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Conventional and biodegradable agricultural microplastics: Effects on soil decomposer animals and protists in three climate zones☆

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ABSTRACT

The effects of microplastics (MPs) in soils have been studied mainly in laboratory experiments, with limited data on soil invertebrates in field. The importance of different natural environmental factors such as climate for responses of soil organism communities to MPs is not yet studied in detail. We tested whether MPs made of conventional low–density polyethylene (PE) and polybutylene adipate terephthalate starch–blend (PBAT) affect soil invertebrates. We studied the effects of these MPs in agricultural fields in three countries (Finland, Germany, and Spain), representing different climatic zones and soils. Community structure of protists and microfauna (through eDNA), abundances of enchytraeids, earthworms, and microarthropods were analysed in soils dosed with two concentrations (0.005 and 0.05% *w*/w in top 10 cm layer) and followed for two growing seasons. Enchytraeids showed over 50% decline in numbers in PE and PBAT MP–dosed soils in Finland and Spain. Earthworms exposed to PBAT MPs slightly increased in biomass and numbers in Finland and Germany. Mites were mostly unaffected, while the abundance of springtails decreased in Finland when exposed to PBAT MPs. Protist community changed in Germany and Finland when exposed to PE MPs. Nematode diversity declined when exposed to PE MPs in Finland and PBAT MPs in Germany. Our results suggest that MPs from agricultural origin can impact invertebrates that form significant part of the soil decomposer community, and effects of MPs on soil fauna can vary by geographical region. Therefore, environmental risk assessments must account for local natural conditions to avoid over– or underestimation.

1. Introduction

Plastics are commonly used in agriculture to store chemicals, construct irrigation systems, wrap silage, and as mulching films to protect crops from pests, weeds, and drought (Bhadauria and Saxena, 2010). Over time, plastics can fragment into microplastics (MPs) due to exposure to ultraviolet light, mechanical forces, and fluctuating temperatures (Thompson et al., 2004; Hartmann et al., 2019; Groß et al., 2025). As a result of the widespread use and slow breakdown of agricultural plastics, MP levels in agricultural soils are steadily rising (Büks and Kaupenjohann, 2020). Globally, in agricultural lands where mulching films are used, MP loads up to 5200 ± 8800 items kg^{-1} soil can be found (Wrigley et al., 2024). To address the environmental concerns associated with conventional plastics, biodegradable alternatives have been developed. Unlike conventional mulching films, biodegradable plastic mulches are intended to be tilled into the soil after use and are

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expected to decompose biologically. To be classified as 'biodegradable' according to ISO 23517:2021 and EN 17033:2018, at least 90% of the material must be converted to CO_2 within a maximum of 24 months. However, in real environmental conditions this process is often slower and incomplete, with plastic residues sometimes persisting in the soil for years (Hale et al., 2020). This challenge is evident both in cold and in arid regions, since both low temperatures and drought inhibit microbial activity and degradation of biodegradable plastics (Ho et al., 1999; Hale et al., 2020).

In agricultural fields, MPs may cause changes in the structure and functioning of soil invertebrate communities, either directly or indirectly by modifying their microenvironments through soil structure, water retention capacity and other soil properties (Wever et al., 2001; Wang et al., 2022b; Saljnikov et al., 2025). Earthworms, enchytraeids and springtails are among decomposers that have key roles in the functioning of agricultural soils and the maintaining of ecosystem services (Edwards and Bohlen, 1996). For example, the ecosystem services provided by earthworms alone are estimated to increase the global grain yield by 6.5% (Fonte et al., 2023). In general, soil structure and aeration, accumulation of soil organic matter, soil erosion, and soil nutrient cycling are highly affected or mediated by soil invertebrates (Wolters, 1991; Bonkowski et al., 2001; Orgiazzi and Panagos, 2018). Further, soil fauna can increase plant growth and plant nutrient content and offer biological control of many plant pests (Wolters, 1991; Edwards and Bohlen, 1996; Knapp et al., 2018). Consequently, potential negative effects of MPs on these functionally important soil organisms may cascade into the ecosystem services they provide.

Despite the widespread contamination of agricultural fields by plastics and the continuous exposure of decomposer communities on MPs, the effects of MPs on soil animals have mostly been studied in laboratory experiments (Jiang et al., 2020; Sobhani et al., 2021; Jemec Kokalj et al., 2022; Forsell et al., 2024). However, the fate and effects of MPs in the field may differ from those in artificial and controlled laboratory conditions. Laboratory studies usually focus on single species, using populations adapted to specific conditions, soil textures are standardized, and conditions such as humidity, temperature, and pH are adjusted to be optimal. Field studies can provide a more holistic view on the possible ecological effects of MPs on the structure and functioning of the soil decomposer community. In the field, organisms form diverse communities that are influenced by ecological interactions, seasonal environmental variations and variable soil properties, and are subjected to indirect MPs effects. Thus far, only few large–scale field experiments on the effects of MPs on soil fauna have been carried out (Lin et al., 2020; Adhikari et al., 2023) In addition, these studies have often focused only on limited number of faunal groups and single study sites.

To provide a holistic view on the potential long–term ecological effects of MPs on the structure and functioning of soil faunal communities, we conducted a 2–year field experiment in three different climate zones in Europe. Instead of using unrealistically high MP concentrations and mono–dispersed MP types as experimental studies are often criticized for (Lenz et al., 2016; Weis and Palmquist, 2021), we used MPs derived from two common, artificially aged, mulching film materials applied at two concentrations, 0.005% and 0.05%, that are considered to be relevant for agricultural fields (Büks and Kaupenjohann, 2020). Earthworms, enchytraeids springtails, mites as well as microfauna and protists (through eDNA) were studied as indicators for agricultural soil health (Görres and Amador, 2021).

We aimed at answering the following research questions: 1) Do MPs derived from commonly used mulching films affect soil invertebrate and protist communities? 2) Are there differences between the effects of conventional and biodegradable MPs and are these effects dose–dependent? 3) Are there differences in the MP effects between climate zones and do effects persist over growing seasons? We hypothesized that although effects on soil invertebrates have been observed at exposure levels 5–500 times higher than concentrations used in the present study (Ding et al., 2021; Weltmeyer and Roß–Nickoll, 2024; Gutiérrez–Rial et al., 2025; Zhang and Ruess, 2025), variation in climatic conditions and other environmental factors may enhance or suppress potential effects of even low MP concentrations on soil decomposers. This field study significantly contributes to our understanding on the impacts of MPs under natural, field conditions, contributing to the development of evidence–based risk management strategies for agricultural plastics.

2. Materials and methods

2.1. Test materials

Pellets made of recycled mulching films consisting of conventional linear low–density polyethylene (PE) or biodegradable polybutylene adipate terephthalate starch–blend (PBAT–BD) were used to produce MPs (<1000 μm) by cryomilling. Of the PE and PBAT MP particles, 90% were below 677 μm and 458 μm, respectively. For more information on the test materials, their production and their properties, is found in Hurley et al. (2024) and Table S1 in the Supplementary Material.

2.2. Experimental design

The field plot experiment was carried out at three study sites located in Finland, Germany and Spain and representing different vegetation and climate zones in Europe (Fig. S1 in the Supplementary material). The study site in Finland was in Jokioinen (60°51′02.4″N, 23°28′03.9″E), the German study site near Bonn (50°37′00.6″N, 6°59′50.7″E) and the Spanish study site in Alcalá de Henares (40°31′41.7″N, 3°17′44.4″W).

At each site, 25 study plots of 3.5 m × 4.5 m were established in an area of 51 m × 51 m, with 5.5 m buffer zones between the plots (Fig. S2). A randomized block design was used, with 5 replicate plots for 5 different treatments: control, PE–low, PE–high, PBAT–low and PBAT–high. No microplastics (controls), 118 g or 1181 g of PE and PBAT MP test materials were added on the plots to reach estimated nominal concentrations of 0.005% ("low") and 0.05% *w*/w ("high"), respectively, assuming soil bulk density of 1.5 g cm^{-3} and mixing depth of 10 cm. MPs were added only at the beginning of the experiment, in spring 2022, and mixed in with the topsoil (1–10 cm) using a rotavator. The concentrations based on the measured soil bulk densities were then calculated to be 0.007% and 0.065% (Finland), 0.006% and 0.055% (Germany), and 0.006% and 0.058% (Spain) (Table S2).

After adding the MPs to the soil, malt barley (*Hordeum vulgare*) was sown. The experiment lasted two growing seasons (2022 and 2023), and barley was grown on the fields in both seasons. In the second year before sowing the barley, the top 10 cm soil layer was mixed with a rotavator. Normal agricultural practices, such as fertilization and treatment with plant protection products when needed, were applied during and between the growing seasons.

2.3. Sampling

Before starting the experiment and applying the MPs, in March (Germany, Spain) and May 2022 (Finland), soil samples were taken from each plot for measuring soil properties and background MP concentrations. At the end of each growing season (July in Spain, August in Germany and September in Finland both in 2022 and 2023), one week after harvesting the barley, soil samples were taken from each plot to measure soil physicochemical properties and MP concentrations. Because of the rather dry growing season, especially in 2022, sampling for soil invertebrates in both years took place later in Autumn. In the first sampling year, samples were randomly taken from one half of the surface of each replicate plot, in the second year from the other half. The sampling scheme is available in Fig. S1.

Earthworms were sampled from the topsoil layer of each plot by digging up two 25 × 25 cm patches of soil with a spade to 10 cm depth and searching it manually. Earthworms from the deeper soil layer were extracted by applying mustard oil solution in the soil after removing the top 10 cm layer. The solution was prepared by mixing 2 mL of allyl isothiocyanate (Aldrich 37,743–0) with 40 mL of isopropanol in the laboratory and diluting this solution with 20 L of water just before application in the field. All collected earthworms were placed in jars with some field soil. In the laboratory, the earthworms were removed from the soil, rinsed with tap water, blotted dry on paper towels and weighted to obtain the fresh weight. The earthworms were then fixated and stored in 70% ethanol for species identification.

All earthworms were identified under a microscope with the help of taxonomic keys (e.g., Krediet, 2019) to the species level, where possible. Juvenile earthworms could not be identified to the species level but were sorted into two groups according to their level of pigmentation and morphological differences in their heads: tanylobic and epilobic. Damaged earthworms that were not identifiable were also counted and sorted into separate groups. All earthworms, including unidentified individuals, were counted into the total numbers and biomass.

Enchytraeid samples were collected by using a soil corer with diameter of 5 cm and sampling depth of 6 cm. The samples were stored at 5 °C before extraction with wet funnels (O'Connor, 1962). The enchytraeids extracted were counted and measured for their length under the microscope.

Microarthropod samples were collected with a soil corer (10 cm diameter in Spain and Germany and 5 cm diameter in Finland, in all cases sampling depth 6 cm) and stored at 5 °C before Tullgren extraction with a temperature gradient. Microarthropods extracted were preserved in 70% ethanol. Springtails were counted and identified to the lowest possible taxonomic resolution, mostly family level, with the help of taxonomic keys (e.g., Hopkin, 2007; Garcelon, 2023) at the Vrije Universiteit Amsterdam. Mites were not identified to the species but only divided into two groups; predatory and non–predatory mites based on the size of their jaws.

For the collection of eDNA samples for assessing the community composition of soil microbes (bacteria and fungi) and the soil microfauna, from each plot 4 soil samples from the top 10 cm soil layer were pooled to one composite sample. The sample was 6–8 mm sieved and transferred into 2 mL Eppendorf tubes for microbial DNA analysis and to 50 mL Falcon tubes for soil microfauna DNA analysis. Description of the method of DNA extraction and analysis (Velmala et al., 2026) is available as Text S1 in the supplementary material.

When sampling, MP cross contamination was prevented by using different sampling equipment for each MP treatment, or by rinsing the equipment with water between the sampling of plots with different MP treatments. MP contamination was also avoided by using jars and equipment made of glass or steel and paper made of 100% cellulose and by wearing cotton clothes.

2.4. Data analysis and bioinformatics

Data on earthworms and soil microarthropods are expressed on a per m^2 basis and for enchytraeids on a soil dry mass basis. Soil chemical and physical properties, earthworm, enchytraeid and soil microarthropod abundances (total and for different taxa or families) and earthworm biomass were analysed using a generalized linear mixed model (GLMM); see Table S3 for a complete overview of all endpoints analysed by GLMM. The fixed effects included treatment, country (Finland, Germany, Spain), year (2022 and 2023), depth (0–10 cm and 10–20 cm), and all their interactions. The random effects of rows and columns within each experiment accounted for spatial variation in the field.

Correlated samples of depths within each plot were modelled using either a homogeneous compound symmetry (CS) or a heterogeneous compound symmetry (CSH) covariance structure through the R–side random effect. Correlations between years within a plot were analysed using the G–side random effect. Unequal variances were allowed for countries, when necessary, based on a likelihood ratio test.

For skewed variables, gamma or log–normal distributions were assumed also to stabilize deviations between countries and treatments. In a few cases, these variables included zero counts of soil invertebrates, which were handled by adding a constant of one to all observations to enable comparison in a log–scale. While this approach facilitates transformation, it may slightly affect interpretation of absolute values. The assumption of normality of residuals was evaluated graphically using residual plots and was found adequate for all models. The residual pseudo–likelihood (RSPL) estimation method was used for skewed variables, while the residual maximum likelihood (REML) method was used for others. The Kenward–Roger method was employed to calculate degrees of freedom.

Treatments were compared with the control in each of the country*depth*year and country*depth combinations to minimize the number of pairwise comparisons, using the Dunnett's test with a significance level (α) of 0.05. The analyses were performed using the GLIMMIX procedure of SAS Enterprise Guide 8.3 (SAS Institute Inc., Cary, NC, USA).

All bioinformatic and statistical analyses of eDNA based communities of protists, and nematodes were performed using R (version 4.3.3, R Foundation for Statistical Computing, Vienna, Austria). Raw 18s rRNA gene sequencing data were first processed using cut adapt to remove and trim primers (Martin, 2011). Subsequently, a pipeline for metazoan 18S rRNA adopted from the dada2 ITS Pipeline Workflow (1.8, Callahan et al., 2016) was used to further process the FASTQ files. Due to the large number of data points, high performance computing was obtained using the Puhti supercomputer at the CSC IT Centre for Science. The Dada2 pipeline was used to check quality, trim sequences, learn error rates, remove redundant sequences, merge pairs, and remove chimeras. Finally, taxonomy was assigned against the PR2 (Protist Ribosomal Reference) Ecosystem SSU rRNA gene database version 5.0.0 (Guillou et al., 2013).

We used the libraries phyloseq (McMurdie and Holmes, 2013, microbiome (Lahti et al., 2017), (Viridis et al., 2024) and ggplot2 (Wickham, 2016) to handle and visualize the amplicon sequence variant (ASV) data. Data was subset to include only the nematode class and the protists (excluding other metazoa, fungi, oomycetes, brown–algae and Streptophyta). Alpha diversity was calculated from the raw ASV data (observed richness and Shannon diversity in microbiome package), and the MP treatment effect was compared separately for each country per year with Dunnett's test (Signorell, 2025). Beta diversity was analysed using Bray–Curtis distances, and significance across treatments was assessed using permutational multivariate analysis of variance (PERMANOVA) via the adonis2 function in the VEGAN package (Oksanen et al., 2025) in R, with 9999 permutations. Bray–Curtis distances were applied to the relative abundance transformed data to capture differences in community composition which emphasizes changes especially in dominant taxa. Moreover, we also calculated the Jaccard distance for presence–absence data, and the results were constant regardless the chosen distance metrics (data not shown). Betadisper was used to test homogeneity of group dispersions prior to analysis. Also, the MP treatment effect on the microarthropod community was assessed with adonis2. Non–metric multidimensional scaling (NMDS, function metaMDS) ordination plots by computing group centroids from NMDS scores were used to visualize the community profiles. Significant differences in the Bray–Curtis distances of each treatment compared to control group were assessed by pairwise t–test with Bonferroni's method to adjust p values for multiple comparisons with a significance level of $\alpha = 0.05$.

3. Results

3.1. Earthworms

There was an increase in the total numbers of earthworms compared to control in the deeper soil layer at the high PE MP concentration in Finland in 2023 ($p = 0.049$) (Fig. 1) and the high PBAT concentration in Germany in 2022 ($p = 0.040$) (Fig. 1). The earthworm numbers were

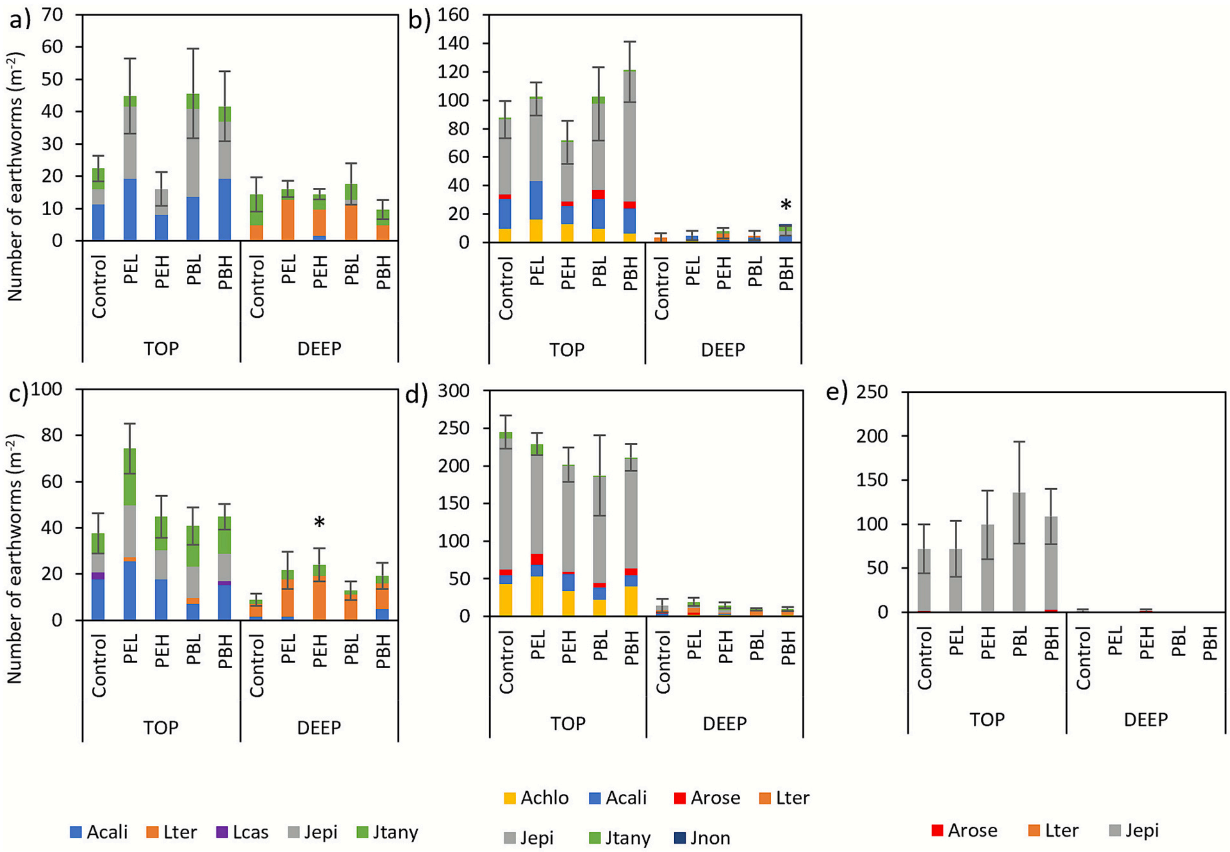

Fig. 1. Abundance of earthworm species in field plots in Finland (a, c) Germany (b, d) and Spain (e) in the autumns of 2022 (a, b) and 2023 (c, d, e). The top 10 cm soil in the plots was dosed with low (L; 0.005%) or high (H; 0.05%) concentrations of biodegradable starch-blended polybutylene adipate *co*-terephthalate (PBAT-BD-MP) or conventional low-density polyethylene (PE-MP) microplastics in spring 2022. PEL = PE low concentration, PEH = PE high concentration, PBL = PBAT low concentration, PBH = PBAT high concentration. Acali = *Aporrectodea caliginosa*, Lter = *Lumbricus terrestris*, Lcas = *Lumbricus castaneus*, Achlo = *Allolobophora chlorotica*, Arose = *Aporrectodea rosea*, Jepi = epilobe juveniles, Jtany = tanylobe juveniles, Jwhole = juveniles that could not be classified as epilobe or tanylobe. Bars represent means and standard errors of total earthworm counts. The asterisks show statistically significant differences when compared to control plot of the corresponding soil layer, analysed using generalized linear mixed model (GLMM), $^{*}p < 0.05$.

significantly affected by country, year, soil layer and their interactions (see Tables S4 and S5).

In Finland, in both 2022 and 2023, the community was dominated by adult *Aporrectodea caliginosa* and *Lumbricus terrestris*, and in 2023 also some adult *Lumbricus castaneus* were found. About half the community (40–62%) consisted of juveniles (Fig. 1). Earthworm numbers were highest in Germany, where also the earthworm community was most diverse. *Allolobophora chlorotica* and *A. caliginosa* were the dominant species, followed by *Aporrectodea rosea* and *L. terrestris*. In both years, a large proportion of the earthworms were juveniles: 53–67% in 2022 and 61–81% in 2023 (Fig. 1). Earthworm abundance in Spain was low and due to the extreme drought conditions, no earthworms were found in 2022. The earthworm community in Spain consisted mainly of epilobe juveniles (97–100%); only in three out of the five replicate plots treated with the low dose of the conventional PE MPs some adult *A. rosea* were found (Fig. 1). The earthworm community was most diverse in Germany with highest number of species (7), in Finland the total number of species discovered was 5 and it was 3 in Spain, when counting the different juvenile types as species.

Earthworm biomasses were significantly affected by the MP treatments ($p = 0.037$), but also by country ($p < 0.0001$), year ($p < 0.0001$) and country * year ($p < 0.0001$) (Fig. 2; Table S5). Earthworm biomass in the deeper soil layer was increased by the high PE MP doses in the Finnish field plots ($p = 0.033$) (Fig. 2), while no difference was detected in the top soil layer. In Spain and Germany, there were no differences in earthworm biomass between the MP treatments (Fig. 2).

3.2. Enchytraeid worms

The total numbers of enchytraeid worms in the field plots were highest in Finland, followed by Germany and lowest in Spain (Fig. 3). In 2022, no enchytraeids were found in the Spanish soil. The GLMM analysis (Table S6) revealed that enchytraeid numbers were affected by the treatments ($p = 0.003$), country ($p < 0.0001$) and year ($p < 0.001$), being overall highest in the control plots, in Finland, and in the second sampling year (2023) (Fig. 3).

In Finland, significantly higher numbers of enchytraeids were found in the control plots compared to the plots treated with the high concentration of PE MPs in 2022 ($p = 0.017$) (Fig. 3). In Spain, the abundance of the enchytraeids was significantly higher in the control plots than at the low concentration of PBAT in 2023 ($p < 0.0001$) (Fig. 3).

Similar responses were observed when analysing total cumulative and average enchytraeids lengths (Fig. 3). GLMM analysis showed that the abundance of 2–4 mm long enchytraeids was decreased by the low PBAT dose ($p = 0.043$) and the high PE dose ($p = 0.011$) in Finland in 2022, and the low PBAT dose ($p = 0.006$) in Spain in 2023 (Fig. 3; Table S6).

3.3. Springtails and mites

For microarthropods, in most cases there were significant effects of the country (Table S7), year and their interaction. In Finland, total springtail numbers were higher in 2023 than in 2022, while in Germany especially mite abundance was higher in 2022 than in 2023 (Fig. 4). In

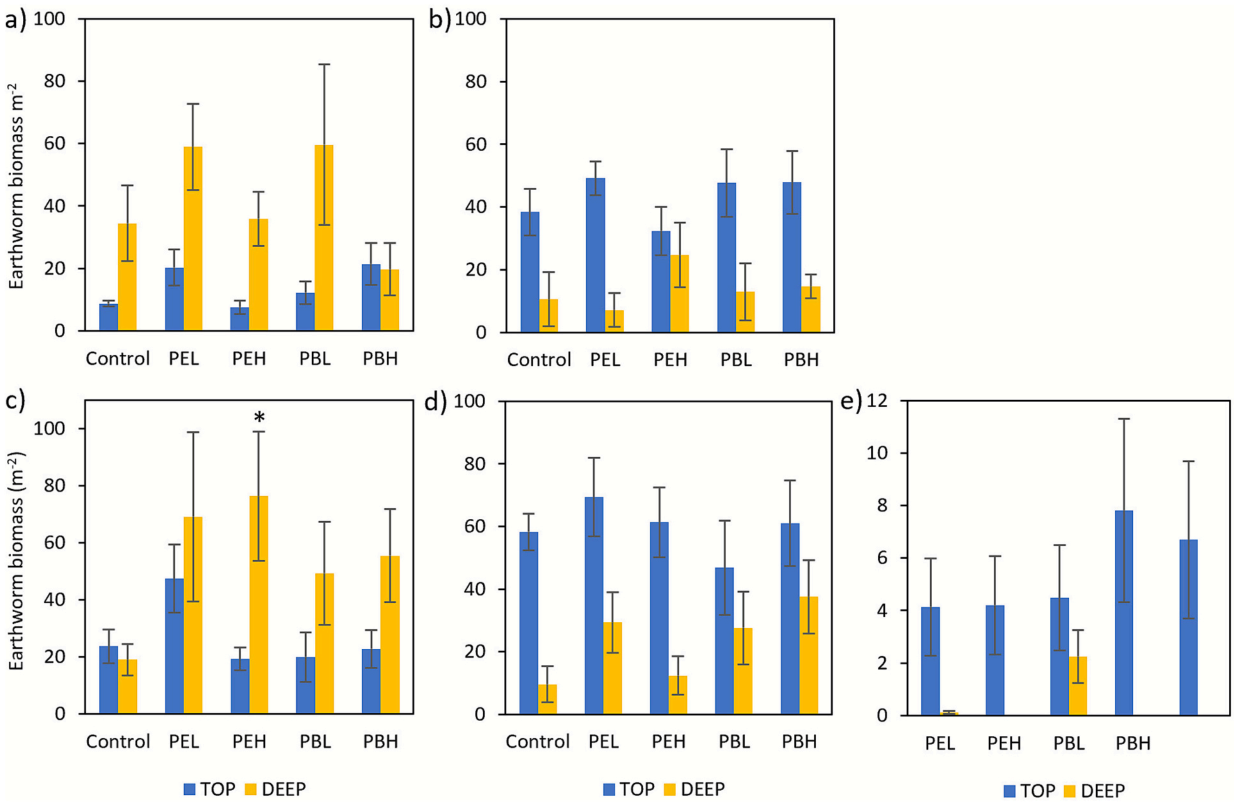

Fig. 2. Earthworm biomass in field plot soils in 0–10 cm (TOP) and deeper than 10 cm (DEEP) layers in Finland (a, c), Germany (b, d) and Spain (e) in the autumns of 2022 (a, b) and 2023 (c, d, e). The top 10 cm soil in the plots was dosed with low (L; 0.005%) or high (H; 0.05%) concentrations of biodegradable starch-blended polybutylene adipate co-terephthalate (PBAT-BD-MP) or conventional low-density polyethylene (PE-MP) microplastics in spring 2022. PEL = PE low concentration, PEH = PE high concentration, PBL = PBAT low concentration, PBH = PBAT high concentration. Bars represent means and standard errors of total earthworm biomass. The asterisks show statistical significant differences when compared to control plot of the corresponding soil layer, analysed using generalized linear mixed model (GLMM), *p < 0.05.

Spain, total number of mites was higer in 2022 than in 2023 (Fig. 4).

In a few cases, MPs did have an effect on soil microarthropods. In Finland, in 2022 the high PBAT treatment caused over 70% decrease in total springtail numbers ($p = 0.005$) (Fig. 4; Table S8), while the low PE treatment reduced the abundance of predatory mites ($p = 0.007$) (Fig. 4; Table S9). In Germany 2022, the PBAT high treatment increased the number of non–predatory mites ($p = 0.008$) (Fig. 4; Table S10).

In springtail communities, the families Onychiuridae/Tullbergiidae, Entomobryidae, Isotomidae and Neelidae were present in all countries (Fig. 4). In addition, Hypogastruridae were found in Finland and Spain, Odontellidae, Bourletielidae and Sminthuridae in Germany and Spain, Katiannidae only in Germany, and Arrhopalitidae and Dicyrtomidae only in Spain. Overall, Isotomidae was among the dominating families in all countries, but other abundant families varied between the countries (Fig. 4).

At the family level, there was a significant MP effect only on Neelidae at the high PE concentration ($p < 0.0001$) and the high PBAT concentration ($p = 0.001$) in 2022 in Germany, and the low PBAT concentration in 2023 in Spain ($p = 0.021$) (Fig. 4; Table S11).

3.4. Protists, nematodes and other microfauna

Soil animal and protist communities differed mainly between countries (Table S12). Unicellular protists and multicellular microfauna, mainly nematodes, were the most abundant organism groups in the field plots (Table S12). Common in the core taxa shared between all three countries were amoeboid protists and nematodes (Table S12). While most shared eukaryotes were cosmopolitan decomposers, few nematode genera included plant parasites that feed on roots. In Germany, PE–MP seemed to heterogenize the community structure of protists during the experiment. Alpha diversity of nematodes, i.e., the observed richness and Shannon diversity, differed only in Finland 2023 between control and PE low ($p < 0.01$), PE high ($p < 0.03$) and PBAT high ($p < 0.03$).

The nematode communities differed clearly between the climate zones (Table S12), country explaining 24% of the variation in their community structure (Permanova $p < 0.01$). There were no significant effects of MPs on the nematode community composition in any country (Permanova $p > 0.05$). However, in the visualization of the ordination analysis (Fig. 5) there was a trend of the PE treatments being grouped closer together compared to the PBAT treatments and the control, hinting at the possibility that PE MPs might have slightly affected the nematode community structure. However, the small number of replicates and high heterogeneity in the environmental factors make interpretation unreliable.

In Germany, the soil protist community was affected by PE low ($p = 0.001$), PE high ($p = 0.04$) and PBAT low ($p = 0.03$) concentrations in 2022, and PE low ($p < 0.0001$) and PE high ($p = 0.02$) concentrations in 2023. In Finland, only PE at high concentration in 2023 showed a significant effect ($p = 0.02$; Fig. 6). These changes reflected a heterogenizing effect on the protist community, with Bray–Curtis dissimilarity higher in Germany (2022) and Finland (2023) compared to controls. In contrast, in Germany in 2023, dissimilarity was lower in PE than in the control.

4. Discussion

Our field experiment with two mulching film–derived MP types (conventional PE and biodegradable starch–PBAT) across three distinct

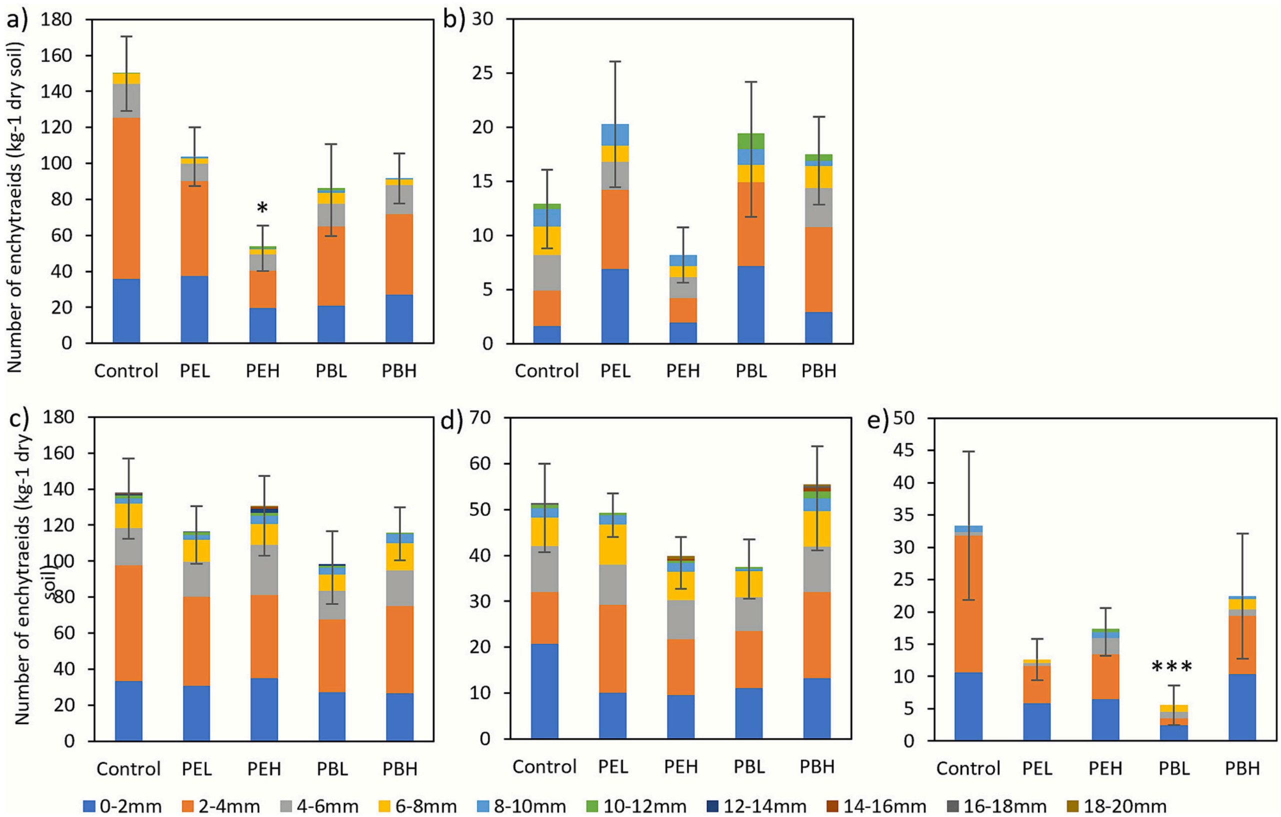

Fig. 3. Total number of enchytraeids in field plot soils in Finland (a, c), Germany (b, d) and Spain (e) in the autumns of 2022 (a, b) and 2023 (c, d, e) in different size classes. The top 10 cm soil of the plots was dosed with low (L; 0.005%) or high (H; 0.05%) concentrations of biodegradable starch-blended polybutylene adipate co-terephthalate (PBAT-BD-MP) or conventional low-density polyethylene (PE-MP) microplastics in spring 2022. PEL = PE low concentration, PEH = PE high concentration, PBL = PBAT low concentration, PBH = PBAT high concentration. Bars represent means and standard errors of total enchytraeid counts. The asterisks show statistical significant differences when compared to control plot of the corresponding soil layer, analysed using generalized linear mixed model (GLMM), *p < 0.05: ***$p < 0.001$.

climate zones, revealed variable effects on soil invertebrates, with responses differing by organism group, MP type, climate zone, and sampling year. Enchytraeids were the most sensitive taxonomic group, while earthworms, mites, springtails and nematodes were less affected. In addition, some community–level effects on protists and nematodes were observed. No clear dose–related responses of soil decomposers to the MPs were found. After the first growing season (2022), in Finland, enchytraeid numbers were reduced by 50% compared to the control in the field plots dosed with the high PE MP concentration (0.05%). An even stronger decline in enchytraeid numbers was observed in Spain at the low (0.005%) PBAT MP concentration. In the previous laboratory tests, mortality of enchytraeids was not observed even at concentrations far exceeding the ones used in our experiment. Similarly, no mortality was recorded when enchytraeids were exposed to the same PE and PBAT materials at concentrations as high as 5% (Šmídová et al., 2024), to PE MPs at 2% (Quigley et al., 2025) and to polyvinylchloride (PVC) MPs at 12% (Lahive et al., 2019). Thus, the MP concentrations used in our experiment are not likely to cause direct lethal effects on enchytraeids. However, in laboratory toxicity tests with the same materials as used in our study, 0.05% of PE and PBAT MPs significantly decreased the reproduction of *Enchytraeus crypticus* in Lufa 2.2 soil (Šmídová et al., 2024). This suggests that disturbance of their reproduction may explain reduced numbers of enchytraeids in our field plots as diminishing reproductive success of enchytraeids can limit their population growth and recovery from environmental stress. Furthermore, as enchytraeids are an extremely important organism group for soil health (Serbource et al., 2025), any decrease in their abundance and activity could negatively impact soil fertility.

In contrast to our study, Lin et al. (2020) observed in their field study that oribatid mites responded negatively to environmentally realistic PE–MP concentrations. On the other hand, Selonen et al. (2020) found no effects of polyester fibres at 0.5% concentration on the oribatid mite *Oppia nitens* in a laboratory experiment. It should be noted that the site studied by Lin et al. (2020) was a mountainous subtropical forest with minimal human activity, while our study sites had been subjected to agricultural practices. It is possible that the populations of agro-ecosystems are more resistant or resilient to chemical and physical disturbances, as such disturbances are more common in agricultural soils compared to the forest soils with less human impact.

In our study, effects of MPs on springtails were observed only in Finland, where the total numbers of springtails decreased significantly in 2022 when exposed to high concentration of PBAT. However, the effect disappeared after one growing season. The change in the effect after one year could mean, that the PBAT degraded during the experiment and the toxicity of MP disappeared. Major difference in the springtail communities between Finland and other countries was eue-daphic groups *Onychiuridae* and *Tullbergiidae* forming a large part of the springtail community in Finland, while these groups were mainly absent in other countries. The sensitivity of these groups to PBAT MPs could be a potential explaining factor, as the abundances in these groups decreased, though the differences were not statistically significant. It is also possible that climatic conditions explain the different responses of springtails between the countries. In addition to the total abundance of springtails in Finland in 2022, the family *Neelidae* showed some effects, but also not consistently in all climate zones and years. These microscopic minute species were only present in low numbers and thus their contribution to the ecosystem processes was apparently small. In standard and prolonged multi–generation laboratory toxicity tests with

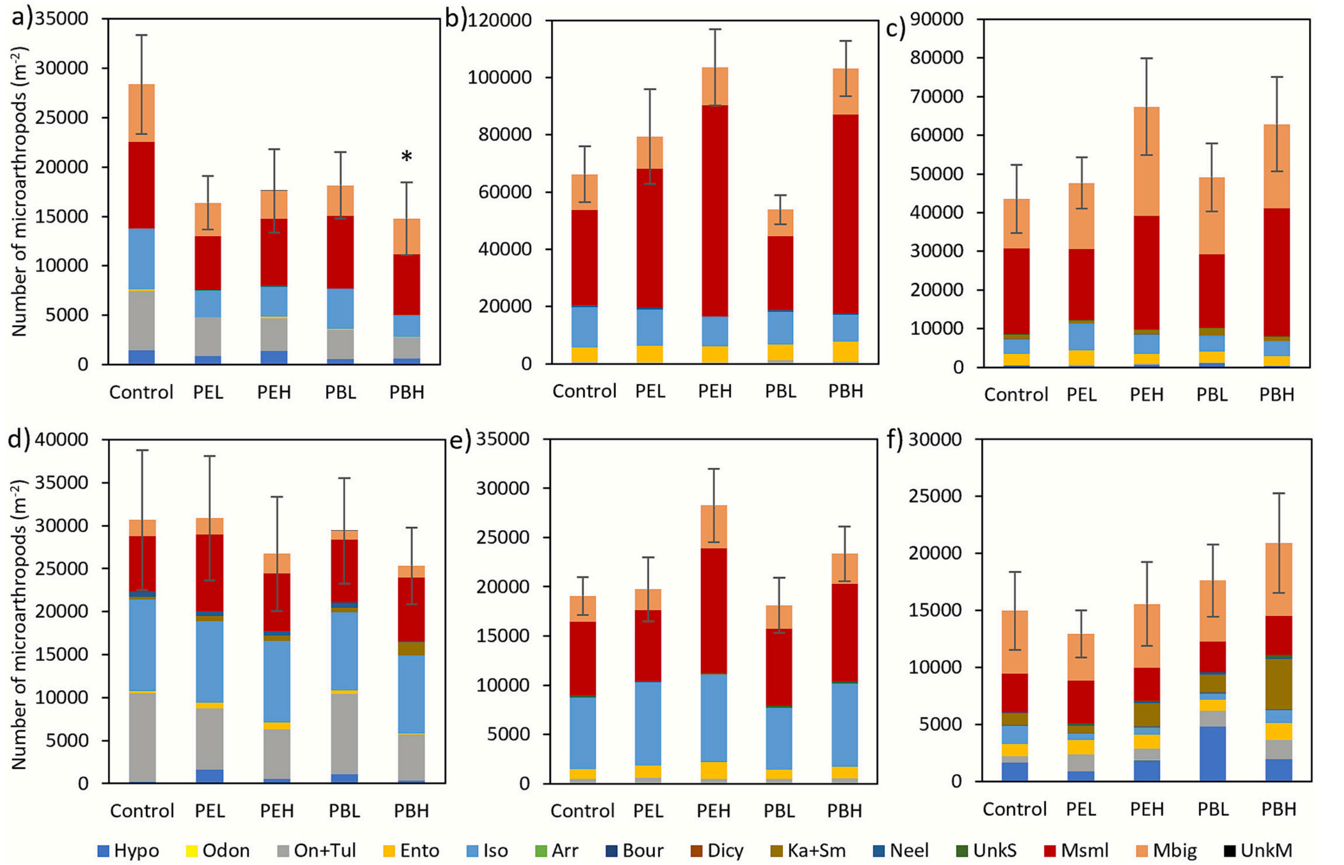

Fig. 4. Number of microarthropods (springtails and mites) in field plot soils in Finland (a, d), Germany (b, e) and Spain (c, f) in the autumns of 2022 (a, b, c) and 2023 (d, e, f). The top 10 cm soil in the plots was dosed with low (L; 0.005%) or high (H; 0.05%) concentrations of biodegradable starch-blended polybutylene adipate co-terephthalate (PBAT-BD-MP) or conventional low-density polyethylene (PE-MP) microplastics in spring 2022. PEL = PE low concentration, PEH = PE high concentration, PBL = PBAT low concentration, PBH = PBAT high concentration. Groups of springtails: Hypo = Hypogasturidae, Odon = Odontellidae, On+Tul = Onychiuridae+Tullbergiidae, Ento = Entomobryidae, Iso = Isotomidae, Arr = Arrhopalitidae, Bour = Bourletielidae, Dicy = Dicyrtomidae, Ka + Sm = Katiannidae+Sminthuridae, Neel = Neelidae, UnkS = Unidentified springtails. Groups of mites: Msml = Mites with small jaws (non-predatory mites), Mbig = Mites with big jaws (predatory mites), UnkM = Unidentified mites. Bars represent means and standard errors of total counts of the individuals. The asterisks show statistically significant differences when compared to control plot of the corresponding soil layer, analysed using generalized linear mixed model (GLMM), *$p < 0.05$.

Folsomia candida, using the same types of MPs as in our field study, no effects on the survival and reproduction were found at concentrations as high as 5% *w*/w (Van Loon et al., 2024, 2025a). Overall, springtails seem not to be very sensitive to mulching film–derived MPs, but the decrease of total springtail numbers caused by PBAT needs further investigation. Furthermore, higher concentrations, different microplastic types, or specific soil conditions may exert harmful effects on springtails, as demonstrated by Ju et al. (2019) for reduced reproduction at concentrations of 0.1% and above, and by Kim and An (2019) for impaired mobility at polystyrene MP concentrations as low as 0.0008% under wet conditions.

We found that the numbers of earthworms in Germany were higher at the high PBAT concentration compared to the control, during the first season after the MP application. These increased densities and biomasses of earthworms may relate to increased nutrition levels. It is possible that earthworms used PBAT MPs or microbes living on these MPs as a food source, as shown for *E. fetida* in the study of Holzinger et al. (2023). Lack of effects during the second year may be related to the decreased amount of PBAT due to gradual degradation and potential consumption of the material. On the other hand, in Finland the higher biomass and numbers of earthworms were evident only at the high PE MP concentration, as there is no evidence that earthworms could utilize PE as a food source. Nevertheless, it is possible that microorganisms colonising the surfaces of the MPs (Moyal et al., 2023) provide nutrition to earthworms even in PE–dosed soil.

Another explanation of the increased numbers and biomass of earthworms could be that the earthworms have avoided the MP–treated topsoil layer by burrowing deeper in the soil profile, since the effects of MPs on earthworms were only observed in the deeper soil layer, where both their numbers and biomasses increased in Germany with PBAT and in Finland with PE. In Germany, topsoil–dwelling (endogeic) species such as *A. caliginosa* and *A. chlorotica* (Edwards and Bohlen, 1996) were found in the deeper soil layer in the high PBAT treatment. Species distribution in the soil profile in Finland also supports our hypothesis. *L. terrestris*, a typical deep burrowing (anecic) earthworm (Edwards and Bohlen, 1996), was the most abundant species in the deeper soil layer, but *A. caliginosa* was also found in the deeper soil layer in the plots with the high PE MP concentration. In both countries, no differences between the treatments in earthworm abundances were observed in the topsoil layer, possibly because the moving deeper in the soil hardly affected the high densities in the top–soil layer but did lead to a significant increase in the low densities in deep soil layer. Although any clear avoidance of MPs has not previously been shown for endogeic and anecic earthworms (Prendergast–Miller et al., 2019; Sanchez-Hernandez, 2024), there is evidence that at least the epigeic species of the genus *Eisenia* avoid high MP concentrations (0.5–4%) (Ding et al., 2021; Khaldoon et al., 2022; Wang et al., 2022a; Van Loon et al., 2025b). Thus, it seems that soil contamination by at least some plastic types may affect the species–specific distribution of earthworm species in the soil profile.

The composition of the nematode community, analysed using eDNA,

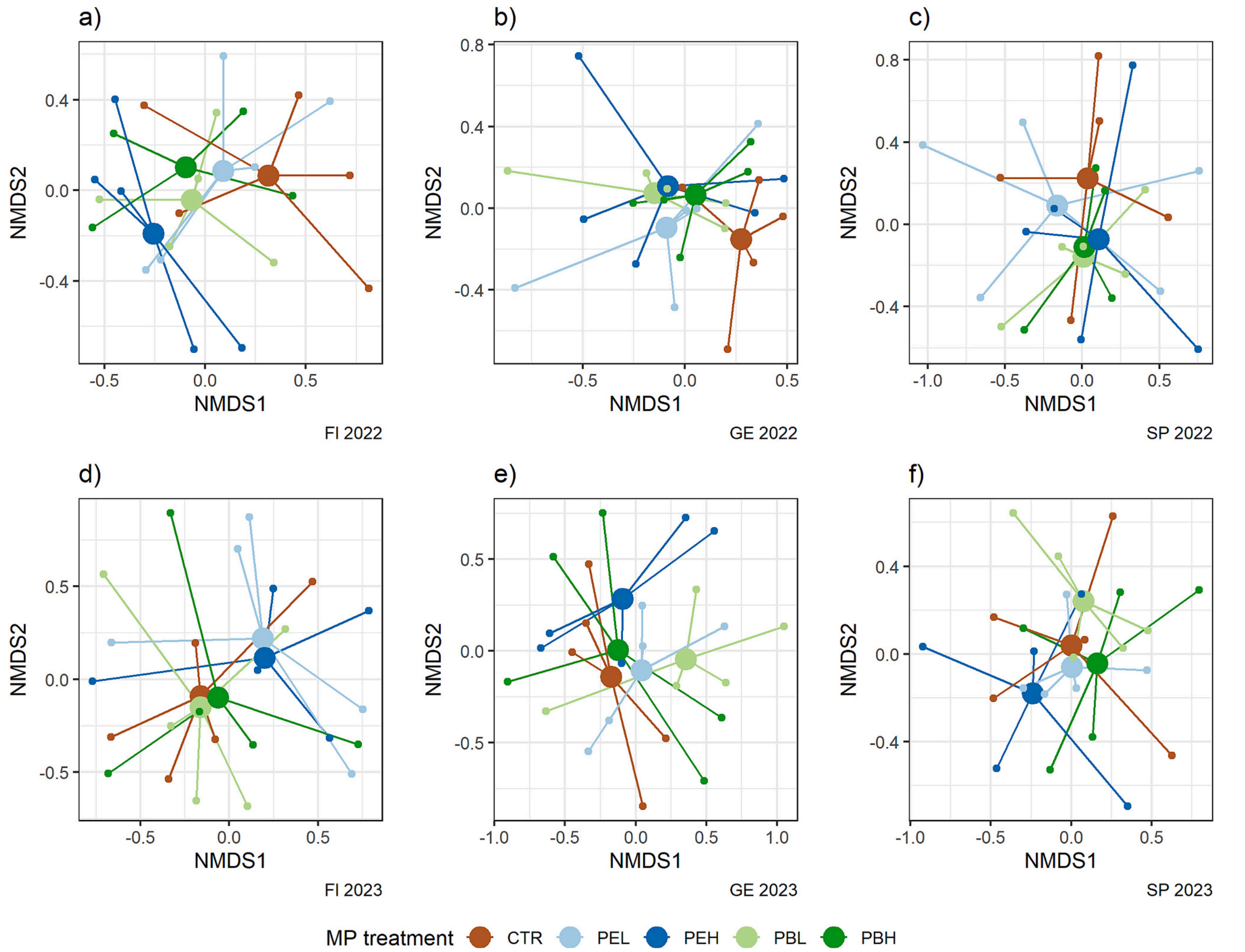

Fig. 5. 2D NMDS ordination on compositional data on nematode communities in the field plot experiments on the effects of microplastics (MP) in Finland (left a, d), Germany (middle b, e) and Spain (right c, f). The experiments were started in spring 2022, when the top 10 cm soil layers were dosed with two concentrations (0.005% (L) and 0.05% (H)) of biodegradable starch-blended polybutylene adipate co-terephthalate microplastics (PBAT-BD-MP; P4) (PBL, PBH) or conventional polyethylene (PE-MP; P3) (PEL, PEH) microplastics. Sampling took place in autumn of 2022 (top) and 2023 (bottom). CTR = control.

did not significantly change with MP treatments in Germany or Spain, but the diversity of nematodes decreased in Finland in 2023 with both PE and high PBAT treatments. MPs have been shown to cause effects on soil nematodes, but the effective concentrations seem to be dependent on the plastic type. An increase in nematode numbers, especially omnivores and fungivores, was observed at 0.1% polyamide (PA) MP contamination in microcosm studies by Liu et al. (2025), with decreasing nematode numbers at higher concentrations. Kim et al. (2020a, 2020b) found that a low MP concentration (0.001%) negatively affected nematode reproduction, but there were great differences between the plastic types used. The most harmful MP types were polyethylene terephthalate (PET) fragments and polyacrylic nitrile (PAN) fibres, while low–density PE, a plastic type most similar to the MPs used in our study, did not induce any effects at concentrations up to 1% (Kim et al., 2020a, 2020b). At a concentration of 0.01% PBAT, no effects on the bacterivore nematode *Caenorhabditis elegans*, were observed. Aged PBAT MPs were more harmful than pristine PBAT particles, but they were not tested in environmentally relevant concentrations (Zhang and Ruess, 2025) Under field conditions, potential effects on nematode numbers can easily fall in the variation caused by other environmental factors and natural heterogeneity of the soil (Burian et al., 2021).

The differences between protist communities between countries were expected as protist diversity and community composition are strongly dependent on climate and soil moisture (Bates et al., 2013). The addition of MPs had opposite effects on the protist community in Germany during the first and the second year; MP tended to heterogenize the protist community in 2022 but homogenize it in 2023. It is possible that the increased heterogeneity of the protist community was due to the MP addition, as microorganisms can colonise the surface of MP particles (Moyal et al., 2023) forming a possible food source for grazing protists. The degradation of PBAT is mainly controlled by biotic processes as the microbial community can utilize it as carbon source, altering their community (Yu et al., 2025). Interestingly, the high PBAT concentration did not show any changes in heterogeneity of the protist community, which indicates that the changes in the protist community were not caused by the addition of a carbon source in the form of MPs. Other mechanisms, such as toxicity, are a possible factor in these changes. Soil protists can take up MPs, and their numbers have been observed to decline when subjected to a concentration of 1% of spherical PS MPs (Kanold et al., 2021). In contrast, soil protist communities showed no changes when exposed to different types of MPs (PS, PP, LDPE) at the 1% concentration in a laboratory experiment (Wiedner and Polifka, 2020). However, there have been cases where effects on soil protists have been observed in low MP concentrations. For example, although soil protists were more resilient to PS MP contamination at a concentration of 0.01% compared to bacteria, families *Gregarinidae* and *Flamella*–lineage sharply

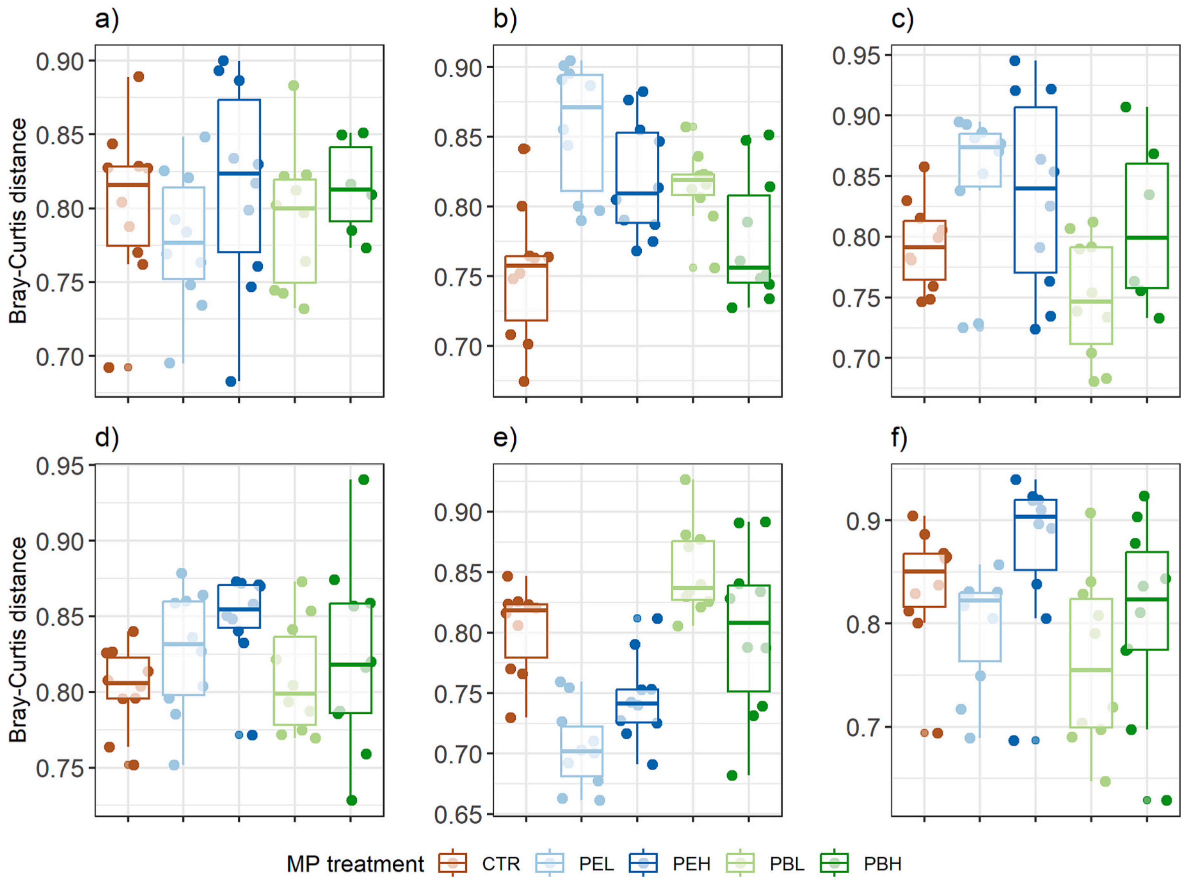

Fig. 6. Boxplot of Bray-Curtis dissimilarity of the protist community in soil treated with (PBAT) and (LDPE) concentrations of 0.005% (L) and 0.05% (H) in soils of field plot experiment in Finland (left a, d), Germany (middle b, e) and Spain (right c, f), in which the top 10 cm soil layers were dosed with two concentrations (0.005% (L) and 0.05% (H)) of biodegradable starch-blended polybutylene adipate co-terephthalate (PBAT-BD-MP; P4) (PBL, PBH) or conventional polyethylene (PE-MP; P3) (PEL, PEH) microplastics (MPs). Sampling took place in the autumns of 2022 and 2023. CTR = control.

declined after 21 days of incubation (Ma et al., 2024). The observed differences in heterogeneity between our field plots may be driven by a decline in certain groups of protists, that are key organisms in decomposition processes. The grazing of microbes by protist and nematodes is important to nitrogen mineralization and nutrient cycling. Nematodes and protist derive large amount of nitrogen from their diet excreting the excess into the soil in chemical forms available for plants (Sylvia et al., 2005). Changes in the nitrogen cycle could affect plant growth, which is extremely important to the productivity of agricultural ecosystems. In our experiment, nitrogen cycle was affected due to the MP addition, as the amount of nitrate and water extractable total nitrogen in soil decreased, as described by Šmídová et al. (2025). However, these changes in nitrogen cycling were only present in Spain, where no significant effects on the protist or nematode communities were observed.

The addition of MPs changed soil pH in the field plots (Šmídová et al., 2025). Compared to the control plots, soil pH was lower in the 0–10 cm soil layer of the high PBAT MP plots in Finland in 2023 and in the 10–20 cm soil layer of the high PBAT MP plots in Germany in 2022 (Šmídová et al., 2025). Further, in Germany, the low PE concentration caused a lower soil pH compared to the controls in the 10–20 cm soil layer in 2022 (Šmídová et al., 2025). In Spain, no significant effects on soil pH were detected (Table S13) (Šmídová et al., 2025). In case of the biodegradable PBAT–MPs, microbial activity may be stimulated by the additional carbon introduced into the soil explaining the change in soil pH. In a mesocosm experiment a similar PBAT–based MP type also caused a change in both soil pH and in microbial community composition and microbial activity (Kim et al., 2025). It is not certain, however, whether the change in soil pH was caused by or did explain the change in microbial activity (Chah et al., 2022). Also the mechanism by which the PE MPs have affected pH in the deeper soil layer in the German field plots remains unclear. Changes in soil pH may indirectly affect soil organisms, for instance by changing the availability of metals and nutrients. In this study, the changes in pH, however, are not likely to be a cause of the effects of the MPs on soil invertebrates, as no changes in the invertebrate community were observed in the treatments with changed pH, expect in Germany with earthworms in 2022 in the deep soil layer. As similiar changes were observed in Finland in 2023 without any changes in pH, likelihood of pH causing the effects on the soil invertebrates is minimal.

MPs seem not always to affect soil biota in a dose–related manner. In our study, the lower PBAT concentration (0.005%) decreased enchytraeid abundance in Spain, but the higher concentration (0.05%) did not. In concordance, missing clear dose–related responses have been reported from several laboratory studies where earthworms and springtails were exposed to MPs derived from mulching films and biodegradable MPs (Ding et al., 2021; Holzinger et al., 2023; Weltmeyer and Roß–Nickoll, 2024; Šmídová et al., 2024; Van Loon et al., 2024, 2025a). The reason behind this phenomenon remains unknown, but it may be related to the mechanisms and pathways how microplastics affect biota. At different MP concentrations the interactions between microplastics, soil and organisms may differ, leading to changes in the indirect effects of the MPs. This hypothesis, however, would need further investigation.

MPs made of conventional PE and biodegradable starch–PBAT blend used in our experiment produced slightly different effects on the soil organisms in different countries, with earthworms being more sensitive to PBAT in Germany and PE in Finland and enchytraeids being more sensitive to PBAT in Spain and PE in Finland. Differences in the effects of MPs derived from PE and starch–PBAT mulching film materials were also observed in several laboratory experiments, negative implications being generally stronger for PE (Forsell et al., 2024; Jemec Kokalj et al., 2024; Šmídová et al., 2024; Saartama et al., 2026). PE MPs reduced the reproduction of the enchytraeid *E. crypticus* (Šmídová et al., 2024) and the earthworm *E. andrei* (Saartama et al., 2026) as well as larval growth and moulting of the mealworm *Tenebrio molitor* (Jemec Kokalj et al., 2024), while the effects of starch–PBAT MPs were smaller or negligible. However, these effects occurred only at a 5% MP exposure concentration, which is currently irrelevant for agricultural soils. Nevertheless, the differences in the impacts have been detected also at environmentally relevant concentrations, as 0.005% of PE decreased the growth of earthworm *E. andrei* in the exposure of the second generation (Saartama et al., 2026), and the same concentration of starch–PBAT MPs increased earthworm growth (Forsell et al., 2024). It is notable, however, that microplastics from both polymer types were found to induce biochemical responses such as oxidative stress in earthworms already in low or moderate concentrations (0.005%–0.1%) (Forsell et al., 2024).

As discussed earlier, positive impacts of PBAT in the present and previous studies may relate to their use as a direct food source of invertebrates, or that of microbes utilised by invertebrates and protists. The negative effects detected on springtails only in Finland and only in the first growing season may, in turn, relate to the differences in the biodegradability of the PBAT MPs between the climatic zones. In cold climatic conditions of Finland, the degradation of PBAT can be slower, leading to higher concentrations of PBAT particles in soil in the first year compared to the other two countries. If the negative effects rise from direct effects of PBAT particles, in other countries and in the second year in Finland the PBAT MP concentrations, and also the effects may have been diminished. It is also possible that the differences between the countries and years relate to the toxicity of harmful degradation products of PBAT (Martínez et al., 2024). If these degradation products of PBAT are short–lived, their highest concentrations may have appeared in Finland already during the first growing season. This could have resulted in combined effects of PBAT and its degradation products already in the first year. However, as the degradation products were not measured, causality between springtail abundance and PBAT MPs in the soil remain speculative. Further, northern climate and soil properties might have added some synergy to the impacts of PBAT MPs.

In our field experiment, differences in soil properties, history of land use, climate and species composition can explain the different impacts of MPs on soil decomposer communities in different countries. Obviously, there were variations in the sensitivity of the different organism groups to the MPs applied. The three different countries in our experiment represent characteristic climate patterns according to the Koeppen–Geiger climate classification. Southern Finland is in the cold zone, with no dry seasons, and with relatively warm summers (Dfb). Germany is in the warm temperate and humid zone with no dry seasons (Cfb) while in Spain temperate climate with dry hot summers prevails (Csa) (Kottek et al., 2006). Compared to the temperate climate of Germany, the dry conditions in Spain and long–lasting freezing temperatures in Finland represent extreme conditions.

The clearest effects were observed on enchytraeids in Finland and Spain. In Spain, soil organisms suffered from high temperatures and frequent droughts while in Finland they experienced short growing seasons and winter frosts. In the mild climate in Germany, the effects of the MPs tested were less severe, and no negative effects were observed on soil invertebrates. Temperature stress has been observed e.g. to increase the toxicity of metals to *E. fetida* (Urionabarrenetxea et al., 2020), hamper detoxification processes in *F. candida*, enhance the toxic effects of fluazinam (Wehrli et al., 2024) and increase toxicity of phenanthrene to *Enchytraeus albidus* (Dai et al., 2023). Hence, it is possible that soil invertebrate communities are more at risk from MP contamination in harsh climates. Ecosystems that have higher species diversity and functional redundancy are typically more resilient to environmental stress factors (Mori et al., 2013). The extreme drought in Spain in 2022 did dramatically reduce the contemporary diversity of the soil invertebrate community, as entire organism groups such as earthworms and enchytraeids, were missing from the soil. In Finland, the diversity of earthworms and springtails was lower when compared to Germany. These natural differences in the soil invertebrate communities together with climatic factors could explain why soil invertebrates were most affected by MPs in Finland and Spain.

The effects of the MPs were not consistent for the whole experimental period, i.e., in some cases effects were only observed either in the first or second year. For example, in Finland the effects of PE MPs on enchytraeids disappeared after one year. This hints to the possibility that the changes in animal numbers caused by low level MP contamination are transient stress factor and enchytraeids can acclimatize to MPs. The presence of enchytraeids and earthworms in the Spanish plots in 2023 after the population collapse in 2022 shows their recovery potential after a heavy disturbance. The lack of any effects on many soil animal groups in Spain is most likely due to the extreme stress of high temperatures and the drought masking any potential effects of the MPs. In addition, the possible decreasing concentrations of PBAT MPs due to degradation processes can partly explain the diminishing or disappearing effects on animals in Germany, where the environmental conditions for PBAT degradation are more optimal than in Finland and Spain.

The environmental hazards of microplastics are ever–increasing and cumulative. Constant use of agricultural plastics will inevitably lead to an increase of soil MP concentrations in the future (Büks and Kaupenjohann, 2020). This means more severe disturbances for whole agroecosystems. Our study highlighted the importance of climatic and other environmental factors for the effects of MPs. In the near future, climate change is increasing the extreme weather phenomena, such as droughts, floods and temperature fluctuations in Europe (Bilgili and Tokmakci, 2025). The combined stress from MPs and extreme weather phenomena can cause unpredictable responses in the soil animal populations and communities. These combined effects urgently need more large–scale and detailed investigations.

5. Conclusions

This study demonstrated that MPs derived from agricultural mulching film material, even at environmentally relevant concentrations, can affect soil invertebrate communities, although the magnitude and nature of these effects varied by organism group, climatic conditions, and sampling year. The mechanisms of the effects can be attributed to toxicity of the MPs themselves, their additives, degradation products or changes caused to soil properties. However, as animals or soil were not tested for MP or additive concentrations, the exact mechanism of the effexts remains uncertain. As soil decomposers are functionally important organisms, earthworms even being ecosystem engineers, even small changes in their numbers or community structure under MP exposure can be reflected in organic matter decomposition activity, nutrient cycling and consequently very likely in plant production of agricultural ecosystems (cf. e.g. Edwards and Bohlen, 1996; Lavelle et al., 2006). In conclusion, this study identifies clear ecological risks of MPs in agricultural soils, establishing a necessary foundation for future controlled, long–term field research to fully quantify their environmental impacts.

CRediT authorship contribution statement

Vili Saartama: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Cornelis A.M. van Gestel:** Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Jari Haimi:** Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Sannakajsa Velmala:**

Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lotte de Jeu:** Writing – review & editing, Methodology, Investigation. **Melanie Braun:** Writing – review & editing, Investigation, Conceptualization. **Janne Kaseva:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Juha-Matti Pitkänen:** Writing – review & editing, Methodology, Investigation. **Derk-Jan Post:** Writing – review & editing, Methodology, Investigation. **Paula Redondo Hasselerharm:** Writing – review & editing, Project administration, Investigation, Conceptualization. **Andreu Rico:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Conceptualization. **Suvi Sutela:** Writing – review & editing, Methodology, Investigation. **Salla Selonen:** Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2026.106885.

Data availability

Data will be available upon publication in Zenodo: https://doi.org/10.5281/zenodo.15622875; https://doi.org/10.5281/zenodo.14825718; https://doi.org/10.5281/zenodo.11068075.

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