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Short Communication

Application of a small scale-terrestrial model ecosystem (STME) for assessment of ecotoxicity of bio-based plastics

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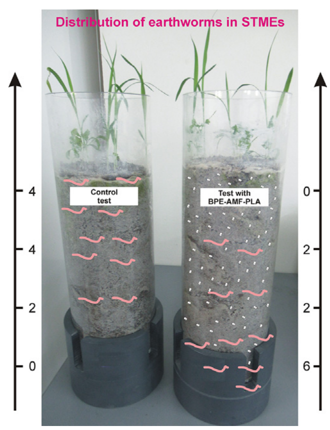
HIGHLIGHTS

- STME is a useful tool to evaluate effect of plastics on soil biota at community level.
- PLA-based plastics did not affect the germination of seeds of higher plants.
- PLA-based plastics did not cause to the mortality of earthworms *Eisenia andrei*.
- PLA-based plastics caused the migration of earthworms to deeper soil layers.
- Earthworm avoidance behaviour by plastics presence in soil found for the first time.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work a small-scale terrestrial model ecosystem (STME) filled with 4 kg of soil mimicking the natural ecosystem was constructed, and then successfully applied to evaluate the effect of bio-based plastics on soil biota at the community level. It was inhabited by higher plants monocotyledonous (*Sorghum saccharatum*) and dicotyledonous (*Lepidium sativum*), and earthworms (*Eisenia andrei*). Two innovative bioplastics based on polylactic acid (PLA) were tested. This work is one of the first studies, in which potential impacts of bioplastic particles on soil organisms were determined at the community level. Owing to the application of the STME the ecotoxicity data for plants and earthworms were simultaneously collected and the mutual interactions might be taken into account.

PLA-based plastics studied did not affect the percentage of seed germination of higher plants that was on average not lower than 88.9%. Neither the length nor fresh mass of shoots of cress were affected. One out of two PLA-based plastics (BPE-RP-PLA) inhibited sorghum growth so that it was statistically significant. PLA-based plastics did not cause to the mortality of earthworms as all ten organisms introduced to each STME survived each experiment. However, the presence of PLA-based plastic particles influenced the depth distribution of earthworms in the STMEs. Most of earthworms (60–70%) exposed to PLA-based plastics migrated downwards to the bottom soil zone, while 80% of the earthworms not exposed to PLA-based plastics lived in the top soil zone of the STME. This avoidance behaviour of earthworms known earlier for other contaminants (e.g. metals, pesticides) was for the first time reported with regard to bioplastic particles present in soil. It is a dangerous phenomenon not only for earthworms but also for the functioning and structure of terrestrial ecosystems. The STME proved to be an appropriate tool to detect it.

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1. Introduction

Plastics belong to one of the most ubiquitous contaminants in the environment (EPA, 2016; Chae and An, 2018; Ng et al., 2018). The main reasons of this phenomenon are a large production of plastics in the world (Plastics Europe, 2021; https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/) and difficulties in the effective management of plastic post-consumer waste. For example about 30% of plastic waste generated globally in 2018 was disposed improperly or leaked into the environment (Conversio Market and Strategy GmbH, 2020). Moreover, plastics are chemically stable and unsusceptible for the biochemical decomposition, what contributes to their accumulation and long-term stay in the environment (Niaounakis, 2013; EPA, 2016). Thus, the bio-based and biodegradable plastics attracted the attention of researchers and manufacturers as a potential replacement for the conventional petroleum-based plastics in the last decades.

Nizzetto et al. (2016) estimated that each year from 125 to 850 tons of microplastics per million inhabitants were introduced to the agricultural soils in Europe at the beginning of the second decade of the 21st century. It was found that microplastics (MPs) and nanoplastics (NPs) influenced the soil-plant system due to their accumulation ability and interactions with other pollutants present in the terrestrial ecosystems (Iqbal et al., 2020; Allouzi et al., 2021). As a consequence, both abiotic and biotic parts of the terrestrial compartment are affected. Plants and invertebrates are the most important functional groups of organisms in the soil ecosystem (Bogum, 2012). Thus, they are also commonly used as model organisms in the ecotoxicological tests (Bogum, 2012).

Khalid et al. (2020) analysed the positive and negative, direct and indirect impacts of MPs and NPs on plants in the terrestrial ecosystems. On the one hand, MPs and NPs could influence seed germination and plant growth by blocking seed pores and limiting water and nutrient uptake through roots (Khalid et al., 2020). On the other hand, in many studies either no effects or slight effects of plastic particles on seed germination, plant biomass, root or stem growth were observed (Qi et al., 2018; Balestri et al., 2019; de Souza Machado et al., 2019; Judy et al., 2019; Lozano and Rillig, 2020; Lozano et al., 2021). For example, Lozano and Rillig (2020) reported that root mass and shoot mass of the plant community (*Festuca brevipila*, *Holcus lanatus*, *Calamagrostis epigejos*, *Achillea millefolium*, *Hieracium pilosella*, *Plantago lanceolata*, and *Potentilla argentea*) increased in the presence of polyester microfibres (length 1.28 ± 0.03 mm, diameter < 30 μm) at the concentration of 0.4% in the soil by about 6% and 90%, respectively. It was most probably combined with the reduction of soil bulk density, improvement of soil aeration, and better penetration of roots in the soil (Lozano and Rillig, 2020). At the same time Bosker et al. (2019) observed the decrease in root growth of *Lepidium sativum* exposed to 50 nm particles of green fluorescent plastic (at the concentration of 10^3–10^7 particles ml^{-1}), and the increase in root growth of this plant in the case of the exposure to 500 nm plastic particles. What is important, the biodegradable plastics might in some cases act on plants even stronger than the petroleum-derived ones (Qi et al., 2018; Balestri et al., 2019).

Plastic particles also influenced the mortality, reproduction, growth and fitness of earthworms (Huerta Lwanga et al., 2016; Jiang et al., 2020; Huerta-Lwanga et al., 2021). However, the results of the ecotoxicological tests using earthworms as model organisms that have been conducted so far are not unequivocal. Neither are those for plants. The majority of works published in this area showed that MPs or NPs (e.g. polyethylene, polystyrene) at concentrations up to 1000 mg kg^{-1} dry soil had no adverse effect on earthworms (Huerta Lwanga et al., 2016; Rillig et al., 2017; Wang et al., 2019).

The terrestrial ecosystem is a complex network of biological, chemical and physical interactions. Therefore, apart from the tests towards the individual species, it is necessary to reflect the functioning of the terrestrial ecosystem at least in the laboratory scale. For this purpose the experiments in a microcosm and/or a mesocosm that involved multiple species are recommended (Bogum, 2012). The number of such works concerning MPs or NPs and their impacts in the soil microcosm/mesocosm systems is very limited. These studies usually dealt with plastic biodegradation and identification of microorganisms responsible for the biochemical decomposition of polymers (Tribedi and Dey, 2017; Thompson et al., 2019; Sun et al., 2022). Hardly any studies focusing on the effect of plastic particles on soil living creatures in the microcosm/mesocosm experiments have been published so far (Boots et al., 2019). Thus, there is a need to design and perform this type of experiments for the soil contaminated with plastics. It will allow for supplementing the results of acute and chronic ecotoxicity tests, and obtaining a more complex description of the impact of plastics on soil biota including the changes in animal behaviour.

The presented study is one of the first attempts filling the gap in the aforementioned area of ecotoxicological tests on plastics in the soil compartment. It was hypothesized that the plastics tested would not affect plant growth and earthworm survival in the microcosm system, but they might act on the behaviour of earthworms. These hypotheses are going to be verified in a small-scale terrestrial model ecosystem (STME) that was constructed to examine and describe the effects of the innovative bio-based plastics on soil biota at the community level in the microcosm experiments.

2. Materials and methods

2.1. Plastic materials

Two bio-based innovative plastic materials were examined in this study. Both are PLA-based polymers and they were selected for testing within the realisation of Bio-plastics Europe Project (Horizon 2020, grant agreement no. 860407). According to the nomenclature adopted in the project these were BPE-AMF-PLA (Bio-Plastics Europe - Agriculture Mulch Film - PolyLactic Acid) provided by NaturePlast SAS (NP, France) and BPE-RP-PLA (Bio-Plastics Europe - Rigid Packaging - PolyLactic Acid) provided by Arctic Biomaterials OY Ltd. (ABI, Finland). Both bio-based plastics were supplied in the form of microparticles by the manufacturers. More information about these materials is collected in Table 1.

The global production capacity of PLA-based bioplastics was the highest in 2020 and due to their favourable properties these materials are forecast to maintain the leader position up to 2025 (European Bioplastics, 2020). Therefore, most of the plastic materials that have been studied within Bio-plastics Europe Project so far are PLA-based compounds.

2.2. Small terrestrial model ecosystem (STME)

The construction of the STME is shown in Fig. 1. It was designed upon the description of the STME presented by Santos et al. (2011). Only the construction of the bottom of the STME, i.e. the rubber rack, was modified in the STME used in this study compared to the STME described in the previous work (Santos et al., 2011).

An individual STME consisted of a cylindrical acrylic glass pipe of the internal diameter 120 mm (wall thickness equal to 5 mm) equipped with the perforated bottom of the thickness equal to 10 mm. This container was mounted in the rubber rack of the total diameter 156 mm (Fig. 1). The space between the perforated bottom and the bottom of the rack enabled collecting the potential leachates during the test. Before each experiment the new layer of geotextile was placed on the perforated bottom of STME and it was removed at the end of the microcosm test. STMEs were located in the acclimatisation chamber FITO 700 (Biogenet, Poland) for the duration of the test.

2.3. Experimental design

In the experiments the reference OECD soil prepared in accordance with method no. 207 (OECD, 1984) containing air-dried quartz sand (85%), kaolin clay (10%), sphagnum peat (5%) and calcium carbonate ($CaCO_3$) to hold an initial pH of 6 ± 0.5 was used (Microbiotests, Belgium). Soil moisture was adjusted to 70% of maximum water holding capacity (WHC) and it was maintained at this level during the microcosm test by adding sterile water once or twice a week.

Table 1
Data on the bio-based plastics tested (provided by the manufacturers).

Acronym of bio-based plastic	Application	Desired properties	Material type	Density g cm^{-3}	Size of granules	Innovation	Material details	Manufacturer
BPE-AMF-PLA[a]	Mulch film	Bio-based and both recyclable and bio-degradable, degrade in controlled fashion	PLA-based	1.26	Length 3 mm; diameter 2.5 mm	Blending of PLA and polyhydroxy butyrate-hydroxyvalerate (PHBV) for controlled degradation, fertilizer added for controlled release	PLA blended with 15% polybutylene adipate terephthalate (PBAT) and <5% process additives, intended to be used for extrusion application	NaturePlast SAS (France)
BPE-RP-PLA[b]	Rigid packaging	Water and oxygen barrier, bio-based and bio-degradable	PLA-based	1.50	Length 3 mm; diameter 2.5 mm	Cold mold, fast cycle time, good heat resistance, food grade	PLA-based mineral filled compound (food grade) for injection molding and potentially sheets for thermoforming	Arctic Biomaterials OY Ltd. (Finland)

[a] BPE-AMF-PLA - Bio-Plastics Europe - Agriculture Mulch Film - PolyLactic Acid.
[b] BPE-RP-PLA - Bio-Plastics Europe - Rigid Packaging-PolyLactic Acid.

The bio-based plastic tested was added to achieve its concentration of 2.5% w/w in the dry soil. The value of plastic concentration used in the experiments was selected on the basis of literature data. The concentration of plastics in the soil in different areas, i.e. agricultural, industrial, floodplain, varies in the wide range from below 0.001% w/w to about 6.75% w/w (Fuller and Gautam, 2016; Scheurer and Bigalke, 2018; Zhang et al., 2018). The values of plastic concentrations tested in the ecotoxicity studies corresponded with these field data and they often were in the range from 0.1% to 7% w/w (Huerta Lwanga et al., 2016; de Souza Machado et al., 2019; Judy et al., 2019; Jiang et al., 2020; Kim et al., 2020).

Each bio-based plastic was treated individually. Apart from the tests with the bio-based plastics, the control runs without the addition of any potential pollutant (only the reference OECD soil) were conducted. All treatments, including the control tests, were replicated three times. Each treatment and each replicate with or without the microparticles of one of the bio-based plastics studied was individually prepared.

Maximum values of WHC were determined in agreement with Annex 2 of the OECD method no. 222 (OECD, 2004). For the OECD reference soil it was 38.4 ± 0.6%, while for the soil containing the plastic particles it was 45.8 ± 1.1% and 43.5 ± 0.9% for BPE-AMF-PLA and BPE-RP-PLA, respectively. Soil bulk density was also measured. It was made by determining the weight after drying and measuring a known volume of soil or the mixture of soil and plastic material using a metal ring pressed into the soil (intact core) (McKenzie et al., 2004). The determination of soil bulk density was performed before placing the soil in the STME. It was made individually for the soil with and without addition of one of the bio-based plastics tested. The OECD reference soil bulk density was at the level of 1.167 ± 0.007 g cm^{-3}, whereas in the case of adding to this soil BPE-AMF-PLA or BPE-RP-PLA the bulk density was 1.044 ± 0.011 g cm^{-3} or 1.011 ± 0.009 g cm^{-3}, respectively.

A 4 kg of the properly prepared soil containing or not containing the bio-based plastic tested was gradually added to the STME. The STME was filled up to about 9 cm from its top. Then, ten depurated (washed with water, wiped and then placed on absorbent paper for a short time) earthworms were located at the soil surface. These were the adult earthworms with clitellum *Eisenia andrei* originating from the synchronized culture of Institute of Environmental Protection – National Research Institute (Warsaw, Poland). They were of homogeneous age and size. The initial mass of the individual organism was at the level of 0.518 ± 0.048 g. No food was added apart from the organic matter present in the soil. After the earthworms buried themselves in the soil (it took about 10 min), the seeds of two plants were sown. Six seeds of monocotyledonous plant *Sorghum saccharatum* (sorghum, series no. SOS041019) and six seeds of dicotyledonous plant *Lepidium sativum* (garden cress, series no. LES260820) were used in each microcosm test. These were standardized seeds provided by Microbiotests (Belgium). Unlike us, Santos et al. (2011) used one plant species, i.e. turnip (*Brassica rapa*) in their study. Earthworms *Eisenia andrei* were selected because this species is recommended to be a model organism in the ecotoxicity tests according to the OECD method no. 207 (OECD, 1984) and OECD method no. 222 (OECD, 2004), while the plant species (*Sorghum saccharatum*, *Lepidium sativum*) were recommended by ISO Standards 18763 standards (ISO, 2016) and OECD method no. 208 (OECD, 2006) for phytotoxicity tests. Moreover, the compatibility of the microcosm tests with other ecotoxicity tests run within Bio-plastic Europe Project was taken into account while selecting the model organisms. Each STME was incubated at 20 ± 0.5 °C with a 16/8 h light dark regime and a relative humidity ~40% in the acclimatisation chamber for 28 days.

With regard to the plants the following endpoints were determined within the microcosm tests: the percentage of seed germination, mass of fresh shoots and length of shoots. In order to measure the length of shoots, the plants were removed gently from the soil at the end of experiment and their images were taken. Then, the shoot length was measured manually with the help of image analysis software NIS ELEMENTS AR software (Nikon, Japan).

At the same time for the earthworms, the mortality, the fresh mass of depurated earthworms and the earthworm depth distribution in the STME were used as endpoints. To determine the distribution of earthworms, the STME was divided into four zones of the similar height (7–8 cm). The soil of each zone was successively removed by hand within approximately 1 min, and the number of earthworms was counted in sequence.

2.4. Statistical analysis

The mean values and the appropriate standard deviations were calculated for each endpoint determined in the microcosm experiments. The differences between the percentage of seed germination as well as the differences between the shoot length/mass in the control run and the run with bio-based plastic (BPE-AMF-PLA or BPA-RP-PLA) were analysed with the use of one-way ANOVA at statistical significance $\alpha = 0.05$. It was made for *Sorghum saccharatum* and *Lepidium sativum* separately. Moreover, the statistical significance of differences: (1) between the body mass of earthworms in the control test and the test with bio-based plastic (BPE-AMF-PLA or BPA-RP-PLA), and (2) between the number of earthworms with regard to each zone of the STME in the control test and the test with bio-based plastic (BPE-AMF-PLA or BPA-RP-PLA) were also checked with the help of one-way ANOVA. The application of ANOVA was preceded by checking the assumptions required for the parametric tests including the normality of data that was verified with help of Kolmogorov-Smirnov test. It was found that the data met the requirements to be analysed by the parametric tests. The statistical analysis of results was performed with the use of MS Excel (Analysis ToolPak) and OriginPro 9.0 (OriginLab).

3. Results and discussion

The percentage of seed germination in the microcosm control tests was at the level of 100 ± 0% and 94.5 ± 9.6% for sorghum and cress, respectively. It indicated that the seeds were of good quality and the germination process ran correctly. According to the manufacturer above 70% of seeds of each plant should germinate to consider the results in the control valid

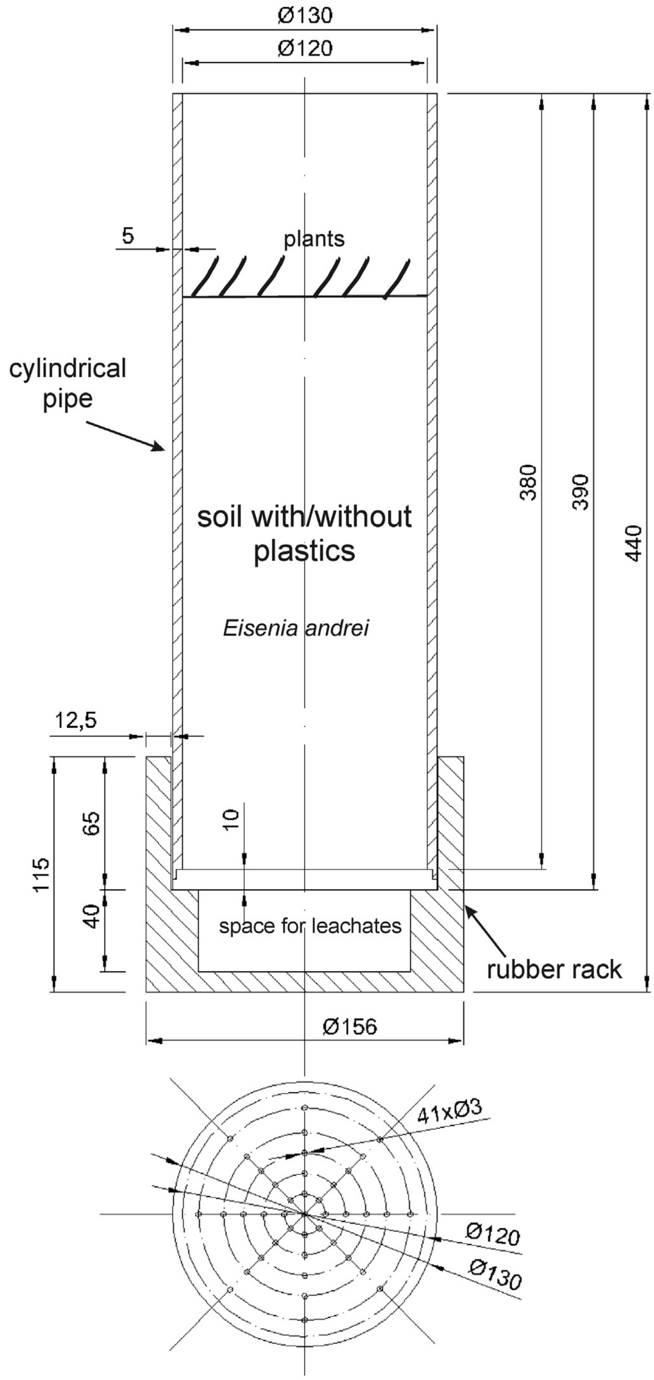

Fig. 1. Vertical cross-section of the small-scale terrestrial model ecosystem (STME).

(Microbiotests, Belgium). None of the two bio-based plastics studied deteriorated the germination of any of two higher plants used as model organisms in the microcosm tests. In each test the level of germination was higher than 80% (Table 2). However, the percentage of seed germination of sorghum was lower in the tests with the bio-based plastic particles in comparison to the control tests (Table 2). The statistically significant difference was confirmed only for the germination of sorghum seeds in the presence of BPE-RP-PLA ($p = 0.0302$), whereas in the other cases no statistically relevant differences in the percentage of seed germination of sorghum or cress between the control test and the test with the bio-based plastic were found ($p > 0.05$).

The shoot length and shoot fresh mass of plants exposed and not exposed to the bio-based plastics are compared in Fig. 2. It is well seen that

Table 2
Percentage of seed germination for two higher plants: *Sorghum saccharatum* (SOS) and *Lepidium sativum* (LES) exposed or not exposed to the bio-based plastic (BPE-AMF-PLA or BPE-RP-PLA) tested.

Tested material	Germination of *Sorghum saccharatum* (%)	Standard deviation for SOS	Germination of *Lepidium sativum* (%)	Standard deviation for LES
BPE-AMF-PLA	94.5	9.6	100	0
BPE-RP-PLA	88.9	9.6	88.9	9.6
Control	100	0	94.5	9.6

the presence of bio-based plastics in the soil did not affect the growth of *Lepidium sativum* but it contributed to the decrease of shoot fresh mass and shoot length of *Sorghum saccharatum*. One way ANOVA was used to check the statistical relevance of these observations. The values of *p* higher than 0.05 (Table 3) proved that the differences between the fresh mass/length of cress shoots exposed or not exposed to the bio-based plastics were not statistically significant. At the same time both the fresh mass and the length of sorghum shoots exposed to BPE-RP-PLA were statistically different from those in the control test. In the case of BPE-AMF-PLA these differences were not statistically significant (Table 3).

Relatively low impact of plastic particles on plant germination and growth reported in this study might be connected with the changes of soil structure induced by the addition of plastics. It was found that the presence of plastic particles in soil usually decreased the soil bulk density (de Souza Machado et al., 2018; Rillig et al., 2019). As a consequence, the penetration resistance for plant roots was lowered and soil aeration was improved (Rillig et al., 2019). In this study the presence of plastic particles in soil caused to the reduction of the soil bulk density from 1.167 ± 0.007 g

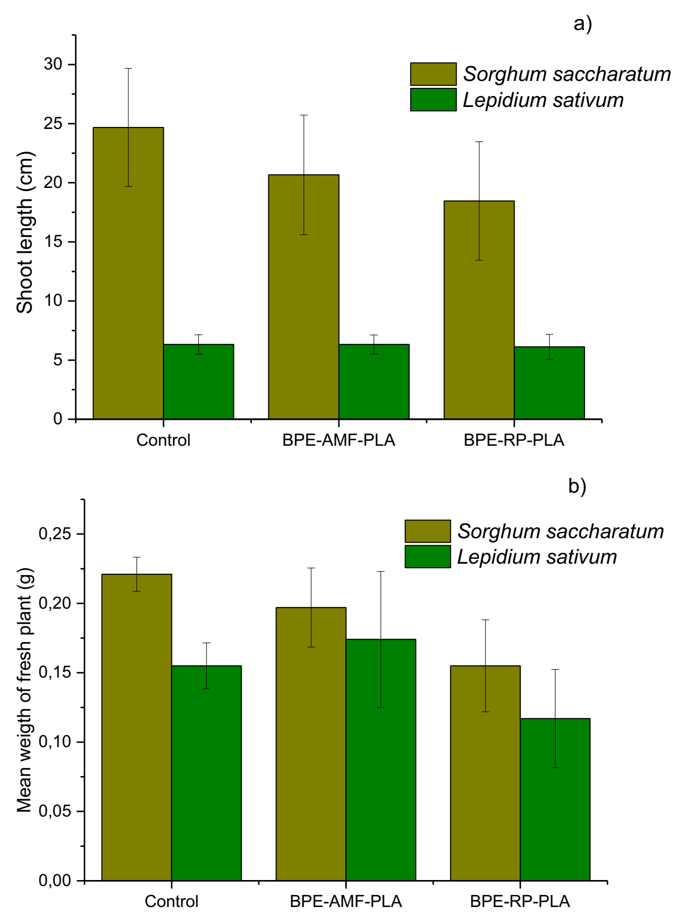

Fig. 2. Effect of bio-based plastic (BPE-AMF-PLA or BPE-RP-PLA) particles on a) shoot length, b) shoot fresh mass. The error bars reflect standard deviations.

Table 3
Statistical evaluation of the differences for the length of shoots and fresh mass of shoots exposed and not exposed to the bio-based plastic (BPE-AMF-PLA or BPE-RP-PLA) tested. Results of one-way ANOVA (*p*-value).

Tested material	*p*-value			
	Sorghum saccharatum		*Lepidium sativum*	
	Shoot fresh mass	Shoot length	Shoot fresh mass	Shoot length
BPE-AMF-PLA	0.151	0.0634	0.373	0.984
BPE-RP-PLA	0.0106	0.00424	0.0879	0.654

cm^{-3} in the reference soil to 1.044 ± 0.011 g cm^{-3} or 1.011 ± 0.009 g cm^{-3} in the case of addition of BPE-AMF-PLA or BPE-RP-PLA, respectively. Thus, the aeration and loosening of soil containing these plastic particles were most probably improved. The bio-based plastics tested did not affect negatively the growth of plants excluding the growth of sorghum exposed to BPE-RP-PLA (Fig. 2, Table 3). It shows that the soil-plant system is subjected to various processes and interactions and it depends not only on soil bulk density but also on plastic composition, plant species, other soil properties and community level effects. As a result plastic particles present in soil may affect plants either positively or negatively (Rillig et al., 2019).

All earthworms survived the microcosm tests in each STME (Fig. 3). It showed that the bio-based plastics even at relatively high concentration 2.5% w/w did not cause to the earthworm mortality. Simultaneously, the earthworm body mass decreased after the experiments including the control tests. There was no statistically significant differences ($p > 0.05$) between the decrease of the earthworm body mass in the control test and the tests with the bio-based plastics (BPE-AMF-PLA or BPE-RP-PLA). The decrease in the earthworm body mass was most probably due to the fact that the reference OECD soil contained relatively small amount of organic matter (5%) and no additional food for earthworms was delivered to the STMEs. Santos et al. (2011) also reported the weight loss of *Eisenia andrei* even in the control test during the experiments in the STME.

The construction of STME applied in this study allowed for the determination of the depth distribution of earthworms. The significant differences in the depth distribution of earthworms between the STMEs containing one of the bio-based plastics and the control STME (without bio-based plastics) were found (Fig. 4). It occurred that the presence of the bio-based plastics favoured the downward movement of earthworms. In the control test most of earthworms (80%) were located in the top zone and the first middle zone, whereas in the microcosm tests with the bio-based plastic particles most earthworms (from 60% to 70%) were found in the bottom zone (Fig. 4). The differences between the earthworm depth distribution in the STMEs containing and not containing the bio-based plastic particles with regard to each zone of the STME were statistically analysed (Table 4). It was found that in three out of four zones of the STMEs they were

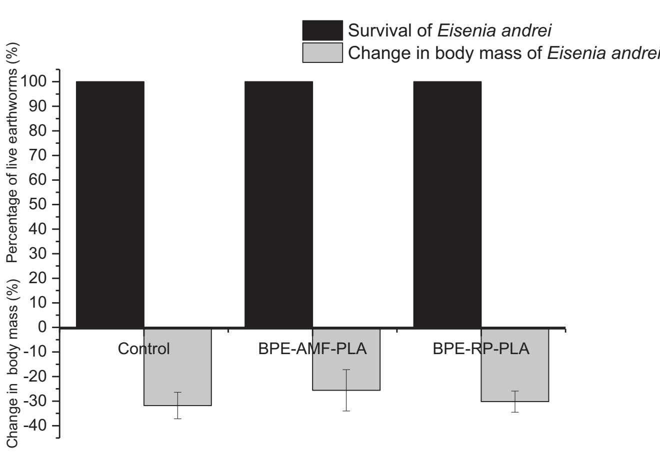

Fig. 3. Effect of bio-based plastic (BPE-AMF-PLA or BPE-RP-PLA) particles on earthworm mortality and body mass.

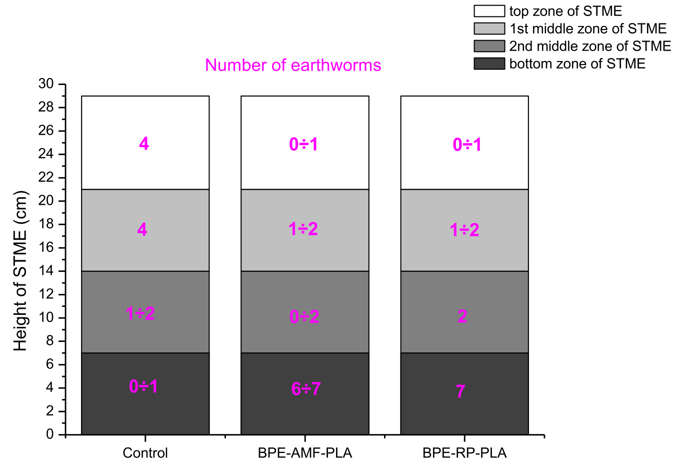

Fig. 4. Depth distribution of earthworms *Eisenia andrei* in the small-scale terrestrial model ecosystems (STMEs): the control test and the tests with the bio-based plastic (BPE-AMF-PLA or BPE-RP-PLA) particles.

statistically significant, while in one of them, i.e. the 2nd middle zone (Fig. 4, Table 4), they were not ($p > 0.05$). This observation was reported for both bio-based plastics tested (Table 4). Statistically confirmed differences in the earthworm depth distribution between the STMEs with and without the bio-based plastic in three out of four zones indicated that the presence of plastic microparticles was the main reason of the avoidance behaviour of earthworms. Santos et al. (2011) reported similar earthworm behaviour in a response to the contamination of soil by pesticides. Normally, earthworm *Eisenia andrei* as an epigeic species are active above the soil surface, and thus they should live in the upper soil layers (Santos et al., 2011; Langdon et al., 2005). Deviations from their natural behaviour indicated that the earthworms were trying to leave the unfavourable conditions. Langdon et al. (2005) informed about the avoidance (escape) behaviour of *Eisenia andrei* that moved towards the lower lead concentration in the soil and the avoidance increased with the increase of lead concentration. This phenomenon of escape from the contaminated soil is dangerous for earthworms that might die out over a longer period of time. It is also dangerous for the whole biotic part of soil ecosystem, in which earthworms play an important role of ecosystem engineers.

4. Conclusions

The STME used in this study mimics in the simplified form the natural soil ecosystem. It is successfully applied for the evaluation of the effect of two innovative bio-based plastics on soil organisms at the community level. Owing to it the data about potential impacts of plastic particles on plants and earthworms are collected simultaneously and the mutual interactions are regarded.

PLA-based plastics studied in this work do not affect seed germination of higher plants and growth of dicotyledonous plant *Lepidium sativum*. The monocotyledonous plant *Sorghum saccharatum* occurs to be more

Table 4
Statistical evaluation of the differences between the earthworm depth distribution in the control tests and the tests with the bio-based plastic (BPE-AMF-PLA or BPE-RP-PLA) with regard to each zone of the small-scale terrestrial model ecosystem (STME). Results of one-way ANOVA (*p*-value).

Zone of STME	*p*-value	
	BPE-AMF-PLA	BPE-RP-PLA
Top zone	0.000562	0.003088
1st middle zone	0.00749	0.00132
2nd middle zone	0.643	0.518
Bottom zone	0.000219	0.0000369

sensitive than *Lepidium sativum* to the soil contamination by bio-based plastic particles in the microcosm tests.

PLA-based plastics do not cause to the mortality of earthworms *Eisenia andrei*. However, the presence of PLA-based plastic particles influences the depth distribution of earthworms. *Eisenia andrei* exposed to PLA-based plastics migrate to deeper soil zones, what is unusual for this epigeic species and it is not observed in the tests without plastic particles in soil. This avoidance behaviour is dangerous for the earthworms and may have serious implications for the structure and functioning of soil ecosystems.

CRediT authorship contribution statement

Ewa Liwarska-Bizukojc - conceptualization, data curation, investigation, visualisation, writing.

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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.

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