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Species-dependent responses of crop plants to polystyrene microplastics☆

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ABSTRACT

Only recently there has been a strong focus on the impacts of microplastics on terrestrial crop plants. This study aims to examine and compare the effects of microplastics on two monocotyledonous (barley, *Hordeum vulgare* and wheat, *Triticum aestivum*), and two dicotyledonous (carrot, *Daucus carota* and lettuce, *Lactuca sativa*) plant species through two complimentary experiments. First, we investigated the effects of low, medium, and high (10^3, 10^5, 10^7 particles per mL) concentrations of 500 nm polystyrene microplastics (PS-MPs) on seed germination and early development. We found species-dependent effects on the early development, with microplastics only significantly affecting lettuce and carrot. When acutely exposed during germination, PS-MPs significantly delayed the germination of lettuce by 24%, as well as promoted the shoot growth of carrot by 71% and decreased its biomass by 26%. No effect was recorded on monocot species. Secondly, we performed a chronic (21 d) hydroponic experiment on lettuce and wheat. We observed that PS-MPs significantly reduced the shoot growth of lettuce by up to 35% and increased its biomass by up to 64%, while no record was reported on wheat. In addition, stress level indicators and defence mechanisms were significantly up-regulated in both lettuce and wheat seedlings. Overall, this study shows that PS-MPs affect plant development: impacts were recorded on both germination and growth for dicots, and responses identified by biochemical markers of stress were increased in both lettuce and wheat. This highlights species-dependent effects as the four crops were grown under identical conditions to allow direct comparison. For future research, our study emphasizes the need to focus on crop specific effects, while also working towards knowledge of plastic-induced impacts at environmentally relevant conditions.

1. Introduction

Microplastics (MPs; >100 nm and <5 mm in size) are persistent in aquatic and terrestrial ecosystems due to the ever-increasing application of plastic in all sectors of society and industry (Wang et al., 2020). While the focus of research to date has been on the quantification and the effects of MPs on aquatic ecosystems, terrestrial ecosystems, especially agricultural soils, have been recognized as a major long-term sink of MPs (Kumar et al., 2020; FAO, 2021). MPs concentration in agricultural soils is already high, e.g., 1.4–4.4 mg/kg (Chile, Corradini et al., 2019), 3.4 mg/kg (Sweden, Ljung et al., 2018) and 0.5 mg/kg (North-western China, Zhang et al., 2018). MPs can enter the soil from various sources, including via landfills, agricultural mulching films, sewage irrigation, the application of biosolids and compost, polymer-based fertilizers and pesticides, and atmospheric deposition (Chae and An, 2018; Guo et al., 2020; Kumar et al., 2020). MPs can have consequences on the soil function, but also on organisms, including invertebrates and plants, living in the terrestrial environment.

Numerous MPs-induced impacts to soil ecosystems have been recorded (Chae and An, 2018, de Souza Machado et al., 2019; Guo et al., 2020; Wang et al., 2020). This may have direct effects on plant health and performance by altering plant root traits, nutrient uptake process

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and thus growth (Wang et al., 2020). Research focusing on the effects of MPs on plants is of great importance since they are at the bottom of the food chain. Moreover, MPs might have impact on the food availability, as crops are an important food source for humans and animals. Previous research demonstrated that nano- and microplastics can be taken up by plant roots (Li et al., 2020) and leaves (Lian et al., 2021; Sun et al., 2021). Studies to date have investigated such impacts, with varying results (Zantis et al., 2023). For example, for corn (*Zea mays* L.), Zhang et al. (2018) detected an increase in root biomass, while Fu et al. (2022) observed a reduction in root biomass. From the overview study by Ng et al. (2018) it became clear that in a range of edible crops different response types can occur being positive, neutral to negative effects. Some suggest that theses impacts may be species-dependent (Gong et al., 2021), size-dependent (Li et al., 2021), or plastic-type-dependent (Meng et al., 2021). One shortcoming highlighted in the review by Zantis et al. (2023) is the large difference in experimental design between studies, making comparison of effect size and even the direction of the impacts among species difficult.

Thus, this study aims to examine the effects of laboratory derived MPs on seed germination, plant growth and biochemical stress indicators on different plant species grown under identical conditions. To investigate this, two experiments were conducted on four commonly cultivated species: two monocotyledonous (barley, *Hordeum vulgare* and wheat, *Triticum aestivum*), and two dicotyledonous (carrot, *Daucus carota* and lettuce, *Lactuca sativa*). The endpoints used within the first, short-term experiment were on seed germination and early development, while the second experiment with an exposure duration of 21 days was used to record growth and biochemical stress indicators. Overall, this study adds knowledge on plastic-induced impacts on a variety of plants species that have a direct contact with the plastic polluted terrestrial compartment.

2. Materials and methods

2.1. Plant species and cultivation

Four common agricultural crop species were studied: two monocotyledonous species, barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) (sourced from Cruydt-Hoeck; Nijeberkoop, The Netherlands), and two dicotyledonous species, carrot (*Daucus carota* L., Summer Carrot Amsterdam) and lettuce (*Lactuca sativa* L., Zwart Duits) (sourced from Koeman Flowerbulbs; Hem, The Netherlands). Protocols for growing conditions were sourced from Lian et al. (2020) and Wu et al. (2020). A base of ¼ Hoagland solution (pH 6.0 ± 0.1; the composition of the Hoagland solution is described in Table S1) was used to provide the seeds and seedlings with nutrients, with different concentrations of MPs added for the treatment conditions. All experiments were performed in a climate room at 21 °C, a 16:8 light-dark cycle and 75% relative humidity.

2.2. Polymer characteristics

Polystyrene (PS) microspheres of 500 nm (Polyscience Polybead Microspheres, Amsterdam, The Netherlands) were used. Both experiments used low, medium, and high concentrations of MPs 10^3, 10^5, 10^7 particles per mL respectively. Stock suspensions of 10^7 particles per mL were prepared in ¼ Hoagland solution and stored at 4 °C until use. Working suspensions of 10^3 and 10^5 were freshly prepared in ¼ Hoagland solution by diluting the stock suspensions of 10^7 particles per mL after sonication for 10 min at 60 Hz.

2.3. Experiment 1 (acute term exposure): seed germination and early development

The study design in this first experiment was based on Bosker et al. (2019). Ten seeds were placed into a Petri dish (Thermo Scientific Sterilin) lined with five filters (Whatman Grade 201). To each Petri dish, 5 mL of treatment suspension were added at the beginning of the experiment. For the control, 5 mL of ¼ Hoagland solution was used. To achieve a critical effect size of 25% at a 95% confidence level, eight replicates were needed. These pre-power calculations were based on experimental results from Bosker et al. (2019) using G*Power (version 3.1.9.7.).

The seeds were checked for germination every 24 h. The experiment was considered complete when 80% of seeds had germinated (Lian et al., 2020). Seeds were assumed germinated when the radicle was at least 2 mm long (Naseer et al., 2001). Once the experiment ended, the root- and shoot length were recorded using a millimetre-scale ruler, and the fresh weight of each seedling was measured. The final endpoints assessed for this experiment were the percentage of seeds germinated [1], shoot length, root length and biomass of the seedlings. To allow for cross-species comparison, germination rate was evaluated by assessing the percentage of seeds germinated at two timepoints, namely halfway through germination (50% of control germinated) and at complete germination (80% of control germinated).

$$\text{Germination rate } (\%) = \frac{\text{number of seeds germinated}}{\text{total number of seeds}} \times 100 \quad [1]$$

2.4. Experiment 2 (long term exposure): plant growth and biochemical analyses

2.4.1. Growth parameters

The second experiment investigated the impacts of MPs on the growth of lettuce and wheat seedlings. We selected one monocot (wheat) and one dicot (lettuce) species because these species are the most common studied species in toxicity testing (Zantis et al., 2023). Seeds were pre-germinated for five days in Petri dishes (Thermo Scientific Sterilin) with filter paper (Whatman Grade 201) using ¼ Hoagland solution. Uniform seedlings were then transferred to 6 mL borosilicate glass tubes (Fisherbrand) filled with respective treatment solutions. The duration of the experiment was 21 days, with treatment solutions replenished every three days to provide enough nutrients to the plant (Lian et al., 2020). Each treatment was performed in eight replicates. At the end of the experiment, the root length, shoot length, seedling wet weight, number of lateral roots (NLR) and number of leaves (NL) were measured.

2.4.2. Biochemical analyses

Fresh shoots of lettuce and wheat were first ground to powder using liquid nitrogen for further analysis. For each treatment, four replicates were taken and analysed in triplicates. For lettuce, not enough material was available after 7 days to analyse all endpoints, this is why we focused on the analysis of chlorophyll content and the lipid peroxidation for day 7. Moreover, for wheat, no samples were analysed after 21 days.

2.4.2.1. Chlorophyll content. Chlorophyll *a* (chl a) and chlorophyll *b* (chl b) levels were assessed spectrophotometrically making use of an established procedure (Warren, 2008). A sample of 0.1 g was ground with 1 mL of 100% methanol, mixed for 1 min and centrifuged for 5 min at 10 000 g in the dark. The supernatant was then removed by pipetting, and the same procedure was repeated until altogether 2 mL of supernatant was collected. For each treatment, four replicates were taken. After running a full absorbance spectrum, the absorbance was measured at 663 nm and 645 nm using a microplate reader (BMG Labtech, ClarioStar) to determine chl a and chl b contents respectively. The following equations were applied to quantify chl a [2], chl b [3] and chl a+b [4].

$$\text{Chlorophyll a } (\text{mg / g FW}) = \frac{(12.7 * A_{663}) - (2.69 * A_{645}) * V}{1000 * W} \quad [2]$$

$$\text{Chlorophyll b } (\text{mg / g FW}) = \frac{(22.9 * A_{645}) - (4.86 * A_{663}) * V}{1000 * W} \quad [3]$$

$$\text{Chlorophyll a} + \text{b (mg / g FW)} = \frac{(8.02 * A_{663}) + (20.20 * A_{645}) * V}{1000 * W} \quad [4]$$

where A is the absorbance at the respective wavelength, V is the volume of extract (mL) and W is the weight of the fresh sample (g).

2.4.2.2. Lipid peroxidation. Lipid peroxidation was measured via lipid peroxidation marker, malondialdehyde (MDA) as described in Hodges et al. (1999). Briefly, 0.25 g of the plant material was homogenized in liquid nitrogen and mixed with 1 mL 0.1% trichloroacetic acid (TCA). After centrifugation, 500 μL aliquots of supernatant were mixed with 500 μL 20% TCA containing 0.5% thiobarbituric acid (TBA). The control sample excluded TBA. The mixtures were heated at 95 °C for 30 min and then immediately cooled in an ice bath. The absorbances were read in the microplate reader (BMG Labtech, ClarioStar) at 532 nm and at 600 nm to subtract non-specific absorption and at 440 nm to subtract sucrose. The results are presented as MDA equivalent (nmol g^{-1} FW).

2.4.2.3. Concentration of salicylic acid and salicylic acid glucosides. Free salicylic acid (SA) and SA-glucosides were extracted and quantified as described in Allasia et al. (2018). Here, 0.25 g of plant material was ground with liquid nitrogen and mixed with 1 mL 70% ethanol with 32 μL anisic acid (15.25 ng μL^{-1}). Following centrifugation pellet was re-extracted with 1 mL of 90% methanol. The supernatants were combined, and methanol and ethanol were evaporated in a vacuum concentrator (Speed Vac, 2–18 CDplus). Then, 65 μL 20% of TCA, and 650 μL of ethyl acetate: cyclohexane (1:1) were added. Following centrifugation, the upper phase was moved into a new tube and the water phase was re-extracted. Pooled upper phase was evaporated in a vacuum concentrator (Speed Vac) to dryness. The dry residue was dissolved in 100 μL 10% methanol with 0.1% trifluoroacetic acid (TFA). This fraction represented free SA. Water phase was mixed with 0.3 mL 12 M HCl and incubated at 80 °C for 1 h. Then, 18 μL of anisic acid (15.25 ng μL^{-1}) was added and samples were extracted twice with 0.9 mL of ethyl acetate: cyclohexane (1:1). Combined upper phases were evaporated to dryness in a vacuum concentrator and dry residue was dissolved with 100 μL 10% methanol with 0.1% TFA. This fraction represented SA-glucosides.

Measurements of free and hydrolyzed SA were conducted in HPLC (Arc HPLC Waters). 20 μL of sample was injected into the HPLC system with a C18 column (Phenomenex, 250 × 4.6 mm, 5 μm) eluted with a methanol gradient from 10% to 82% at flow rate 1 mL min^{-1} at temperature of 30 °C. Eluent contained 0.1% TFA to maintain the protonated form of carboxylic acids. Quantification of SA and hydrolyzed SA was conducted with fluorescence detector (excitation at 305 nm and emission at 407 nm). SA concentrations were expressed as μg SA g^{-1} FW.

2.4.2.4. Total phenolic content. Total phenolic content was measured as in Herald et al. (2012). Briefly, 0.3 g of plant material was ground with liquid nitrogen and mixed with 80% methanol. Mixtures were incubated for 1 h at room temperature. Following centrifugation, 25 μL of supernatant was mixed with 75 μL of water and 25 μL of Folin-Ciocalteu reagent. After 6 min, 100 μL 7.5% Na_2CO_3 was added, mixed and samples were incubated in the dark for 90 min. The absorbance at 765 nm was measured with a microplate reader (BMG Labtech, ClarioStar). As a standard gallic acid was used. Total phenolic content was expressed as μg gallic acid (GA) equivalent g^{-1} FW.

2.5. Statistical analysis

Statistical analysis was performed in R studio (Version, 2023.03.0 + 386). Results are presented as means ± standard error (SE). The results were screened for normality and homogeneity of variance, using Shapiro-Wilk test and Levene's test respectively. A one-way ANOVA at alpha level 0.05 difference in means test was performed to evaluate if results were statistically significant at a 95% confidence level. If the ANOVA was significant ($p < 0.05$), a Tukey's post hoc test was performed to determine significant effects between treatments. Adjusted p-values are presented in this study.

3. Results

3.1. Impact of microplastics on seed germination and early development

There were significant differences in germination and early development between plant species exposed to PS-MPs (Table 1). The germination rate was only significantly reduced for lettuce. PS-MPs significantly delayed the germination of lettuce at the highest concentration (ANOVA; $p = 0.008$). After 24 h of exposure, when approximately half of the control was germinated, the percentage of seeds germinated was 24% lower than the control when exposed to the high PS-MPs concentration (Table 1). Once full germination was reached, this effect disappeared and there was no significant difference in germination between treatments. Seed germination of carrot, wheat and barley were not affected ($p > 0.05$) by PS-MPs exposure.

Focusing on early development (Table 1), shoot length and wet weight were significantly impacted only for carrot. The shoot length of carrot was 71% longer than the control when exposed to the medium concentration ($p = 0.04$; Fig. 1A). The wet weight of carrot was 26% lighter than the control when exposed to the high concentration ($p = 0.04$; Fig. 1B). No significant effects ($p > 0.05$) of PS-MPs were observed on the early development of lettuce, wheat, and barley.

3.2. Impact of microplastics on plant growth

After 21-day exposure, PS-MPs impacted lettuce and wheat growth differently (Table 2). Shoot length and wet weight were significantly impacted only for lettuce. PS-MPs significantly promoted the shoot growth of lettuce at low concentration (ANOVA; $p = 0.01$; Table 2). The shoot length was 35% higher than the control when exposed to the low concentration (Fig. 2A). In contrast, PS-MPs significantly inhibited shoot elongation at medium ($p = 0.01$) and high concentrations ($p = 0.01$; Table 2). The shoot length of lettuce was 33% and 35% lower than the control when exposed to the medium and high concentrations respectively (Fig. 2A). No significant effect on shoot length of wheat was observed ($p > 0.05$; Fig. 2B). Furthermore, PS-MPs significantly increased the biomass of lettuce seedlings when exposed to the low ($p = 0.04$), medium ($p = 0.04$), and high concentrations ($p < 0.001$; Table 2). The wet weight was increased by 35% at the low, 36% at the medium, and 64% at the high concentration (Fig. 2C). No significant effect was observed on the biomass of wheat seedlings ($p > 0.05$; Fig. 2D). In addition, there were no effects on root length, number of lateral roots, or number of leaves for wheat ($p > 0.05$; Table 2). Although not statistically significant, we observed an increase in the root length ($p = 0.09$) and an increase in the number of lateral roots ($p = 0.29$) of lettuce seedlings. In fact, relative to the control the root length of lettuce was increased by 23% when exposed to the low concentration, 18% at the medium concentration and 20% at the high concentration of MPs (Table 2).

3.3. Impact of microplastics on biochemical markers

3.3.1. Chlorophyll content

PS-MPs induced a significant decrease in the overall (chl a & b) chlorophyll content of lettuce seedlings at day 7, but no significant differences were observed after 14 days and 21 days. After 7 days, all treatments showed a decrease in chlorophyll *a* content in lettuce. The low and medium concentrations significantly decreased the chlorophyll *a* level by 27% (ANOVA, $p = 0.03$) and 26% ($p = 0.04$; Fig. 3A) respectively. Similarly, a reduction in chlorophyll *b* content by approximately 25% was recorded in both low ($p = 0.03$) and medium ($p = 0.04$; Fig. 3C) concentrations. Overall, the total chlorophyll (chl a+b)

Table 1
The effects of low, medium, and high 500 nm PS-MPs concentrations (10^3, 10^5, 10^7 particles mL^{-1}) on the seed germination (%) at early germination (50% of control germinated) and at the end of germination (80% of control germinated), and on the early development, including root length, shoot length and wet weight of lettuce (*Lactuca sativa*), carrot (*Daucus carota*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) buds. All values are presented as mean ± SE. Different letters indicate significant differences (ANOVA & Tukey's post-hoc test, $p < 0.05$) between treatments. There are no halfway germination values for wheat as halfway and complete germination occurred on the same day (day 2).

Plant type	Plant species	Concentration (particles/mL)	Germination (%)		Root length (mm)	Shoot length (mm)	Wet weight (mg)
			Halfway germination (50%)	Complete germination (80%)			
Monocot	Barley	Control	58 ± 4.5	80 ± 1.9	17 ± 1.4	18 ± 3.0	131 ± 5.3
		10^3	60 ± 5.7	79 ± 3.0	20 ± 3.3	21 ± 4.7	133 ± 7.4
		10^5	65 ± 4.2	75 ± 3.8	18 ± 2.4	20 ± 3.7	126 ± 6.2
		10^7	64 ± 3.8	81 ± 2.3	23 ± 2.9	25 ± 4.9	141 ± 7.8
	Wheat	Control		81 ± 5.5	13 ± 1.3	6 ± 0.7	79 ± 2.7
		10^3		73 ± 7.7	10 ± 1.1	5 ± 0.7	76 ± 2.7
		10^5		81 ± 3.0	13 ± 1.4	6 ± 0.9	82 ± 2.5
		10^7		71 ± 6.4	9 ± 1.0	5 ± 0.4	74 ± 3.0
Dicot	Carrot	Control	60 ± 7.6	78 ± 4.9	2 ± 0.1	1 ± 0.2 **a**	4 ± 0.2 **a**
		10^3	73 ± 3.7	83 ± 3.1	2 ± 0.2	2 ± 0.5 **ab**	3 ± 0.2 **ab**
		10^5	73 ± 5.6	75 ± 5.4	2 ± 0.4	3 ± 0.8 **b**	4 ± 0.3 **a**
		10^7	53 ± 8.8	59 ± 9.3	2 ± 0.1	1 ± 0.2 **a**	3 ± 0.3 **b**
	Lettuce	Control	44 ± 5.3 **a**	88 ± 4.1	2 ± 0.1	4 ± 0.2	5 ± 0.2
		10^3	38 ± 5.9 **ab**	86 ± 4.6	2 ± 0.1	4 ± 0.2	5 ± 0.2
		10^5	40 ± 3.3 **a**	89 ± 3.5	2 ± 0.1	4 ± 0.2	4 ± 0.3
		10^7	20 ± 2.8 **b**	79 ± 4.4	2 ± 0.1	4 ± 0.2	5 ± 0.2

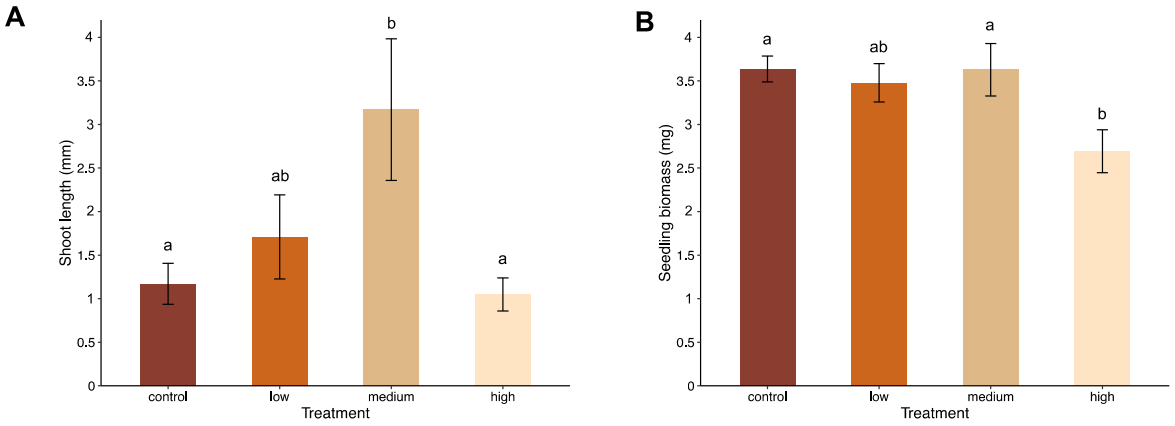

Fig. 1. The mean ± SE shoot length [A] and bud biomass [B] of carrot (*Daucus carota*) after exposure to low, medium and high 500 nm PS-MPs concentrations (10^3, 10^5, 10^7 particles mL^{-1}). Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. Error bars represent standard errors.

Table 2
The effects of low, medium and high 500 nm PS-MPs concentrations (10^3, 10^5, 10^7 particles mL^{-1}) on the root length, shoot length, seedling biomass, number of lateral roots and number of leaves of lettuce (*Lactuca sativa* L.) and wheat (*Triticum aestivum* L.) after 21 days. All values are presented as mean ± SE. Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments.

Plant species	Concentration (particles/mL)	Root length (mm)	Shoot length (mm)	Wet weight (mg)	Number of lateral roots (pcs)	Number of leaves (pcs)
Wheat	Control	113 ± 7.1	275 ± 5.4 ab	611 ± 33.9	8 ± 0.3	3 ± 0.2
	10^3	110 ± 5.9	271 ± 4.9 ab	619 ± 18.9	8 ± 0.6	4 ± 0.2
	10^5	117 ± 4.1	304 ± 16.8 a	605 ± 34.6	9 ± 0.7	3 ± 0.2
	10^7	105 ± 4.9	253 ± 6.0 b	574 ± 18.9	8 ± 0.4	4 ± 0.1
Lettuce	Control	100 ± 8.5	6 ± 0.7 a	324 ± 30.1 a	21 ± 3.3	8 ± 0.4
	10^3	123 ± 7.0	8 ± 0.3 b	438 ± 34.1 b	28 ± 4.1	7 ± 0.2
	10^5	119 ± 6.4	4 ± 0.3 c	439 ± 21.4 b	22 ± 4.6	8 ± 0.3
	10^7	121 ± 5.6	4 ± 0.3 c	531 ± 27.2 b	30 ± 3.0	8 ± 0.2

content in lettuce was also decreased by approximately 26% in both low ($p = 0.03$) and medium ($p = 0.04$) treatments (Fig. 3E).

On the other hand, total chlorophyl content in wheat seedlings showed more varied results over time, but also across chlorophyll *a* and *b*, showing a more complex response pattern. The chlorophyll *a* content in wheat seedlings after 7 days was decreased at the lowest concentration (ANOVA, $p = 0.03$; Fig. 3B). For both medium ($p = 0.7$) and high ($p = 0.2$) concentrations, an increase in chlorophyll *a* content was noted,

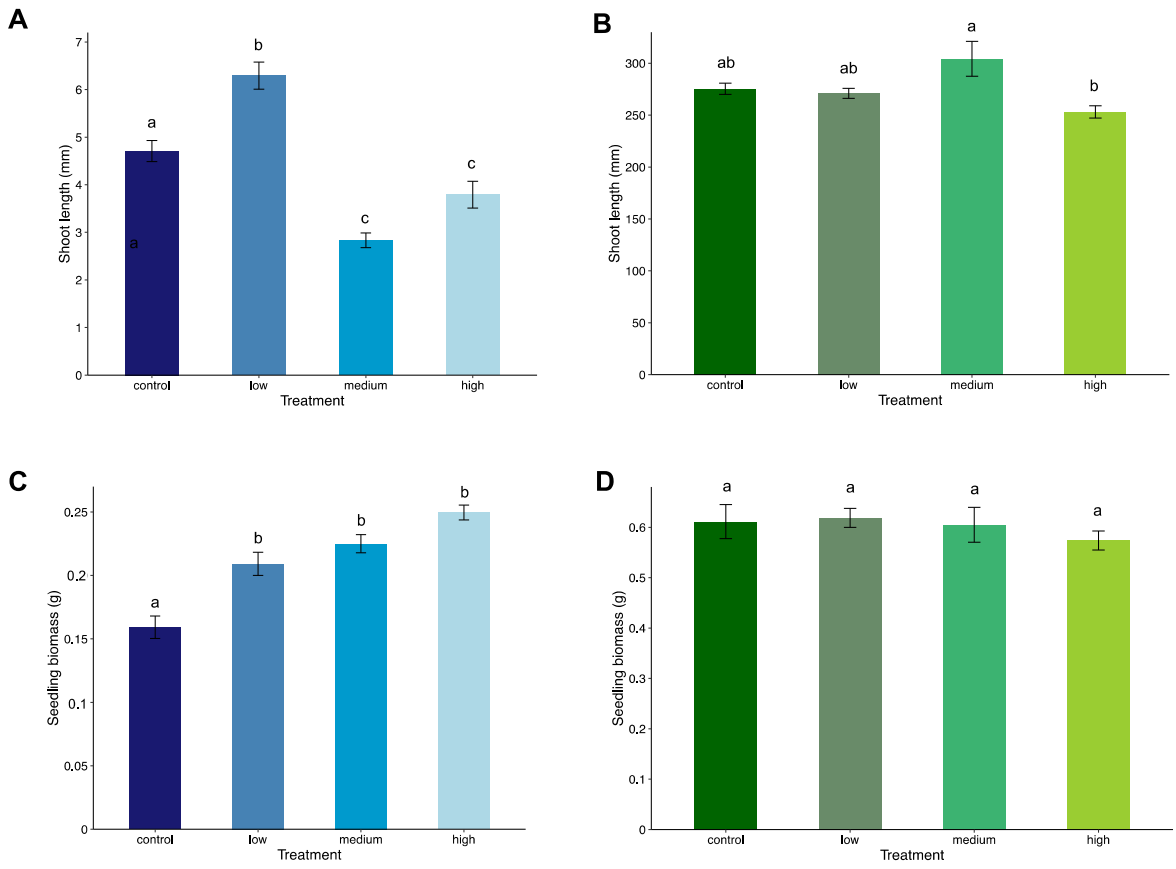

Fig. 2. The mean ± SE shoot length [A: lettuce, B: wheat] and seedling biomass [C: lettuce, D: wheat] of lettuce (Lactuca sativa) and wheat (*Triticum aestivum* L.) after 21 days of exposure to low, medium and high 500 nm PS-MPs concentrations (10^3, 10^5, 10^7 particles mL^{-1}). Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. Error bars represent standard errors.

but was not significant. After day 14, a decrease in chlorophyll *a* content in wheat seedlings was observed (Fig. 3B). However, this was only significant for the highest concentration ($p = 0.008$), decreasing the chlorophyll *a* content by 18% (Fig. 3B). Focusing on chlorophyll *b*, an increase after 7 days was detected in all treatments. Chlorophyll *b* content increased about 37% after addition of low and medium ($p = 0.02$) PS-MPs concentrations (Fig. 3D). After 14 days, a decrease in chlorophyll *b* content was observed in the low treatment ($p = 0.004$), while an increase was detected in the high treatment ($p = 0.008$; Fig. 3D). Overall, the total chlorophyll content in wheat seedlings was increased in the medium ($p = 0.02$) and high ($p = 0.01$) treatments after 7 days (Fig. 3F). However, the opposite effect was observed after 14 days, where the total chlorophyll content of wheat seedlings was reduced by about 15% at the lowest concentration ($p = 0.02$; Fig. 3F).

3.3.2. Lipid peroxidation

For both lettuce and wheat, we found an elevation in MDA levels indicating alterations in the lipid peroxidation process. On day 7, an increase in the MDA level in lettuce seedlings was detected of 101% for the medium (ANOVA, $p = 0.002$) and 69% for the high ($p = 0.03$) treatments compared to the control (Fig. 4A). After 14 days, all treatments significantly increased the level of MDA by 50% (low, $p < 0.001$), 27% (medium, $p = 0.04$) and 54% (high, $p < 0.001$). After 21 days, the medium PS-MPs concentration increased the MDA level by 76% ($p < 0.0001$; Fig. 4A). In wheat, a similar response was observed, with increased MDA levels compared to the control. After 7 days, an increase by 30%, 20% and 26% was recorded for the low ($p = 0.0004$), medium ($p = 0.01$) and high ($p = 0.001$; Fig. 4B) treatments respectively. An even more pronounced difference was found after 14 days, where the low, medium and high treatments increased MDA levels by about 74% ($p < 0.0001$), 63% ($p < 0.0001$) and 57% ($p < 0.0001$) respectively (Fig. 4B).

3.3.3. Salicylic acid (SA) and salicylic acid glucosides (HSA) level

PS-MPs increased the level of SA in both lettuce and wheat seedlings. For lettuce, the level of SA was two times higher in response to the high treatment (ANOVA, $p = 0.0002$) and three times higher in the low treatment ($p < 0.0001$) compared to the control after 14 days (Fig. 5A). The medium treatment even increased the SA level by 315% ($p < 0.0001$). The trend was maintained after 21 days where in response to the low and medium treatments, SA levels increased by about 164% ($p < 0.0001$) and 292% ($p < 0.0001$). For wheat, an increase in the level of SA was observed both after 7 and 14 days, with the medium concentration showing the strongest response (Fig. 5B). After 7 days, the level of SA was increased by about 62% for the low ($p = 0.004$), by 160% for the medium ($p < 0.0001$) and by 114% for the high treatment ($p < 0.0001$; Fig. 5B). After 14 days, a similar trend to the observations after 7 days of exposure is observed across all treatments ($p < 0.0001$; Fig. 5B).

The build-up of the hydrolyzed form of salicylic acid was induced by PS-MPs within all tested concentrations in both lettuce and wheat seedlings. After 14 days, the HSA level in lettuce seedlings was increased by about 29% at the medium treatment (ANOVA, $p = 0.0002$) and 24% at the high treatment ($p = 0.001$; Fig. 5C). The HSA level was increased for all treatments after 21 days by 18% for the low ($p = 0.008$), 31% for medium ($p = 0.0001$) and 17% for high ($p = 0.01$) treatments (Fig. 5C). In wheat, a gradual accumulation of HSA was detected across treatments after 7 days (Fig. 5D). The HSA level in wheat seedlings was increased by about 18%, 29% and 32% for the low ($p = 0.02$), medium ($p < 0.001$) and high ($p < 0.001$) concentrations (Fig. 5D). After 14 days, even though an increase is seen across all treatments, it was only significant for the highest treatment ($p = 0.01$; Fig. 5D).

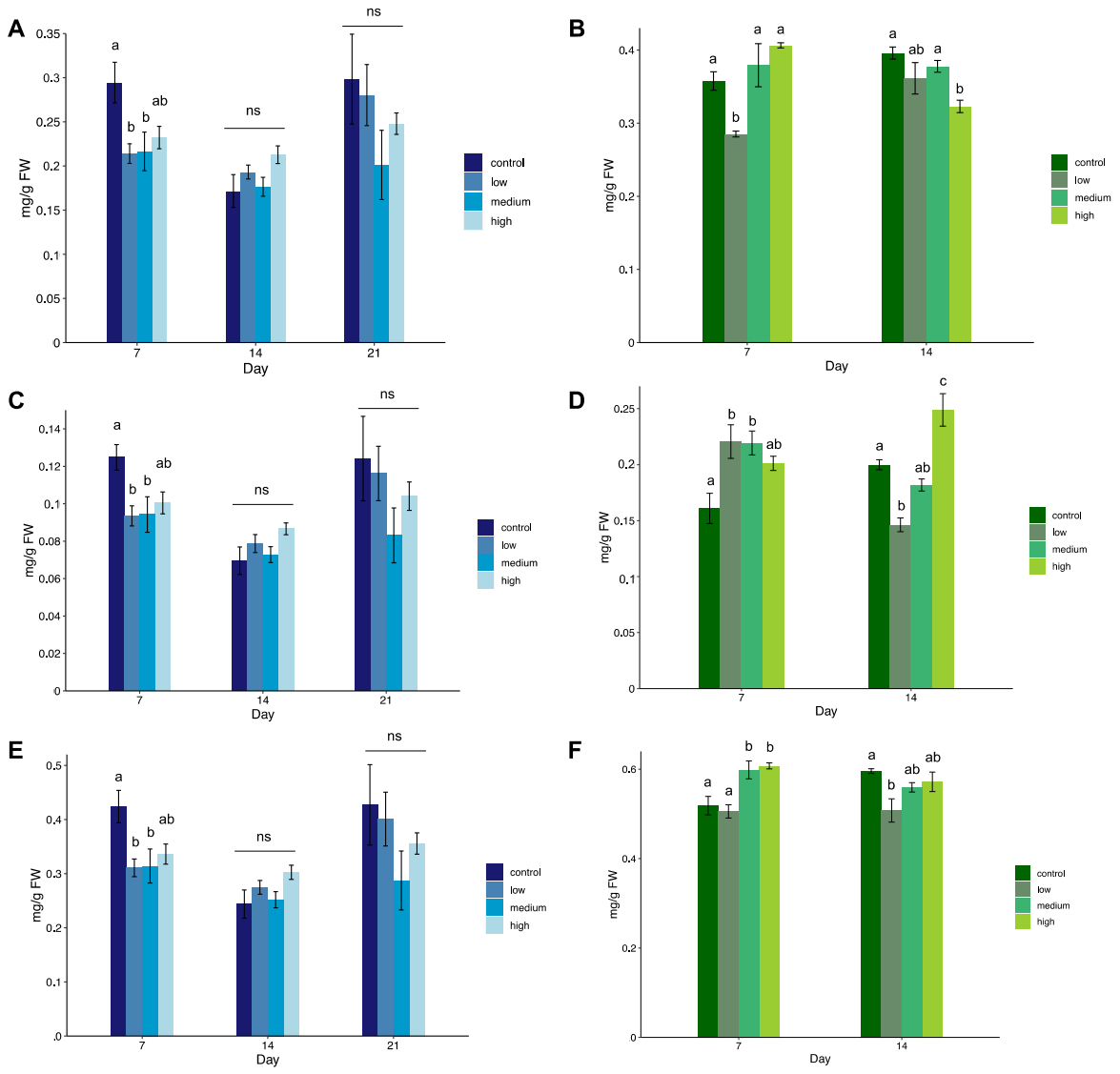

Fig. 3. The mean ± SE chlorophyll *a* [A: lettuce, B: wheat], chlorophyll *b* [C: lettuce, D: wheat] and total chlorophyll content [E: lettuce, F: wheat] of lettuce (*Lactuca sativa*) and wheat (*Triticum aestivum* L.) seedlings after exposure to low, medium and high 500 nm PS-MPs concentrations (10^3, 10^5, 10^7 particles mL^{-1}) over time. Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. Error bars represent standard errors.

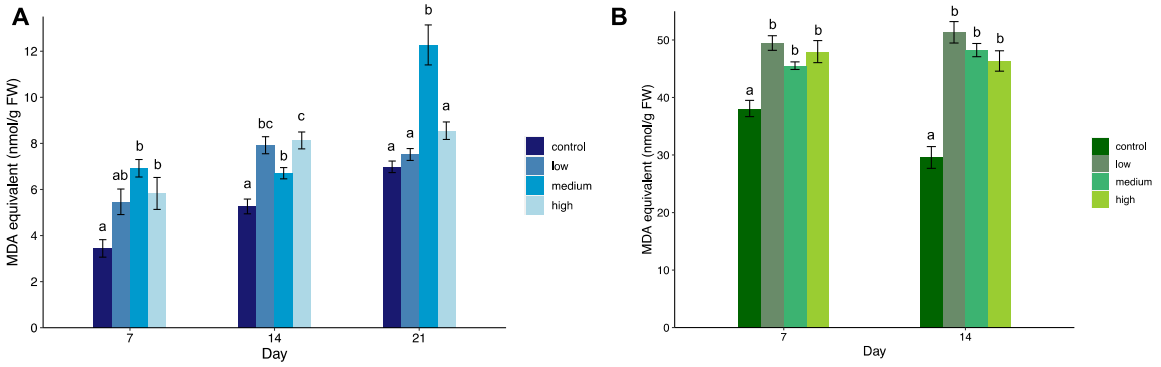

Fig. 4. The mean ± SE lipid peroxidation of [A] lettuce (*Lactuca sativa*) and [B] wheat (*Triticum aestivum* L.) seedlings after exposure to low, medium and high 500 nm PS-MPs concentrations (10^3, 10^5, 10^7 particles mL^{-1}) over time. Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. Error bars represent standard errors.

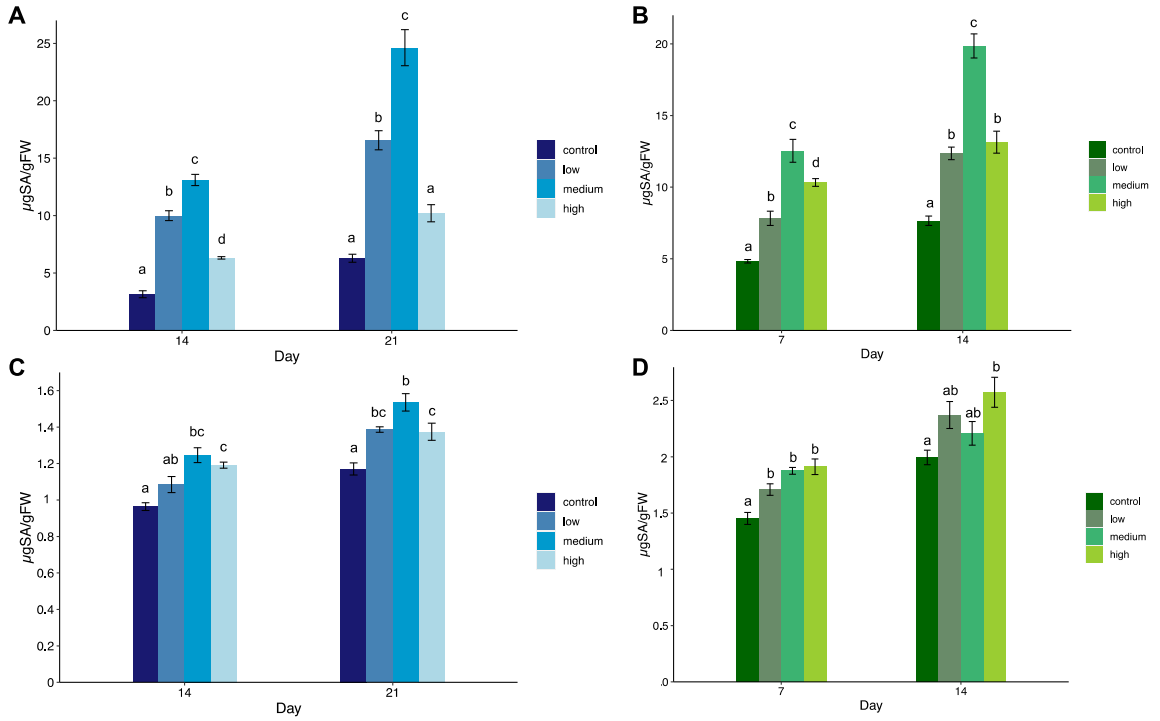

Fig. 5. The mean ± SE salicylic acid [A: lettuce, B: wheat] and hydrolyzed form of salicylic acid [C: lettuce, D: wheat] content of lettuce (*Lactuca sativa*) and wheat (*Triticum aestivum* L.) seedlings after exposure to low, medium and high 500 nm PS-MPs concentrations (10^3, 10^5, 10^7 particles mL^{-1}). Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. Error bars represent standard errors.

3.3.4. Total phenolic content (TPC)

A consistent increase in the total phenolic content was observed in lettuce seedlings. The TPC level was increased after 14 days by 109% for the medium (ANOVA, $p = 0.001$) and 387% for the high treatment ($p < 0.0001$; Fig. 6A). After 21 days, a similar trend as for 14 days is reported. The low, medium and high treatments increased the TPC level by 141% ($p < 0.0001$), 231% ($p < 0.0001$) and 142% ($p < 0.0001$) respectively (Fig. 6A). For wheat seedlings, the TPC levels varied between concentrations and over time (Fig. 6B). After 7 days, an increase in TPC level in wheat seedlings was observed for the low ($p < 0.0001$) and medium ($p = 0.004$) treatments. For the high concentration, a non-significant decrease was detected ($p = 0.2$; Fig. 6B). After 14 days, all three treatments showed an increase in TPC levels. The low treatment increased the TPC level by 25% ($p = 0.002$), medium by 54% ($p < 0.0001$) and high by 63% ($p < 0.0001$; Fig. 6B).

4. Discussion

This study examines the effects of PS-MPs on the plant performance of four crops species, allowing to compare *if* and to *what extent* effect size and the direction of the MPs-induced impacts among species differ. Significant impacts by PS-MPs on both germination and early development and plant growth were recorded for dicots, while no significant effects were observed on monocots. In contrast, responses in biochemical biomarkers were consistent between the dicotyledonous lettuce and the monocotyledonous wheat.

Seed germination and early development showed that both monocot species were not impacted by PS-MPs. For the dicots, PS-MPs significantly delayed the germination of lettuce at the highest concentration, but no effect was observed on carrot. From our meta-analysis (Zantis et al., 2023) we learned that in only 18% of studies a significant effect in the germination of monocots was observed, while it was 61% for dicots.

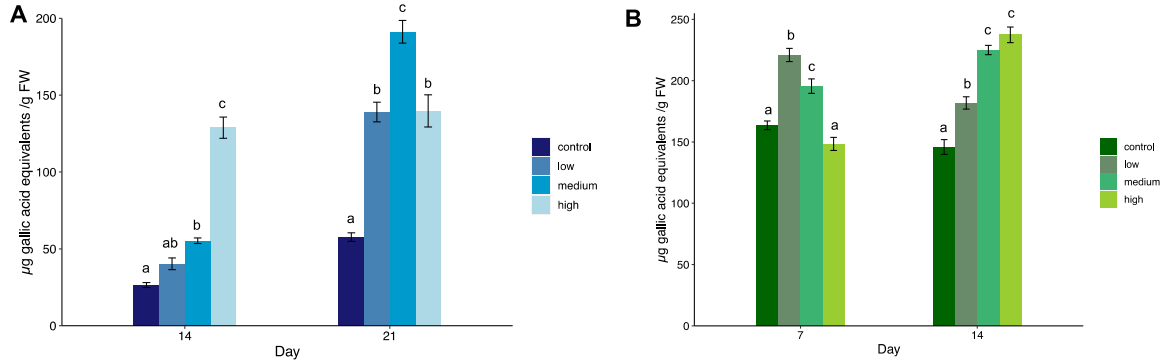

Fig. 6. The mean ± SE total phenolic content in [A] lettuce (*Lactuca sativa*) and [B] wheat (*Triticum aestivum* L.) seedlings after exposure to low, medium and high 500 nm PS-MPs concentrations (10^3, 10^5, 10^7 particles mL^{-1}) over time. Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. Error bars represent standard errors.

The most likely explanation for the difference in responses during germination between monocot and dicot species is related to seed size, since monocot seeds are usually much larger than dicot seeds. For example, in terms of weight, 1000-grain weight of lettuce is about 0.7 g (Souza et al., 2019), while for wheat it is around 40 g (Shahwani et al., 2014). Plant-contaminant interactions is thought to be facilitated by the higher surface-to-volume ration of a small seeded species (Cañas et al., 2008). Our findings suggest that plant species may have a moderating effect on how germination rate is impacted, as significant results were only found for lettuce and not for carrot, nor for both monocots wheat and barley. Moreover, sensitivity of germination to MPs may be species-specific, for other reasons than species type or seed size, as carrot was not affected while lettuce was.

Variation between individual dicot species were also observed. First, for the seed germination, lettuce germination was delayed by PS-MPs, which is in line with Gong et al. (2021) which exposed lettuce seeds to 0.1 and 5 μm PS. The accumulation of MPs on the seed capsule and root surface is hypothesized to slow down the water uptake by the plant and thus delays seed germination (Calero et al., 1981). However, no impact on the germination of carrots was observed, which cannot be compared to as no study has investigated the effects of MPs on the seed germination and early development of carrots. Second, even though an effect was recorded on lettuce germination, physical traits, such as root length, shoot length, and biomass, were not impacted by PS-MPs in this study. Previous research has shown a reduction in root length of lettuce (Gong et al., 2021; Martín et al., 2021). In contrast to lettuce, we did observe adverse effects during the early development of carrots: an increase in shoot elongation and a decrease in the seedling total biomass. Such an opposite effect was also reported in the dicotyledonous mung beans (*Vigna radiata*; Wang et al., 2021). The simultaneous increase in shoot elongation and decrease in biomass of carrot presents contradictory results when both parameters are growth characteristics. For wheat, at least, another study has also observed no significant effect on the germination when exposed to PS-MPs (Gong et al., 2021). Moreover, no effects were recorded on the early development of both monocots. These results vary compared to two other studies. Gong et al. (2021) recorded no significant effect on root length and an increase in shoot length on wheat seedlings, while Lian et al. (2020) observed an increase in root length. Interestingly, both studies have used PS and the same size (0.1 μm). This emphasizes that experimental design, in this case the size of the experimental unit and number of replicates, might impact results for the same plant species and type of MPs.

During chronic exposure, growth was only impacted for lettuce, while no effect was observed for wheat. This is in line with other studies, which also found no effect on the shoot height of wheat exposed to 0.5 and 0.2% w/w PE (Guo et al., 2022; Liu et al., 2021) and 0.001% PS (Ren et al., 2022). In contrast, we found an increase at the low concentration and a decrease at the medium and high concentrations of MPs on the shoot length of lettuce. Most other studies have noticed a clear decrease in the shoot length of lettuce (Lian et al., 2021; Gao et al., 2021). At higher concentrations, plants may experience reduced water and/or nutrient availability due to higher levels of MPs blockage. According to the theory of Poorter et al. (2012) this leads to an increased nutrient allocation to the roots to optimize their resource use in response to the stressor, which comes to the detriment of the shoots, meaning a decrease in shoot length. In addition, the effect on the chlorophyll content varied between monocots and dicots. Chlorophyll compounds were consistently decreased in lettuce, which is also the main pattern seen in other studies (Dong et al., 2021; Lian et al., 2021; Xu et al., 2022). This was explained that due to lipid peroxidation chloroplast organs are damaged, thus leading to a decrease in chlorophyll levels in plants exposed to MPs (Bagheri et al., 2019). On other hand, results varied in wheat seedlings. Other studies observed that chlorophyll content in wheat was either increased or not impacted by the exposure to MPs (Zantis et al., 2023).

Biochemical markers showed consistent responses to MPs for both lettuce and wheat. Both stress indicators and defence mechanisms were up-regulated in lettuce and wheat when exposed to PS-MPs. In this study, MDA levels were studied as a biomarker for lipid peroxidation (Awasthi et al., 2018). In both lettuce and wheat, levels of MDA were up-regulated by the exposure to PS-MPs, which is consistent with other studies (Zantis et al., 2023). Consequently, oxidative stress can also cause cell damage and the disfunction of cell components (Demidchik, 2015). Next, salicylic acid is a plant hormone involved in the activation of the plant's defence mechanisms against pathogen infections (Lefevere et al., 2020). Across all treatments, we observed an up-regulation in salicylic acid levels in both lettuce and wheat exposed to PS-MPs. It is known that SA levels increase when the plant is exposed to stress (Lefevere et al., 2020). Finally, phenolic compounds play an important role in the development of the plant, but also in the defence responses of the plant responsible for antioxidant activity (Pratyusha, 2022). An accumulation of phenolic compounds is usually a sign that the plant is under stress (Chowdhary et al., 2022). This was also observed within our study, where the total phenolic content was increased in both exposed lettuce and wheat seedlings. Overall, within both monocot and dicot species exposed to PS-MPs, a higher stress level within the plant cells and also an increase in the activity of the plant's defence mechanisms against oxidative stress were detected. This high alert status within the plants may also have consequences on the growth characteristics (Zhang et al., 2020b), as we saw for example a decrease in shoot length for lettuce. Moreover, plants are rarely exposed to a single contaminant but rather to a combination of stressors, highlighting the need to expose plants to multiple stressors (Wang et al., 2014; Zantis et al., 2023). Therefore, there is a need for more elaborate experiments, in which plants are exposed to a combination of plastic particles and other stressors, for example environmental stressors (heat, drought) or chemical (metals, pesticides).

Since this study was limited to crops grown hydroponically in laboratory conditions and under controlled conditions, it will be essential for future studies to expose plants under more environmentally realistic conditions. An important question remains how the results from the lab-based studies can be extrapolated to the field. Moreover, this study used the standard PS spheres as a model compound. However, research on MPs in soils has shown that polyethylene, polyvinyl chloride, and polyethylene terephthalate are the most abundant polymers in the terrestrial ecosystems (Qi et al., 2020), which are also commonly used in agricultural plastic products (FAO, 2021). Using weathered particles and a combination of microplastics rather than laboratory derived PS spheres, as used in this study, is essential to mimic what can be found under realistic field conditions. All these factors would provide more environmentally realistic assessment of the potential risks of MPs to crop growth and food safety (Zhang et al., 2020a; Nelis et al., 2023) compared to the lab-based initial screenings in hydroponic solutions making use of PS spheres.

5. Conclusions

This study investigates the effects of PS-MPs on the germination, early development, plant growth and biochemical response of four common crops. MPs had significant inhibitory effects on the crop development of dicots, both during germination and seedling growth phases, with differences between species. Even though differences in responses on growth parameters were found between species, stress indicators and defence mechanisms were up-regulated for all species during PS-MPs exposure. Moreover, differences were observed during germination and early development among dicot species. This highlights that the effects of MPs on plant species are difficult to generalize. Some responses, be it during short- or long-term exposure, were observed for all tested species. Although a variety of responses were observed for the apical endpoints with different impacts, the biochemical markers showed a similar trend for all plants species. For both monocot and dicot species, biochemical markers indicate that the plants were stressed due

to the exposure to MPs, which may have further consequences on growth, functioning and yield. Moreover, this raises the question of what would occur if plants were exposed to several stressors (e.g., heat, metals) as in farmlands a wide range of other contaminants are present in the soil. To conclude, research on the differences of crop specific impacts of MPs continues to be of great importance. In addition, testing under more environmentally realistic conditions is necessary to use these results to make realistic interpretations for field scenarios.

Author contribution statement

Laura J. Zantis: Conceptualization, Methodology, Investigation, Writing- Original draft preparation, Visualization, Annebelle Rombach: Conceptualization, Methodology, Investigation, Writing- Original draft preparation, Sylwia Adamczyk: Investigation, Methodology, Writing- (Reviewing and Editing), Sannakajsa M. Velmala: Writing- (Reviewing and Editing), Bartosz Adamczyk: Investigation, Methodology, Writing- (Reviewing and Editing), Martina G. Vijver: Conceptualization, Writing- (Reviewing and Editing), Willie Peijnenburg: Conceptualization, Writing- (Reviewing and Editing)Thijs Bosker: Conceptualization, Methodology, Writing- (Reviewing and Editing), Supervision.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interestsLaura J. Zantis reports financial support was provided by Horizon Europe. Sylwia Adamczyk reports financial support was provided by Horizon Europe. Sannakajsa M. Velmala reports financial support was provided by Horizon Europe. Bartosz Adamczyk reports financial support was provided by Horizon Europe. Thijs Bosker reports financial support was provided by Horizon Europe. Martina G. Vijver reports financial support was provided by European Research Council. Willie Peijnenburg reports financial support was provided by Horizon Europe.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122243.

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