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Ecotoxicological impact of Albendazole and low-density polyethylene microplastics on the collembola *Folsomia candida* (Willem, 1902)

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ABSTRACT

Plastics offer significant benefits but also pose serious environmental concerns, especially in areas of intensive livestock production where they frequently coexist in soil with veterinary drugs such as albendazole (ABZ), a broad-spectrum anthelmintic. ABZ can enter soil ecosystems either directly through cattle excretion or indirectly via the application of contaminated manure, raising concerns about its potential harmful effects on soil health. Collembola play a vital role in soil ecosystems through organic matter decomposition and nutrient cycling and the species *Folsomia candida* is widely used in ecotoxicological studies due to its sensitivity to pollutants. This study examined the individual and combined effects of 28-day exposure to low-density polyethylene (LDPE) microplastics (MPs) at 0 and 0.1 *w*/w% (0 and 1000 mg kg^{-1}), alone or in combination with ABZ at 0, 0.0001, 0.1, and 1 w/w % (0, 1, 1000, and 10,000 mg kg^{-1}) on *F. candida* in a sandy soil (LUFA 2.2.). Results showed that LDPE alone had no lethal or reproductive effects on *F. candida*, while ABZ, either alone or with MPs, significantly reduced the insect reproduction, pointing at ABZ as the key driver of the adverse effects. Reproductive inhibition was observed even at 1 mg kg^{-1}, suggesting that ABZ may affect soil mesofauna at concentrations that can occur in manure-amended agricultural soils. The absence of differences in *F. candida* survival and reproduction suggests no synergistic effects between ABZ and MPs, nor any potential co-action mechanisms influencing ABZ behaviour under the specific experimental conditions, including soil substrate characteristics.

1. Introduction

Since the mid-20th century, plastics have contributed substantially to advances in health care, food preservation, and resource efficiency. However, their persistence and widespread use have also led to increasing environmental contamination, posing a major threat to both aquatic and terrestrial ecosystems (Guzzetti et al., 2018). Agricultural soils represent one of the main sinks for plastic residues, which can enter through compost and organic fertilizers, irrigation systems, and the extensive use of plastic mulching films (Okoffo et al., 2021; Piehl et al., 2018). Once introduced into soil, plastic materials gradually degrade under the combined influence of ultraviolet radiation, mechanical abrasion, and chemical weathering, producing micro- and nano-plastic fragments (MPs) (Gigault et al., 2021). These small plastic particles have a high surface-to-volume ratio and a strong tendency to adsorb other substances, allowing them to act as carriers for organic pollutants and potentially altering contaminant transport and fate in soil (Chang et al., 2022; Zhang et al., 2024). The extent of these interactions depends on both the properties of the plastics and the characteristics of the surrounding soil—such as porosity, organic matter content, and pH—which together determine the mobility, persistence, and bioavailability of MPs and associated contaminants (Li et al., 2021, 2023; Meng et al., 2023; Zhang et al., 2024).

Agricultural soils often receive complex mixtures of contaminants derived from livestock manure and crop protection products. Despite their frequent co-occurrence with MPs, little is known about how these

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pollutants interact in soil ecosystems. Recent studies have only begun exploring such interactions; for instance, Saldi et al. (2025) reported that different microplastic types can influence the bioavailability and toxicity of the veterinary drug albendazole and the fungicide pyraclostrobin in the collembola *Folsomia candida*. These findings highlight that polymer type and environmental conditions modulate contaminant behaviour and underscore the need to investigate MP–organic pollutant systems in more detail. Among the contaminants commonly detected in agricultural soils, veterinary pharmaceuticals deserve particular attention because of their potential ecological effects and widespread use in livestock farming. Albendazole (ABZ), a broad-spectrum anthelmintic, is one of the most frequently applied compounds and is largely excreted in unmetabolized or minimally transformed form (Lagos et al., 2023). Application of contaminated manure therefore introduces ABZ into agricultural soils, where it may persist and affect non-target organisms Aksit et al., 2015). Laboratory studies have demonstrated that ABZ is toxic to various terrestrial species, including *Eisenia foetida* (Gao et al., 2015) and arbuscular mycorrhizal fungi (Gkimprixi et al., 2023). However, to date, no studies have directly investigated the effects of ABZ on soil mesofauna, leaving a gap in understanding its ecological risks. Springtails (Collembola) are critical to soil ecosystems due to their role in organic matter decomposition, nutrient cycling, biodiversity maintenance, and microbial community activity (Potapov et al., 2023). The parthenogenetic species *F. candida* is widely used in soil ecotoxicology because of its sensitivity to contaminants, ecological relevance, and ease of laboratory rearing. Its reproduction, survival, and behaviour serve as valuable indicators of soil health and are included in standardized assays recommended by international guidelines (OECD, 2016; ISO, 2014).

In the present work, we explored the individual and combined effects of low-density polyethylene (LDPE) and ABZ on *F. candida* in a sandy soil (LUFA 2.2). LDPE is among the most widely used polymers in agriculture, especially for mulch films, irrigation systems, and greenhouse structures. Its high resistance to degradation leads to long-term persistence and accumulation in soils, where its residues may co-occur with veterinary pharmaceuticals in mixed crop–livestock systems. In agricultural soils, LDPE can persist for years, fragmenting gradually into irregular particles within the sub-millimeter to millimeter range depending on the extent of environmental degradation (Piehl et al., 2018; Scheurer and Bigalke, 2018;). In this study, we used LDPE fragments derived from agricultural films to represent larger secondary particles at an early fragmentation stage. These fragments correspond to coarse microplastics commonly detected in soil environments and were selected to ensure consistency with materials used in other studies (Meng et al., 2024; Saldi et al., 2025; Špela et al., 2025). This study aims to investigate whether LDPE MPs influences the bioavailability and toxicity of ABZ and to provide new insights into the potential ecological risks associated with co-contamination by persistent plastics and veterinary drugs in soil ecosystems.

2. Materials and methods

2.1. Test organisms

F. candida was reared under dark conditions at 20 ± 1 °C, 80 % humidity. Cultures were maintained in glass boxes with a 2 cm plaster of Paris/graphite (10:1 *w*/w) substrate and fed dried granulated yeast (*Saccharomyces cerevisiae*) twice a week. To obtain synchronized juveniles, adults were allowed to lay eggs in Petri dishes for two days before being removed. Eggs were kept at 20 ± 1 °C in the dark until hatching, and 9–12 days old juveniles were used for experiments (ISO, 2014; OECD, 2016).

2.2. Test chemical and test soil

The LDPE MPs used in this study were provided by Wageningen University and Research (MINAGRIS project). LDPE MPs were derived from 30 μm thick plastic films, cryomilled, and sieved as described by Meng et al. (2024). Particle size distribution, analyzed with a Mastersizer 3000, ranged from 271 ± 2 μm (10th percentile) to 780 ± 15 μm (90th percentile), with a median mean diameter of 466 ± 4 μm and a density of 0.937 g/cm^3. These sizes align with microplastic fragments found in soils, where particles less than 500 μm in diameter are known to dominate (Scheurer and Bigalke, 2018). ABZ (purity ≥98 %) was purchased from Sigma Aldrich (CAS 54965-21-8), and acetone (purity ≥99.8 %) from CARLO ERBA Reagents (CAS 67–64-1). All experiments were conducted using LUFA 2.2 standard soil (LUFA Speyer), a sandy loam with 1.84 % ± 0.48 organic carbon, 0.21 % ± 0.05 nitrogen, pH of 5.66 ± 0.23 (0.01 M $CaCl_2$), cation exchange capacity of 9.46 ± 1.33 meq/100 g, and maximum water holding capacity of 46.6 % ± 3.5 (g/100 g).

2.3. Test soil preparation

LDPE MPs were mixed with LUFA 2.2 soil 48 h before ABZ treatment at a final concentration of 0.1 *w*/w% (1000 mg kg^{-1}). This concentration falls within the 0.062–28 % range tested on soil invertebrates in previous studies (Huerta Lwanga et al., 2017). Negative controls for LDPE MPs were included.

ABZ was applied at 0.0001 w/w% (1 mg kg^{-1}mg kg^{-1}, LD), 0.1 w/w% (103 mg kg^{-1}, MD), and 1 w/w% (104 mg kg^{-1}, HD), with LD reflecting expected environmental exposure in manure-amended soils (Gkimprixi et al., 2023), while MD and HD were used to assess dose-dependent effects. ABZ was applied via acetone solution (20 mL for LD/MD, 200 mL for HD) to 10 g of dry soil, left to evaporate for 24–48 h, then transferred to Petri dishes containing plaster of Paris/graphite (10:1). Soil moisture was adjusted to 60 % water-holding capacity. Acetone controls (20–200 μL) showed no significant effects (data not shown).

2.4. Experimental design and ecotoxicological test

Ecotoxicity was assessed following modified OECD guidelines for the collembola reproduction test (OECD 232, 2016). Tested groups were control group with soil only "CTRL", group with soil treated with Albendazole "ABZ", group with LDPE MP only "MP", group treated with a mixture of LDPE MPs and Albendazole "MP + ABZ". A total of 3 experimental tests were conducted, one per ABZ concentration (HD, MD, LD), each testing 6 plates per group for a total of 36 replicates per experiment. Ten synchronized juveniles were placed in glass Petri dishes with 10 g of moistened LUFA 2.2 soil and fed with dried baker's yeast. Dishes were maintained at 20 ± 1 °C, 80 % ± 1 RH, with a 12-hour light-dark cycle. After 28 days, soil was gently stirred with water and transferred to a larger container for springtail floatation. Adults, juveniles, and eggs were counted using a Zeiss Discovery V8 stereomicroscope equipped with a camera.

2.5. Statistical analysis

Data for each experimental concentration (HD, MD, LD) were analyzed independently. Non-normal data distributions were confirmed using Shapiro–Wilk tests (*p*-values in Supplementary Table S1), followed by Kruskal–Wallis tests (Bonferroni-adjusted) and Dunn's post-hoc comparisons ($\alpha = 0.05$). Data were further modeled using Generalized Linear Mixed-Effect Models (GLMMs) (Brooks et al., 2017). Statistical analyses and graphing were conducted in R (v. 4.4.0; R Core Team, 2023), with models built using the *lme4* and *glmmTMB* packages (Bates et al., 2015) and lognormal family functions. The response variables were the specific count (adults, juveniles, eggs), while treatment type (CTRL, ABZ, MP, MP + ABZ) and ABZ concentration (LD, MD, HD) were fixed effects; plate number was included as a random effect. Model diagnostics were assessed using quantile–quantile plots. Pairwise comparisons were performed using post-hoc Tukey tests, and results are reported as mean estimates with 95 % confidence intervals. Statistical differences among treatments are indicated using Compact Letter Display notation (Piepho, 2018).

3. Results

All ecotoxicological tests met the OECD 232 (2016) validity criteria for untreated controls at each concentration. Results of the Kruskal–Wallis test (Bonferroni-adjusted) followed by Dunn's post-hoc comparisons are reported in Supplementary Tables S2–4 for Albendazole concentrations (low, medium, and high dose) and shown in the violin plots of Fig. 1. Adult survival did not differ significantly among treatments. However, Albendazole-treated plates, with or without low-density polyethylene microplastics, exhibited a significant reduction in *F. candida* juveniles and eggs at all concentrations. The treatment effect increased with rising Albendazole concentration.

Quantile–quantile (Q–Q) plots showing no significant deviations are presented in Supplementary Fig. S1. Results of the modelling are shown in Fig. 2 for each count (adults, juveniles, eggs). As already highlighted by the Kruskal-Wallis test, adult counts do not show significant differences among treatments and concentrations (Fig. 2a), with a slight increase of the mean prediction value for Albendazole concentration equal to 0.1 % (medium dose). On the other hand, juvenile and egg counts differed significantly among treatments (Fig. 2b–c). Groups sharing the same letter are not significantly different, whereas those with different letters are. At each Albendazole concentration, the Albendazole and Albendazole + microplastics treatments (labelled “a”) showed lower counts than the control and microplastics-only groups (labelled “b”). The effect of Albendazole increased with concentration, especially for juveniles, as also highlighted by the bar plots of mean values per treatment (Supplementary Fig. S1).

4. Discussion

The results indicate that exposure to low-density polyethylene microplastics alone did not induce lethal or reproductive effects on the collembola, confirming previous studies involving linear low-density polyethylene plastic fragments (Van Loon et al., 2024). This absence of effects may be explained by the relatively large particle size of the plastics used in this study. The microplastics were produced from 30 μm agricultural films by cryomilling and sieving, resulting in a size distribution of approximately 271–780 μm (median ≈ 466 μm), comparable to that used in previous studies (Meng et al., 2024; Saldi et al., 2025; Špela et al., 2025). This range corresponds to coarse low-density polyethylene fragments, consistent with the upper size classes of microplastics typically reported in soils (Scheurer and Bigalke, 2018). Because our experiment was conducted without macrofauna (e.g. earthworms) capable of ingesting and fragmenting relatively large microplastics (Meng et al., 2024) or any physical disturbance, further degradation of low-density polyethylene particles was unlikely, and the particle-size distribution was expected to remain stable over the 28-day exposure. Ingestion is also the main route of exposure to toxic substances for Collembola (Papa et al., 2023), and adverse effects from plastic fragments have been reported only when particle sizes fall within the species' edible range, which for *F. candida* is <66.0 ± 10.9 μm (Kim and An, 2019). The median microplastic size used here was well above this threshold, making ingestion highly unlikely and effectively preventing direct particle-mediated effects. In addition, *F. candida* may have actively avoided low-density polyethylene fragments because of their low palatability. Springtails preferentially feed on organic substrates such as fungi, bacteria, and decomposing plant matter (Jørgensen et al., 2008); unpalatable materials like inert plastics are typically ignored, further minimizing exposure.

In contrast, exposure to Albendazole alone or in combination with plastic fragments produced clear ecotoxicological effects, reflected in impaired reproduction. The veterinary pharmaceutical was the principal driver of toxicity. Adult survival remained high across treatments, indicating no lethal effects, whereas reproduction (juvenile and egg production) declined sharply with increasing Albendazole concentration. This dose-dependent inhibition highlights Albendazole's strong reproductive toxicity even at environmentally relevant concentrations.

Low-density polyethylene microplastics alone caused no measurable changes in any endpoint, and their combination with Albendazole did not enhance toxicity. The higher medians observed in the microplastics plus Albendazole groups suggest a minor attenuation of the veterinary drug effects, particularly at medium and high concentrations, where adult counts were consistently higher than in the corresponding Albendazole-only treatments, although differences were not statistically significant. For juveniles, this attenuation was much less evident, with only minimal shifts in central tendency. This trend may reflect weak adsorption of the moderately hydrophobic compound (log P ≈ 3.2) onto low-density polyethylene microplastics surfaces, which could slightly reduce its bioavailability in soil. Similar adsorption-driven moderation of toxicity has been reported for other organic pollutants associated with microplastics (Hüffer et al., 2019; Jiang et al., 2020; Li et al., 2024). This interpretation aligns with the findings of Saldi et al. (2025), who observed that low-density polyethylene had limited capacity to enhance the toxicity of *co*-occurring organic pollutants compared with more reactive biodegradable polymers.

Although reproduction in *F. candida* was impaired, parthenogenesis remained functional. The endosymbiont *Wolbachia* is essential for parthenogenesis in this species (Frati et al., 2004) and its strong disruption typically results in severe reproductive failure (Asgharian et al., 2014; Negri et al., 2010; Negri, 2012). The partial reduction in eggs and juveniles observed here therefore aligns more with direct Albendazole toxicity to the host rather than a major loss of symbiont function. Nevertheless, an indirect contribution of *Wolbachia* effects cannot be completely ruled out, given its broader influence on arthropod reproduction and the ability of the veterinary drug and its metabolite Albendazole sulfone to target this symbiont (Serbus et al., 2012). Further analysis dissecting potential interactions between Albendazole and *Wolbachia* physiology will help clarify whether symbiont modulation contributes alongside direct host toxicity.

Regarding the sub-lethal effects observed in *F. candida* exposed to the environmentally relevant dose of 1 mg kg^{-1} Albendazole, it is worth mentioning that in areas of intensive livestock the veterinary drug in soil may reach concentrations up to an order of magnitude higher (Belew et al., 2021), suggesting a significant impact on springtail species and the ecosystem services they provide in the long term.

Overall, these findings indicate that while Albendazole poses a clear reproductive risk to *F. candida*, low-density polyethylene microplastics of the size and type tested did not enhance its toxicity, suggesting that their combined presence in soil is unlikely to produce additive effects under comparable conditions. Further studies are needed to assess whether different polymer characteristics, particle sizes, or soil properties may alter these interactions.

CRediT authorship contribution statement

Bartolo Forestieri: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Diego Voccia:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Lucrezia Lamastra:** Writing – review & editing, Supervision, Data curation. **Esperanza Huerta Lwanga:** Writing – review & editing, Validation, Project administration, Methodology. **Dimitrios G. Karpouzas:** Writing – review & editing, Validation, Project administration, Methodology. **Cristina Nuzzi:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Formal analysis. **Ilaria Negri:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

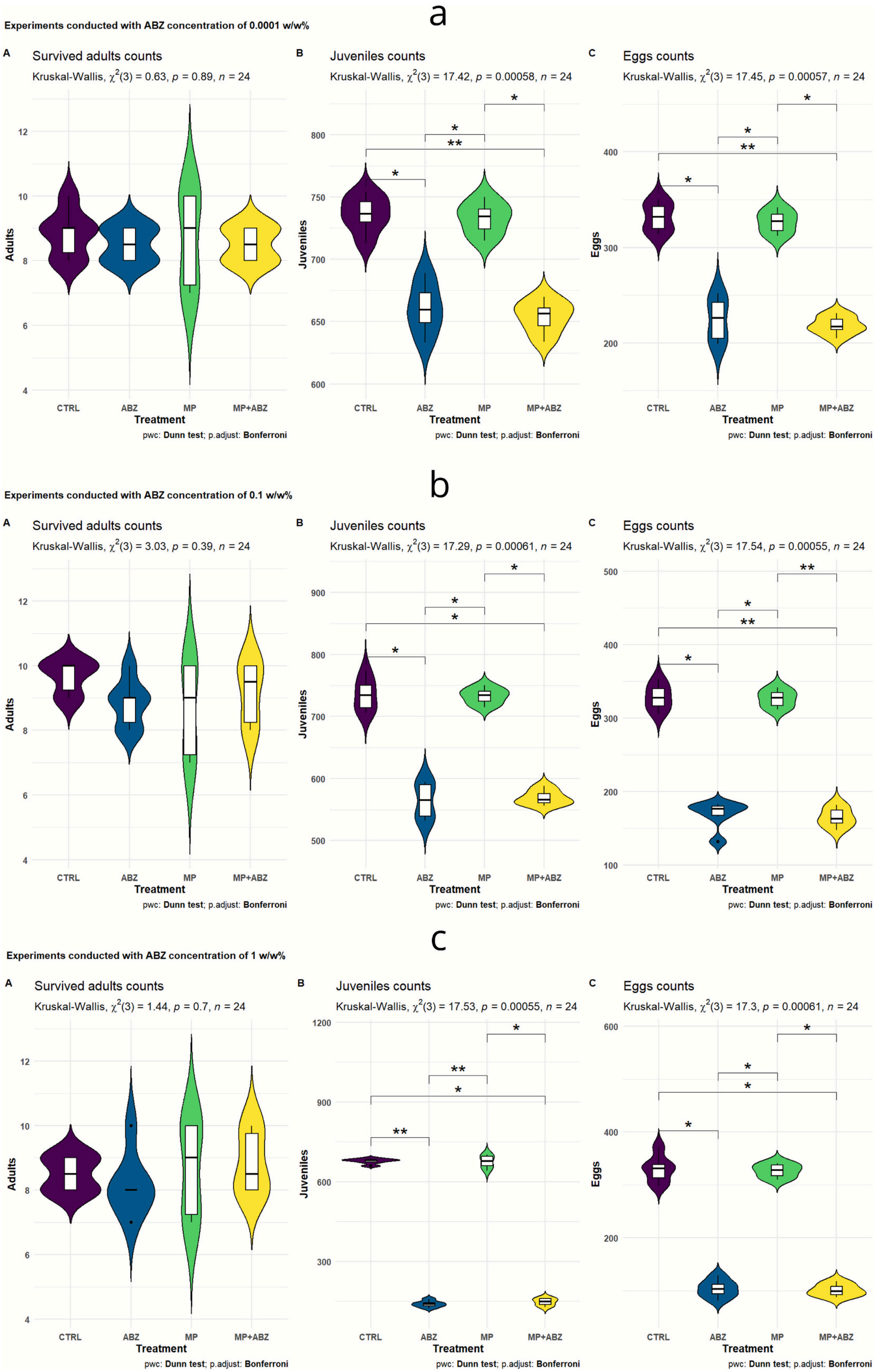

Fig. 1. Violin plots of the counts data with significant differences among groups resulting from the Kruskal-Wallis test highlighted. Results for ABZ concentration of (a) 0.0001 %, (b) 0.1 %, (c) 1 %. A-B-C letters in each figure represent survived adults counts, juveniles, and eggs respectively. Treatments: control group (CTRL), Albendazole only (ABZ), LDPE microplastic only (MP), combination of Albendazole and LDPE microplastic (MP + ABZ).

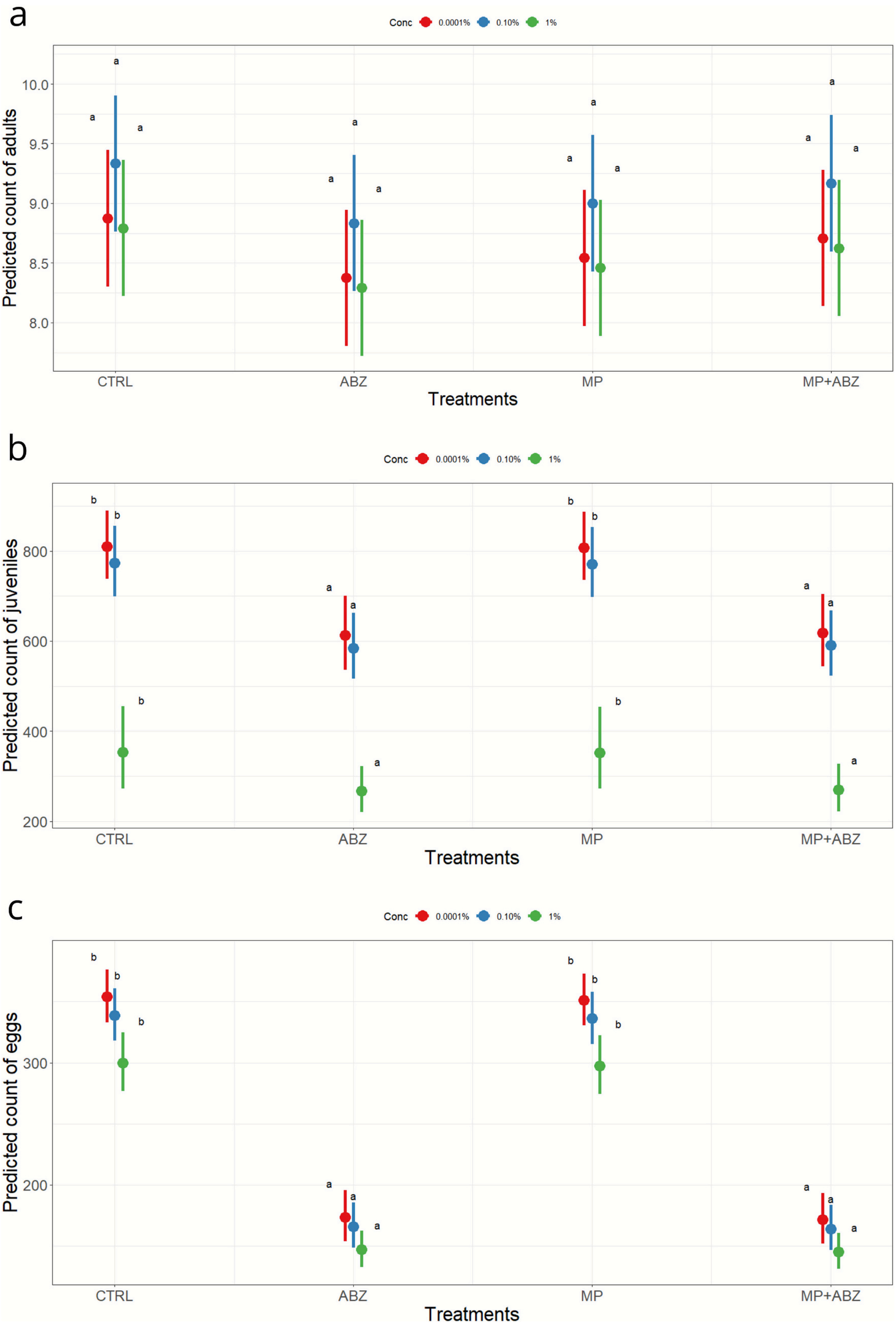

Fig. 2. Model predictions for the counts of (a) adults, (b) juveniles, (c) eggs. Different colors represent different ABZ concentrations (red: 0.0001 %, blue: 0.1 %, green: 1 %). Treatments: control group (CTRL), Albendazole only (ABZ), LDPE microplastic only (MP), combination of Albendazole and LDPE microplastic (MP + ABZ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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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.2025.106764.

Data availability

Data will be made available on request.

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