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Original Research

Impact of Polyethylene and Polylactic Acid Microplastics on Seed Germination and Seedling Development of *Trifolium repens*

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Abstract

Microplastics (MPs) are emerging contaminants with uncertain effects on plants, particularly ornamentals. We tested how polyethylene (PE), polylactic acid (PLA), and a constant-total-dose 1:1 (w/w) mixture of PE and PLA (MIX; 25 μm) at 0.1% (w/v), 0.5% (w/v), and 1.0% (w/v) influence seed germination and early growth of white clover (*Trifolium repens*) in Petri-dish assays. Although these exposure levels exceed typical background levels in bulk soils, they were used to represent worst-case/high-exposure scenarios to elucidate potential mechanisms and effect thresholds in a 7-day assay. Endpoints included germination potential and final germination rate, root traits (radicle elongation inhibition rate and root-to-shoot ratio), seedling biomass (fresh/dry mass), and water content. Responses generally followed a dose-dependent hormetic pattern, with low-dose PE increasing final germination rate, while higher exposures impaired root development and altered biomass allocation. Notably, the high-dose MIX treatment reduced the root-to-shoot ratio by ~37% relative to the control, suggesting disrupted allocation and/or disproportionate root impairment under intense MP stress. Water relations were polymer-specific: PE reduced seedling water content across all tested levels, consistent with physical blockage and/or impaired root water transport, whereas PLA and MIX caused significant declines mainly at higher concentrations. Overall, polymer identity and concentration jointly regulated early establishment, supporting the "low-dose stimulation, high-dose inhibition" framework and underscoring the need to consider both biodegradable and conventional MPs in ecological risk assessment.

Keywords: biodegradable microplastics, non-biodegradable microplastics, seed germination, seedling growth, *Trifolium repens* L.

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Introduction

Microplastics (MPs) – plastic fragments or fibers <5 mm – are regarded as one of the most serious emerging pollutants in the 21st century, having widely infiltrated the atmosphere, water bodies, and soil environment [1-3]. Sources of MPs include the breakdown of traditional non-biodegradable plastics (such as PE) and the residual fragments of biodegradable plastics (such as PLA) [4]. The global surge in research on agricultural waste-based bioplastics (e.g., PLA) has further raised concerns about the environmental fate of biodegradable microplastic residues [5]. Due to their extremely small size, microplastics can enter soil via atmospheric deposition, surface runoff, and other pathways, accumulating in the rhizosphere and even within plant tissues [6-8]. Microplastics affect plant growth through both physical blockage (e.g., interfering with water and nutrient uptake, damaging root tips) and chemical toxicity (e.g., adsorbing other pollutants, causing changes in plant resource allocation) [9-12]. The phytotoxic effects of microplastics are closely related to their material composition: non-degradable PE, due to its high stability, can persist in the environment and mainly inhibits root elongation through mechanical action [13, 14], whereas degradable PLA releases acidic monomers and nanofragments as it degrades [15, 16], which may impose osmotic stress on plants (the specific toxic mechanisms of PLA degradation products remain unclear [17-19]). Notably, because biodegradable microplastics degrade faster than traditional plastics, their residence time in the environment is shorter, but their continuous input may lead to more persistent and extensive pollution effects [20, 21].

Recent studies have documented the growth-inhibiting effects of microplastics on various plants, including legumes [21-23], wheat, and other crops as well as chenopod species, often showing a dose-dependent pattern: low concentrations of microplastics might activate plant defense systems, while high concentrations cause irreversible growth damage [24, 25]. A scientometric analysis of global agricultural waste-based bioplastic research has also confirmed the rapid development of biodegradable plastic (PLA) research in recent years [5]. Yet research on the stress responses of ornamental herbaceous plants, especially regarding combined pollution by degradable and non-degradable microplastics, remains insufficient [26, 27]. The complexity of plant-microplastic interactions is not yet fully elucidated. From an environmental ecotoxicology perspective, clarifying the toxicological pathways and combined pollution effects of microplastics on ornamental lawn plants is important for improving ecological risk assessment and understanding the hazards of microplastic pollution; it also provides a basis for developing urban ecological restoration strategies [28, 29].

White clover (*Trifolium repens* L.) is a perennial legume [21, 23] with significant ecological and landscape value [30, 31]. It is a key component of cool-season turf and is cultivated in over 80 countries worldwide [25, 26]. This widespread cultivation makes it likely to encounter microplastic-contaminated environments, yet research on how white clover responds to emerging pollutants like microplastics is extremely limited. An in-depth study of white clover's response mechanisms to microplastic stress can not only improve the theoretical framework of plant-microplastic interactions but also provide practical guidance for vegetation restoration in contaminated areas. Previous research has noted that, within certain thresholds, microplastics may exhibit a "low-dose promotion and high-dose inhibition" effect on seed germination, known as hormesis. For instance, low concentrations (0.01%) of PE combined with cadmium stress were reported to enhance peroxidase activity in *duckweed (Lemna minor*) [32]. Similarly, De Silva et al. [21] found in a 7-day study that microplastic accumulation in *Lemna* was positively correlated with exposure concentration: PE particles adhering to seed surfaces blocked stomata and impeded water and nutrient uptake, and as concentration increased, seed germination activity, root length, seedling height, and biomass all declined. Another study reported that low concentrations of PLA microplastics could alter rhizosphere ion balance and increase soil organic carbon, thereby modestly promoting plant growth [33]. Overall, however, there are still many gaps regarding the combined pollution effects of typical biodegradable (PLA) and non-biodegradable (PE) microplastics in the environment [27]. In particular, it remains unclear how these microplastics interact to affect the early growth stages of white clover (e.g., seed germination and seedling root configuration). This knowledge gap limits our ability to use plants like white clover as bioindicators for assessing the ecological risks of microplastic pollution in urban soils.

Based on the above, we hypothesized that both PE and PLA microplastics can exert toxic effects on white clover and that the degree of harm depends on microplastic type and concentration. To test this hypothesis, we exposed white clover seeds to PE and PLA microplastics of the same particle size (25 μm) at multiple concentrations (0.1% (w/v), 0.5% (w/v), 1.0% (w/v)), including a combined treatment (MIX) prepared as a constant-total-dose 1:1 (w/w) mixture of PE and PLA. To identify toxic effects and dose–response relationships in the 7-day assay, we used 0.1-1.0% (w/v) microplastics to represent worst-case exposure. This helps determine the upper threshold of phytotoxicity and supports future soil studies using environmentally realistic concentrations. Notably, while high for bulk soil, these concentrations may occur locally in hotspots such as degradable mulch areas. By measuring seed germination parameters (germination potential and germination rate), root phenotypic indices (root-to-shoot ratio and radicle elongation inhibition rate), seedling biomass (fresh and dry weight) [29], and water content [34, 35], we aimed to: (1) reveal the hormesis effect of "low-dose stimulation and high-dose inhibition" caused by different microplastic types and concentrations on the germination vigor of white clover seeds; (2) clarify how microplastic stress interferes with plant resource allocation (e.g., shifts in biomass allocation toward roots) and the resulting ecological adaptation strategies in white clover; and (3) provide a scientific basis for evaluating the stress tolerance of ornamental lawn species and guidance for vegetation restoration and management in microplastic-contaminated areas. By examining a range of physiological responses under these treatments, this study seeks to elucidate plant-microplastic interaction mechanisms, contribute to the assessment of plant germplasm diversity under pollutant stress, and suggest adaptive measures to mitigate microplastic damage. Overall, our findings offer a theoretical foundation for evaluating terrestrial plants' stress responses and adaptive evolution in the face of new pollutants such as microplastics [16, 28, 36, 37].

Materials and Methods

Experimental Material

Plant material: White clover (*Trifolium repens* cv. "Haifa") was selected as the experimental plant due to its widespread use in lawns and potential exposure to soil pollutants. Seeds were purchased in September 2023 from Bai Lv Forage Seed Company (China) and stored at 4ºC for one year to stabilize viability. Before the experiment, seeds with a full, smooth appearance and no signs of disease or pests were selected.

Microplastic materials: The microplastic particles used were polyethylene (PE) and polylactic acid (PLA), both with a diameter of 25 μm, provided by Zoomlion Plasticizing Company. Microplastic treatment suspensions of different concentrations were prepared by adding a measured mass of particles to ultrapure water and thoroughly mixing on a vortex oscillator. As shown in Table 1, we established a control (0% microplastic) and a series of treatments with 0.1%, 0.5%, and 1.0% (w/v) for PE and PLA. The MIX treatment was prepared as a constant-total-dose 1:1 (w/w) mixture of PE and PLA (e.g., 0.1% (w/v) MIX = 0.05% (w/v) PE + 0.05% (w/v) PLA, and analogously for the other levels). Each suspension was prepared fresh, ensuring the microplastics were well dispersed in the water before use.

Seed Germination Experiment

The germination test was carried out in accordance with the Chinese National Standard "Seed Testing Protocol – Germination Test (GB/T 3543.4-1995)" [38]. Before sowing, the surface of the white clover seeds was sterilized by soaking in 10% sodium hypochlorite (bleach) solution for 5 min, followed by thorough rinsing with deionized water (5 times) and air-drying on clean filter paper. After disinfection and drying, 50 healthy seeds were placed evenly in each glass Petri dish (9 cm diameter) per treatment [32]. Each dish was lined with two layers of filter paper and moistened with an appropriate amount of deionized water prior to seeding.

Experimental Design

Microplastic treatments were applied at the time of sowing. Immediately after the seeds were placed in the Petri dishes, 5 mL of the corresponding microplastic suspension (at the designated concentration for that treatment) was added to each dish, ensuring the seeds and filter paper were fully in contact with the treatment solution. A control group (no microplastics added) and 9 microplastic treatment groups were set up: PE at 0.1% (w/v), 0.5% (w/v), 1% (w/v); PLA at 0.1% (w/v), 0.5% (w/v), 1.0% (w/v); and MIX (PE+PLA, constant total concentration at 0.1% (w/v), 0.5% (w/v), 1.0% (w/v), prepared as 1:1 w/w, i.e., 0.05+0.05, 0.25+0.25, and 0.5+0.5 % (w/v) for PE+PLA, respectively). Each treatment was replicated in 4 Petri dishes (50 seeds per dish). All dishes were placed in an incubator at a constant 25ºC in darkness to germinate. The germination period was 7 days, during which no light was provided to simulate soil-covered seed conditions.

Germination Observation and Index Calculation

The germination process was monitored for 7 days, covering the key early developmental stages of white clover seeds: emergence of the radicle (usually ~24-48 h after sowing), cotyledon unfolding (~3 days), and primary true leaf development (~7 days) [39]. Every 24 h, the number of newly germinated seeds in each dish was recorded. Throughout the experiment, the moisture of the filter paper substrate was monitored by weighing

Table 1. Microplastic treatment concentrations (% (w/v)).

Treatment	PE (% w/v)	PLA (% w/v)	MIX (PE+PLA, % w/v, total constant)
Control	0	0	0 + 0
Low	0.1	0.1	0.05 + 0.05
Medium	0.5	0.5	0.25 + 0.25
High	1	1	0.5 + 0.5

the dishes; any water lost to evaporation was replenished promptly to maintain adequate moisture in all treatments [33, 34]. A seed was considered germinated when its radicle reached approximately 2 mm in length [35]. When no new seeds had germinated for 3 consecutive days, germination was deemed essentially complete, and the final germination count was recorded. The 7-day incubation thus encompassed the period from radicle emergence to the development of the first true leaves in the seedlings.

Using the daily germination data, we calculated standard germination indices. Germination potential was defined as the percentage of seeds that germinated within a set early period (3 days in this experiment), reflecting the initial vigor and uniformity of germination. Germination rate (final germination percentage) was the proportion of seeds that had germinated by the end of the 7 days, indicating the overall germination capacity. In addition, for each treatment, we computed the relative germination potential and relative germination rate, expressed as the ratio of that treatment's value to the corresponding value in the control (control = 1.00).

At the end of the germination trial (Day 7), 8 germinated seedlings were randomly selected from each Petri dish (for a total of 32 seedlings per treatment) for morphology measurements. The primary root (radicle) length and shoot (embryonic axis) length of each seedling were measured using a ruler or digital caliper. From these measurements, we derived 2 key indices: the Elongation Inhibition Rate (EIR) and the Root-to-Shoot ratio (R/S). EIR quantifies the degree to which a treatment inhibits seedling elongation, and was calculated separately for roots and shoots as the percentage reduction in length compared to the control (i.e., how much shorter the radicle or shoot was under stress relative to the control). The root-to-shoot ratio was calculated as the length of the root (radicle) divided by the length of the shoot (hypocotyl and cotyledonary node), reflecting the plant's biomass allocation strategy in early growth. Together, EIR and R/S offer insight into the stress response at both the organ level and whole-plant level.

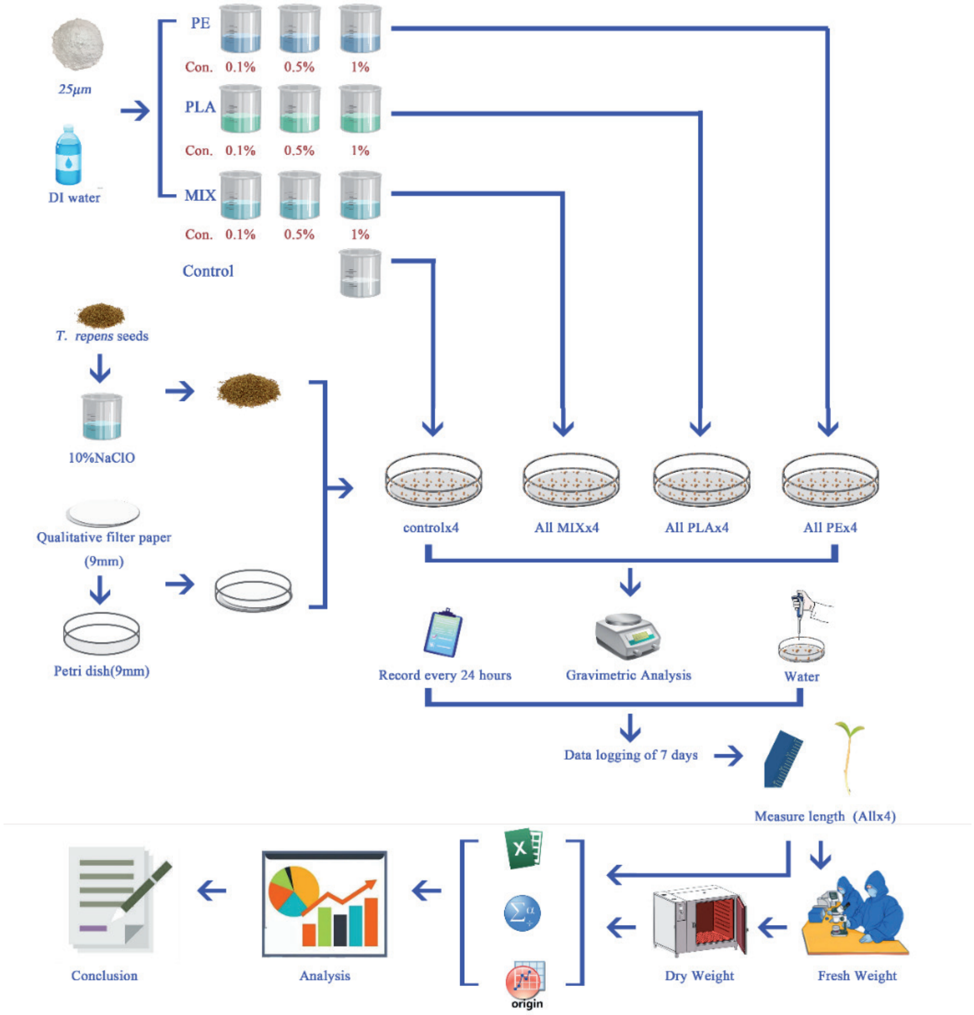

Fig. 1. Experimental workflow of the study.

Experimental Procedure

The overall experimental workflow is illustrated in Fig. 1. In summary: seeds were disinfected and sown in Petri dishes → microplastic suspensions of different concentrations were added → dishes were incubated at 25°C in the dark for 7 days → germination was observed daily, and water was replenished as needed → on Day 7, germination indices were calculated and seedlings were collected for morphological and biomass measurements → data were analyzed for treatment effects.

Data Analysis

After the experiment, all data were collated using Microsoft Excel 2016. Statistical analyses were performed with SPSS 27. Since the primary objective of this study was to compare each microplastic treatment (different types and concentrations) against the control and among themselves, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for multiple comparisons among all 10 treatment groups (Control + 9 treatments). This method is advantageous for direct pairwise comparisons, enabling clear interpretation of dose-response relationships and treatment-specific effects, which directly supports the testing of our hypothesis. Although two-way ANOVA could reveal interactions between microplastic type and concentration, the present study focused on individual treatment effects; thus, one-way ANOVA was more consistent with our experimental design and research objectives. The significance level was set at $P<0.05$. All results are reported as mean ± standard error (SE). Graphs were plotted using Origin 2024 (OriginLab Corp.) software.

Results

Effects of Microplastics on Seed Germination

Germination potential is an important indicator of initial seed vigor and the rate of germination. Fig. 2 shows that white clover seed germination potential responded to microplastic treatments (PE, PLA, MIX) in a pattern of first increasing and then decreasing with concentrations. PE treatments tended to slightly enhance germination potential at low concentrations compared with the control, followed by a decline at higher concentrations. In the PE treatment, germination potential at 0.1-1.0% (w/v) did not differ significantly from the control ($P\geq0.05$). In the PLA treatments, germination potential at 0.1% (w/v), 0.5% (w/v), 1.0% (w/v) was higher than the control ($P<0.05$), with no significant differences among PLA concentrations ($P\geq0.05$). For the MIX treatments, germination potential showed significant variation only at the medium concentration: 0.5% (w/v) was significantly higher than both the control and the 1.0% concentration ($P<0.05$), while no significant differences were observed between 0.1% and the control, or between 0.1% and other MIX concentrations ($P\geq0.05$).

Germination rate is another key indicator for evaluating seed germination under stress conditions [40]. The effects of the microplastic treatments on the final germination rate are presented in Fig. 3.

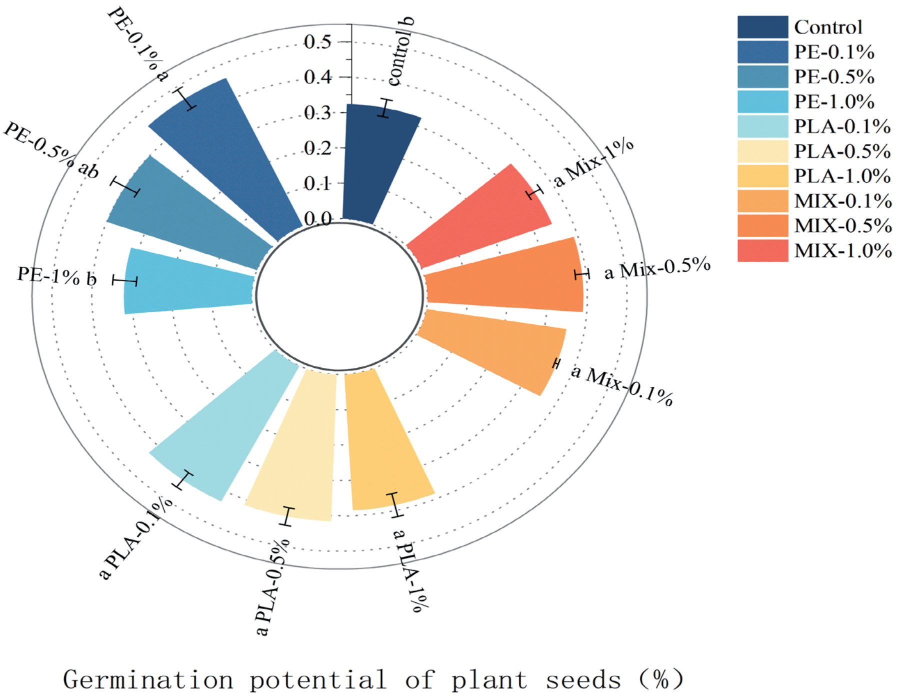

Fig. 2. Germination potential of white clover seeds under different MP treatments.

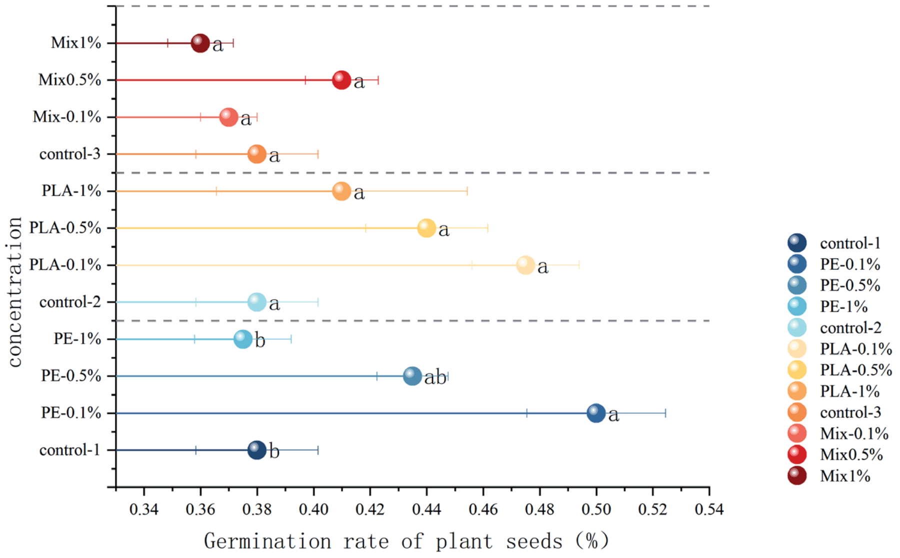

Fig. 3. Germination rate of white clover seeds under different MP treatments.

A similar hormetic trend was observed: germination rates increased at low microplastic levels and then declined at higher levels. In the PLA and MIX groups, the germination rates at 0.1% (w/v), 0.5% (w/v), and 1.0% (w/v) were not significantly different from each other ($P \geq 0.05$). Notably, the 0.1% (w/v) PE treatment yielded the highest germination rate and was significantly greater than the control ($P<0.05$). There was no significant difference among the three PLA treatment concentrations ($P \geq 0.05$), although the 0.1% (w/v) PLA treatment did result in a slightly higher germination rate than the control. For the MIX treatments, the germination rate was maximal at 0.5% (w/v) MIX, but this was not statistically different from the other MIX concentrations.

To more directly compare treatments with the control, relative germination indices were calculated (with the control = 1.00) and are summarized in Table 2. In the PE treatment group, none of the concentrations caused a significant change in relative germination *potential* compared to the control ($P \geq 0.05$). However, the relative germination *rate* was significantly increased under 0.1% (w/v) and 0.5% (w/v) PE ($P<0.05$ vs. control). In the PLA group, all concentrations (0.1%-1.0% (w/v)) showed relative germination potentials significantly higher than the control ($P<0.05$). For the MIX group,

Table 2. Effects of different types and concentrations of microplastics on relative germination potential and relative germination rate of *Trifolium repens* seeds (control = 1.00).

Microplastic treatment	Concentration (% w/v)	Relative germination potential	Relative germination rate
PE	0 (Control)	1.00±0.00a	1.00±0.00a
	0.1	1.46±0.18a	1.33±0.11 b
	0.5	1.26±0.12 a	1.07±0.07 b
	1	0.99±0.09 a	0.99±0.04 a
PLA	0 (Control)	1.00±0.00 b	1.00±0.00 b
	0.1	1.33±0.04 a	1.26±0.05 b
	0.5	1.28±0.05 a	1.17±0.07 ab
	1	1.18±0.03 a	1.08±0.07 ab
MIX (PE+PLA, % w/v, total constant)	0 (Control)	1.00±0.00 b	1.00±0.00 a
	0.1	1.11±0.07 ab	0.98±0.06 a
	0.5	1.21±0.04 a	1.06±0.04 a
	1	0.96±0.05 b	0.95±0.03 a

Different lowercase letters in the same column indicate significant differences at $P<0.05$.

only the 0.5% MIX treatment produced a relative germination potential significantly above the control value (P<0.05).

Effects of Microplastics on Seedling Root Systems

During seed germination and early seedling growth, the radicle (primary root) is critical for water and nutrient uptake, and root development can be very sensitive to environmental stress. Two indicators were used to evaluate root responses in our experiment: Elongation Inhibition Rate (EIR) and Root-to-Shoot ratio (R/S). EIR reflects the extent to which external stress (such as chemical toxins, salinity, or heavy metals) inhibits the elongation of the radicle (or the embryonic axis), and R/S (based on either biomass or length) reflects the plant's strategy of resource allocation between belowground (root) and aboveground (shoot) parts. Together, analyzing EIR and R/S provides insight into the plant's stress response both at the organ level and in overall growth strategy.

Our results show that microplastic exposure significantly affected root elongation and allocation. As seen in Fig. 4, the radicle elongation inhibition rate in each microplastic treatment group was significantly different from the control (P<0.05). Under low concentrations of microplastics, the EIR of the seedlings was slightly lower than (or similar to) that of the control. In contrast, high concentrations, especially 1.0% (w/v) PE and 1.0% (w/v) MIX (constant total concentration), led to a marked increase in EIR, indicating that high doses significantly inhibited radicle elongation. Correspondingly, there were notable effects on the root-to-shoot ratio of the seedlings. Under 0.5% (w/v) PLA treatment, the average R/S reached its highest value among treatments, whereas with 1.0% (w/v) MIX (prepared as 0.5% (w/v) + 0.5% (w/v) PE+PLA), the R/S dropped to the lowest value (approximately 37% lower than the control). Changes in the root-to-shoot ratio (R/S) can be interpreted in two complementary ways. The increase in R/S under moderate stress (e.g., 0.5% (w/v) PLA) could represent an adaptive allocation strategy, where the plant invests more resources into root development to enhance water and nutrient foraging under perceived stress [36]. However, the significant decrease in R/S observed under high-concentration MIX (1.0% (w/v)) suggests an alternative, less adaptive explanation. This decline may primarily reflect disproportionate damage to the root system, where root growth is more severely inhibited than shoot growth due to its direct and prolonged contact with the MPs [41, 42]. Under such extreme stress, the plant's capacity for adaptive resource allocation may be overwhelmed, leading to a breakdown in normal growth patterns and a visible toxic effect on the most exposed organ.

Visible differences in seedling morphology further illustrated these effects (Fig. 5). In the high-concentration microplastic treatment groups, white clover seedlings showed clear signs of growth inhibition compared to the control. The length of the radicle decreased significantly, the overall seedling height was reduced, and seedlings

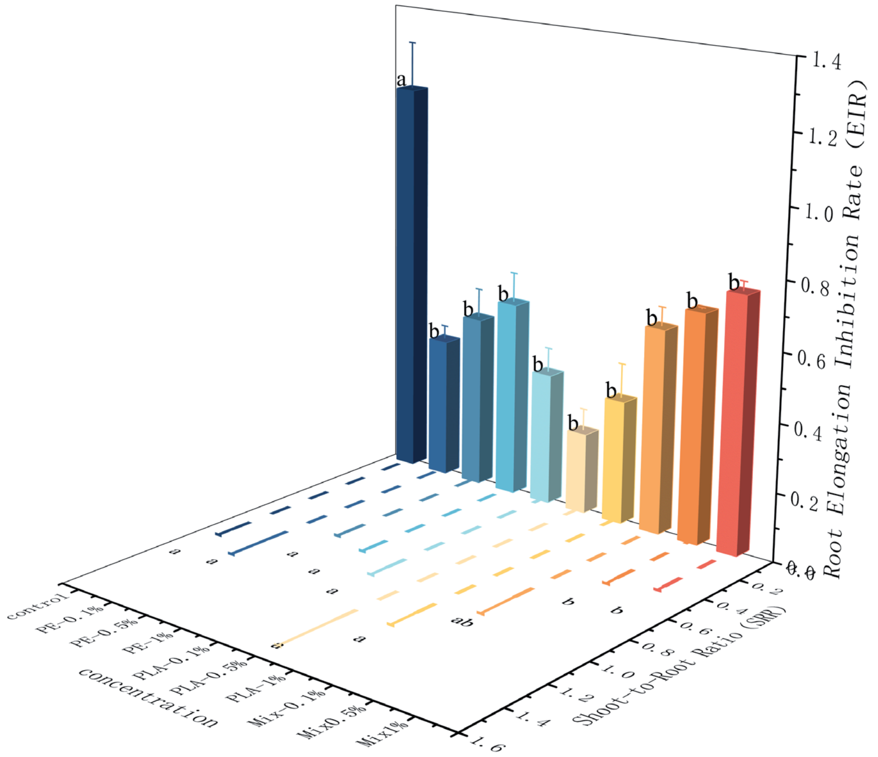

Fig. 4. Root elongation inhibition rate of white clover (T. repens) seedlings under different MP treatments.

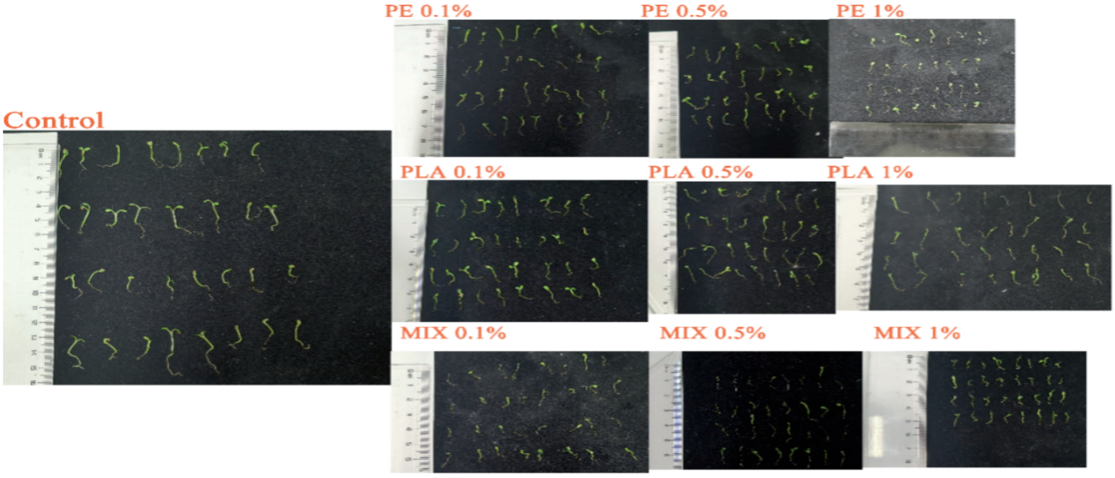

Fig. 5. Phenotypic comparison of T. repens seedlings under different treatments.

had fewer and smaller leaves. These phenotypic changes vividly reflect the inhibitory impact of high microplastic levels on both root development and above-ground growth.

We also compared the root-to-shoot ratios among all treatments in greater detail (Fig. 6). For most individual microplastic treatments (particularly at low and medium concentrations), the R/S did not differ significantly from the control ($P \geq 0.05$). However, an interesting trend emerged for the MIX treatments: although differences among the 0.1% (w/v), 0.5% (w/v), and 1.0% (w/v) MIX treatments were not significant; at the higher concentrations (0.5% (w/v) and 1% (w/v)), the MIX groups tended to have a much lower R/S than any of the pure PE or pure PLA treatments. In fact, at 0.5% (w/v) and 1.0% (w/v), the MIX R/S was significantly lower than the R/S of all other treatment groups ($P<0.05$, excluding control). This indicates that the combined presence of a degradable and non-degradable microplastic at high concentration has a more severe disruptive effect on the plant's biomass distribution between roots and shoots than either type of microplastic alone.

Effects of Microplastics on Seedling Biomass and Water Content

Seedling biomass (fresh and dry weight) is an important parameter for evaluating plant growth and stress impact, as it reflects overall growth accumulation. Fresh weight (FW) is particularly sensitive to acute stress – stresses like drought or salinity often cause immediate reductions in FW due to cell dehydration and growth arrest – whereas dry weight (DW) reductions usually manifest after a longer stress duration, indicating sustained impacts on photosynthesis, metabolism, or resource reallocation [43]. We measured both FW and DW of 7-day-old seedlings to assess microplastic stress severity.

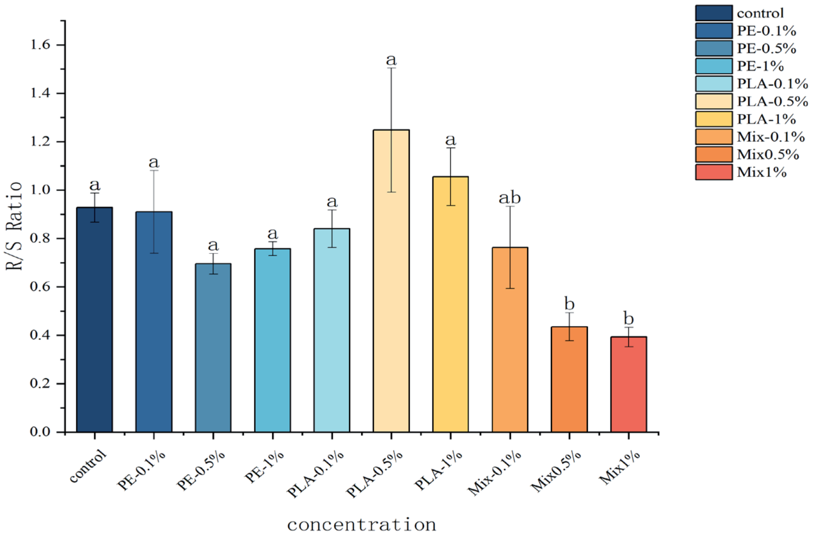

Fig. 6. Root-to-shoot ratio of white clover seedlings under different MP treatments.

The effects of microplastics on white clover seedling biomass are shown in Fig. 7. All microplastic treatments (PE, PLA, and MIX at low, medium, and high concentrations) caused a decrease in average seedling fresh weight compared to the control (0% (w/v)). In the PE treatments, seedling FW decreased by approximately 41.1%, 38.6%, and 46.2% at 0.1, 0.5, and 1.0% (w/v), respectively (all $P<0.05$ vs. control). The PLA treatments reduced FW by about 26.1%, 43.9%, and 46.9% at low, medium, and high concentrations ($P<0.05$). Similarly, the MIX treatments led to FW reductions of roughly 45.9%, 42.3%, and 48.9% at the three concentrations ($P<0.05$). There were also significant differences in fresh weight among the treatment groups ($P<0.05$), suggesting that microplastic type and dose both influence the magnitude of fresh weight loss. In contrast to the consistent FW reductions, seedling dry weight showed a clear threshold response. Only the highest concentration (1% (w/v)) treatments caused a significant decrease in dry weight compared to the control ($P<0.05$). At low and medium concentrations (0.1 and 0.5% (w/v)), the seedling dry weights under PE, PLA, and MIX were not significantly different from the control ($P\geq0.05$). This indicates that white clover seedlings were able to maintain dry matter accumulation under mild microplastic stress, and significant biomass loss in terms of dry weight occurred only once the microplastic exposure reached a high level (the toxicity threshold).

Water content (WC) in plant tissues is another important physiological indicator of stress. Water deficit conditions often cause plants to close stomata, reducing CO_2 assimilation and thereby inhibiting photosynthesis [40]; simultaneously, water stress can disrupt enzyme activities and metabolic processes, and in severe cases, lead to wilting or even plant death [44]. In this study, we measured the relative water content of white clover seedlings under each treatment (Fig. 8). The results demonstrate that PE microplastics had a direct and significant impact on plant water status: at all tested concentrations of PE, the seedling water content was significantly lower than in the control ($P<0.05$). For the PLA and MIX treatments, a significant reduction in water content was observed only beginning at the higher concentrations. In contrast, the low-concentration PLA (0.1% (w/v)) and the low/medium MIX (0.1% (w/v) and 0.5% (w/v)) did not significantly affect seedling water content ($P\geq0.05$). The consistent reduction in seedling water content under all PE treatments, even at low concentrations, is a striking result that points to a mechanism distinct from that of PLA. We propose two non-mutually exclusive pathways. First, a physical mechanism is highly plausible: hydrophobic PE particles may adhere to the seed coat and root surface [9, 21], forming a physical barrier that directly impedes water imbibition by the seed and water uptake by root hairs. This aligns with PE's inert nature and lack of significant chemical toxicity at low doses. Second, a physiological mechanism could involve PE-induced damage to root cells, impairing their hydraulic conductivity and water transport capacity. While our data cannot definitively separate these, the fact that water content was reduced even at 0.1% (w/v) PE, a concentration that did not inhibit root elongation, lends more support to the initial physical blockage hypothesis. High concentrations would then exacerbate both physical and physiological damage. These results suggest that PE microplastics

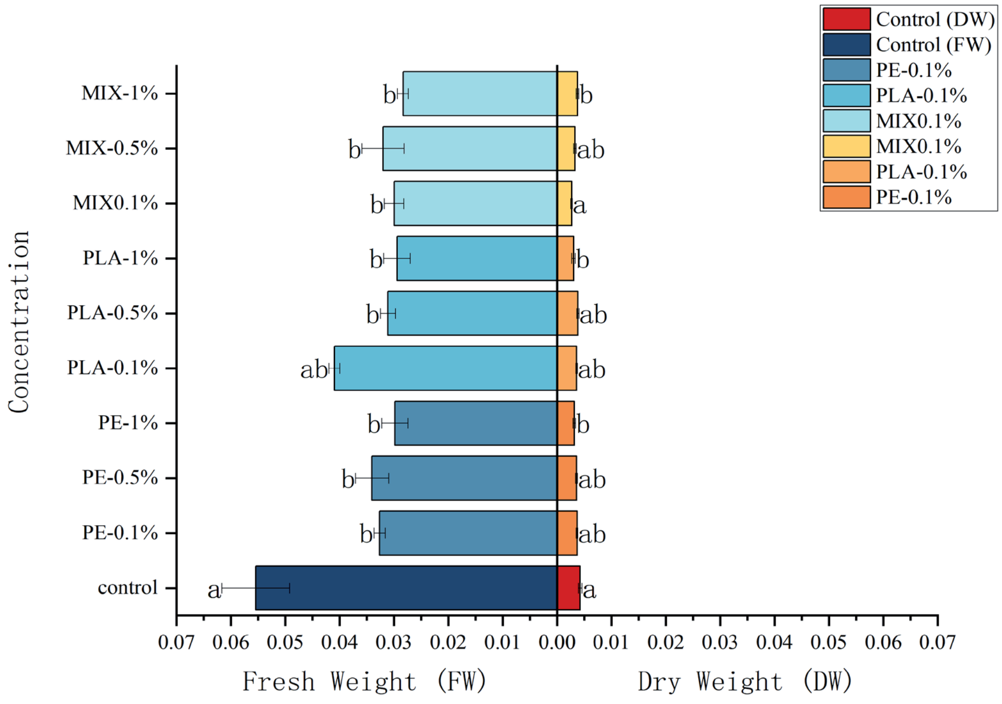

Fig. 7. Seedling fresh weight and dry weight of white clover (*T. repens*) under different MP treatments.

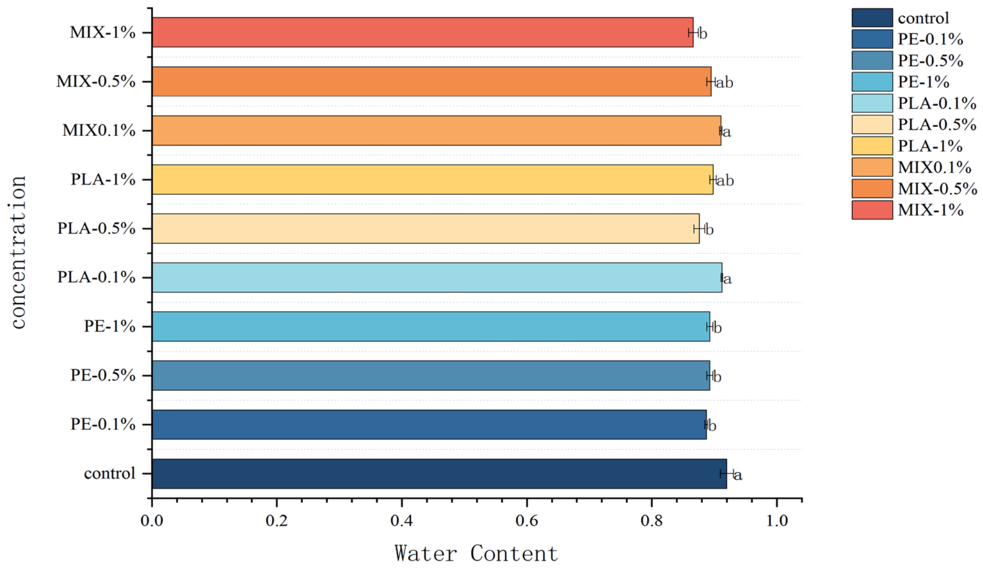

Fig. 8. Water content of white clover seedlings under different MP treatments.

impose water stress on plants even at low levels, whereas PLA (and combined PE+PLA) microplastics only interfere with water uptake or retention after their concentration (or perhaps their degradation by-products) exceed a certain threshold.

Discussion

Effects on Seed Germination

Seed germination is one of the most sensitive stages in a plant's life cycle to environmental stresses, and germination success directly affects population establishment and renewal. Our results demonstrated that microplastics can promote the germination vigor of white clover seeds at low concentrations but inhibit it at high concentrations, a response consistent with the classic hormesis effect. This "low-dose stimulation and high-dose inhibition" phenomenon under microplastic stress has also been reported for other plant-pollutant interactions. For example, Zeng et al. [45] observed that the impact of microplastics on Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) seed germination depended on microplastic type and dose, with certain microplastics stimulating germination at low doses but inhibiting it at high doses [46, 47]. In our study, exposure to 0.1% (w/v) PE significantly increased the germination rate of white clover, which aligns with findings in other species under PE stress. Raveendra et al. [48] reported that low doses of PE microplastics promoted the germination rate of black gram (*Vigna mungo*) and tomato (*Solanum lycopersicum*) seeds, whereas higher doses significantly inhibited germination. On the other hand, some studies have found contrasting results: De Silva et al. [21] observed that even a 0.1% (w/v) concentration of PE microplastics significantly inhibited the germination of pea (*Pisum sativum*) seeds. Similarly, Wang et al. [49] found that under 13 μm PE microplastic stress at 0.05% (w/v) and 0.1% (w/v), the germination index of soybean (*Glycine max*) seeds decreased significantly, while the germination index of mung bean (*Vigna radiata*) was relatively unaffected under the same conditions. These discrepancies suggest that different plant species vary in their sensitivity to microplastic stress, and whether microplastics have a stimulatory or inhibitory effect on seed germination depends on factors such as the plant species, microplastic type, particle size, and concentration [12, 45, 50, 51].

The observed low-dose stimulation, high-dose inhibition pattern aligns with the hormesis model [52]. It should be emphasized that the stimulatory effect induced by low concentrations is not a direct beneficial effect of microplastics, but rather a short-term compensatory or overcompensatory response of plants to mild stress. Mild stress caused by low levels of microplastics can activate the plant defense system, enhance antioxidant capacity and stress-related responses, and endow plants with temporarily improved growth performance. As the concentration increases, the stress exceeds the tolerance threshold of plants; the compensatory effect disappears and is replaced by significant inhibition, indicating a clear concentration threshold for this response [45].

Effects on Seedling Root Development

The root system is the primary organ for water and nutrient acquisition, and its growth and development are directly influenced by soil pollutants. Our results showed that among the treatments, 1% (w/v) PLA had the most severe impact on white clover seedling roots, significantly reducing both root length and root biomass. This finding is consistent with the report by Liang et al. [41], who found that various microplastic types (PE, PLA, PVC) all had toxic effects on the root development of adzuki bean (*Vigna angularis*) seedlings, with PLA causing the largest reduction in root biomass. In field soil experiments, Sridharan et al. [42] confirmed that introducing microplastics can mechanically damage seed and seedling roots through particle abrasion and adhesion, significantly inhibiting root growth; among the microplastics tested (PVC, polypropylene, and PLA), PVC caused the greatest reduction in root length, while PLA notably hindered root elongation. Pinto-Poblete et al. [53] similarly reported that exposure to microplastics (and to cadmium, as well as their combination) significantly reduced the root biomass of strawberry plants (*Fragaria × ananassa*, $P<0.05$). Additionally, Esterhuizen et al. [54] observed in a germination experiment with perennial ryegrass (*Lolium multiflorum*) that 7 days of microplastic exposure (using various aged polyethylene MPs) significantly shortened the seedling root length ($P<0.05$). In summary, different types, concentrations, and sizes of microplastics all tend to negatively affect plant root systems, primarily manifested as: (i) reduced root biomass; (ii) significantly shortened root length; and (iii) mechanical root damage and particle adhesion impeding root growth [41, 42]. This further indicates that microplastics have effectively become a new type of soil abiotic stress factor capable of severely inhibiting normal root development.

To ensure consistent experimental conditions, only 25 μm microplastics were used in this study [55, 56]. Previous studies have confirmed that particle size is an important parameter regulating the toxic effects of microplastics. Smaller particles, especially nanoscale particles, have a larger specific surface area and are more likely to release additives and degradation monomers [7, 13, 46], as well as to affect plants by blocking seed pores or invading root tissues. In contrast, larger particles tend to exert more physical impacts, including root tip abrasion and physical barrier formation on the seed surface, which interfere with normal water and gas exchange [45]. Future studies should establish a gradient of particle sizes to systematically elucidate the physical and chemical mechanisms underlying the phytotoxicity of microplastics.

One of the important findings of this study is that the mixed treatment (MIX) exerted a more significant negative impact on seedling growth than exposure to either PE or PLA alone, especially with regard to the stronger inhibition of the root-to-shoot ratio at high concentrations, indicating a synergistic toxic effect between PE and PLA particles [19, 46, 49]. The specific mechanisms can be summarized as follows: First, physically, the two different types of particles can form a denser and heterogeneous barrier layer around the seeds and roots [9], which more strongly impedes the normal transport of water and nutrients compared with a single plastic layer. Second, chemically, hydrophobic PE particles can adsorb polar degradation products released from PLA, forming localized high-toxicity zones on the root surface and enhancing the overall toxicity [18]. Third, stress induced by PLA (e.g., osmotic and pH stress) weakens seedling vigor and increases their susceptibility to the physical blockage effects of PE. Such complex interactions highlight the necessity for further research on the environmental effects of microplastic combined pollution.

Effects on Seedling Biomass and Water Content

The results of this study indicate that both degradable and non-degradable microplastics can decrease the biomass accumulation and water content of seedlings once their concentration exceeds a certain level. Zang et al. [57] pointed out that the concentration of microplastics in soil determines the dose-effect relationship of 1 μm PVC and PE particles on the shoot and root biomass of wheat seedlings. Wang et al. [58] reported that both a biodegradable microplastic (PBAT) and a conventional non-biodegradable microplastic (PE) reduced the biomass of lettuce (*Lactuca sativa*) seedlings, with the magnitude of the effect being concentration-dependent [9]. Liang et al. [41] compared the effects of three microplastics (PE, PLA, PVC) on red kidney bean (*Phaseolus vulgaris*) and noted that all three types inhibited plant biomass accumulation, with PLA having the strongest inhibitory effect. In addition, Lu et al. [59] found that for two submerged aquatic plants, PLA microplastics at 5 $mg \cdot L^{-1}$ significantly decreased plant fresh weight. Furthermore, Pinto-Poblete et al. [53] observed that in strawberries subjected to combined microplastic and heavy metal pollution, plant biomass and water content both showed significant declines. These studies collectively highlight the complex mechanisms by which microplastics influence plant growth. The severity of microplastic-induced growth inhibition is closely tied to the material type of the microplastic and its environmental concentration [47, 51, 60]. Key plant indicators – such as germination vigor, biomass, and tissue water content – can all be altered under microplastic stress to varying degrees [61, 62]. Generally, when the intensity of external stress approaches the upper threshold of what plants can tolerate, plants may activate certain self-regulation mechanisms (e.g., osmotic adjustment, antioxidant production) to maintain cellular homeostasis and protect physiological functions [52, 63]. However, this regulatory capacity is limited; once microplastic stress exceeds the plant's tolerance threshold, it leads to irreversible damage to growth and development.

While our results suggest that PLA toxicity may be linked to its degradation products (e.g., acidic oligomers) [16, 17], we acknowledge that no direct chemical measurements of the exposure medium (e.g., pH changes, quantification of lactic acid or other breakdown compounds) were performed in this study.

This is a limitation of our work. The inferred role of degradation products is therefore based on the existing literature, and the observed stronger effects of PLA compared to PE at higher concentrations [18]. Future studies should combine plant bioassays with analytical chemistry to definitively link the presence and concentration of specific PLA degradation products to the observed phytotoxic effects.

It is important to consider that our findings are based on simplified Petri-dish assays, which lack the complexity of a natural soil matrix. In soil systems, the observed effects might be mitigated or altered by several factors [11, 51]. Soil particles could dilute the concentration of MPs in the immediate vicinity of the seed, and the soil's buffering capacity might neutralize acidic degradation products from PLA. Additionally, soil microorganisms could form biofilms on MPs' surfaces, potentially modifying their toxicity or leading to biodegradation [64]. Conversely, the soil environment might exacerbate effects by introducing combined stressors (e.g., drought, co-pollutants). Therefore, while our controlled experiment provides crucial mechanistic insights into the direct phytotoxicity of MPs, these results serve as a foundation for hypothesis-driven research in more ecologically relevant soil-based systems.

Our study focused on the critical but brief early seedling stage (7 days). An important question is whether these observed effects persist, intensify, or diminish during later developmental stages. It is plausible that some effects, such as the initial stimulation at low doses, might be transient and disappear as the plant matures. Conversely, early damage to the root system, as seen with high MPs concentrations, could have lasting consequences, impairing nutrient and water acquisition throughout the plant's life cycle and ultimately affecting biomass accumulation and reproduction [53, 54]. Long-term studies extending to the vegetative and reproductive stages are therefore essential to fully assess the ecological consequences of microplastic pollution on plant fitness.

While white clover is an ecologically important model species, it is crucial to exercise caution when extrapolating these findings to other plant taxa. Plant responses to microplastics are likely species-specific and depend on various traits [11, 42, 48]. For instance, species with large seeds may have more resources to buffer against early stress, while those with small seeds might be more vulnerable to physical blockage by MPs [23, 42, 48]. Root architecture and growth rate will also influence the degree of contact and damage. Therefore, our results provide a valuable benchmark for a leguminous herb, but comparative studies across diverse functional groups (e.g., grasses with fibrous roots, tap-rooted crops) are needed to build a more comprehensive and generalizable understanding of microplastic phytotoxicity.

Conclusions

This study examined the impact of polyethylene (PE), polylactic acid (PLA), and their constant-total-dose 1:1 mixture (MIX) on the germination and early growth of *Trifolium repens* under 0.1% (w/v), 0.5% (w/v), and 1.0% (w/v). Low concentrations (0.1% (w/v)) tended to promote seed germination, while higher concentrations (≥0.5% (w/v)) inhibited early growth, consistent with a "low-dose stimulation, high-dose inhibition" hormesis framework. This was observed in both PE and PLA treatments, with MIX showing similar trends. Microplastics, especially PLA at 0.5% (w/v), increased the root-to-shoot ratio, whereas 1.0% (w/v) reduced the ratio markedly, suggesting disrupted allocation and/or disproportionate root impairment. PE microplastics reduced seedling water content across all concentrations, indicating a direct disruption in water uptake, likely through physical blockage. PLA and MIX treatments caused significant water loss only at higher concentrations, highlighting material-specific impacts on plant water status. In conclusion, both biodegradable and non-biodegradable microplastics affect seedling growth, with low concentrations stimulating and high concentrations inhibiting plant development. These findings underscore the importance of considering microplastic type and concentration in environmental risk assessments and highlight potential strategies for mitigating microplastic pollution in urban ecosystems. Future research should focus on soil-based systems, longer exposure durations, and a wider range of plant species to validate and extend these findings.

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Data Availability Statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding authors.

Conflict of Interest

The authors declare no conflict of interest.

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