microplastics

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Earthworm (*Eisenia andrei*)-Mediated Degradation of Commercial Compostable Bags and Potential Toxic Effects

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Abstract: The availability of compostable plastic bags has increased greatly in the past few years, as it is perceived that this type of bags will be degraded after disposal. However, there are some knowledge gaps regarding the potential effects on the soil ecosystems. We assessed the rate of degradation of samples of four different types of commercial compostable bags in vermicomposting systems with the earthworm species *Eisenia andrei*. We also evaluated the biological response of *E. andrei* (survival and reproduction) to microplastics (MPs) from fragments of the plastic bags (<2000 µm) and assessed seedling emergence in common garden cress (*Lepidium sativum* L.) exposed to micronized plastic (<250 µm) and the respective leachate, following OECD and ISO guidelines, respectively. The rate of degradation differed significantly depending on the type of plastic rather than the substrate in the vermicomposting system. This finding suggests that the degradation process is more dependent on the microbial community colonizing the different plastic types than on earthworm activity. Regarding the biological response of the soil system, *L. sativum* seedling emergence was not significantly affected; however, earthworm reproduction was affected, suggesting that although compostable, some of the formulations may potentially be toxic to soil fauna.

Keywords: degradable plastics; additives; ecotoxicity; model organisms; terrestrial ecosystems

Citation: Mendes, L.A.; Beiras, R.; Domínguez, J. Earthworm (*Eisenia andrei*)-Mediated Degradation of Commercial Compostable Bags and Potential Toxic Effects. *Microplastics* **2024**, *3*, 322–338. https://doi.org/10.3390/microplastics3020020

Academic Editor: Nicolas Kalogerakis

Received: 20 March 2024
Revised: 14 May 2024
Accepted: 3 June 2024
Published: 8 June 2024

1. Introduction

Plastic pollution is a worldwide issue that has resulted from consumption patterns in daily life. The disposal of single-use plastic items, including bags, is causing the rapid and ever-increasing accumulation of plastic debris in aquatic and terrestrial ecosystems, including soil [1]. Plastic manufacturers have followed two different routes with the aim of tackling this problem [2]. One is the introduction of reusable plastic bags, which decreases the frequency of disposal and reduces the influx of plastic debris in soil. Another is the development of biodegradable or compostable bags, which can be rapidly fragmented and degraded, as they are made from polymers such as poly (butylene adipate co-terephthalate) (PBAT) or polylactic acid (PLA) that undergo weathering via hydrolysis, mechanical and enzymatic activity, leading to their eventual disappearance [3]. As the weathering process leads to the polymer chains being broken down into oligomers and monomers that are more easily transformed by microbes [4], this appears to be the best option [5]. Therefore, many manufacturers have introduced bags made of these polymers into the market, and labeled them with national and European certificates of compostability as a commercial advantage for their products [6].

However, the fragmentation and degradation of plastic items will result in the formation of microplastics (MPs) of different shapes and sizes [7]. In addition, the weathering of plastics will lead to the release of chemical additives and degradation products through leaching [8]. Biodegradable plastics are quite complex in terms of their chemical composition [9,10], and despite the exponential increase in the number of new products of

this type, their ecological effects remain widely unknown, which raises concern about the environmental safety of the by-products of discarded compostable bags [11].

Furthermore, these compostable bags may reach liquid and solid waste treatment facilities and undergo degradation in other matrices, such as sewage sludge (SS) or organic solid wastes, which, upon disposal, eventually end up in the soil [12]. This leads to scenarios in which compostable bags are subjected to abiotic-related degradation processes before reaching the soil. As such, studies have been performed on soil [13,14] and on compost, using a thermophilic phase [15,16], showing a clear effect on plastic surface [13], significant decrease in weight [16], presence of oligomers and particle size [15].

However, these studies have not linked abiotic and microbial degradation with what occurs once these materials reach terrestrial ecosystems, where plastics are further processed by soil-dwelling organisms such as earthworms [7,17]. Earthworms are known to be able to transform organic matter (OM) and contribute to soil microbial turnover, as they can process different types of organic waste, e.g., SS, and non-recyclable solid waste, such as spent coffee grounds (SCGs), into material that can potentially be used as fertilizer [18,19]. The role of earthworms in transporting and transforming plastic particles has also been demonstrated, and the abundance of MPs has been found to be altered in vermicomposting systems, suggesting that they can alter MPs and plastic adjuvant availability in soil systems [20–22].

There is, therefore, a need to investigate the earthworm-mediated degradation of plastics in intermediate matrices prior to disposal in the soil system and also to assess the potential toxicity of plastic degradation products to soil fauna and flora. Several studies published in the last decade have used toxicity tests following ISO and OECD guidelines to assess the environmental impact of plastic particles by using model species. This includes the study of the response of *Eisenia fetida/andrei*, as representative earthworms, to conventional polyethylene (oxidative stress and internal lesions) and polyester microfibers, as well as to PLA-based bioplastics (effect on reproduction) [23–25]. *Lepidium sativum* L., as representative terrestrial plants, was also studied, with growth inhibition and physiological parameters shown to be altered in response to conventional polyethylene terephthalate (PET) and polycarbonate (PC) [26–28] but also to PLA-based bioplastics [29]. Nonetheless, to our knowledge, few or no studies have used both types of model species to assess the toxicity of plastics or, specifically, of compostable polymers. Another factor for consideration is the potential source of toxicity. To date, most studies conducted in terrestrial ecosystems have overlooked the leaching from plastic structures due to weathering, resulting in the release of compounds such as phthalates and flame retardants that may be toxic to soil organisms [30,31].

The aim of the present study was to assess how samples of different types of certified compostable plastic bags are degraded in vermicomposting systems with two distinct substrates (SS and SCGs). The study also aimed to assess the toxicity of the degradation products, i.e., MPs of different sizes and plastic leachates, to the model plant species *L. sativum* and the model earthworm species *E. andrei* following ISO 18763 and OECD 222 standard guidelines [32,33], respectively. We hypothesized that the combination of the composition of the substrate and the composition of the plastic bags would have a role in the degradation rate of the latter [34,35]. On the other hand, we hypothesized that the presence of polylactic acid (PLA) in the plastic bag composition can potentiate negative effects on *E. andrei* survival and reproduction [23] and that higher degradation can result in higher toxicity due to the release of plasticizers and other unknown by-products of plastic bag degradation into soil [36]. The data obtained were examined by multivariate analysis, to identify key chemical components of compostable bags that should be carefully considered during manufacture due to the potential effects on soil fauna and flora during the decomposition of the compostable plastics.

2. Materials and Methods

2.1. Source, Preparation and Characterization of Commercial Compostable Plastic Bags

Plastic bags showing in their labels compostability certifications were bought from on-line suppliers and local markets and were given identification numbers, for inclusion in the LABPLAS project sample database: 069_LPB-Bag_Pat-GW (hereafter 069), 070_LBP_BagBioTuf_PHA (070), 072_LBP_Bagbrown (072) and 073_LBP_BagBio100 (073). The plastic composition was provided by the supplier and also checked by Fourier Transformed Infra-Red Spectroscopy coupled with Attenuated Total Reflectance (FTIR-ATR) through a Nicolet 6700 FT-IR spectrometer coupled with a Smart Orbit diamond (Thermo Electron Corporation, Waltham, MA, USA). Further characterization was performed for phthalates through Gas Chromatography–Mass Spectroscopy (GC-MS) using an Agilent 5975C TAD series chromatograph (Agilent Technologies, Santa Clara, CA, USA) (methodology published by Abril et al. [37]). All assessments were performed at Centro de Apoio Científico-Tecnolóxico á Investigación (CACTI, UVigo).

Prior to the different tests (degradation or toxicity), the plastic bags were prepared and fragmented according to the test purpose. For the degradation tests, each plastic bag was cut with scissors into square pieces of approximately 25 cm^2 in area, and the dry weight was then determined. For the earthworm toxicity test, the samples were further fragmented into pieces in which the largest dimension was less than 2000 µm. For the seedling emergence test, the plastic fragments were micronized into particles of less than 250 µm in size to ensure interaction with the organism in an Ultracentrifugal Mill (ZM 200; Retsch Verder Scientific, Haan, Germany) at 16,000 rpm. Dry ice was added during the micronization process to prevent heating.

To distinguish the potential toxicity of the plastic particles themselves and their leachates, <250 µm fragments were added to distilled water at a concentration of 10 g/L, and the suspensions were thoroughly mixed to extract water-soluble components, as previously described [38].

2.2. Degradation Test

To assess how the plastics degrade under vermicomposting conditions, previously weighed and identified samples were placed within the surface layer of two ongoing active vermicomposting systems, one containing SS obtained from a local wastewater treatment plant (WWTP) (Moaña, Pontevedra, Spain) and another containing SCGs obtained from a local cafeteria (Faculty of Biology, University of Vigo). The plastic sheet location within the vermicomposting system was marked for sampling time, and the plastic bag ID was marked with a plastic stick. The main characteristics of each substrate are presented in Table 1.

Table 1. Main characteristics of the initial substrates in each vermicomposting system used to assess the degradation of the plastic samples. EC: electric conductivity; OM: organic matter.

	SS	**SCGs**
pH	5.48 ± 0.12	6.76 ± 0.08
EC (µS/cm)	592 ± 1.7	124 ± 11
% OM	58.1 ± 0.2	93.4 ± 3.8
% humidity	77.3 ± 1.2	71.1 ± 2.0

Each vermicomposting system comprised 680 L containers with a surface area of 1 m^2 and a depth of 50 cm, with a bottom layer of vermicompost obtained from the respective original material, serving as a bed, prior to fresh SS or SCG addition, similar to other studies performed by the group [39]. Each system contained approximately 6000 individuals/m^2 (5990 ± 53 for SS and 6212 ± 100 for SCGs) of *E. andrei* at the start of the experiment. Earthworm activity was continued by adding fresh substrate material (SS or SCGs) every two weeks, also promoting upward mobility and interaction with the deposed plastic

sheets, and the moisture was maintained by spraying the material with water 3 times a week.

After 15, 30, 60, 90 and 120 days, each vermicomposting system was locally sampled for the corresponding ID and time and at each sampling time by retrieving a vertical sample of the system, minimizing the mixing of vermicomposting system layers. For each sampling time, 4 pieces of each type of plastic were retrieved, washed and dried prior to weighing.

The plastic material was examined for tears, deformation and softening, and the weight loss, calculated as the difference between the initial and final weights, was recorded as indicator of degradation.

2.3. Lepidium sativum Seedling Emergence Test

The *L. sativum* seedling emergence test was conducted in glass Petri dishes (ø = 80 mm) lined with Whatman #1 filter paper, in an adaptation of the ISO guidelines [32]. Before the start of the test, powdered plastic was added directly to Petri dishes followed by 1 mL of distilled water and 3 mL of a LUFA 2.2 soil extract (1:5 weight/Volume). Soil extracts were used to simulate the existing interstitial soil–water interface for root development and predict more realistically plastic–plant interactions. Other Petri dishes were spiked with leachate solutions by adding 1 mL of serial dilutions of the original 10 g/L leachate solution and 3 mL of soil extract. In addition, control dishes were prepared with 1 mL of distilled water and 3 mL of soil extract. The resulting concentration ranges of powdered plastic were 0.5, 1.25, 2.5 and 12.5 g/L, and the concentration ranges of plastic leachate were 0.25, 0.5, 1.25 and 2.5 g/L. In each replicate (n = 3), groups of 30 *L. sativum* seeds were added to all dishes, which were then held in the dark at room temperature for 7 days. The germinated seeds were counted on days 1, 2, 3 and 7, and the root and shoot lengths in each germinated seed were measured after 7 days with the use of a caliper (metric scale). Seeds were considered to have germinated when the shoot was longer than 1 mm. The test validity was confirmed when, in the control, the seedling emergence at the end of the test was higher than 70% and the average root length was greater than 30 mm.

The germination index (GI) was calculated after 7 days based on the relative seed germination (RSG), i.e., the ratio of germinated seeds under test and control conditions, and the relative root growth (RRG), i.e., the ratio of the average root length (in mm) of germinated seeds under test and control conditions (Equation (1)). The relative shoot growth (RShG), i.e., the ratio of the average shoot length (in mm) of germinated seeds under test and control conditions, and the root–shoot ratio (RSR), i.e., proportion between the average root length (in mm) and shoot length (mm) under a given condition (test or control), were also calculated.

$$GI = RRG \times RSG \quad (1)$$

The germination index (GI) was calculated from the relative root growth (RRG) and the relative seed germination (RSG).

2.4. Chronic Toxicity Test with Eisenia andrei

The response of *E. andrei* to soil spiked with the different plastics was assessed following the OECD standard guidelines [33], with some modifications. Prior to the start of the test, LUFA 2.2 soil was spiked by adding plastic fragments to dry soil, mixing thoroughly and adding distilled water to at least 50% of the water holding capacity of the soil. Two concentrations of plastics were tested: 2 and 10 g/kg dry weight (dw). Soil without added plastic was used as control.

For the toxicity test, *E. andrei* specimens were retrieved from culture systems in which they had been fed continuously with SCGs, and they were acclimatized to a batch of fresh LUFA 2.2 soil for one week. At the start of the test, groups of 10 mature specimens of *E. andrei*, each with a well-developed clitellum and weighing 304 ± 5 mg (average ± standard error), were thoroughly washed and placed in replicate glass vials (n = 3) containing at least 300 g of soil. The tests were carried out at 20 ± 2 °C under a photoperiod of 16:8 h light–darkness for 8 weeks. Pre-moistened non-spiked SCGs (7 g) were spread across the

soil surface of the test system as a food source, and water was replenished weekly. After 4 weeks, the surviving adult earthworms were removed, counted, washed and weighed to determine any change in body mass. After 8 weeks, the numbers of juveniles and cocoons were sorted by spreading the soil over a white tray, sorted with tweezers and counted with the aid of a magnifying glass.

The OECD guideline test validity was confirmed when the adult earthworm mortality in the control boxes was less than 10% after 4 weeks; after 8 weeks, the coefficient of variation of reproduction was less than 30%, and more than 30 juveniles were produced per replicate.

2.5. Statistical Analysis

The Shapiro–Wilk test was used to check the normality of the data. For the degradation test data, for each vermicomposting condition, two-way ANOVA was used to detect any significant differences ($p < 0.05$) between sampling times and plastic types, as well as sampling times and substrate used for each polymer, while three-way ANOVA was performed to additionally assess the role of the substrate in plastic degradation, together with Tukey's post hoc test.

For the ecotoxicological assessment, one-way ANOVA, together with Dunnet's post-hoc test, was used to detect significant differences ($p < 0.05$) between the control and test conditions.

Principal component analysis (PCA) was used to correlate *E. andrei* toxicity data and plastic degradation data and identify key factors to explain the toxic effects on the organisms. All analyses were performed by using SigmaPlot, version 14.0.

3. Results

3.1. Characterization of Commercial Plastic Bags

The plastic bag material characterization provided by the supplier is presented in Table 2, jointly with the data acquired through FTIR-ATR. The FTIR-ATR analysis identified all compostable materials as polyesters containing a terephthalate group. This is compatible with the PBAT composition provided by the several brands analyzed. In addition, other components were also detected, namely, talc in 070 and other ester groups in 070, 072 and 073 compatible with the aliphatic polyesters PLA and PHA. Through GC-MS, phthalates were identified in extracts obtained from the materials, with the highest concentration of the latter (in μmol/g) being observed in 070 (Table 2).

3.2. Degradation of Commercial Plastic Bags

The maximum degradation recorded in the vermicomposting system was 24.8 ± 0.5% (mean ± standard error), corresponding to bag 073 after 120 days in SCGs (Figure 1). While a significant weight loss corresponding to plastic bag degradation was recorded in all systems after 90 days, in direct contrast, the degradation of bag 070 was almost non-existent (<2%). The degradation of bag 069 reached a maximum after 90 days in SCGs (17.3 ± 0.6%) while for bag 072, maximum degradation was reached after 120 days in SS (16.2 ± 2.9%).

A comparison of the two vermicomposting systems (SCGs and SS) revealed small but significant differences ($p < 0.05$) in the degradation time of the materials: 070 after 120 days, 072 and 073 after 15 days (SS > SCGs) and 073 after 90 and 120 days (SCGs > SS) (detailed information on significant differences observed when performing three- and two-way ANOVAs in Tables S2 and S3). In addition, the degradation of bag 069 in SCGs reached a plateau between 90 and 120 days (Figure 1).

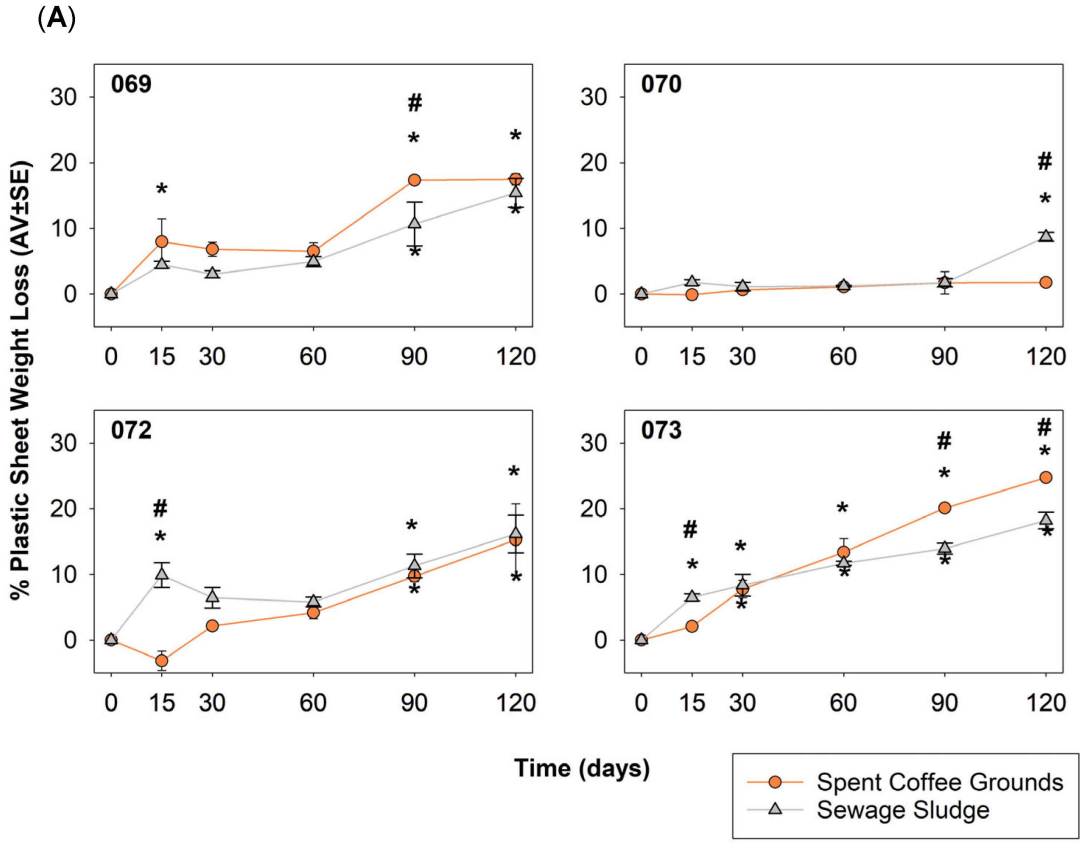

Figure 1. (**A**) Degradation of the 4 types of plastic films in vermicomposting systems, represented as % weight loss. Significant differences compared with the initial timepoint (T = 0) are indicated by *, while differences between vermicomposting systems are identified by #. (**B**) Visual appearance of sheets from each of the 4 types of plastics sampled after 120 days in a vermicomposting system with sewage sludge, post-washing and drying steps, from left to right: 069, 070, 072 and 073.

Table 2. Commercial plastic bag identification (ID) codes, physical appearance and composition provided by the supplier and checked by FTIR-ATR analysis and phthalate concentration complemented by GC-MS analysis.

Short Code ID	Full Code ID	Appearance	Composition According to Supplier	FTIR-ATR Analysis	% Match	Σ Phthalates (µmol/g)	Certification/Labeling
069	069-LPB-Bag Pbat		PBAT with cornstarch	Terephthalate polyester	86.60	2768	Certified EN-13432 and "home" compostable by TÜV (Austria) [40]
070	070_LBP_BagBioTuf		PBAT + PHA	Terephthalate polyester + talc + other esters	83.37	3876	BPI certified compostable. Conforms to ASTM D6400 Standard [41]
072	072_LBP_BagBrown		Mater-Bi + cornstarch	Terephthalate polyester + other esters	84.32	2255	Certified compostable by TÜV (Austria) (S2096) [42]
073	073_LBP_BagBio100		PBAT with potato starch	Terephthalate polyester + other esters	85.18	1037	Certified EN-13432 and "home" compostable by TÜV (Austria) [40]

PLA: polylactic acid; PBAT: polybutylene adipate-co-terephthalate; PHA: polyhydroxyalkanoates.

3.3. Response of Lepidium sativum to Microplastics and Leachates from Commercial Plastic Bags

The *L. sativum* seedling test fulfilled the performance criteria outlined in the ISO guidelines, with percentage germination reaching 81.2 ± 0.8% (mean ± standard error) after 7 days under control conditions and the root length control reaching 60.4 ± 1.5 mm (mean ± standard error) in the control replicates. The results contradict the hypothesis that the degradation of compostable plastic could lead to the release of toxic compounds to plants. No overall effect on seedling emergence after 7 days was observed when the *L. sativum* seeds were exposed to either powdered microplastics (<250 µm) or leachates from the four types of plastic bags, even at a concentration of 12.5 g/L. No significant differences in RSG were observed after 7 days (Figure S2). However, a significant decrease ($p < 0.05$) in RRG was observed for exposure to 0.25 and 0.5 g/L of bag 073 as leachate (Figure 2) and a significant decrease in GI was observed for the same material at 0.25 mg/L (Figure 3) (detailed information in Table S4). Regarding RRG, there was a positive tendency with the increase in concentration from 0.25 to 2.5 g/L, while the GI values for higher concentrations (2.5 g/L) were as high as the control.

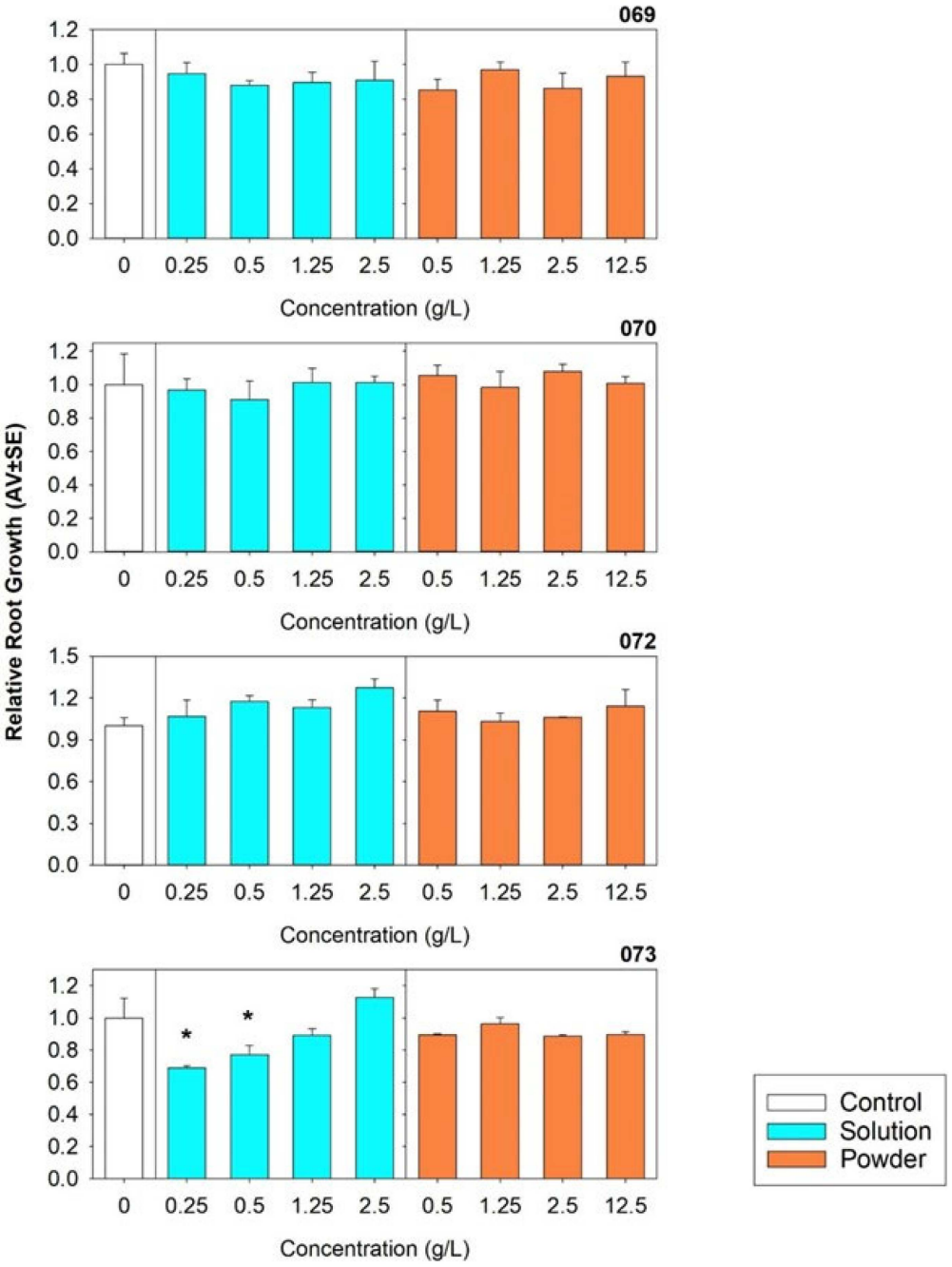

Figure 2. Representation of relative root growth (RRG) in *L. sativum* after exposure for 7 days to powdered microplastics (<250 μm) and to leachates from 4 types of plastic bags. Values are expressed as the ratio between the mean root length in seedlings growing under test conditions and the mean root length in control seedlings after 7 days (mean ± standard error). Significant differences relative to the control ($p < 0.05$) are indicated by *.

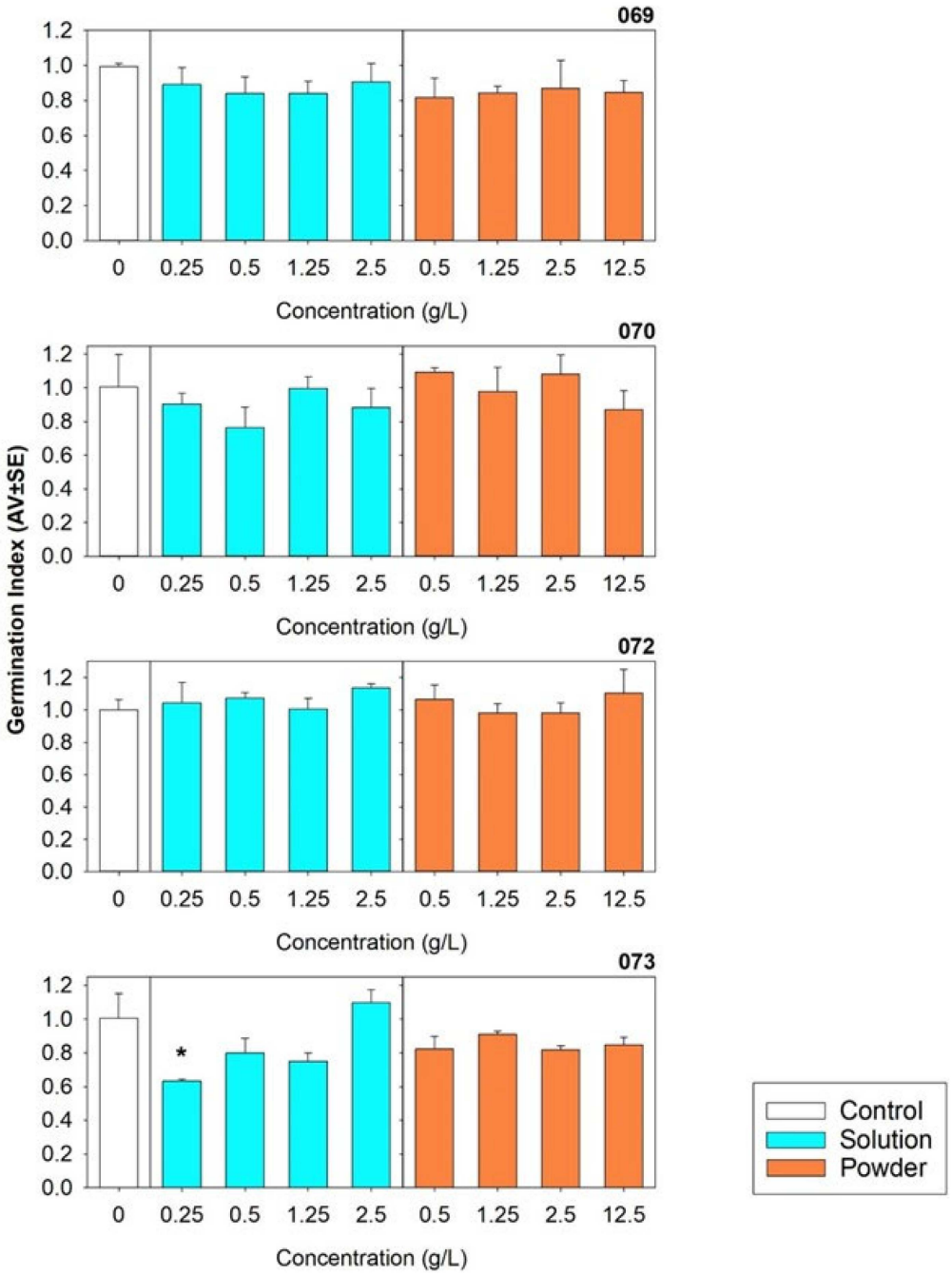

Figure 3. Representation of germination index (GI) of *L. sativum* after 7 days of exposure to powdered microplastics (<250 µm) and to leachates from 4 types of plastic bags. Values are expressed as the germination index, i.e., relative seed germination (RSG) multiplied by relative root growth (RRG) after 7 days (mean ± standard error). Significant differences relative to the control ($p < 0.05$) are indicated by *.

3.4. Earthworm Response to Microplastics from Commercial Plastic Bags

The chronic toxicity test fulfilled the validity criteria outlined by the OECD standard guideline [33], with adult mortality of 6.7% in controls, the number of juveniles produced reaching 35.3 ± 1.8 in controls and a coefficient of variation of 8.6%.

As expected, the survival of *E. andrei* adults was not affected after 28 days. However, after 56 days, there was a significant decrease in the numbers of juveniles and cocoons produced ($p < 0.05$) relative to the controls (detailed information in Table S5). For the exposure to the highest concentration of fragments for all plastics, the number of juveniles

produced was significantly lower than in the controls. This was also observed for exposure to bags 070 and 072 at a concentration of 2 g/kg. The number of cocoons produced was also significantly lower for exposure to bags 069 and 073 at a concentration of 10 g/kg (Figure 4).

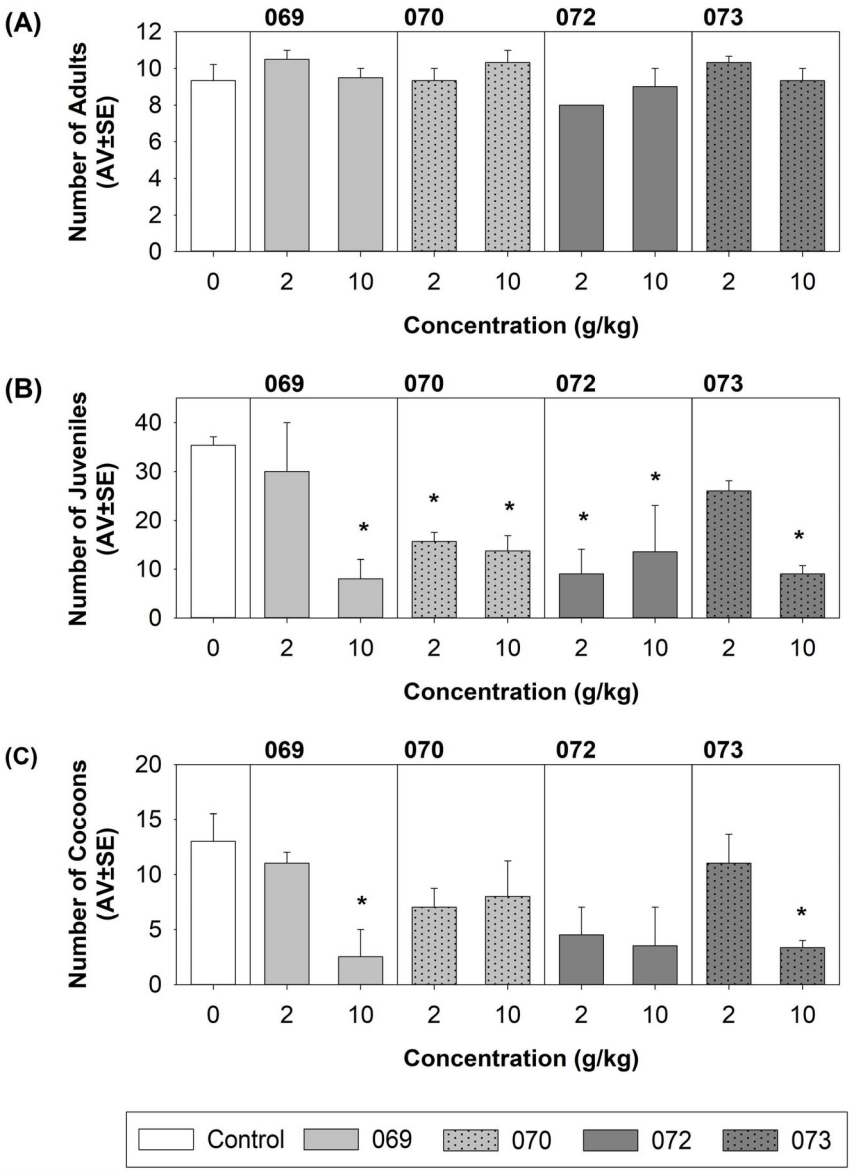

Figure 4. (**A**) *E. andrei* survival, represented by the number of adults after 28 days, (**B**) *E. andrei* reproduction, represented by the number of juveniles after 56 days and (**C**) the number of cocoons after 56 days of exposure to microplastics (<2000 µm) from 4 types of plastic bags. Significant differences relative to the control ($p < 0.05$) are indicated by *.

3.5. Multivariate Analysis

The PCA identified two principal components (eigenvalue > 1), which together represented 77.1% of the variance (PC1: 54.5%; PC2: 22.6%). For PC1, the main factors (loadings $\geq |0.7|$) were bag characterization (terephthalate group and other ester groups), earthworm response (juveniles) and degradation in SCGs and SS, whilst for PC2, bag characterization, in particular the use of additives (talcum and phthalate sum), was the most important factor. Regarding the bag characterization, the presence of the terephthalate group was correlated with degradation in SS after 120 days. On the other hand, the same factor, together with other esters and the sum of phthalates, was negatively correlated the

reproduction of *E. andrei*, similar to the plastic concentration. In addition, the degradation results at all timepoints in different vermicomposting systems were highly correlated (Figure 5, Table S1).

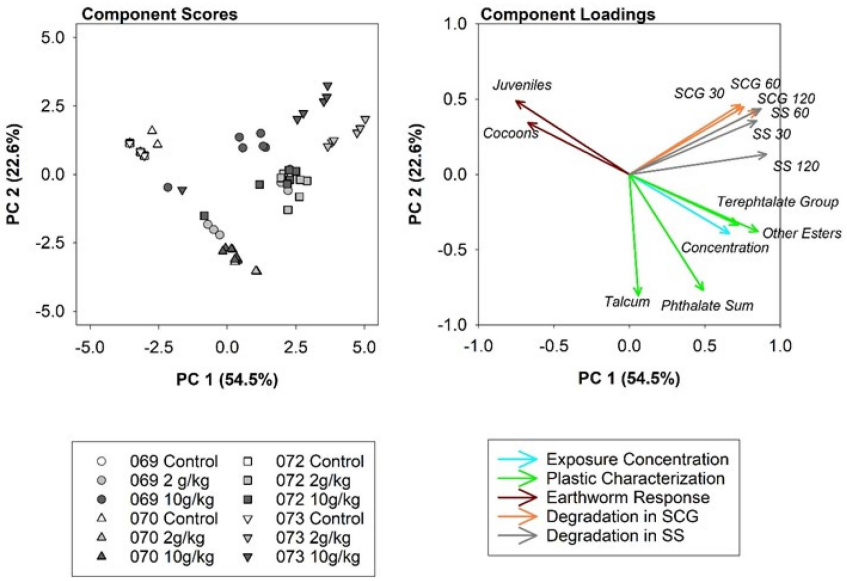

Figure 5. Representation of the component scores and loadings of the 2 principal component axes (eigenvalue > 1) obtained from the principal component analysis of *E. andrei* reproduction data and exposure concentration, plastic composition (according to FTIR-ATR and GC-MS analyses) and degradation data at 30, 60 and 120 days.

4. Discussion

The aims of the present study were to examine whether the composition of compostable plastic bags and the composting substrate determine how the bags degrade and to assess the potential toxicity of plastic and its degradation products to soil organisms. When SS or SCGs were used as vermicomposting substrate, the rate of degradation of the plastic bag samples after 120 days (measured as weight loss) reached approximately 20%, with the highest value being observed for 073 in SCGs (25%). However, one of the plastics (070) was not significantly degraded even after 120 days (Figure 1). This could be attributed to the composition of the plastic. According to the manufacturer, bag 070 is made of the biodegradable polymer PHA, but the FTIR analysis also detected talc as a major component. None of the other bags tested in this study contained talc (Table 2). Mineral talc is used in plastics as a functional filler to improve the rigidity, impact strength, flexural modulus and thermal stability of the final product [43]. Biodegradable polymers in particular need additives to improve their mechanical properties, and talc is frequently added, contributing to lower rates of degradation [44,45].

On the other hand, the highest rate of degradation was observed in the plastic containing potato starch, PBAT (according to the manufacturer), with additional ester groups (determined by FTIR), which was highly correlated in the PCA. The addition of potato starch to biodegradable polymers such as PBAT has been shown to increase the degradation rate [46], and the degree of degradability increases as the percentage of starch increases [47,48].

Weight loss has been used as an endpoint in previous studies intended to assess degradability of biopolymers in aquatic (e.g., [49–51]) and terrestrial [52] environmental compartments. Another study examining the presence and abundance of MPs in vermicomposted SS reported an increase in MPs in a similar range to the weight loss values obtained in the present study (approximately 20%) [53].

Furthermore, few differences between the degradation rates according to the substrates employed were observed, despite clear differences in their physical–chemical characteri-

zation, namely, in pH, in electrical conductivity and more so in the percentage of organic content (much higher in SCGs). This indicates that the impact of earthworm activity, given its high density (6000 individuals per m^2) in a continuous vermicomposting system can override the influence of the used substrate. A significant level of degradation was observed after 15 days in SS spiked with bags 072 and 073, while in SCGs, significant differences were only observed in later stages. As both materials are aliphatic–aromatic co-polyesters, carboxylesterase activity may differ significantly in each substrate in this early stage (15 days). Previous studies have shown that carboxylesterase activity in earthworms (*Eisenia fetida*) is enhanced by the presence of plastics [35] and can also alter the microbial communities of organic waste [39,54]. This is important, given that the role of bacteria in the degradation of MPs in different substrates has become the focus of several studies [55]. Indeed, the incubation of different types of MPs in WWTP effluents has been shown to enhance the selection of some bacterial strains [56].

Regarding the potential toxicity of degradation products, no dose–response effects were observed for *L. sativum* after exposure to <250 µm MPs (Figures 2 and 3). In contrast, polycarbonate and polystyrene MPs inhibited seedling germination, with a particular effect on root and seedling length after 7 days under similar conditions [26,27]. In addition, significant effects have also been observed in seed germination tests performed in soil spiked with PET [28,57]. However, exposure to PLA did not induce changes in seed germination but affected root growth [29,58]; short-term exposure (3 days) to leachates from plastic bags only induced changes in morphology but did not affect germination [59]. This corroborates our observations at lower concentrations of leachates for 073 of a decrease in root growth but not in seedling emergence. On the other hand, no effects were observed when exposing poly(3-caprolactone) with adipate-modified starch in rice plantlets grown in a soil microcosm over 14 days and for *L. sativum* exposed to PLA-based (similar to 072) plastics in a soil mesocosm system for 28 days [14,60]. The different levels of response in the aforementioned studies suggest that the type of exposure matrix (leachate vs. powder) has a relevant role in the toxicity of plastics due to the release and availability of additives used in their manufacturing.

Regarding the response of *E. andrei* exposed to plastic bag fragments, no effects on survival were observed (Figure 4A). This was expected given the demonstrated resilience of earthworms in plastic-polluted environments, e.g., including SS [61], and the low mortality observed in standard toxicity tests in soil [62]. However, reproduction performance (measured as the number of juveniles and cocoons produced after 56 days) was affected by plastic concentration, as revealed by the PCA (Figures 4B,C and 5 and Table S2). While the number of juveniles was significantly affected at the highest concentration tested (10 g/kg, equivalent to 1% weight/weight), this was only true for bags 070 and 072 at 2 g/kg (0.2% *w*/*w*). This indicates that the use of additives, such as PHA or talc (in 070), as well as other esters (in 070 and 072) or phthalates (070 and 072—see Table 1) may affect the reproductive process [63]. The mechanisms underlying plastic toxicity remain unknown, given the uncertainty regarding the source of toxicity (particle size, leaching of adjuvants and vector for other contaminants) [64].

Interesting enough is the fact that exposure to 072, which is Mater-Bi and starch, induced effects on reproduction, while another study did not show significant differences in this parameter when using soil in which Mater-Bi-based plastics were already biodegraded [65]. Differences in using "fresh" particles versus degraded plastics may be key to explaining the observed toxicity.

Nonetheless, it appears that earthworms can ingest plastic particles [66], thus potentially affecting their internal functioning, e.g., through lesions or induction of stress at the cellular level [24,67], the latter as a result of the release of additives (phthalates) or even other plasticizers, such as Bisphenol A [68,69], and potentially affecting their reproductive capacity [70,71]. Earthworms may produce non-viable cocoons in response to exposure to plastics or their chemical additives, not covalently bond to the polymer chains. This would explain why the number of cocoons produced by *E. andrei* exposed to bag 070 was not

significantly lower than in the control worms, even though fewer juveniles were produced. Previous studies have shown that PLA-based plastics do not induce mortality but can significantly decrease the number of juveniles produced [23]. On the other hand, the lower toxicity to *E. andrei* of starch- and PBAT-based bags is consistent with the findings of a study in which no significant effects were observed on survival and growth of *Lumbricus terrestris* exposed to PBAT microplastics [72].

The least degradable bag (070) was also considered one of the most toxic to earthworms, although seedling emergence in *L. sativum* was not affected. Given that it was shown that these biodegradable plastics contain phthalates and other substances as additives, it could be hypothesized that their low degradation rate can lead to a small but continuous release of more available hazardous substances over time, thus having a larger effect on long-term reproduction.

This indicates that the production of truly biodegradable plastics demands that not only the polymeric matrix (e.g., PLA) but also major chemical additives (talc, other ester and phthalates) be susceptible to microbial and enzymatic degradation in a safe manner.

5. Conclusions

This study addressed the lack of information regarding how the composition of compostable plastic bags affects the degradation of plastic under vermicomposting conditions and the potential toxicity of the fragmentation and degradation products to soil organisms. It was observed that additives, more than the biodegradable polymeric matrix, can modulate the degradation rate of compostable bags in vermicomposting systems.

As for the potential impact of the degradation of compostable bags on soil biota, it should be highlighted that negative effects were observable even at lower concentrations when organisms were exposed to plastics with a low degradation rate. As the low degradation rate should indicate a low release rate of additives (with or without toxic potential) to the soil system, this represents an issue that should attended to. Although the biological mechanisms underlying the effects on *E. andrei* reproduction in response to plastic exposure remain unclear, a closer look at the additive composition of compostable bags could be a way to explain and explore the benefits and mitigate the hazardous potential of these products to soil systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microplastics3020020/s1, Figure S1: Representation of the degradation of the 4 types of plastic films, represented as % weight loss, in two vermicomposting systems, one containing spent coffee grounds and another containing sewage sludge, Figure S2: Representation of the Relative Seed Germination (RSG) in *L. sativum* after exposure to powdered microplastics (< 250 µm) and leachates of 4 types of plastic bags for 7 days. Values are expressed as number of germinated seeds relative to control after 7 days (mean ± standard error), Table S1: Descriptors of the principal component analysis model (component loadings, explained variance and component fitted correlation). Component loadings and correlation > |0.7| marked in bold. Table S2: Descriptors of the three-way ANOVA performed on the plastic weight loss in vermicomposting systems. DF–Degrees of Freedom, SS–Sum of Squares, MS–Mean of Squares. $p < 0.05$ values highlighted in bold. Table S3: Descriptors of the two-way ANOVA performed on the plastic weight loss in vermicomposting systems for each plastic type (069, 070, 072, 073). DF–Degrees of Freedom, SS–Sum of Squares, MS–Mean of Squares. $p < 0.05$ values highlighted in bold. Table S4: Descriptors of the one-way ANOVAs performed on the *Lepidium sativum* germination test parameters (Relative Seed Germination, Relative Root Growth, Relative Shoot Growth, Root to Shoot Ratio, Germination Index) after exposure to plastic bag as powder and as leachate, for each plastic type (069, 070, 072, 073). DF–Degrees of Freedom, SS–Sum of Squares, MS–Mean of Squares. $p < 0.05$ values highlighted in bold. Table S5: Descriptors of the one-way ANOVAs performed on the *Eisenia andrei* survival and reproduction test parameters (Number of surviving adults, number of juveniles and number of cocoons) after exposure to plastic bag as powder and as leachate, for each plastic type (069, 070, 072, 073). DF–Degrees of Freedom, SS–Sum of Squares, MS–Mean of Squares. $p < 0.05$ values highlighted in bold.

Author Contributions: Conceptualization, L.A.M. and J.D.; Methodology, L.A.M.; Validation, L.A.M. and J.D.; Formal Analysis, L.A.M.; Investigation, L.A.M.; Resources, J.D.; Data Curation, L.A.M.; Writing—Original Draft Preparation, L.A.M.; Writing—Review and Editing, L.A.M., J.D. and R.B.; Visualization, L.A.M.; Supervision, J.D. and R.B.; Project Administration, R.B.; Funding Acquisition, J.D. and R.B. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Spanish Ministerio de Ciencia e Innovación (PID2021-124265OB-100), Xunta de Galicia (grant number ED431C 2022/07), and MCIN/AEI and European Union Next Generation_EU (project TED2021-129437B-100). L.A.M. acknowledges support from the European Union Horizon 2020 Programme-funded LABPLAS—Land-Based Solutions for Plastics in the Sea—project (grant agreement number 101003954) through the award of a post-doctoral research contract and from the IDI&CA Programme-funded PLASCOGEN project (IPL/2021/PLASCOGEN_ESTeSL). Funding for the open access charge was provided by Universidade de Vigo/CISUG.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: The authors are grateful to Alejandro Vilas for assistance in preparing the microplastics used in the current study and to Sara López-Ibáñez for providing information on the tested materials.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Pinto da Costa, J.; Paço, A.; Santos, P.S.M.; Duarte, A.C.; Rocha-Santos, T. Microplastics in Soils: Assessment, Analytics and Risks. *Environ. Chem.* **2019**, *16*, 18. [CrossRef]
2. Prata, J.C.; Silva, A.L.P.; da Costa, J.P.; Mouneyrac, C.; Walker, T.R.; Duarte, A.C.; Rocha-Santos, T. Solutions and Integrated Strategies for the Control and Mitigation of Plastic and Microplastic Pollution. *Int. J. Environ. Res. Public Health* **2019**, *16*, 2411. [CrossRef] [PubMed]
3. Flury, M.; Narayan, R. Biodegradable Plastic as an Integral Part of the Solution to Plastic Waste Pollution of the Environment. *Curr. Opin. Green Sustain. Chem.* **2021**, *30*, 100490. [CrossRef]
4. Witt, U.; Einig, T.; Yamamoto, M.; Kleeberg, I. Biodegradation of Aliphatic-aromatic Copolyesters: Evaluation of the Final Biodegradability and Ecotoxicological Impact of Degradation Intermediates. *Chemosphere* **2001**, *44*, 289–299. [CrossRef] [PubMed]
5. García-Depraect, O.; Bordel, S.; Lebrero, R.; Santos-Beneit, F.; Börner, R.A.; Börner, T.; Muñoz, R. Inspired by Nature: Microbial Production, Degradation and Valorization of Biodegradable Bioplastics for Life-Cycle-Engineered Products. *Biotechnol. Adv.* **2021**, *53*, 107772. [CrossRef] [PubMed]
6. Syberg, K.; Nielsen, M.B.; Westergaard Clausen, L.P.; van Calster, G.; van Wezel, A.; Rochman, C.; Koelmans, A.A.; Cronin, R.; Pahl, S.; Hansen, S.F. Regulation of Plastic from a Circular Economy Perspective. *Curr. Opin. Green Sustain. Chem.* **2021**, *29*, 100462. [CrossRef]
7. Accinelli, C.; Abbas, H.K.; Bruno, V.; Khambhati, V.H.; Little, N.S.; Bellaloui, N.; Shier, W.T. Field Studies on the Deterioration of Microplastic Films from Ultra-Thin Compostable Bags in Soil. *J. Environ. Manag.* **2022**, *305*, 114407. [CrossRef] [PubMed]
8. Xu, Z.; Xiong, X.; Zhao, Y.; Xiang, W.; Wu, C. Pollutants Delivered Every Day: Phthalates in Plastic Express Packaging Bags and Their Leaching Potential. *J. Hazard. Mater.* **2020**, *384*, 121282. [CrossRef] [PubMed]
9. Uribe-Echeverría, T.; Beiras, R. Acute Toxicity of Bioplastic Leachates to Paracentrotus Lividus Sea Urchin Larvae. *Mar. Environ. Res.* **2022**, *176*, 105605. [CrossRef]
10. Rosenboom, J.-G.; Langer, R.; Traverso, G. Bioplastics for a Circular Economy. *Nat. Rev. Mater.* **2022**, *7*, 117–137. [CrossRef]
11. Barbale, M.; Chinaglia, S.; Gazzilli, A.; Pischedda, A.; Pognani, M.; Tosin, M.; Degli-Innocenti, F. Hazard Profiling of Compostable Shopping Bags. Towards an Ecological Risk Assessment of Littering. *Polym. Degrad. Stab.* **2021**, *188*, 109592. [CrossRef]
12. Guo, J.-J.; Huang, X.-P.; Xiang, L.; Wang, Y.-Z.; Li, Y.-W.; Li, H.; Cai, Q.-Y.; Mo, C.-H.; Wong, M.-H. Source, Migration and Toxicology of Microplastics in Soil. *Environ. Int.* **2020**, *137*, 105263. [CrossRef] [PubMed]
13. Weng, Y.-X.; Jin, Y.-J.; Meng, Q.-Y.; Wang, L.; Zhang, M.; Wang, Y.-Z. Biodegradation Behavior of Poly(Butylene Adipate-Co-Terephthalate) (PBAT), Poly(Lactic Acid) (PLA), and Their Blend under Soil Conditions. *Polym. Test.* **2013**, *32*, 918–926. [CrossRef]
14. César, M.E.F.; Mariani, P.D.S.C.; Innocentini-Mei, L.H.; Cardoso, E.J.B.N. Particle Size and Concentration of Poly(ε-Caprolactone) and Adipate Modified Starch Blend on Mineralization in Soils with Differing Textures. *Polym. Test.* **2009**, *28*, 680–687. [CrossRef]

15. Wohlleben, W.; Rückel, M.; Meyer, L.; Pfohl, P.; Battagliarin, G.; Hüffer, T.; Zumstein, M.; Hofmann, T. Fragmentation and Mineralization of a Compostable Aromatic–Aliphatic Polyester during Industrial Composting. *Environ. Sci. Technol. Lett.* **2023**, *10*, 698–704. [CrossRef]
16. Ruggero, F.; Onderwater, R.C.A.; Carretti, E.; Roosa, S.; Benali, S.; Raquez, J.-M.; Gori, R.; Lubello, C.; Wattiez, R. Degradation of Film and Rigid Bioplastics during the Thermophilic Phase and the Maturation Phase of Simulated Composting. *J. Polym. Environ.* **2021**, *29*, 3015–3028. [CrossRef]
17. Lwanga, E.H.; Beriot, N.; Corradini, F.; Silva, V.; Yang, X.; Baartman, J.; Rezaei, M.; van Schaik, L.; Riksen, M.; Geissen, V. Review of Microplastic Sources, Transport Pathways and Correlations with Other Soil Stressors: A Journey from Agricultural Sites into the Environment. *Chem. Biol. Technol. Agric.* **2022**, *9*, 20. [CrossRef]
18. Gómez-Brandón, M.; Domínguez, J. Recycling of Solid Organic Wastes Through Vermicomposting: Microbial Community Changes throughout the Process and Use of Vermicompost as a Soil Amendment. *Crit. Rev. Environ. Sci. Technol.* **2014**, *44*, 1289–1312. [CrossRef]
19. Domínguez, J.; Gómez-Brandón, M.; Martínez-Cordeiro, H.; Lores, M. Bioconversion of Scotch Broom into a High-Quality Organic Fertiliser: Vermicomposting as a Sustainable Option. *Waste Manag. Res.* **2018**, *36*, 1092–1099. [CrossRef]
20. Rillig, M.C.; Ziersch, L.; Hempel, S. Microplastic Transport in Soil by Earthworms. *Sci. Rep.* **2017**, *7*, 1362. [CrossRef]
21. Huerta Lwanga, E.; Mendoza Vega, J.; Ku Quej, V.; Chi, J.D.L.A.; Sanchez del Cid, L.; Chi, C.; Escalona Segura, G.; Gertsen, H.; Salánki, T.; van der Ploeg, M.; et al. Field Evidence for Transfer of Plastic Debris along a Terrestrial Food Chain. *Sci. Rep.* **2017**, *7*, 14071. [CrossRef]
22. Ragoobur, D.; Huerta-Lwanga, E.; Somaroo, G.D. Reduction of Microplastics in Sewage Sludge by Vermicomposting. *Chem. Eng. J.* **2022**, *450*, 138231. [CrossRef]
23. Liwarska-Bizukojc, E.; Bernat, P.; Jasińska, A. Effect of Bio-Based Microplastics on Earthworms *Eisenia andrei*. *Sci. Total Environ.* **2023**, *898*, 165423. [CrossRef]
24. Rodriguez-Seijo, A.; Lourenço, J.; Rocha-Santos, T.A.P.; da Costa, J.; Duarte, A.C.; Vala, H.; Pereira, R. Histopathological and Molecular Effects of Microplastics in *Eisenia andrei* Bouché. *Environ. Pollut.* **2017**, *220*, 495–503. [CrossRef] [PubMed]
25. Tourinho, P.S.; Loureiro, S.; Talluri, V.S.S.L.P.; Dolar, A.; Verweij, R.; Chvojka, J.; Michalcová, A.; Kočí, V.; van Gestel, C.A.M. Microplastic Fibers Influence Ag Toxicity and Bioaccumulation in *Eisenia andrei* but Not in *Enchytraeus crypticus*. *Ecotoxicology* **2021**, *30*, 1216–1226. [CrossRef] [PubMed]
26. Bosker, T.; Bouwman, L.J.; Brun, N.R.; Behrens, P.; Vijver, M.G. Microplastics Accumulate on Pores in Seed Capsule and Delay Germination and Root Growth of the Terrestrial Vascular Plant *Lepidium sativum*. *Chemosphere* **2019**, *226*, 774–781. [CrossRef] [PubMed]
27. Pflugmacher, S.; Sulek, A.; Mader, H.; Heo, J.; Noh, J.H.; Penttinen, O.-P.; Kim, Y.; Kim, S.; Esterhuizen, M. The Influence of New and Artificial Aged Microplastic and Leachates on the Germination of *Lepidium sativum* L. *Plants* **2020**, *9*, 339. [CrossRef] [PubMed]
28. Pignattelli, S.; Broccoli, A.; Piccardo, M.; Felline, S.; Terlizzi, A.; Renzi, M. Short-Term Physiological and Biometrical Responses of *Lepidium sativum* Seedlings Exposed to PET-Made Microplastics and Acid Rain. *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111718. [CrossRef]
29. Liwarska-Bizukojc, E. Effect of Innovative Bio-Based Plastics on Early Growth of Higher Plants. *Polymers* **2023**, *15*, 438. [CrossRef]
30. Okoffo, E.D.; O'Brien, S.; Ribeiro, F.; Burrows, S.D.; Toapanta, T.; Rauert, C.; O'Brien, J.W.; Tscharke, B.J.; Wang, X.; Thomas, K.V. Plastic Particles in Soil: State of the Knowledge on Sources, Occurrence and Distribution, Analytical Methods and Ecological Impacts. *Environ. Sci. Process. Impacts* **2021**, *23*, 240–274. [CrossRef]
31. Yu, M.; van der Ploeg, M.; Lwanga, E.H.; Yang, X.; Zhang, S.; Ma, X.; Ritsema, C.J.; Geissen, V. Leaching of Microplastics by Preferential Flow in Earthworm (*Lumbricus terrestris*) Burrows. *Environ. Chem.* **2019**, *16*, 31. [CrossRef]
32. *ISO 18763:2016*; ISO Soil Quality—Determination of the Toxic Effects of Pollutants on Germination and Early Growth of Higher Plants. ISO (International Organization for Standardization): Geneva, Switzerland, 2016.
33. OECD. *OECD Guidelines for the Testing of Chemicals (222/2016) Earthworms Reproduction Test (Eisenia fetida/Eisenia andrei), Section 2*; OECD Publishing: Paris, France, 2016. [CrossRef]
34. Sanchez-Hernandez, J.C.; Capowiez, Y.; Ro, K.S. Potential Use of Earthworms to Enhance Decaying of Biodegradable Plastics. *ACS Sustain. Chem. Eng.* **2020**, *8*, 4292–4316. [CrossRef]
35. Sáez, J.A.; Pedraza Torres, A.M.; Blesa Marco, Z.E.; Andreu-Rodríguez, F.J.; Marhuenda-Egea, F.C.; Martínez-Sabater, E.; López, M.J.; Suarez-Estrella, F.; Moral, R. The Effects of Agricultural Plastic Waste on the Vermicompost Process and Health Status of *Eisenia fetida*. *Agronomy* **2022**, *12*, 2547. [CrossRef]
36. Estoppey, N.; Castro, G.; Slinde, G.A.; Hansen, C.B.; Løseth, M.E.; Krahn, K.M.; Demmer, V.; Svenni, J.; Tran, T.-V.-A.T.; Asimakopoulos, A.G.; et al. Exposure Assessment of Plastics, Phthalate Plasticizers and Their Transformation Products in Diverse Bio-Based Fertilizers. *Sci. Total Environ.* **2024**, *918*, 170501. [CrossRef] [PubMed]
37. Moreno Abril, S.I.; Pin, A.O.; Beiras, R. Effects of Primary Leachates of Conventional and Alternative Plastics in *Cyprinodon variegatus* Fish Larvae: Endocrine Disruption and Toxicological Responses. *Environmental Pollution* **2024**, *347*, 123717. [CrossRef] [PubMed]
38. Almeda, R.; Gunaalan, K.; Alonso-López, O.; Vilas, A.; Clérandeau, C.; Loisel, T.; Nielsen, T.G.; Cachot, J.; Beiras, R. A Protocol for Lixiviation of Micronized Plastics for Aquatic Toxicity Testing. *Chemosphere* **2023**, *333*, 138894. [CrossRef]

39. Domínguez, J.; Aira, M.; Crandall, K.A.; Pérez-Losada, M. Earthworms Drastically Change Fungal and Bacterial Communities during Vermicomposting of Sewage Sludge. *Sci. Rep.* **2021**, *11*, 15556. [CrossRef]
40. *CEN EN-13432:2000*; Requirements for Packaging Recoverable through Composting and Biodegradation—Test Scheme and Evaluation Criteria for the Final Acceptance of Packaging. European Commission: Brussels, Belgium, 2000.
41. *ASTM D6400*; Standard Specification for Labeling of Plastics Designed to Be Aerobically Composted in Municipal or Industrial Facilities. ASTM International: West Conshohocken, PA, USA, 2012.
42. *ÖNORM S 2096-1*; Material Flow Analysis—Part 1: Application in Waste Management—Concepts. Austrian Standards International: Vienna, Austria, 2005. Available online: https://www.austrian-standards.at/en/shop/onorm-s-2096-1-2005-01-01~p1453654 (accessed on 2 June 2024).
43. Mak, S.L.; Wu, M.Y.T.; Tang, W.F.; Li, C.H.; Chan, T.W. A Review on Mechanical Properties Modification of Polymer with Talc Powder. In Proceedings of the 2021 IEEE International Symposium on Product Compliance Engineering-Asia (ISPCE-ASIA), Taipei, Taiwan, 30 November–1 December 2021; pp. 1–5. [CrossRef]
44. Tolga, S.; Kabasci, S.; Duhme, M. Progress of Disintegration of Polylactide (PLA)/Poly(Butylene Succinate) (PBS) Blends Containing Talc and Chalk Inorganic Fillers under Industrial Composting Conditions. *Polymers* **2021**, *13*, 10. [CrossRef]
45. Surendren, A.; Pal, A.K.; Rodriguez-Uribe, A.; Shankar, S.; Lim, L.-T.; Mohanty, A.K.; Misra, M. Upcycling of Post-Industrial Starch-Based Thermoplastics and Their Talc-Filled Sustainable Biocomposites for Single-Use Plastic Alternative. *Int. J. Biol. Macromol.* **2023**, *253*, 126751. [CrossRef]
46. Pokhrel, S.; Sigdel, A.; Lach, R.; Slouf, M.; Sirc, J.; Katiyar, V.; Bhattarai, D.R.; Adhikari, R. Starch-Based Biodegradable Film with Poly(Butylene Adipate-Co-Terephthalate): Preparation, Morphology, Thermal and Biodegradation Properties. *J. Macromol. Sci. Part A* **2021**, *58*, 610–621. [CrossRef]
47. Imam, S.H.; Gordon, S.H.; Shogren, R.L.; Tosteson, T.R.; Govind, N.S.; Greene, R.V. Degradation of Starch–Poly(β-Hydroxybutyrate-Co-β-Hydroxyvalerate) Bioplastic in Tropical Coastal Waters. *Appl. Environ. Microbiol.* **1999**, *65*, 431–437. [CrossRef] [PubMed]
48. López-Ibáñez, S.; Beiras, R. Is a Compostable Plastic Biodegradable in the Sea? A Rapid Standard Protocol to Test Mineralization in Marine Conditions. *Sci. Total Environ.* **2022**, *831*, 154860. [CrossRef] [PubMed]
49. Mergaert, J.; Anderson, C.; Wouters, A.; Swings, J.; Kersters, K. Biodegradation of Polyhydroxyalkanoates. *FEMS Microbiol. Lett.* **1992**, *103*, 317–321. [CrossRef] [PubMed]
50. Volova, T.G.; Boyandin, A.N.; Vasil'ev, A.D.; Karpov, V.A.; Kozhevnikov, I.V.; Prudnikova, S.V.; Rudnev, V.P.; Xuân, B.B.; Dũng, V.V.; Gitel'zon, I.I. Biodegradation of Polyhydroxyalkanoates (PHAs) in the South China Sea and Identification of PHA-Degrading Bacteria. *Microbiology* **2011**, *80*, 252–260. [CrossRef]
51. Beltrán-Sanahuja, A.; Casado-Coy, N.; Simó-Cabrera, L.; Sanz-Lázaro, C. Monitoring Polymer Degradation under Different Conditions in the Marine Environment. *Environ. Pollut.* **2020**, *259*, 113836. [CrossRef] [PubMed]
52. Madbouly, S.A.; Schrader, J.A.; Srinivasan, G.; Liu, K.; McCabe, K.G.; Grewell, D.; Graves, W.R.; Kessler, M.R. Biodegradation Behavior of Bacterial-Based Polyhydroxyalkanoate (PHA) and DDGS Composites. *Green Chem.* **2014**, *16*, 1911–1920. [CrossRef]
53. Cui, G.; Lü, F.; Hu, T.; Zhang, H.; Shao, L.; He, P. Vermicomposting Leads to More Abundant Microplastics in the Municipal Excess Sludge. *Chemosphere* **2022**, *307*, 136042. [CrossRef] [PubMed]
54. Domínguez, J.; Aira, M.; Kolbe, A.R.; Gómez-Brandón, M.; Pérez-Losada, M. Changes in the Composition and Function of Bacterial Communities during Vermicomposting May Explain Beneficial Properties of Vermicompost. *Sci. Rep.* **2019**, *9*, 9657. [CrossRef] [PubMed]
55. Yuan, J.; Ma, J.; Sun, Y.; Zhou, T.; Zhao, Y.; Yu, F. Microbial Degradation and Other Environmental Aspects of Microplastics/Plastics. *Sci. Total Environ.* **2020**, *715*, 136968. [CrossRef]
56. Martínez-Campos, S.; González-Pleiter, M.; Fernández-Piñas, F.; Rosal, R.; Leganés, F. Early and Differential Bacterial Colonization on Microplastics Deployed into the Effluents of Wastewater Treatment Plants. *Sci. Total Environ.* **2021**, *757*, 143832. [CrossRef]
57. Pignattelli, S.; Broccoli, A.; Piccardo, M.; Terlizzi, A.; Renzi, M. Effects of Polyethylene Terephthalate (PET) Microplastics and Acid Rain on Physiology and Growth of *Lepidium sativum*. *Environ. Pollut.* **2021**, *282*, 116997. [CrossRef] [PubMed]
58. Liwarska-Bizukojc, E. Phytotoxicity Assessment of Biodegradable and Non-Biodegradable Plastics Using Seed Germination and Early Growth Tests. *Chemosphere* **2022**, *289*, 133132. [CrossRef] [PubMed]
59. Balestri, E.; Menicagli, V.; Ligorini, V.; Fulignati, S.; Raspolli Galletti, A.M.; Lardicci, C. Phytotoxicity Assessment of Conventional and Biodegradable Plastic Bags Using Seed Germination Test. *Ecol. Indic.* **2019**, *102*, 569–580. [CrossRef]
60. Liwarska-Bizukojc, E. Application of a Small Scale-Terrestrial Model Ecosystem (STME) for Assessment of Ecotoxicity of Bio-Based Plastics. *Sci. Total Environ.* **2022**, *828*, 154353. [CrossRef] [PubMed]
61. Rorat, A.; Suleiman, H.; Grobelak, A.; Grosser, A.; Kacprzak, M.; Płytycz, B.; Vandenbulcke, F. Interactions between Sewage Sludge-Amended Soil and Earthworms—Comparison between *Eisenia fetida* and *Eisenia andrei* Composting Species. *Environ. Sci. Pollut. Res.* **2016**, *23*, 3026–3035. [CrossRef] [PubMed]
62. Ding, W.; Li, Z.; Qi, R.; Jones, D.L.; Liu, Q.; Liu, Q.; Yan, C. Effect Thresholds for the Earthworm Eisenia Fetida: Toxicity Comparison between Conventional and Biodegradable Microplastics. *Sci. Total Environ.* **2021**, *781*, 146884. [CrossRef]
63. Zimmermann, L.; Dombrowski, A.; Völker, C.; Wagner, M. Are Bioplastics and Plant-Based Materials Safer than Conventional Plastics? In Vitro Toxicity and Chemical Composition. *Environ. Int.* **2020**, *145*, 106066. [CrossRef] [PubMed]

64. Hodson, M.E.; Duffus-Hodson, C.A.; Clark, A.; Prendergast-Miller, M.T.; Thorpe, K.L. Plastic Bag Derived-Microplastics as a Vector for Metal Exposure in Terrestrial Invertebrates. *Environ. Sci. Technol.* **2017**, *51*, 4714–4721. [CrossRef]
65. Sforzini, S.; Oliveri, L.; Chinaglia, S.; Viarengo, A. Application of Biotests for the Determination of Soil Ecotoxicity after Exposure to Biodegradable Plastics. *Front. Environ. Sci.* **2016**, *4*, 68. [CrossRef]
66. Wang, L.; Peng, Y.; Xu, Y.; Zhang, J.; Liu, C.; Tang, X.; Lu, Y.; Sun, H. Earthworms' Degradable Bioplastic Diet of Polylactic Acid: Easy to Break Down and Slow to Excrete. *Environ. Sci. Technol.* **2022**, *56*, 5020–5028. [CrossRef]
67. Yu, H.; Shi, L.; Fan, P.; Xi, B.; Tan, W. Effects of Conventional versus Biodegradable Microplastic Exposure on Oxidative Stress and Gut Microorganisms in Earthworms: A Comparison with Two Different Soils. *Chemosphere* **2022**, *307*, 135940. [CrossRef] [PubMed]
68. Novo, M.; Verdú, I.; Trigo, D.; Martínez-Guitarte, J.L. Endocrine Disruptors in Soil: Effects of Bisphenol A on Gene Expression of the Earthworm Eisenia Fetida. *Ecotoxicol. Environ. Saf.* **2018**, *150*, 159–167. [CrossRef] [PubMed]
69. Novo, M.; Muñiz-González, A.B.; Trigo, D.; Casquero, S.; Martínez Guitarte, J.L. Applying Sunscreens on Earthworms: Molecular Response of *Eisenia fetida* after Direct Contact with an Organic UV Filter. *Sci. Total Environ.* **2019**, *676*, 97–104. [CrossRef] [PubMed]
70. Kwak, J.I.; An, Y.-J. Length- and Polymer-Dependent Ecotoxicities of Microfibers to the Earthworm *Eisenia andrei*. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2022**, *257*, 109354. [CrossRef] [PubMed]
71. Kwak, J.I.; An, Y.-J. Microplastic Digestion Generates Fragmented Nanoplastics in Soils and Damages Earthworm Spermatogenesis and Coelomocyte Viability. *J. Hazard. Mater.* **2021**, *402*, 124034. [CrossRef]
72. Meng, K.; Lwanga, E.H.; van der Zee, M.; Munhoz, D.R.; Geissen, V. Fragmentation and Depolymerization of Microplastics in the Earthworm Gut: A Potential for Microplastic Bioremediation? *J. Hazard. Mater.* **2023**, *447*, 130765. [CrossRef]