. Very wet vermicompost is difficult to store and very expensive to transport (because of its weight) and is also difficult to handle and apply. The moisture content obviously also has an important impact on the price of vermicompost when sold by weight, and vermicompost is therefore generally sold by volume. One of the most difficult technical obstacles to resolve during vermicompost production is drying the material. In most vermicomposting facilities, vermicompost is dried outdoors to reduce the initial water content, from about 75–85% to about 45–50%; however, this is a costly, slow process. It is commonly argued that dry vermicompost can be difficult to re-wet, with significant loss of its biological properties. However, further studies are necessary to confirm this finding.

2.13 Vermicomposting as an Ecological Engineering Technique for Improving Soil Health and Sustainability in Vineyards: A Case Study

Grape is the largest fruit crop in the world, with more than 7 million ha of harvested area and an annual worldwide production of about 80 million tonnes, around 80% of which is used to make wine. The wine sector is very important in economic, social and environmental terms. Winemaking generates millions of tons of grape marc annually as a by-product. Grape marc (also known as bagasse and pomace) consists of the skin, pulp, and seeds that remain after pressing the grapes to obtain the must for making wine. It is a valuable resource and is used to produce ethanol, grape seed oil, bioactive compounds, and animal feed. It can also be used as a nutrient-rich organic soil amendment; however, when applied to soils without prior treatment, it can damage crops due to its acidity and high content of phytotoxic polyphenols. Excessive accumulation of this waste at specific times and in particular areas is problematical, leading to inadequate management, including uncontrolled disposal, dumping on fields without prior treatment, burning and other environmentally unfriendly solutions.

Vineyards are usually managed intensively to maximize wine production, which reduces soil biodiversity, decreases soil fertility and causes environmental problems such as changes in primary production and nutrient cycling, reduction of above-ground biodiversity, high soil erosion rates, groundwater eutrophication and contamination, and global warming.

Soils are among the most biodiverse habitats on Earth, with extremely diverse and complex biological communities that play a key role in the functioning of natural ecosystems. Although soil fauna has a strong influence on soil ecosystems and regulates many important processes, the key steps in the cycling of the main elements are governed by microorganisms. Intense land use generates serious environmental problems and interferes with soil biological processes. Intensive agriculture, which involves conventional tillage and massive, repeated fertilization and pesticide application—and which is associated with low plant diversity—has very negative effects on soil biodiversity, promoting simpler food webs composed of smaller organisms and fewer functional groups. Less diverse communities have been shown to be less resilient to stress than more diverse communities owing to changes in functional capacities. Therefore, intensification of agriculture and the consequent biodiversity loss together lead to environmental problems, such as changes in primary production and nutrient cycling, reduced surface biodiversity, eutrophication of water bodies, and global warming. However, production levels are lower in most sustainable systems than in intensive systems, and they must therefore be optimized.

The discrepancy between high agricultural yields and ecosystem sustainability can only be overcome by major modifications to ecosystem processes. While "green revolution" approaches focus on external manipulation, the internal manipulation of ecosystems has an enormous potential to improve yields, but with fewer

Fig. 2.4 Summary of the ongoing "EWINE" project, started in 2012, and undertaken to close a cycle in which grape pomace is converted into vermicompost, which is then applied to the vineyards as a biostimulant treatment

environmentally negative consequences. Vermicomposting accelerates the decomposition and stabilization of organic matter, substantially modifying the physical, chemical, and biological properties. Vermicompost, the final product of vermicomposting, is a biologically stable, very fine, porous material with a high water retention capacity, a low C/N ratio and a high nutrient content. It also contains complex microbial communities that have a wide range of beneficial effects on the soil-plant system. Vermicompost and its liquid derivatives have been shown to increase the growth and productivity of many crops. These effects occur independently of the nutrients provided and may be due to biological mechanisms derived from the associated microbial activity.

Based on those premises, in 2012, we started the current "EWINE" project, in which the combined and synergistic activity of earthworms and microorganisms during vermicomposting are studied for development of an integrated cycle to convert the waste generated by the wine industry into vermicompost, which is applied to the vineyards as a biostimulant treatment (Fig. 2.4).

Pilot-scale vermicomposting of the residual by-products generated in the wine industry (mainly bagasse and bagasse distilled from white and red grapes) was applied and studied with the aim of producing a high quality vermicompost which meets the requirements demanded by the legislation regulating the production of fertilizers in general, and sustainable, organic and ecological agriculture in particular. The biofertilizer and biopesticide effects of the vermicompost were studied in vineyards in two different geographical areas and two different appellations of origin.

Raw and distilled grape marc derived from white and red wine varieties were processed in pilot-scale vermireactors, yielding a high quality organic, polyphenol-free fertilizer and grape seeds. Earthworm population density and structure and earthworm biomass were monitored or determined monthly. Samples of the processed grape marc and the vermicompost were collected periodically and analysed to determine their chemical and biological properties.

Vermicomposting substantially reduced (by 50–70%) the biomass of grape marc, and the process yielded a nutrient-rich, microbiologically active and stabilized peat-like material that is easily separated from the seeds by sieving. The separation and removal of the seeds eliminates the residual polyphenol-associated phytotoxicity in the vermicompost. The seeds can then be easily processed to obtain polyphenol-rich extracts and fatty acid-rich seed oil. Moreover, the vermicomposting process produces large numbers of earthworms that can be processed as fish bait and as a source of protein for animal feed.

We have also studied the effects of earthworms on the process and the changes during vermicomposting of important biological parameters in microbial communities, such as their abundance, composition, structure, diversity, and activity.

The efficacy of vermicomposting as a low-cost, environmentally safe solution for the treatment and valorization of raw and distilled grape marc, together with an in-depth characterization of the final vermicompost, including its chemical, biochemical and microbiological properties is presented in detail in Chap. 6 of this book.

The vermicompost derived from the different wine varieties was applied in solid and liquid formulations to the grapevines in the vineyards where the grapes were harvested to make wine, and the grape marc was obtained.

The effects of the vermicompost on the vineyard soil and in the grapevines were studied during several crop seasons in different vineyards of two different denominations of origin in two biogeoclimatic areas: Rías Baixas, a Spanish *Denominación de Origen Protegida* (DOP) (Protected Designation of Origin) for wines located in the southwest of Galicia (Spain) and Ribeira Sacra, a DOP for wines from the south of the province of Lugo and in the north of the province of Ourense, Galicia (Spain). The vineyard experiments were conducted in the commercial Albariño vineyard *Terras Gauda*, located in O Rosal, Pontevedra (*Vitis vinifera* cultivar Albariño, DOP Rías Baixas), and in the commercial Mencía vineyard *Adegas Moure*, located in A Cova, O Saviñao, Lugo (*Vitis vinifera* cultivar Mencía, DOP Ribeira Sacra).

Fertilization with vermicompost significantly improved grape production in the two denominations of origin and in the two biogeoclimatic areas. We have collected data on nine vintages or harvest between 2014 and 2022, and although the results vary depending on the year, region, grape variety, and age of the plants, in all cases grapevines treated with vermicompost produced significantly more grapes than untreated grapevines.

In the wineries where the vineyard experiments were conducted, wine was made from grapes fertilized with vermicompost derived from grape marc. *Terras Gauda* (Albariño) and *Adegas Moure* (Mencía) made wines elaborated with grapes from vines fertilized with vermicompost derived from grape marc, and control wines elaborated with grapes from vines from the same experimental plot but not treated with vermicompost. Otherwise, both wines were made following the same standard procedures of the wineries.

Blind wine tastings carried out at both wineries revealed notable differences in organoleptic properties (e.g. overall increased complexity, expression, freshness and balance, with better visual intensity and taste persistency) that led to the wine made using grapes from the treated vines better ratings and reviews.

We used metataxonomic approaches to characterize the bacteriota and mycobiota of the must and finished wine from Albariño and Mencía grapevines treated with vermicompost derived from grape marc and controls (standard fertilization) during 2 consecutive years. We found statistically significant, important differences in composition, structure, and predicted metabolic functions of the microbiota.

Our findings suggest an important beneficial role of vermicompost supplementation in the vineyards, improving grape productivity and quality of the wine.

The research has led to important advances in the application of vermicomposting technologies in the wine sector. Both wineries where the experiments have been performed have built vermireactors and they currently process all of their grape marc by vermicomposting and apply the vermicompost to the vineyards.

2.14 Conclusion

This chapter summarizes research on vermicomposting conducted in the Soil Ecology Laboratory, University of Vigo, and it represents an up-date of the original text entitled "State-of-the-Art and New Perspectives on Vermicomposting Research", written in 2004 and included in the book Earthworm ecology. The detailed research has been published in scientific articles that can be consulted in the research group's webpage (http://jdguez.webs.uvigo.es/).

Acknowledgements For their contributions to the vermicomposting research programme developed by the Soil Ecology Laboratory (University of Vigo), I thank Manuel Aira, Fernando Monroy, Cristina Lazcano, María Gómez Brandón, Luis Sampedro, Alberto Velando, Marta Lores, Robert W. Parmelee, Patrick J. Bohlen, Scott Subler, Rola M. Atiyeh, Juan Carlos Sánchez Hernández, Alfredo Ferreiro, Julio Eiroa, Andrea Tato, Domingo Pérez, Jose Carlos Noguera, Sim Yeon Kim, Salustiano Mato, Fuencisla Mariño, Xavier Freire, Ramón Eixo, David Cereijo, Lucía Senra, Aida Costas, Sara Rodríguez, Salomé Álvarez, Pablo G. Porto, Lorena San Miguel, Belen Longa, Julia Arnold, Hans Zaller, Rafael Zas, Michelle Webster, Jose Angel Gago, Cristóbal Pérez, Anxo Conde, Gonzalo Mucientes, Hugo Martínez, Alberto Da Silva, Daniel Fernández Marchán, Luis A. Mendes, Celestino Quintela Sabarís, Gustavo Schiedeck, Daniel Pazzini, Natielo Almeida Santana, Nariane de Andrade, Zaida Inês Antoniolli, Afrânio Guimaraẽs, Mario Domínguez-Gutiérrez, Isabelle Barois, Marcos Pérez Losada, Keith A. Crandall, and all other temporary collaborators in vermicomposting science and technology.

I also thank the vintners at Terras Gauda and Abadía da Cova, particularly Emilio Canas and Evaristo Rodríguez, for providing access to their vineyards and wineries and for their continued partnership and interest in the application of vermicomposting to the wineries in the long-term EWINE project.

This chapter is dedicated to the memory of Clive A. Edwards, a brilliant scientist, friend and mentor who inspired my dedication to earthworm ecology and vermicomposting.

Funding This study was partly co-funded by the Spanish Ministerio de Ciencia e Innovación (PID2021-124265OB-100 and TED2021-129437B-I00), the Xunta de Galicia (ED431C 2022/07) and the European Union's Horizon 2020 research and innovation programme (LABPLAS_101003954).