Integrated assessment of the chemical, microbiological and ecotoxicological effects of a bio-packaging end-of-life in compost

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Integrated assessment of the chemical, microbiological and ecotoxicological effects of a bio-packaging end-of-life in compost

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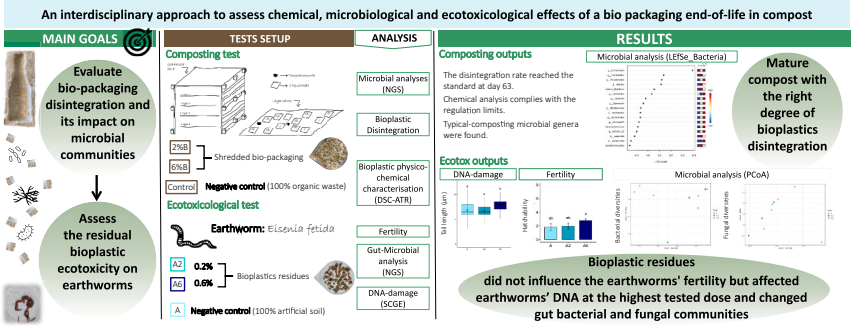
HIGHLIGHTS

- Ecotoxicity tests assessed bio-packaging residues and their impact on earthworms.
- The bio-packaging reached the disintegration rate required by the standards
- The fungal and bacterial communities found indicate proper composting process.
- Bioplastic residues didn't impact earthworm fertility but affected DNA at high doses.
- Bioplastic residues influenced gut bacterial and fungal communities.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study aimed to i) assess the disintegration of a novel bio-packaging during aerobic composting (2 and 6 % tested concentrations) and evaluate the resulting compost ii) analyse the ecotoxicity of bioplastics residues on earthworms; iii) study the microbial communities during composting and in 'earthworms' gut after their exposure to bioplastic residues; iv) correlate gut microbiota with ecotoxicity analyses; v) evaluate the chemico-physical characterisation of bio-packaging after composting and earthworms' exposure.

Both tested concentrations showed disintegration of bio-packaging close to 90 % from the first sampling time, and compost chemical analyses identified its maturity and stability at the end of the process.

Ecotoxicological assessments were then conducted on *Eisenia fetida* regarding fertility, growth, genotoxic damage, and impacts on the gut microbiome. The bioplastic residues did not influence the earthworms' fertility, but DNA damages were measured at the highest bioplastic dose tested. Furthermore bioplastic residues did not significantly affect the bacterial community during composting, but compost treated with 2 % bio-packaging exhibited greater variability in the fungal communities, including *Mortierella*, *Mucor*, and *Alternaria* genera, which can use bioplastics as a carbon source. Moreover, bioplastic residues influenced gut bacterial communities, with *Paenibacillus*, *Bacillus*, *Rhizobium*, *Legionella*, and *Saccharimonadales* genera being particularly abundant at 2 % bioplastic concentration. Higher concentrations affected microbial composition by favouring different genera such as *Pseudomonas*, *Ureibacillus*, and *Streptococcus*.

For fungal communities, *Pestalotiopsis* sp. was found predominantly in earthworms exposed to 2 % bioplastic residues and is potentially linked to its role as a microplastics degrader. After composting, Attenuated Total Reflection analysis on bioplastic residues displayed evidence of ageing with the formation of hydroxyl groups and amidic groups after earthworm exposure.

1. Introduction

Plastics have become an environmental emergency worldwide causing severe diffuse pollution (Li et al., 2021), mainly due to their recalcitrance and persistence in the environment and, accumulation in living organisms in the form of fragments less than 5.0 mm in length (micro-plastics, MPs) or inferior to 100 nm (nano-plastics, NPs). The community has taken action with international or national plans, investments by private companies, and global and local associations' initiatives to limit plastic pollution (Schnurr et al., 2018). In Europe, the progressive reduction of single-use plastic production, representing the most impactful artefacts (Báreková et al., 2021), was promulgated by a 2019 EU directive (European Parliament, 2019). In addition, it encouraged the development and use of new materials that could fulfil conventional plastics' functions and biodegrade in adequate times (Emadian et al., 2017).

In this context, bioplastics, i.e. polymer compounds that are functionally similar to synthetic plastics but considered more environmentally sustainable, were introduced in the past decades (Atiwesh et al., 2021). Bioplastics comprise a vast family of materials and could be classified mainly as bio-based, biodegradable or with both characteristics. According to European standards, bio-based plastics are made at least partially from biological resources (Bátori et al., 2018). On the other hand, bioplastics, defined as biodegradable, must biodegrade at definite timescales, with precise temperature and ventilation in industrial conditions, as accurately described by specific EU regulations (European Committee for Standardization, 2007, 2000). The packaging sector is a driving force in bioplastic object manufacturing; in 2021, 43 % of the production of bioplastic materials is concerned with bio-based packaging and 50 % with biodegradable packaging (European Bioplastics, 2022).

The growing demand for sustainable alternatives for packaging from renewable resources, not fossil hydrocarbon-based, is strongly recommended to reduce waste and recycle waste materials (Babalis et al., 2013; UNEP, 2021). Using biopolymers from biomass and crop residues leads to both economic savings and lower environmental impacts. Cereal industry byproducts could fit perfectly into circular economy projects; some examples are starch-based and cellulosic materials, which are among the most often produced byproducts (Gadhave et al., 2018; Rossi et al., 2020).

Although bioplastics save more non-renewable energy than conventional plastics and emit fewer greenhouse gasses, they could have negative environmental impacts such as water eutrophication and acidification (Bohlmann, 2004; Folino et al., 2020; Rameshkumar et al., 2020; Weiss et al., 2012).

Moreover, studies found that bioplastics could interfere with natural environments, especially in marine ecosystems (Brizga et al., 2020; Mastrolia et al., 2022; Napper and Thompson, 2019), while few reports exist on their effects on the terrestrial environment (Boots et al., 2019; Liwarska-Bizukojc, 2021). Bioplastics can enter the environment following different routes, mainly summarised in the incorrect disposal by consumers, the use of bioplastic mulch films, the distribution of amendments, which may contain bioplastic residues such as compost, sewage sludge, digestate, etc. (Bandini et al., 2022a; Liwarska-Bizukojc, 2021; Van Schothorst et al., 2021).

Bioplastics and their residues entering the soil ecosystem are subjected to chemical, physical, and biological transformations, while interacting with microorganism, plants and earthworms. *Eisenia fetida* (*E. fetida*) earthworm is commonly employed in ecotoxicity standard analysis with various endpoints (behavioural response, lethality, growth and fertility) (ISO-17512-1, 2008; OECD, 2016, 2004). Moreover, due to their ability to ingest and transport soil particles, these annelids are considered appropriate organisms for the study of plastic and bioplastic pollutant particles.

Recently, some local wine companies proposed introducing and potentially using bio-packaging consisting of compressed straw and bioplastic film for their environmentally friendly products (Rossi et al., 2020). Limited research is available on this new class of bio-packaging and its effect on fungi and bacteria during composting. Furthermore, most of the articles studied the ecotoxicological effects of plastics, and little is known about the possible impacts of bioplastics on soil organisms and microorganisms (Fan et al., 2022). The present work was developed to shed light on this literature gap, offering a complete end-of-life view and the effects on the environment, also analysing the change of the material from a chemical point of view.

More in detail, this study aimed to evaluate a novel bio-packaging material's early disintegration and its effect on the microbial dynamics involved during aerobic composting. Secondly, bioplastic residues and their impacts on earthworms were evaluated. *Eisenia fetida* was used to assess the genotoxicity damage, reproductive activity, and influence on their gut microbiota. Furthermore, bioplastics were chemically characterised in each step to evaluate their degradation evolution after composting and after exposure to earthworms.

2. Materials and methods

2.1. Tested bio-packaging

The bio-packaging used for the composting process was a prototype suggested for wine bottle delivery. It was made of compressed straw covered externally by a bioplastic multilayer film (Supplementary materials, Fig. S1). The bioplastic film was composed of an aliphatic-aromatic copolyester based on the monomers 1.4-butanediol, adipic acid and terephthalic acid in the polymer chain, and it is characterised by 12 μm total thickness. The bio-packaging ratio of bioplastic to straw fibres was 1 to 5 (*w*/w).

The bio-packaging was chopped up manually for the experiment into large pieces of approximately 2 × 5 cm (Supplementary materials, Fig. S2) to simulate the mechanical stresses due to industrial composting processes such as shredding, turning, screening, etc., and as already reported (Bandini et al., 2022a, 2022b).

2.2. Experimental design and composting

The starting substrate assembled to make the compost pile was a mixture of organic materials, all screened and plastic/bioplastic-free. The mixed substrate (MS) comprised, in equal proportion, domestic food waste from the organic fraction of the municipal waste separate collection system, plus other vegetable waste from the local fruit and vegetable market, and waste from maintenance of the urban green areas such as tree-shrub pruning residues, twigs, grass, and leaves, acting as a bulking agent. A portion of manure was added to the mixture to allow fermentation to begin.

A 1-m^3 compost pile with the MS was assembled outdoors on a concrete platform covered by a waterproof wooden roof to prevent precipitation interference (Supplementary Materials, Fig. S3). The entire pile was turned over in the first week and at each sampling time to keep the compost properly aerated and homogenous. The MS was amended with the shredded bio-packaging at different concentrations, 2 and 6 % w/w, or left unamended as negative control (100 % MS). Each of these three treatments was arranged in 16 replicates represented by mesh bags with 1 mm holes containing the equivalent of 1 kg dry substrate. These two concentrations were chosen based on the latest Italian waste reports and current bioplastic production trends. The percentage of bioplastics in organic waste was 3.7 % (w/w) in the latest Italian report, and two different percentages, a lower and a higher one, were selected to predict future trends, considering the continuous increase of these materials.

The compost pile was divided into four main layers, namely layers 1, 2, 3 and 4 from bottom to top. In each layer, a total of 12 bags were randomly placed, respectively adding 4 replicated bags for each treatment. This design was employed to obtain 4 destructive replicas per treatment from each of the 4 layers. The 4 layers were sampled respectively at day 21 (Sample 1; S1), 42(Sample 2; S2), 63(Sample 3; S3) and 84 (Sample 4; S4) after the beginning of the aerobic composting. During the trial, the temperature was monitored to check accurately the progress of the composting. Two temperature probes (Watchdog 1000, Ecosearch srl, Italy) per layer were located respectively in a lateral and central position of the pile's core to estimate the potential temperature gradient. Data loggers were used to record the temperature measurements every 15 min.

2.3. Biodegradation and compost analyses

At each sampling time, the bags' contents were sieved at 2 mm, and the bioplastics residues were cleaned with water, weighed, and compared with the initial sample to evaluate the disintegration status of the bioplastic with the following formula according to the UNI EN 16929:2021 Regulation (ISO 16929, 2021):

$$D = \frac{m_i - m_r}{m_i} \times 100$$

where *mi* represents the initial dry mass of the bioplastic tested, and *mr* is the dry mass of the persistent bioplastic with a diameter higher than 2 mm.

At the end of the composting process, total elements (Cd, Cr, Cu, Fe, K, Mn, Ni, Pb, Zn and Hg) were extracted following the protocol in aqua regia by Kasassi et al. (2008) with some modifications as reported in a previous study (De Bernardi et al., 2022). According to EPA 6010D (U.S. EPA., 2014), the analysis of the elements was carried out by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent mod. 5800).

Other analyses made at the end of the composting were Total Organic Carbon, following the standard method by Walkley and Black (1934); Total phosphorous (Olsen method), pH ($CaCl_2$) and nitrogen analysis were conducted following Italian regulation (Ministero delle Politiche Forestali, 2013).

Finally, Organic nitrogen content was calculated as the difference between the total fraction (Kjeldahl method) and the N-NH_3 measured by titration digestion and steam distillation.

2.4. Ecotoxicological tests with Eisenia fetida

Eisenia fetida earthworms were purchased from the company Lombricoltura Bella Farnia (Sabaudia, Italy) and then reared in the laboratory at 20 ± 1 °C in organic compost and fed with organic oats and vegetables. Adults with wet body weights between 300 and 600 mg were selected and acclimatised for a week in an artificial soil (ART) as described by the Organization for Economic Cooperation and Development (OECD, 2004). ART soil was characterised by 3 % organic matter content, 6.5 pH (H_2O), and nutrient content such as N and P, respectively of 0.6 and 7.1 g kg^{-1}.

A 28-day test was set up to evaluate the ecotoxicological effects of bioplastic residues obtained at the end of the composting processes on earthworms and separately added to an artificial soil (A).

In detail, a total of three treatments were tested: the unaltered artificial soil (A) that was used as control, and two bioplastics treatments, namely A2 and A6, in which the A soil was mixed respectively with all the bioplastic residues from the 2 and 6 % bio-packaging additions, that represented quantitatively 0.2 and 0.7 % of the final mixture tested.

Three replicates of each treatment were prepared, and each consisted of 500 g of the substrate and ten adult earthworms. At the beginning of the tests and after 28 days of exposure, the gut of one earthworm per replicate was stored for metagenomic analysis.

The analysis of earthworms' reproductive capacity and other sublethal endpoints (i.e. unusual behaviour and body anomalies) was performed following the n. 222 OECD guidelines (OECD, 2016). All treatments were kept at a controlled temperature (20 ± 1 °C) and water content (60 %) for 56 days. After 28 days, adults were weighed and removed from the containers while the substrate containing juveniles and cocoons was incubated for another four weeks. One adult from each replicate was used for the genotoxicity assessment with the SCGE test, also known as Comet Assay; similarly, three test earthworms were euthanised with 70 % ethanol, and their gut was dissected and used for the analysis of the intestinal microbiome; protocols were the same as reported in a previous study (Marini et al., 2024).

After 56 days, the number of juveniles and cocoons per replicate was recorded. The growth rate (GR %) was calculated as the percentage of weight change between the experiment's start and end (28 days).

Significant differences between treatments in the applied tests were assessed using the Tukey post-hoc parametric test where ANOVA assumptions were respected; in other cases, non-parametric Kruskal–Wallis and post-hoc Dunn tests were employed. Statistical analyses were performed in R software version 4.3.1. A compact letter display

was constructed using the cld function of the R companion package to show significant differences ($\alpha = 0.05$). Treatments not sharing any letters were significantly different. When lowercase letters were not reported, no statistical differences were found between groups.

2.5. Microbiological analyses

2.5.1. DNA extraction

Microbiological molecular analyses were performed on compost at different stages of the process (21, 42, 63 and 84 days after the beginning of the aerobic composting), as described in paragraph 2.1, and on the gut of earthworms after exposure to bioplastics residues. For each sample, the total DNA was extracted with DNeasy PowerSoil (Qiagen), according to the manufacturer's protocol. Extracted DNA was quantified with a Quant-iTTM HS ds-DNA assay kit (Invitrogen, Paisley, UK) with a QuBit 2.0 fluorometer (Invitrogen, Paisley, UK).

2.5.2. DNA amplification and Illumina high-throughput sequencing

The bacterial V3-V4 region of the 16S ribosomal RNA (rDNA) gene was amplified by PCR using the universal primers 343F (5′-TACGGRAGGCAGCAG-3′) and 802R (5′-TACNVGGGTWTCTAATCC-3′). The thermal profile comprised an initial denaturation, followed by 20 cycles of denaturation, annealing and extension, as previously described (Bandini et al., 2021; Vasileiadis et al., 2012, 2015). The fungal Internal Transcribed Spacer 1 (ITS1) region of ribosomal RNA (rRNA) was amplified using the universal primers ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-2 (5′-GCTGCGTTCTTCATCGATGC-3′) (White et al., 1990) with a similar thermal cycle. A two-step PCR was performed to sequence multiple samples in a single run using forward-indexed primers. The products of the second step were multiplexed as a single pool for bacteria and fungi separately and purified, and the sequencing process was performed by Novogene UK (Cambridge, UK). More detailed information is provided in the supplementary materials (Supplementary Information, Section 1).

2.5.3. Sequence data preparation and analyses

The TruSeq DNA sample preparation kit was employed for amplicon preparation (REF 15026486, Illumina Inc., San Diego, CA), and the Novaseq 6000 Illumina instrument (Illumina Inc., San Diego, CA) was used to obtain 250 bp paired-end reads. Illumina barcode demultiplexing and base calling were performed with the MiSeq Control Software version 2.3.0.3, RTA v1.18.42.0, and CASAVA v1.8.2 (Bortolini et al., 2016). Raw sequences were aligned with the 'pandaseq' script (Bartram et al., 2011) with a minimum overlap of 30 bp between read pairs and a maximum of two mismatches allowed. After the filtration, trim, and denoising of the demultiplexed sequences of each sample, the chimeric sequences were recognised and removed using the QIIME™ 2 vsearch plugin to acquire the feature table of amplicon sequence variants (ASV) (Bolyen et al., 2019). For bacteria, the QIIME™ 2 feature-classifier plugin was used to align the ASV sequences with a Silva trained classifier, which was trimmed to the V3-V4 region bound by the 338F/806R primer pair. Similarly, the UNITE classifier, trained with release 29.11.2022 (10.15156/BIO/2483915), was applied for ITS taxonomy identification,.

2.6. Bio-packaging physico-chemical characterisation

The physico-chemical characterisation of the biopolymer under study was carried out on the pristine samples at time zero (T0-pristine), therefore before the composting process, on the bioplastic residues found at the end of the composting for both concentrations tested (2 % C and 6 % C) and finally on the residual bioplastics after they had been in contact with earthworms in the ecotox test which lasted 56 days (2 % E and 6 % E).

For thermal characterisation, TA Instruments DSC (Differential Scanning Calorimetry) Q20 (New Castle, Delaware, USA) was used in order to analyse about 8 mg of each sample with the following thermal cycle (performed under nitrogen atmosphere with a rate of 10 °C/min): a first heating step was performed from 25 °C to 220 °C, followed by an isothermal step at 220 °C for 3 min. Afterwards, a cooling scan from 220 to 25 °C and a second heating ramp from 25 °C to 220 °C have been done.

For spectroscopic analysis, Attenuated Total Reflectance (ATR) analysis was carried out using a Perkin-Elmer Frontier FT-IR spectrophotometer in the range of 500–4000 cm^{-1} (16 scans, 4 cm^{-1} resolution, diamond crystal). Three measures were performed on different points of each sample and the mean spectrum was evaluated.

3. Results and discussions

3.1. Composting test

The temperature recorded by each probe during the 84-day compost process (Supplementary materials, Fig. S4) showed the sudden temperature drops corresponding to the turnings. Although highly variable temperature trends have been measured between the outermost side (probes at edge, B) and the innermost side (probes at centre, C) of the pile, 60 °C was reached in all the layers for at least two weeks, a threshold value for the activation of degradative processes as described in the regulations.

The disintegration values measured ($n = 4$) at each sampling time (21, 42, 63 and 84 days) were 87.10 ± 8.60, 90.70 ± 1.46, 92.28 ± 1.80 and 91.41 ± 2.98 % for treatment with 2 % of bio-packaging and 90.05 ± 0.98, 90.31 ± 1.63, 92.00 ± 5.61 and 93.13 ± 2.17 % for treatment about 6 % of bio-packaging (data not shown). Values close to 90 % of disintegration were achieved in both treatments already at the first sampling time. The standard provides for exceeding 90 % of disintegration at the end of composting, a parameter acquired in both treatments from the second sampling time (42 Days).

Metal analysis of the compost sampled from the control bags and from the test bags with 2 and 6 % bio-packaging at the end of the composting process are shown in Table S1. Pb, Cd, Cu, Ni, Zn and Hg were found in concentrations below the thresholds indicated by the Legislative Decree of 2010 (Gazzetta Ufficiale 75/2010).

The organic matter measured on the treatments at the end of composting was 20.14 ± 5.73 %, 18.89 ± 1.71 % and 19.78 ± 1.78 % for MS, 2 % and 6 %, respectively. No significant differences in organic matter content between treatments ($n = 6$, Kruskal Wallis and Dunn post hoc test) were found. No unpleasant smell and pH values around 8.0 were assessed in all samples; therefore, the required standards have been achieved (UNI CEN/TES 16202:2013). Other parameters measured on compost samples were N (11.2 g kg^{-1}) and P (3.65 g kg^{-1}); the data measured were similar to values reported in the literature (Balaguer et al., 2016; Khater, 2012). The pH values obtained at the end of composting are in line with those of a mature compost, which should generally fluctuate between 7 and 9. Temperature and pH registered are known to favour the biodegradation of bioplastics by microorganisms during the composting process, which is considered the best technique for the disposal of this material (Beltrán-Sanahuja et al., 2021; Shruti and Kutralam-Muniasamy, 2019).

The quantity of organic matter measured is slightly low, but this depends a lot on the starting materials and can be interpreted as an indicator of a good composting process for transforming organic matter into CO_2 (Azim et al., 2018).

The C/N ratio, calculated as organic carbon/organic nitrogen, resulted in 10.25. This value was consistent with the ratio measured in other papers (Canditelli et al., 2022) and is below 25, the "compost stability limit" suggested by environmental agencies (Ministero delle Politiche Forestali, 2013). As reported by other authors, bioplastics do not affect the compost's final quality negatively (Canditelli et al., 2022; Lavagnolo et al., 2020).

3.2. Bacterial and fungal communities' dynamics during composting with bioplastics

Microbial communities are crucial during composting process to degrade organic matter (Xu et al., 2019). The presence of plastics and bioplastics in soil affects the diversity, richness and composition of soil microbial communities (Hou et al., 2021; Huang et al., 2019; Ng et al., 2021; Ren et al., 2020). Especially, when composted, fragmented polymers decrease the richness and diversity of bacteria at thermophilic conditions (Sun et al., 2021).

In our work, bacterial amplicons resulted in 4.706.292 high-quality sequences and a minimum of 25.000 reads per sample. Fig. 1b shows the dendrogram grouping composting samples, based on a distance measure between data points in the clustering input (Brian Curtis Index). The samples clustered differently depending on the percentage of bioplastics and sampling time. Notably, the T0 sampling, performed before starting composting, shows a completely different bacterial community, indicating that the fermentation process of the organic matter occurred. On the other hand, S2 for both 2 % and 6 % bioplastics treatment was the most distinctive, while for S3 and S4 less differences in bacterial dynamics were found. Control samples showed no significant differences during the process, demonstrating that the differences in the bacterial community found in the other samples are probably due to the treatment and thus the presence of the bioplastics. In Fig. 1a, OTUs are summarised and compared to their abundance at genus taxonomic levels based on the annotation. Abundances of bacterial genera typical of composting, such as *Bacillus* sp., *Thermoactinomyces* sp., *Gluconobacter* sp., *Brevibacillus* sp., *Paenibacillus* sp., were identified, and their presence suggests that the process was carried out correctly. Furthermore, no pathogenic bacteria genera were identified. Going into more detail, *Leuconostoc* sp. was mostly abundant at T0 and decreased during composting. These mesophilic bacteria are typical for organic household waste, together with *Lactobacillus* and *Pseudomonas* sp. (Alfreider et al., 2002; Andrews et al., 1994). On the other hand, the SBR1031 genus, which was mostly abundant in 2 % treatment during all the sampling times, due to their ability to grow again during the cooling stage of the process is related to the maturity of the compost (Wang et al., 2022). Moreover, the presence of *Ureibacillus*, *Lysinibacillus* and *Limochordaceae* genera leads to an appropriate temperature environment, enhancing the amino acid and carbohydrate metabolism function and promoting the degradation of the organic matter (Wang et al., 2022). However, no particular differences were noted between the treatments, and the presence of bioplastic pieces in different percentages appears to have had no impact on the bacterial community during composting. Bacteria, such as *Solibacillus* sp., *Pseudomonas* sp. and *Phenylobacterium* sp., and fungi, such as *Aspergillus* sp., *Trametes* sp. and *Phanerochaete chrysosporium*, are responsible for organic materials disintegration into carboxyl acids, amino acids, polyphenols and sugars reduction (Jiang et al., 2021a; Wang et al., 2018).

Although the bacterial community plays a crucial role in the composting process, the fungal community can decompose several compounds, such as lignocellulose (Xie et al., 2021a), due to high metabolic versatility. The effect of plastic pollution and its fragmentation on the biological treatment of organic waste has been widely assessed, but little is known about the influence of bioplastics and bioplastics residues on the composting process and the fungal community involved. However, in a recent study by Zhou et al. (2022), micro-plastics and -bioplastics added during thermophilic composting decreased the diversity and richness of the fungal community and increased the relative abundance of phytopathogenic fungi.

In the current work, fungal amplicons resulted in 2.723.326 high-quality sequences and a minimum of 15.000 reads per sample. Fig. 2b shows the distance measure among fungal communities in compost samples treated with different percentages of bioplastics. As for bacteria,

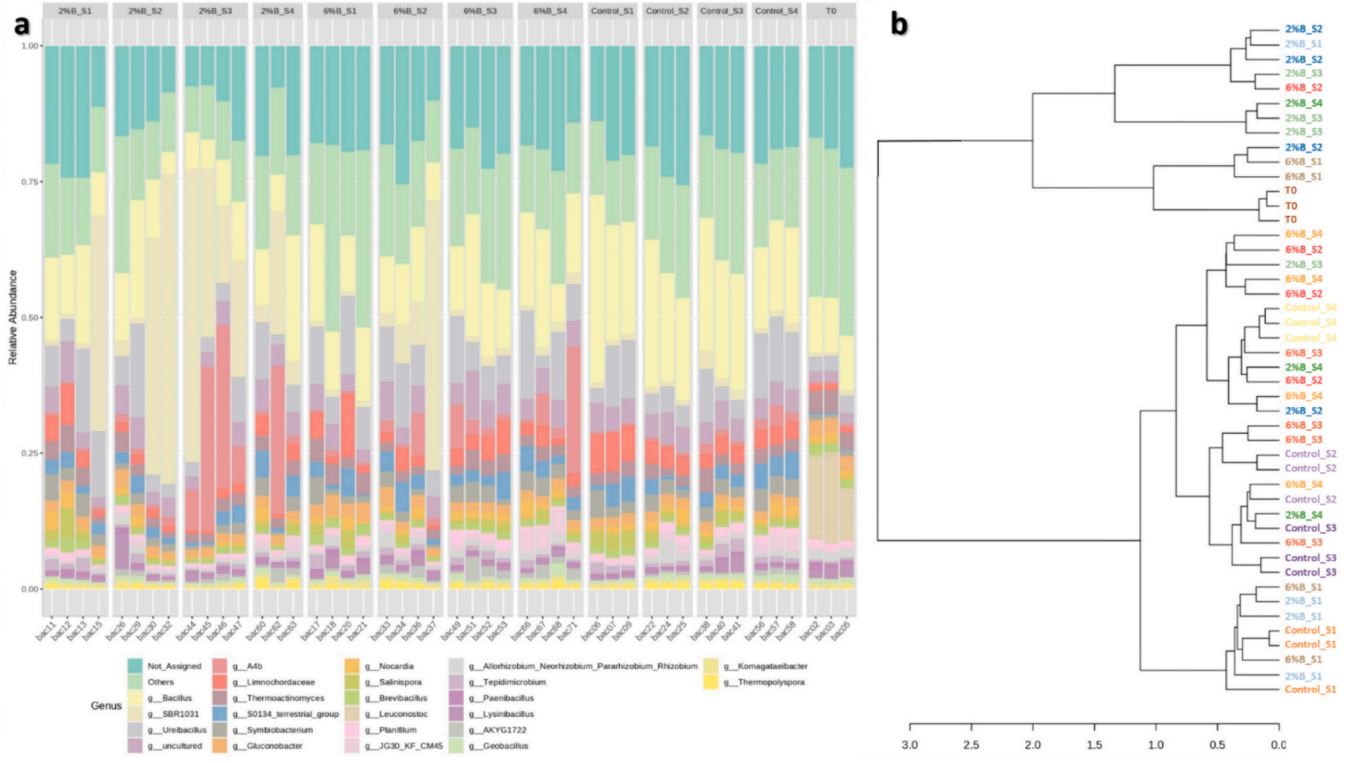

Fig. 1. a) Taxonomic distribution of bacterial communities in compost at different process stages using the average linkage algorithm at the genus level. b) Phylogenetic tree showing the relationship between the different compost treatments. Legend: "Control_*Sn*", control treatment at different sampling times S1, S2, S3 and S4; "2%B_*Sn*", 2 % of bioplastic treatment at different sampling times S1, S2, S3 and S4; "6%B_*Sn*", 6 % of bioplastic treatment at different sampling time S1, S2, S3 and S4.

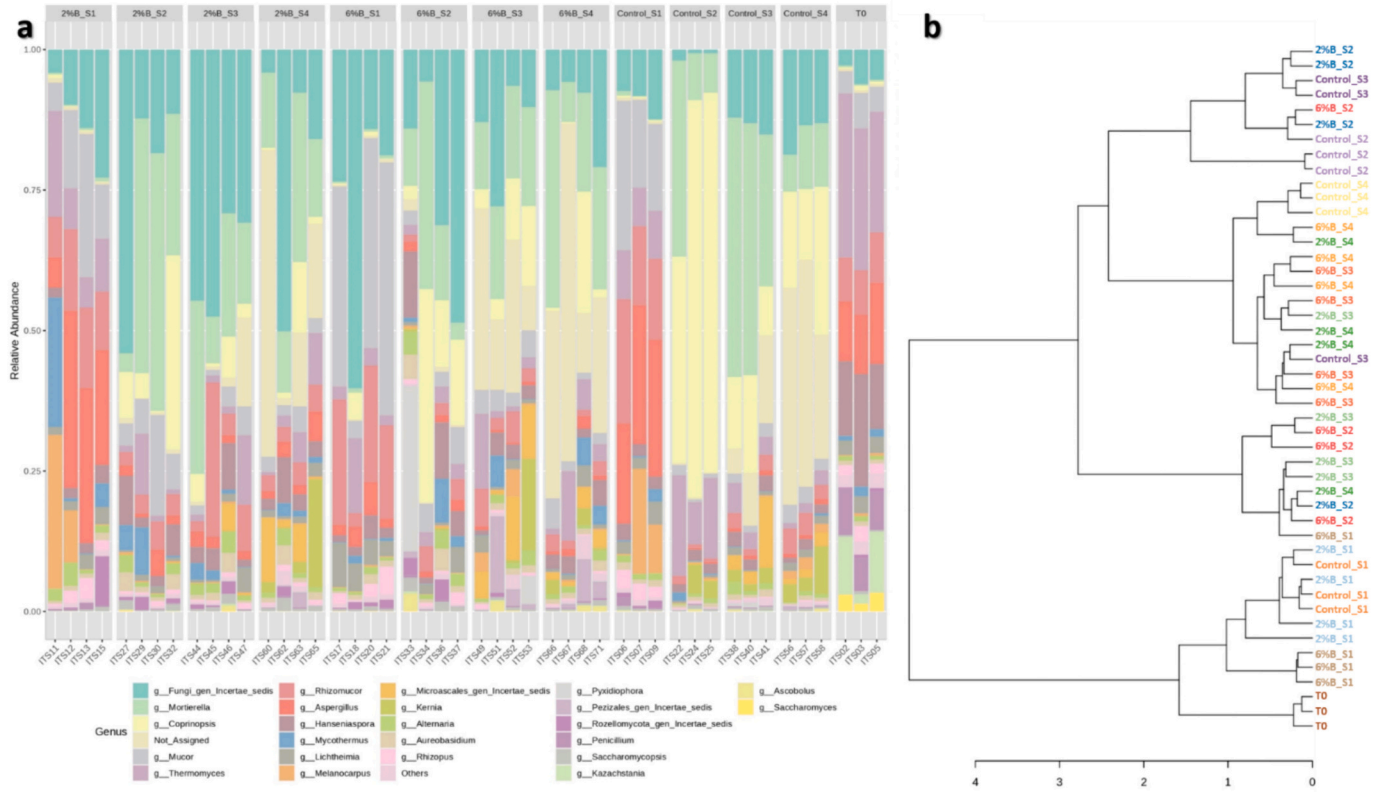

Fig. 2. a) Taxonomic distribution of fungal communities in compost at different stages of the process using the average linkage algorithm at the genus level. b) Phylogenetic tree showing the relationship between the different compost treatments. Legend: "Control_*Sn*", control treatment at different sampling times S1, S2, S3 and S4; "2%B_*Sn*", 2 % of bioplastic treatment at different sampling times S1, S2, S3 and S4; "6%B_*Sn*", 6 % of bioplastic treatment at different sampling time S1, S2, S3 and S4.

T0 clustered separately, while S1 for 2 % bioplastics treatment and the control showed similarities. On the other hand, S2 and S3 for the three treatments (2 %, 6 % and control) showed a distinctive distance measure based on the Brian Curtis Index. The general pattern discovery at the genus level is shown in Fig. 2a and reports *Aspergillus* sp. as one of the most abundant genera at S1 for 2 % bioplastics, control, and at T0. *Mortierella* and *Mucor* genera were found from S2 in every sample, but their abundance decreased at later sampling times, as for *Rhizomucor* sp. whose abundance decreased during composting in 2 % bioplastic treatment. *Mortierella* and *Mucor* genera were found in previous works on the organic fraction of municipal solid waste composting in the presence of bioplastics (Bandini et al., 2022b; Zhou et al., 2022). Species belonging to *Mortierella* sp. were isolated from soil samples and exploited to enhance the biodegradation of bioplastics at low temperatures (Urbanek et al., 2021). The *Alternaria* genus was identified mainly in the samples with 2 % bioplastic, particularly in the first three sampling times. Its abundance in the remaining samples analysed was lower, assuming that its presence is favored by the presence of a certain percentage of bioplastic used as a carbon source. Some species of this genus are reported in the literature as PCL (polycaprolactone) and PBS (polybutylene succinate) degraders (Abdel-Motaal et al., 2019; Qi et al., 2010). In conclusion, compost treated with 2 % bioplastics showed a higher variability for fungal communities, and the most significant difference was found for the fungal communities during composting with bioplastics.

3.3. Ecotoxicological effects of bioplastics residues on earthworms

The statistical analysis did not highlight significant differences in the Growth Rate (GR) between treatments A (−11.83 ± 2.47), A2 (−11.34 ± 1.18), and A6 (−10.61 ± 2.83). Even in a study by Huerta-Lwanga et al. (2021) conducted on *Lumbricus terrestris*, where PLA (polylactic acid) was tested at different concentrations (0.1, 0.25, 0.5, 0.75, 1, 3 and 5 %), no significant differences were found regarding the percentage biomass change between treatments.

In a previous paper (Boots et al., 2019), *Aporrectodea rosea* earthworms exposed to microplastics such as PLA at a ratio of 0.1 % significantly reduced their biomass but always more moderately than those exposed to non-biodegradable plastics HDPE (high-density polyethylene).

The absence of mortality in tests with earthworms exposed to bioplastic particles (0.2 % in A2 and 0.7 % in A6 treatment) was also observed in other studies (Liwarska-Bizukojc, 2022; Rodríguez et al., 2023). Conversely, mortality was observed on *Lumbricus terrestris* exposed to 1 % (*w*/w) micro or macro particles from starch-based mulch in the study by Qi et al. (2018). Other authors (Ding et al., 2021) also found dead earthworms when subjected to PLA and PPC (biodegradable polypropylene carbonate) microparticles, but in the latter, doses were considerably higher (mortality started at 12.5 % w/w). The mortality absent in our study and instead recorded in the studies of Qi and Ding can be motivated by the fact that in the latter, the concentrations tested are higher, and several species have different capacities to break down plastic and microplastics (Khaldoon et al., 2022).

In Fig. 3, fertility outputs such as cocoon and juvenile production and hatchability values were reported for each treatment.

Regarding added bio-packaging residues (0.2 % in A2 and 0.7 % in A6 treatment), no differences with the control (artificial soil without bioplastic residues A) were found, suggesting no adverse effects of these particles on earthworms' fertility. As discussed previously, some experiments report an unfavourable effect of bioplastics on earthworms in terms of survival and reproductive activity, while other papers didn't measure any adverse effects; however, comparing results is complicated

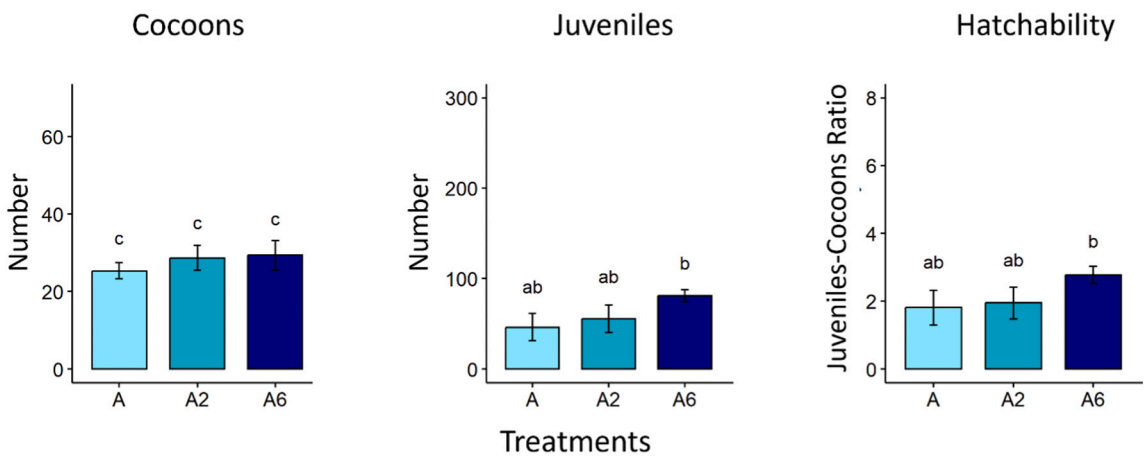

Fig. 3. Fertility parameters from reproduction trial (56 days). Different lowercase letters represent significant differences between treatments according to the ANOVA-Tukey parametric test ($n = 3$). Treatments codes legend: artificial soil (A), artificial soil with bio-packaging at lower concentration (A2), artificial soil with bio-packaging at higher concentration (A6).

due to different experimental designs (heterogeneity of substrates, earthworm species, and tested bioplastic) (Chah et al., 2022).

As regards fertility, on the one hand, some studies found a stimulation due to the bioplastics addition in the substrate: Qi et al. (2018) measured an enhanced production of juveniles by bioplastics compared to non-degradable plastics tested (low-density polyethylene), and Holzinger et al. (2023) estimated a stimulation of reproduction when *E. fetida* was exposed for eight weeks in an artificial soil amended with 1 % and 2.5 % (*w*/w) biodegradable polymers (poly-(L-lactide): PLLA and PCL).

Otherwise, in a work on *E. andrei* (Sforzini et al., 2016), no effects of MaterBi® residues were measured on survival and reproduction rates; this perhaps also because the material was not added as is but after a six-month composting process. This ageing condition of the bioplastic materials tested approaches and aligns with the reproduction outputs of the present study. It must be taken into consideration that the weathering on bioplastics brings chemical changes that usually reduce toxicity in earthworms (Ji et al., 2021), as reported by Ferreira-Filipe et al. (2022), where the same bioplastic material does not produce adverse effects on *E. andrei* if subjected to weathering. Furthermore, the invasiveness of untouched bioplastic instead of the weathered one was perceived by earthworms, which preferred to swallow the latter in a study focusing on the biofilm ingestion behaviour of *L. terrestris* (Zhang et al., 2018).

Fig. 4 shows the results of Comet Assay on the earthworm' coelomocytes after 28 days of exposure. This test is highly indicative of genotoxic damages occurred during exposition to a toxicant.

According to Comet assay results, a certain DNA-damage response is observable in earthworms subjected to bio-packaging residues added to 0.7 % (A6) compared to control and treatment with 0.2 % residues (A2). Bioplastics' size and dose strongly influence toxicity effects on earthworms (Fan et al., 2022; Jiang et al., 2021b; Mo et al., 2023). Most studies focus on microparticles of plastic (non-bioplastic type), and where bioplastics were employed, they were studied in terms of oxidative stress or by calculating integrated biomarker response indexes induced in earthworms instead of the use of comet assay (Sanchez-Hernandez et al., 2020). Despite this, it is known that oxidative stress (such as excessive ROS accumulation) induces DNA damage in earthworm coelomocytes (Guo et al., 2023; Mincarelli et al., 2016).

Baihetiyaer et al. (2023a, 2023b) found that micro-bioplastic (PLA) induced DNA damage at 1 % w/w exposure on *E. fetida*. In another paper that combined the PS (polystyrene)/PLA effect alone or in combination with Cd exposure on the same earthworm species, authors didn't find an adverse effect on DNA about PLA alone, but comet assay revealed that both microparticles exacerbate the toxicity of Cd in earthworms (Shang et al., 2023).

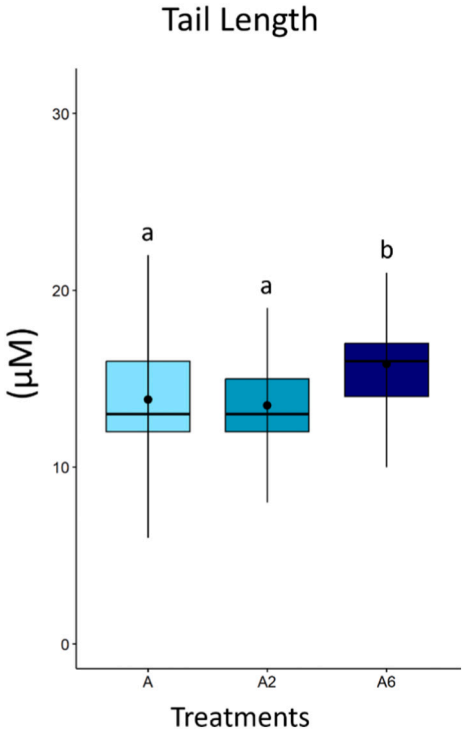

Fig. 4. Tail Length measured in earthworms' coelomocytes from each treatment after 28 days of exposure. Different lowercase letters represent significant differences between treatments according to the ANOVA-Tukey parametric test. Treatments codes legend: artificial soil (A), artificial soil with bioplastics at lower concentration (A2), artificial soil with bioplastics at higher concentration (A6).

3.4. Bacterial and fungal dynamics in earthworm's gut exposed to bioplastics residues

Earthworms feed on soil rich in microorganisms which are digested in the gut due to specific enzymes and an environment of anaerobic, neutral pH and high humidity (Baihetiyaer et al., 2023b). The earthworms' gut microbiota plays a crucial role in their metabolism and promotion of nutrient transformation (Baihetiyaer et al., 2023b). Microorganisms colonising the gut maintain the activity of the immune system and defend the organism against external pollutants (Jin et al.,

2017; Round et al., 2010; Zhou et al., 2019), such as MPs. Negative effects of MPs on soil organisms were correlated to an alteration in gut microbiome or microbiome dysbiosis after their ingestion (Ju et al., 2019; Li et al., 2022; Xie et al., 2021b). Changes in gut microbiome after MPs exposure occurred in dependence on polymer type (Rohrbach et al., 2023), especially conventional polymers rich in additives such as polystyrene (PS) and polyethylene (PE) (Ji et al., 2021). However, the literature mainly reports studies on MPs of conventional versus biodegradable polymers without comparison, suggesting that further investigation is urgently required (Boots et al., 2019; Ding et al., 2021; Ferreira-Filipe et al., 2022; Vaccari et al., 2022; Yu et al., 2022; Zhang et al., 2018).

In our study, bacterial amplicons from earthworms' guts resulted in 183.000 high-quality sequences and a minimum of 9.000 reads per sample. Fig. S6 (Supplementary Materials) shows the dendrogram based on the distance between data points in the clustering input. Earthworms treated with A had a distinct bacterial community compared to those treated with bioplastics in different percentages. The gut environment can activate spore-forming bacteria (Koubová et al., 2010), such as some *Bacillus* species, or other Gram-negative facultative anaerobic bacteria, such as *Aeromonas* and *Pseudomonas* (Hong, 2011; Hong et al., 2011). *Bacillus*, *Streptomyces* and *Pseudomonas* genera were mainly detected in earthworms treated with compost (Koubová et al., 2015). These genera are involved in nitrogen fixation and organic matter degradation, and some species of *Bacillus* genera are MPs degraders according to the literature (Auta et al., 2017; Xiang et al., 2023). In general, the structure of the bacterial communities in the gut of earthworms exposed to different percentages of bioplastics was quite distinct from the bacterial communities during composting, including the final sampling time (S4). Our results were in line with a recent study in which earthworms exposed to PLA biodegradable MPs and/or imidacloprid reported significant differences in the structure of the bacterial communities in *E. fetida* gut and surrounding soil (Baihetiyaer et al., 2023b). This result may be due to the gut environment, which stimulates microbial communities with greater metabolic capacities.

Linear discriminant analysis effect size (LEfSe) was performed to find differences in taxa among samples treated differently. LEfSe combines the standard tests for statistical significance (Kruskal-Wallis test and pairwise Wilcoxon test) with linear discriminate analysis. The analysis was performed under the following conditions: the *p*-value for the factorial Kruskal–Wallis test among classes was 0.1 and the threshold on the logarithmic LDA score for discriminative features was 2.0. Fig. 5 shows the LEfSe graphical summary at the genus level and highlights how the type of sample (A or A combined with different percentages of bioplastic) influenced the abundance of certain genera of bacteria. The genus *Comamonas* includes cellulolytic bacteria and was only found in A samples (Shweta, 2012; Vijayakumar et al., 2009; Wang et al., 2008; Wonnapinij et al., 2022). On the other hand, when A was mixed with bioplastic residues, other genera were more abundant, indicating a different effect on gut bacterial communities depending on the concentration. For example, in samples with 2 % bioplastic, *Paenibacillus*, *Bacillus*, *Rhizobium*, *Legionella* and *Saccharimonadales* genera were very abundant compared to the others. *Paenibacillus* sp. is a common host of the earthworm gut and lives in symbiosis with it, producing enzymes for digesting organic compounds, such as chitin, cellulose and pectin (Parthasarathi et al., 2007; Pathma and Sakthivel, 2012). On the other hand, *Bacillus* sp. is a well-known thermophilic bacteria able to biodegrade plastics in compost (Skariyachan et al., 2018, 2015), and its presence was mainly detected in samples with 2 % bioplastic and less frequently in samples with 6 %. In contrast, several bacterial genera, such as

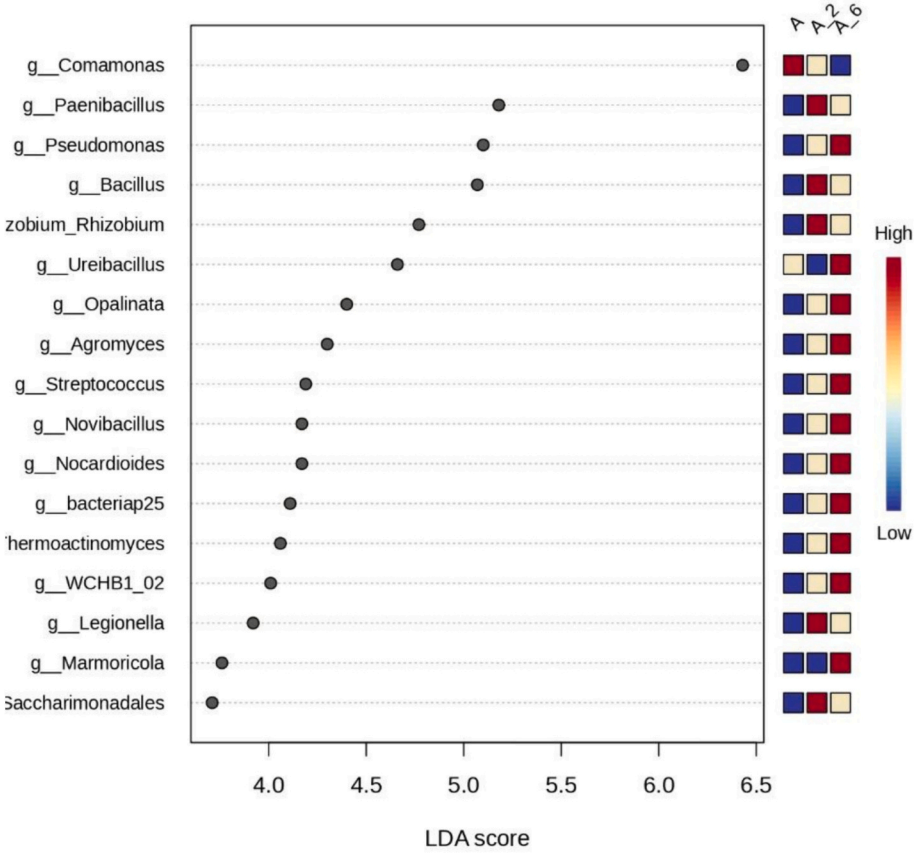

Fig. 5. Graphical summary of LEfSe analyses. Significant taxa (genus level) are ranked in decreasing order by their LDA scores (x-axis). The mini heatmap to the right of the plot indicates whether the taxa are higher (red) or lower (blue) in each group. Legend: artificial soil (A); artificial soil with bioplastics at lower concentration (A2); artificial soil with bioplastics at higher concentration (A6).

Pseudomonas, *Ureibacillus*, *Opalinata*, *Agromyces*, *Streptococcus*, etc., were identified in greater abundance or only in earthworms exposed to 6 % bioplastic. Some of these bacteria are typical of the composting process and earthworms' gut (Brito-Vega and Espinosa-V, 2009; Liu et al., 2013; Wonnapinij et al., 2022), and were also identified in our monitoring shown in Fig. 1a. Moreover, *Paenibacillus*, *Pseudomonas* and *Bacillus* have been identified as potential MPs and NPs degraders (Maddela et al., 2023), suggesting that bioplastics to which earthworms have been exposed may stimulate their presence, especially in higher percentages.

Fungal amplicons for earthworms' gut resulted in 497.091 high-quality sequences and a minimum of 12.000 reads per sample. Fig. S7 (Supplementary Materials) summarises and compares the most abundant fungal genera found in gut samples based on the annotation. *Aspergillus* sp., which was found also in compost, were most abundant in samples A and A2. On the other hand, *Curvularia* sp. was mainly found in A6 and was also reported in the literature as widely present in the gut of earthworms during composting (Parthasarathi et al., 2007). *Mortierella* sp. was most reported in earthworms treated with bioplastics (Yu et al., 2022). This saprophytic fungus is widely detected in earthworms and material rich in organic matter (Sapkota and Nicolaisen, 2018).

The linear discriminant analysis effect size (LEfSe) was performed to find differences in fungal taxa under the following conditions: the *p*-value for the factorial Kruskal–Wallis test among classes was 0.1 and the threshold on the logarithmic LDA score for discriminative features was 2.0. In this analysis (Fig. 6), the significant genera were different and less than those shown in Fig. S7. Moreover, the significant genera were identified mainly in the samples exposed to A. *Bjerkandera* sp. was the most abundant genus identified in the control samples slightly less in samples exposed to 6 % bioplastics, and its presence in the digestive tract of *E. fetida* has already been reported in the literature. This fungus was previously isolated from the gut of *E. fetida* (Byzov et al., 2009) and was also inoculated to accelerate the waste material degradation (Moran-Salazar et al., 2016). *Bjerkandera adusta* was selected in previous studies due to its ability to produce versatile peroxidase, for both lignine and manganese peroxidase enzymatic activities (Wang et al., 2002). Its wide presence in earthworm gut is therefore associated with good activity and could be considered a positive indicator, and consequently, it could be assumed that bioplastic residues reduce its growth. On the other hand, *Pestalotiopsis* sp. was mainly found in the gut of earthworms exposed to 2 % bioplastics residues. One of the few references in the literature reports the species *Pestalotiopsis microspora*, isolated from wastewater, as an MPs degrader (Dey et al., 2021; Gaur et al., 2022), and its presence could be linked to this feature. In conclusion, bibliographic references for fungi associated with the gut of *E. fetida* and the possible interaction with MPs of fossil and bio-based origin are still limited. As fungi are mainly aerobic, research usually does not focus on their dynamics in earthworm's gut, but in this study, some differences and changes in the fungal community due to the presence of bioplastic residues were highlighted. Further studies and investigations are certainly needed to better understand the interactions and effects on the health of the earthworm and the microbial dynamics of its digestive tract.

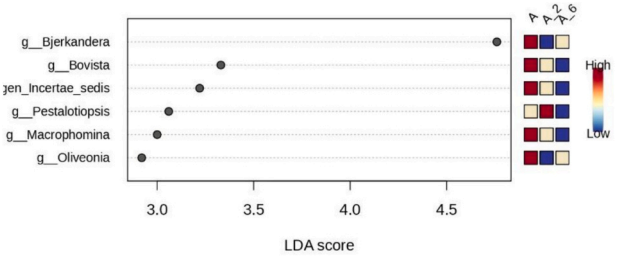

Fig. 6. Graphical summary of LEfSe analyses. Significant taxa (genus level) are ranked in decreasing order by their LDA scores (x-axis). The mini heatmap to the right of the plot indicates whether the taxa are higher (red) or lower (blue) in each group. Legend: artificial soil (A); artificial soil with bioplastics at lower concentration (A2); artificial soil with bioplastics at higher concentration (A6).

Principal coordinate analysis (PCoA) for bacterial and fungal communities is represented in Fig. 7a and b, respectively. This Beta-Diversity analysis, based on Bray-Curtis Index, measures the distances or dissimilarity between each sample pair to evaluate the impacts of the treatment of the different substrates on *E. andrei* gut microbiota. The results for the bacterial community (Fig. 7a) showed that the two axes of PCoA exhibited 53.3 % and 23.9 % of the total variation, respectively. The matrix reflects the negative clusterisation of A samples, while A2 and A6 grouped separately, positively correlated to Axis 1. A6 and A2 reported a wider variability between replicates. The PCoA for the fungal community (Fig. 7b) showed a 35.1 % total variation between samples for Axis 1 and 26 % for Axis 2. In this case, the samples clustered more homogeneously, without showing any separation between the different treatments, confirming the microbial trend discussed above.

3.5. Exploring the nexus between ecotoxicity and gut microbiota dynamics of earthworms exposed to bioplastics residues

Since ‘earthworm's gut environment changes when exposed to pollutants (Ma et al., 2017), the microbial communities are considered important indicators to evaluate MPs effects. On the other hand, the presence of earthworms influences plastic residues, their stability, fate, transport and behaviour (Manzoor et al., 2022). Indeed, at the end of the exposure test with earthworms (56 days), the 2 mm bioplastics residues were recovered and weighed (an example reported in Fig. S5). The reduction percentage of residues from the start of the ecotoxicological test to the end of 56 days was36.29 ± 9.53 and 31.43 ± 6.73, corresponding to treatments A2 and A6, respectively.

The present study aimed to correlate the gut responses of earthworms with the toxicity analyses carried out and to understand possible interactions. The differentiation in the gut microbiome, as well as the damage in the DNA of the earthworms exposed to bioplastic residues, can additionally change compost fertility or other compost properties. The relationship between MPs-earthworm-gut microbiota is extremely complex and has been the subject of several studies in recent years, and the effects are still unclear (Yu et al., 2022). However, studies demonstrated that the increased abundance of *Proteobacteria* plays a pathogenic role in gut inflammation (Shin et al., 2015) and is associated with the production of lipopolysaccharides which trigger inflammation, mucosal barrier disruption and intestinal permeability (Zhang et al., 2021). In our study, genera belonging to Proteobacteria, such as *Acinetobacter*, *Pseudomonas*, *Sphingomonas* and *Dickeya*, were mainly found in earthworms exposed to bioplastics residues, suggesting a role for these materials in promoting them or transporting them within the organism by acting as vectors.

Future perspective consider more in-depth investigations monitoring earthworms' responses, by evaluating the stress level, and behaviours, by mapping the structure and topography of their burrials.

3.6. Physico-chemical analyses of materials

3.6.1. Differential Scanning Calorimetry (DSC) results

Table S2 (Supplementary Materials) shows the main results of DSC analysis from cooling and second heating of pristine samples and after composting and treatment with earthworms. In particular, crystallisation temperature T_{CR}, melting temperatures of the single polymer T_{M1} and T_{M2}, crystallisation enthalpy ΔH_{CR} and melting enthalpy ΔH_M are reported. T_{CR} and T_{M1} increase with higher polymer percentages in compost, while T_{M2} is present only in pristine polymer; on the other hand, both ΔH_{CR} and ΔH_M decrease after composting and treatment with earthworms because of degradation. During the cooling stage, crystallisation is faster since the chains result shorter after composting, so T_{CR} increases as ΔH_{CR} decreases, considering that the degradation rate of the PBAT component is greater concerning one of the higher crystalline PLA (Nomadolo et al., 2022). Amorphous polymers, such as

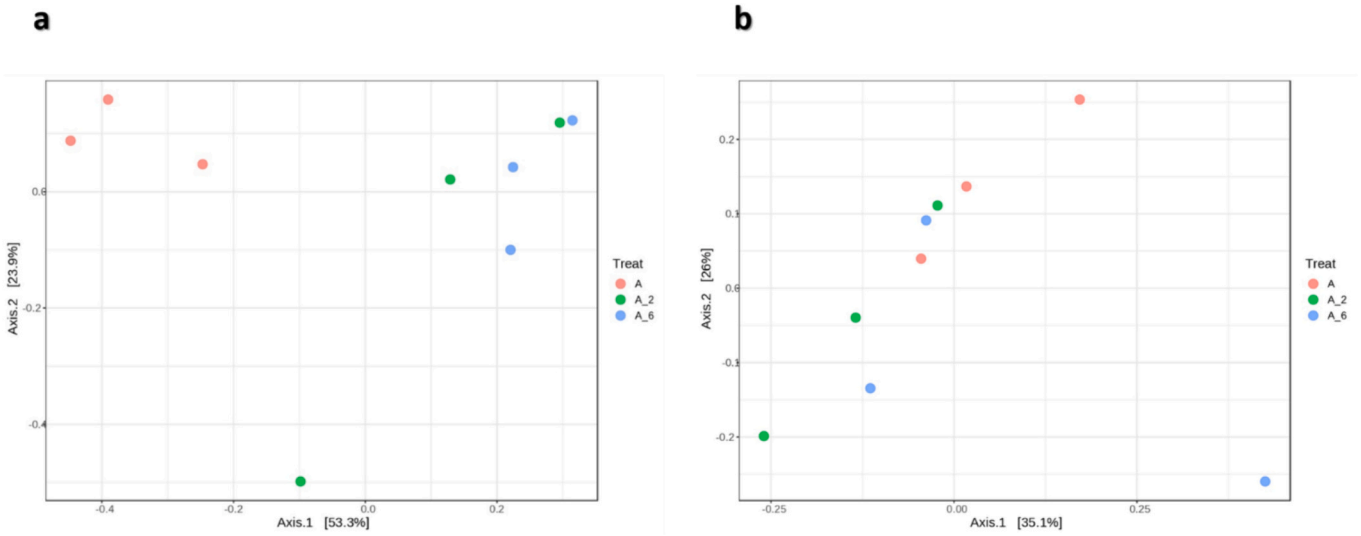

Fig. 7. Principal Coordinate Analysis (PCoA) of bacterial (a) and fungal (b) diversities in earthworms' gut exposed to different treatments. Legend: artificial soil (A); artificial soil with bioplastics at lower concentration (A2); artificial soil with bioplastics at higher concentration (A6).

PBAT, are indeed more sensitive to biodegradation and this results in T_{CR} closer to the crystallisation temperature of the crystalline region, as PLA one (Kijchavengkul et al., 2010). Regarding melting, the DSC curve of the pristine sample is complex with 4 melting points consisting of 2 main sharp peaks at 126 °C and 152 °C and 2 broader ones at 87 °C and 144 °C since there are two crystalline phases in the blend (Palsikowski et al., 2018); after ageing, the peaks of PLA disappears due to the loss of crystallinity, while the T_{M1} increases but its signal is less intense.

3.6.2. *Attenuated Total Reflectance (ATR) results*

Fig. 8a shows the ATR spectra of the pristine sample and after composting with 2 % and 6 % of compost, while Fig. 8b shows the ATR spectra of the pristine sample and after treatment with earthworms. T0 spectra are characterised by the peaks of both PBAT and PLA. Some of the main peaks of these polymers are reported in Tables S3 and S4 (Supplementary Materials). At high wavenumbers, composting leads to chain scission and ester linkages, a wide band appears between 3640 and 3100 cm^{-1} approximately due to the formation of hydroxyl groups —OH (Nomadolo et al., 2022; Sedničková et al., 2018; Tammer, 2004), a likely formation of a layer of protein on the surface (Sedničková et al., 2018), while the bands at 2920 cm^{-1} and 2850 cm^{-1} become wider (Myalenko and Fedotova, 2023). Moreover, the PLA peak at 1450 cm^{-1} separates into a double peak at 1454 cm^{-1} and 1447 cm^{-1}, especially at 6 % of compost, an indication of the shift in the absorption of asymmetric bending mode of CH_3 groups, while the peak ratio between PLA (1745 cm^{-1}) and PBAT (1710 cm^{-1}) related to CO stretching drops (Tabasi and Ajji, 2015) due to hydrothermal ageing and hydrolysis of PLA (Palsikowski et al., 2018).

Regarding treatment with earthworms, the presence of amine groups and resulting NH stretching is assessed by a band between 3600 and 3465 cm^{-1} with a maximum at 3530 cm^{-1} and between 3465 and 3335 cm^{-1} with a maximum at 3405 and a peak at 600 cm^{-1} (Tammer, 2004), as well as stretching of —OH groups in depolymerised PLA with a wide peat at 3340 (Meng et al., 2023). Furthermore, a peak related to the amidic group appears between 1450 and 1400 cm^{-1}, probably due to residues of earthworms on the surface (Ruggero et al., 2020).

4. Conclusions

Disintegration levels above 90 % were reached after 63 days of aerobic composting. Furthermore, the compost chemical analyses and the presence of several fungal and bacteria genera typical of composting environments indicates the correct progress of the composting process and the reached maturity of the substrate. Microbial diversity analysis suggests that different bioplastic concentrations may affect the fungal community more than the bacterial community.

The biopolymer residues after composting did not influence the earthworms' reproductive activity, but limited damage at the DNA level

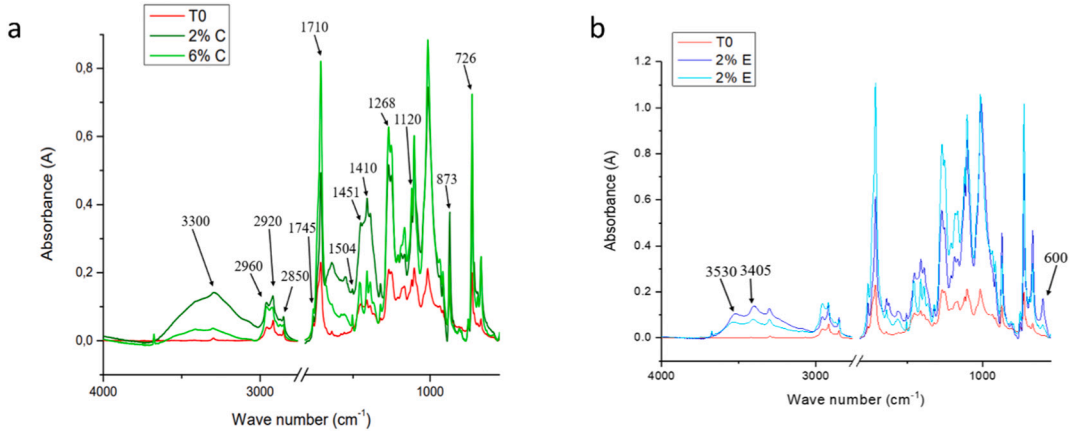

Fig. 8. a) ATR analysis on the pristine sample and after composting; b) ATR analysis on the pristine sample and after treatment with earthworms.

was observed at the highest dose after twenty-eight days of exposure.

Mixing artificial soil with bioplastic residues altered gut bacterial communities, with *Paenibacillus*, *Bacillus*, *Rhizobium*, *Legionella*, and *Saccharimonadales* being abundant at 2%B, while higher concentrations (6%B) favored *Pseudomonas*, *Ureibacillus*, and *Streptococcus*. *Pestalotiopsis* sp. was predominant at 2%B for fungal communities, suggesting its potential role as an MPs degrader.

The increased presence of Proteobacteria, known for their role in gut inflammation through lipopolysaccharide production, was prominent in earthworms exposed to bioplastic residues, suggesting a potential association between bioplastics and the proliferation or transportation of Proteobacteria within the organism, prompting the need for further investigation into earthworm responses and behaviour. ATR analysis showed significant changes in sample spectra due to hydrothermal ageing and hydrolysis of samples after composting and detection of amine groups after treatment with earthworms.

Our study presents the first interdisciplinary evidence of the impact of bioplastic residues on earthworm gut ecology, particularly shedding light on fungal community dynamics. The potential risk of bioplastics during aerobic composting with the organic fraction of municipal solid waste must be further explored to determine the safe threshold amount of bioplastics, and a robust risk assessment is still needed. Although the actual mechanisms of the response of *E. andrei* in the present work require further studies, our findings could improve the understanding of the ecotoxicological risks of biodegradable plastic and its residues.

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CRediT authorship contribution statement

Arianna De Bernardi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Enrica Marini:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Francesca Tagliabue:** Formal analysis, Data curation. **Cristiano Casucci:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Gianluca Brunetti:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Filippo Vaccari:** Validation, Software, Investigation, Formal analysis, Data curation. **Gabriele Bellotti:** Investigation, Formal analysis, Data curation. **Vincenzo Tabaglio:** Investigation, Funding acquisition, Formal analysis, Data curation. **Andrea Fiorini:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Alessio Ilari:** Writing – review & editing. **Chiara Gnoffo:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Alberto Frache:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Eren Taskin:** Writing – review & editing, Methodology. **Uberson Boaretto Rossa:** Writing – review & editing. **Elisângela Silva Lopes Ricardo:** Writing – review & editing. **Amarildo Otávio Martins:** Writing – review & editing. **Daniele Duca:** Writing – review & editing. **Edoardo Puglisi:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Ester Foppa Pedretti:** Writing – review & editing, Supervision, Funding acquisition. **Costantino Vischetti:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing 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.

Data availability

Data is contained within the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.175403.

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