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Comparing the impact of microplastics derived from a biodegradable and a conventional plastic mulch on plant performance

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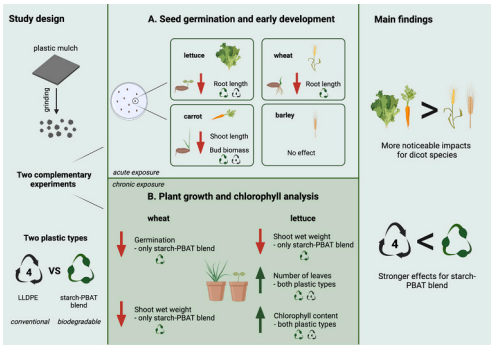
HIGHLIGHTS

- Conventional and biodegradable MPs impacted early development of lettuce and carrot.
- Limited chronic effects recorded on plant growth of barley and lettuce.
- Chronic exposure to MPs resulted in increased chlorophyll content in lettuce.
- Biodegradable plastics induced more adverse effects than conventional plastics.

GRAPHICAL ABSTRACT



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ABSTRACT

Agricultural lands have been identified as plastic sinks. One source is plastic mulches, which are a source of micro- and nano-sized plastics in agricultural soils. Because of their persistence, there is now a push towards developing biodegradable plastics, which are designed to undergo (partial) breakdown after entering the environment. Yet, limited research has investigated the impacts of both conventional and biodegradable plastics on distinct plants. Moreover, comparisons among studies are difficult due to differences in experimental design. This study directly compares the effects of artificially weathered conventional polyethylene (PE) and starch-based biodegradable polybutylene adipate terephthalate (PBAT) on four food crops, including two monocots (barley, *Hordeum vulgare*, and wheat, *Triticum aestivum* L.) and two dicots (carrot, *Daucus carota*, and lettuce, *Lactuca sativa* L.). We investigated the effects of environmentally relevant low, medium, and high (0.01 %, 0.1 %, 1 % *w/w*) concentrations of PE and starch-PBAT blend on seed germination (acute toxicity), and subsequently on plant growth and chlorophyll through a pot-plant experiment (chronic toxicity). Germination of all species was not affected by both plastics. However, root length was reduced for lettuce and wheat seedlings. No other effects were recorded on monocots. We observed a reduction in shoot length and bud wet weight of carrot seedlings for

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the highest concentration of PE and starch-PBAT blend. Chronic exposure resulted in a significant decrease in shoot biomass of barley and lettuce. Additionally, a positive increase in the number of leaves of lettuce was observed for both plastics. Chlorophyll content was increased in lettuce when exposed to PE and starch-PBAT blend. Overall, adverse effects in dicots were more abundant than in monocots. Importantly, we found that the biodegradable plastic caused more commonly adverse effects on plants compared to conventional plastic, which was confirmed by a mini-review of studies directly comparing the impact of conventional and biodegradable microplastics.

1. Introduction

Microplastics (MPs) are persistent within aquatic and terrestrial ecosystems (Kumar et al., 2023; Sridharan et al., 2021). While initially research revolved around the quantification and impacts of MPs in aquatic ecosystems, it is becoming increasingly evident that terrestrial ecosystems, particularly agricultural soils, serve as substantial long-term repositories for MPs (FAO, 2021; Kumar et al., 2020). MPs are detected in agricultural soils around the globe, with examples such as 0.00014–0.00044 % *w*/w in Chile (Corradini et al., 2019), and 0.004 % w/w in China (Li et al., 2020a), and up to 0.24 % w/w in industrial sites (Büks and Kaupenjohann, 2020). MPs infiltrate agricultural soils from diverse sources, including landfills, sewage irrigation, the application of biosolids and compost, polymer-based fertilizers and pesticides, as well as atmospheric deposition (Jin et al., 2022; Kumar et al., 2020; Tian et al., 2022). Agricultural mulching films are another specific type of application releasing high amounts of micro- and nanoplastics (MNPs). These mulching films are often used to conserve soil moisture and regulate soil temperature to enhance crop production (Liu et al., 2021; Tian et al., 2022) and avoid the need for herbicide application (Salama and Geyer, 2023). However, over time, films degrade into MPs, which can accumulate in the soil and surrounding ecosystems (Jin et al., 2022; Tian et al., 2022), thus potentially harming organisms in the environment.

Given the persistency of synthetic MPs, there has been a push towards the development of biodegradable plastic materials (Fan et al., 2022; Touchaleaume et al., 2016). One of these substitutes is polybutylene adipate terephthalate (PBAT) blended with biobased materials such as starch or cellulose (Burford et al., 2023). PBAT is often used in the manufacturing of mulching films as an alternative to conventional polyethylene (PE; Gross and Kalra, 2002; Touchaleaume et al., 2016). Similar to conventional mulching films, these biodegradable materials break down in the environment forming biodegradable MPs (bio-MPs). Nonetheless, the effects of the biodegradable MPs are very poorly understood (Fan et al., 2022; Zantis et al., 2023a), as they are even less studied than conventional plastics.

Numerous studies have documented impacts of MPs on soil ecosystems (Qiu et al., 2022; Shafea et al., 2022). These effects can directly influence plant health and performance by modifying plant root characteristics, nutrient absorption processes, and consequently growth (Huang et al., 2021; Li et al., 2022). Investigating effects of MPs on plants is of importance given their pivotal role in the food chain. Furthermore, MPs may exert an influence on food availability since crops represent a crucial food source for all organisms (FAO, 2022). Previous research has shown that nano- and microplastics can be taken up by plant roots (Li et al., 2020b) and leaves (Lian et al., 2021; Sun et al., 2021). In addition, a recent systematic review on the impacts of MNPs on plants showed that adverse effects are commonly observed at environmentally realistic levels (Zantis et al., 2023a). Moreover, nanoplastics can increase oxidative stress in plant cells and significantly affect plant defence mechanisms (Adamczyk et al., 2023). This raises concern in terms of large-scale food production, as these induced effects potentially reduce yields, and the possibility to transfer MPs to humans (Hofmann et al., 2023; Neto et al., 2023).

Studies on the comparison, within the same study, between a conventional and a biodegradable plastic are limited (Zantis et al., 2023a). In addition, most of these studies perform testing with only one or two plant species. Moreover, comparisons among studies which have used either conventional or biodegradable plastics are difficult due to differences in experimental design (Zantis et al., 2023a). Here we directly investigate and compare the impacts of conventional and biodegradable MPs sourced from plastic mulch on the seed germination and the growth of four terrestrial plants grown under identical conditions. To explore these effects, we conducted two experiments involving four commonly cultivated species: two monocotyledonous plants (barley, *Hordeum vulgare*, and wheat, *Triticum aestivum*) and two dicotyledonous plants (carrot, *Daucus carota*, and lettuce, *Lactuca sativa*). The first experiment focused on seed germination and early development (acute), while the second experiment was used to record growth and chlorophyll content (chronic; only on lettuce and barley). Finally, we conducted a mini-review comparing our results with all other studies directly comparing conventional to biodegradable microplastics. As a result, our study contributes to the understanding of different types of MPs on plant performance. Moreover, it adds valuable new data as well as a synthesis of current knowledge into the use of biodegradable plastics as an alternative to conventional plastics on commonly grown crops directly exposed to contaminated agricultural soils.

2. Materials and methods

2.1. Plant species and cultivation

Four agricultural crop species were studied. Two monocotyledonous species; common wheat (*Triticum aestivum* L.; sourced from Cruydt-Hoeck, Nijeberkoop, The Netherlands) and barley (*Hordeum vulgare*, Fennica; sourced from Boreal, Finland), and two dicotyledonous species, carrot (*Daucus carota,* Summer Carrot Amsterdam) and lettuce (*Lactuca sativa* L., Zwart Duits) (sourced from Koeman Flowerbulbs; Hem, The Netherlands) were tested. Both experiments were performed in a climate room at 21 °C, a 16 h light cycle and 75 % relative humidity.

2.2. Polymers characteristics

A conventional and a biodegradable plastic type were tested in this study: low linear density polyethylene (LLDPE) and polybutylene adipate terephthalate (PBAT) starch blend respectively. The two materials used here are test materials that have been used in several studies within the PAPILLONS project and are given the following identifiers M-rPE-black-A0 or P3 (LLDPE) and M-rBIO-black-A0 or P4 (starch-PBAT blend). Both MPs were obtained from grinding commercially available re-pelleted mulching films commonly used in agriculture. Shredded MPs were then cryomilled. The size distribution of both MPs used in this study can be found in the Supplementary Table S1. Three environmentally realistic exposure levels were used in both experiments. The following concentrations were chosen for both experiments: 0.01 % *w*/w, 0.1 % w/w and 1 % w/w. We will refer to these concentrations as low, medium and high concentrations in the manuscript. These concentrations were chosen based on Nizzetto et al. (2024), which stated that concentrations between 0.004 % w/w and 0.4 % w/w are environmentally plausible for biodegradable plastic residues in soils. Thus, our low and medium concentrations are within this range, and the high concentration is a projection of future levels in the soil (Nizzetto et al.,

2024).

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

The germination experiment was based on Zantis et al. (2023b). Briefly, ten seeds were placed in a 90 mm Petri dish (Thermo Scientific Sterilin) containing five filter papers (Whatman Grade 201). The corresponding amount of PE and starch-PBAT blend respectively was added to reach low, medium and high treatment concentrations. No MPs were added for the control treatment. Then, 5 mL of ¼ Hoagland solution (pH 6.0 ± 0.1) were added. The composition of the Hoagland solution is described in Table S2. Each treatment had eight replicates, and seed germination was recorded every 24 h. Seeds were considered germinated when the radicle was at least 2 mm long (Naseer et al., 2001). Based on a pilot study, lettuce, wheat and barley seeds were germinated for four days, and carrot seeds for ten days. Once the experiment ended, the shoot and root length (mm) were measured using a millimetre-scale ruler, and the fresh biomass (g) of individual seedlings was recorded. From this data, the following endpoints could then be calculated: percentage of seed germination, shoot length, root length and seedling fresh biomass. To allow for cross-species comparison, germination rate was evaluated by assessing the percentage of seeds germinated at two timepoints, namely halfway through germination (half of the full germination time) and at complete germination. Seedling samples were then frozen at −80 °C for further chlorophyll analysis.

2.4. Experiment 2: plant growth in a soil system (chronic exposure)

The second experiment focused on the plant growth of one monocot (barley) and one dicot (lettuce) in MPs-contaminated soil. Lufa 2.2 standard natural soil (Lufa Speyer, Germany) was used for this experiment (see Table S3 for soil characteristics provided by the supplier). First, the soil was dried for 48 h in an oven at 40 °C. MPs were mixed in within the soil to reach low, medium and high concentrations. MPs and dry soil were mixed for 5 min in a bucket by stirring with a metal spoon. Then, ¼ Hoagland solution was slowly added to prevent MPs to coagulate until the soil moisture content reached 70 %.

Seedlings were grown in 100 mL beakers (Fisherbrand Griffin Beaker), and approximately 70 g of wet soil was added within each beaker. For the control, wet soil without MPs was used. Each treatment was performed in eight replicates. Five seeds were added to each beaker and trimmed down randomly to three seedlings after five days. Seed germination was recorded for the first five days (see Section 2.3 for more information). Seedlings were watered every two days with ¼ Hoagland solution by adjusting the weight back to the original weight on day 0. Wheat seedlings were grown for 14 days, and lettuce seedlings for 21 days.

At the end of the experiment, all three seedlings were carefully removed from the soil and the following endpoints were measured: shoot length, shoot fresh biomass, root length, root fresh biomass and number of leaves. One seedling was then used to determine the specific leaf area (SLA, [formula 1]), and then dried for 24 h at 60 °C to record dry weight. The total leaf area for the SLA was determined using ImageJ (version 1.53t). The other two seedlings were saved at −80 °C to determine chlorophyll content.

$$\textit{Specific leaf area}\left(\frac{cm^2}{mg}\right) = \frac{\textit{Total leaf area}\ (cm^2)}{\textit{Total leaf dry weight}\ (mg)} \quad (1)$$

To determine chlorophyll content in the leaves, fresh shoots of lettuce and barley were first ground to powder using liquid nitrogen. For each treatment, eight replicates were taken and analysed in triplicates. 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 mins 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. 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).

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

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

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

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).

2.5. Statistical analysis

Statistical analysis was performed in R studio (Version, 2023.03.0 + 386; R Core team, 2023). Results are presented as means ± standard error (SE). The measured parameters were screened for normality and homogeneity of variance, using Shapiro-Wilk test and Levene's test respectively. For some endpoints, one of these assumptions was violated. For these, instead of using non-parametric tests, we have chosen to use parametric tests as these are more powerful compared to non-parametric tests. In these cases, *p* values below and excluding 0.05 were considered significant, while values between 0.05 and 1 were treated as non-significant. First, a two-way ANOVA in means test was performed to evaluate if there was a statistically significant interaction between *plastic type* and *concentration*. If yes, a Tukey post hoc test was performed to determine significant effects between treatments. If not, then a one-way ANOVA ($\alpha = 0.05$) difference in means test was performed to evaluate if results were statistically significant at a 95 % confidence level with a combined explanatory variable (plastic type and concentration). If the ANOVA was significant, a Tukey post hoc test was performed (TukeyHSD function) to determine significant effects between treatments. More information about statistical analyses can be found in Table S4. All datasets will be made available on Zenodo (https://doi.org/10.5281/zenodo.10361834).

3. Results

3.1. Experiment 1: seed germination and early development

No significant interaction was found between *plastic type* and *concentration* for all endpoints of this experiment (based on two-way ANOVAs; Table S4). All the following statistical analyses were performed using a one-way ANOVA. There was no significant effect on the seed germination of any tested species exposed to PE or starch-PBAT blend respectively ($p > 0.05$; Table 1).

For the two dicot species we found species-dependent effects on physical traits. No significant effect was recorded on the shoot length ($p = 0.58$) and fresh weight ($p = 0.37$) of lettuce buds (Table 1). Root length of lettuce buds was significantly affected by PE and starch-PBAT blend ($p < 0.0001$; Fig. 1B). Only the low concentration of PE decreased the root length by 25 % ($p = 0.03$), while all three treatments of starch-PBAT blend negatively affected the lettuce root length. The low, medium and high starch-PBAT blend treatment significantly reduced the root length by 35 % ($p < 0.001$), 34 % ($p < 0.001$) and 27 % ($p = 0.02$) respectively. For carrot buds, the shoot length was significantly reduced by both the PE and the starch-PBAT blend. The high PE concentration reduced the shoot length by approximately 40 % ($p = 0.02$), while the medium and high starch-PBAT blend concentration decreased the shoot

Table 1
The effects of low, medium, and high PE and starch-PBAT blend concentrations (0.01, 0.1, 1 % *w*/w) on the seed germination (%) at early germination and at the end of germination, 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. Lettuce, barley and wheat seeds were germinated for four days, and carrot seeds for ten days. All values are presented as mean ± SE. Different letters indicate significant differences (ANOVA & Tukey's post-hoc test, $p < 0.05$) between treatments.

Plant type	Plant species	Treatment (% w/w)	Germination (%)			
			Halfway germination	Complete germination	Shoot length (mm)	Wet weight (mg)
Dicot	Carrot	Control	85 ± 6.5	88 ± 4.1	20 ± 0.9 a	0.008 ± 0.0005 **a**
		PE 0.01	69 ± 8.3	87 ± 4.7	19 ± 0.9 ab	0.007 ± 0.0005 **ab**
		PE 0.1	74 ± 7.3	81 ± 4.8	17 ± 0.8 abc	0.005 ± 0.0004 **abc**
		PE 1	66 ± 6.5	76 ± 5.0	12 ± 0.6 bc	0.004 ± 0.0003 **bc**
		PBAT 0.01	73 ± 4.1	81 ± 1.3	20 ± 1.1 a	0.007 ± 0.0005 **abc**
		PBAT 0.1	65 ± 3.3	84 ± 3.2	11 ± 0.7 c	0.004 ± 0.0003 **bc**
		PBAT 1	63 ± 4.9	84 ± 5.3	11 ± 0.7 c	0.004 ± 0.0003 **c**
	Lettuce	Control	93 ± 2.5	98 ± 1.6	5 ± 0.2	3 ± 0.1
		PE 0.01	90 ± 1.9	93 ± 1.6	4 ± 0.2	2 ± 0.1
		PE 0.1	89 ± 4.0	96 ± 1.8	4 ± 0.3	3 ± 0.1
		PE 1	91 ± 2.3	93 ± 1.6	4 ± 0.2	3 ± 0.1
		PBAT 0.01	80 ± 5.7	87 ± 6.0	4 ± 0.3	3 ± 0.1
		PBAT 0.1	88 ± 4.9	95 ± 3.8	5 ± 0.2	3 ± 0.1
		PBAT 1	86 ± 4.3	94 ± 3.7	5 ± 0.2	3 ± 0.1
Monocot	Barley	Control	75 ± 5.3	79 ± 4.4	13 ± 0.8	120 ± 2.7
		PE 0.01	74 ± 3.2	79 ± 3.5	16 ± 0.9	135 ± 3.5
		PE 0.1	71 ± 2.3	76 ± 4.2	15 ± 0.8	117 ± 3.3
		PE 1	85 ± 4.2	88 ± 4.1	15 ± 0.9	124 ± 3.5
		PBAT 0.01	74 ± 4.2	80 ± 5.3	15 ± 0.9	125 ± 3.3
		PBAT 0.1	78 ± 5.9	80 ± 6.5	15 ± 0.9	116 ± 4.0
		PBAT 1	76 ± 4.2	85 ± 2.7	17 ± 1.3	125 ± 4.0
	Wheat	Control	45 ± 9.3	89 ± 4.0	10 ± 0.7	86 ± 1.8
		PE 0.01	46 ± 7.3	95 ± 2.7	8 ± 0.3	85 ± 1.3
		PE 0.1	35 ± 5.0	96 ± 1.8	7 ± 0.3	83 ± 1.4
		PE 1	48 ± 5.9	86 ± 3.2	8 ± 0.4	81 ± 1.7
		PBAT 0.01	43 ± 6.2	90 ± 4.6	9 ± 0.5	92 ± 1.5
		PBAT 0.1	40 ± 5.7	93 ± 2.5	8 ± 0.3	77 ± 1.7
		PBAT 1	51 ± 5.5	94 ± 2.6	9 ± 0.4	84 ± 1.6

length by 45 % and 56 % respectively ($p = 0.005$ and $p = 0.003$). In addition, the fresh biomass of carrot buds was also significantly reduced ($p < 0.001$; Table 1). The high PE and starch-PBAT blend treatment decreased the wet weight of carrot buds by 51 % and 55 % respectively compared to the control ($p = 0.03$ and $p = 0.01$). As the fresh weight data was not normally distributed and the p value is close to 0.05, the effect observed by the medium starch-PBAT blend treatment on the fresh weight on carrot buds should be interpreted with caution ($p = 0.049$). For root length, a significant effect was detected by the ANOVA ($p = 0.008$; Table S4), however no statistical significance was found between treatments based on the Tukey post-hoc test because of the large variance within treatments, especially the control (Fig. 1A).

For both monocot species, no significant effect by PE and starch-PBAT blend was recorded, except for the root length of wheat (Table 1). A decrease by all treatments of PE and starch-PBAT blend on the root length of wheat buds was observed (Fig. 1D). This decrease was however only significant for the medium concentration of starch-PBAT blend ($p = 0.02$), which reduced the root length by 48 % compared to the control. For wheat, no significant effect of PE and starch-PBAT blend on the shoot length was observed ($p = 0.35$). The bud wet weight was reduced for most treatments (Table 1). A statistically significant effect was detected by the ANOVA ($p = 0.003$; Table S4), however the Tukey post-hoc test was only significant between 0.01 % and 0.1 % *w*/w starch-PBAT blend treatments ($p < 0.001$). No effect was observed for barley on shoot length ($p = 0.67$) and bud wet weight ($p = 0.19$; Table 1). Even though the ANOVA showed a significant effect on the root length ($p = 0.043$; Table S4), no effect was observed between treatments by the Tukey post-hoc test (Fig. 1C) probably due to the high variance. All effects of this experiment are summarized in Table 2.

3.2. Experiment 2: pot plant experiment

No significant interaction was found between *plastic type* and *concentration* for all endpoints of this experiment, except for the halfway germination (day 2) and shoot dry weight of barley and the number of leaves of lettuce. For these three endpoints, an interactive effect was found but, importantly, no clear pattern was observed. For all other endpoints, a one-way ANOVA was performed combining both *plastic type* and *concentration*.

Germination of lettuce was not affected by both the PE and starch-PBAT blend ($p > 0.05$; Table 3). For barley, a significant interaction between *plastic type* and *concentration* was determined for germination after two days (Two-Way ANOVA, $p = 0.007$; Table S4). Even though an overall decrease in germination rate was noted for barley seeds, only the highest treatment of starch-PBAT blend was significantly different from the control after two days ($p = 0.04$; Table 3). In addition, a simple main effects analysis showed that the concentration of both plastic types had a significant effect on germination ($p = 0.02$; Table S4). A statistically significance difference was also observed by the ANOVA at the final seed germination ($p = 0.023$; Table S4). Nonetheless, no statistically significance was found between treatments after the post hoc analysis, even though there was still a 25 % reduction for the highest treatment of starch-PBAT blend in germination. No significant effect on the seed germination of barley for the other treatments was recorded (Table 3).

Exposure to the (biodegradable) plastic particles resulted in some impacts on growth parameters of lettuce (dicot). Significant effects on shoot fresh weight and number of leaves of lettuce seedlings were observed after 21 days of exposure. The shoot fresh weight of lettuce decreased when exposed to all three starch-PBAT blend treatments. However, it was only statically significant at the highest concentration ($p = 0.015$; Fig. 2A). Moreover, a significant interaction between *plastic type* and *concentration* was determined for the number of leaves (Two-Way ANOVA, $p = 0.003$). The number of lettuce leaves increased for all treatments compared to the control and was statistically significant for PE at the highest concentration ($p = 0.036$), and starch-PBAT blend at the low ($p = 0.002$) and medium ($p = 0.036$) concentrations (Table 4). In

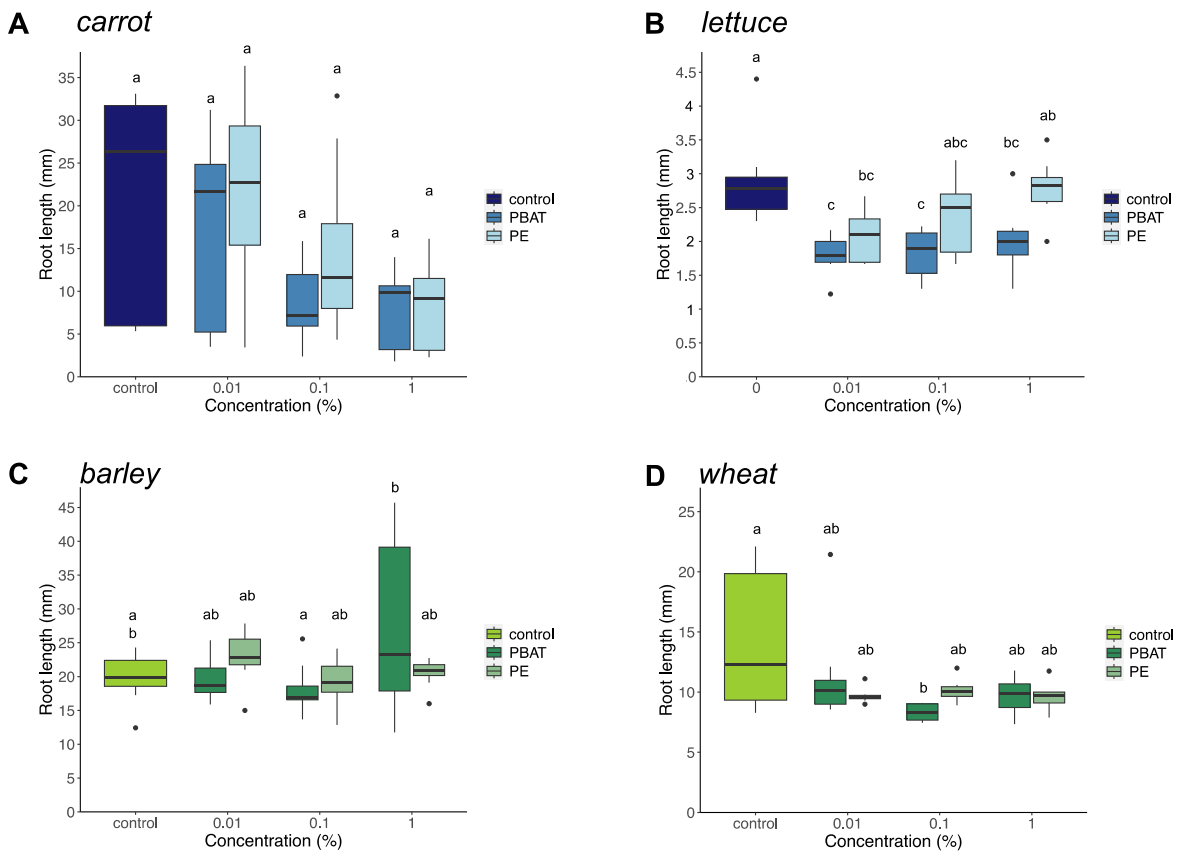

Fig. 1. The mean ± SE root length of carrot (*Daucus carota*, [A]), lettuce (*Lactuca sativa*, [B]), barley (*Hordeum vulgare,* [C]) and wheat (*Triticum aestivum* L., [D]) buds after exposure to low, medium and high PE and starch-PBAT blend concentrations (0.01, 0.1, 1 % *w*/w). Lettuce, barley and wheat seeds were germinated for four days, and carrot seeds for ten days. Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. Please note the high variance in the controls of carrot (A) and wheat (D).

addition, a simple main effects analysis showed that concentration had a statistically significant effect on the number of lettuce leaves ($p = 0.0003$; Table S4). No significant effects on shoot length ($p = 0.22$), root length ($p = 0.48$), root fresh biomass ($p = 0.13$; Table 5), the shoot and root dry weight ($p > 0.05$) and SLA of lettuce ($p = 0.29$; Table 4) were observed. The chlorophyll content was significantly impacted for lettuce. Both PE and starch-PBAT blend significantly increased the chlorophyll *a* content. Low and medium PE treatments increased the chlorophyll *a* content ($p = 0.02$ and $p > 0.0001$ respectively; Fig. 3A). The same trend was seen at the low and medium starch-PBAT blend concentration ($p = 0.007$ and $p = 0.003$ respectively). Chlorophyll *b* was only increased by the medium PE concentration ($p = 0.002$; Fig. 3B). Overall, the total chlorophyll (chl *a* + *b*) content in lettuce was also increased for both PE and starch-PBAT blend medium concentrations ($p < 0.001$ and $p = 0.009$; Fig. 3C).

For barley, only shoot biomass was impacted by treatments. All treatments decreased the shoot fresh weight of barley ($p < 0.001$; Table 4). However, the decrease was only significant when exposed to the medium ($p < 0.05$) and high ($p < 0.005$) concentrations of starch-PBAT blend (Fig. 2B). A significant effect on root length ($p = 0.02$) was only observed when comparing 0.01 % PE and 0.1 starch-PBAT blend ($p = 0.028$; Table S4). For root fresh weight ($p = 0.07$), a decrease in the treatments is observed, which was however not statistically significant (Table 4). No significant effect on barley was recorded on the shoot length ($p = 0.18$), root fresh weight ($p = 0.07$), number of leaves ($p = 0.44$; Table 4), root dry weight of barley ($p = 0.77$) and SLA ($p = 0.62$; Table 4). Even though a significant interaction between *plastic type* and *concentration* was determined for the shoot dry weight (Two-Way ANOVA, $p = 0.02$), a Tukey post-hoc test did not reveal any significant differences between treatments (Table 4). In addition, no effects of both plastic types were observed on the chlorophyll *a* ($p = 0.9$; Fig. 3D), chlorophyll *b* ($p < 0.05$; Fig. 3E) and total chlorophyll content ($p = 0.9$; Fig. 3F) of barley seedlings. All effects of this experiment are summarized in Table 5.

4. Discussion

We investigated the impact of traditional polyethylene (PE) and a starch blend of polybutylene adipate-*co*-terephthalate (PBAT) on the growth of four common crop species. Our results showed that MPs exposure affected early development and plant growth of both dicot species more than the two monocot species used in this study. Moreover, impacts induced by the starch-PBAT blend were more pronounced than the PE MPs.

While no effect was recorded on seed germination of both monocots and dicots, dicots were more strongly impacted than monocots during their early development. This difference was also observed in our previous study comparing the impacts of polystyrene (PS) MPs on acute effects of these four crops (Zantis et al., 2023b). This pattern is most likely explained by seed size, as dicot seeds are usually much smaller than monocot seeds (Zantis et al., 2023b). The interaction between plants and contaminants is supposed to be easier for small-seeded species because they have a higher ratio of surface area to volume (Cañas et al., 2008). For dicots, both lettuce and carrot were negatively impacted by exposure to both types of MPs. Variations between individual dicot species were also observed. The root length of lettuce was decreased for all starch-PBAT blend concentrations and the lowest PE treatment, while no effect was observed on carrot. Roots are sensitive to stress, which might have consequences on the crop yield and plant health in the long-term (Karlova et al., 2021; Li et al., 2021a). Although

Table 2
Summary of acute effects of different concentrations (0.01 %, 0.1 % and 1 % *w*/w) of PE and starch-PBAT blend on the seed germination, root length, shoot length and biomass of lettuce, carrot, barley and wheat. Lettuce, barley and wheat seeds were germinated for four days, and carrot seeds for ten days. A downward (↓) and the red colour signifies decrease in the endpoint, while no effect (–) was shown as in blue.

		Plastic	Concentration (% w/w)	Germination	Root length	Shoot length	Biomass
Monocot	Barley	PE	0.01	–	–	–	–
			0.1	–	–	–	–
			1	–	–	–	–
		PBAT	0.01	–	–	–	–
			0.1	–	–	–	–
			1	–	–	–	–
	Wheat	PE	0.01	–	–	–	–
			0.1	–	–	–	–
			1	–	–	–	–
		PBAT	0.01	–	–	–	–
			0.1	–	↓	–	–
			1	–	–	–	–
Dicot	Carrot	PE	0.01	–	–	–	–
			0.1	–	–	–	–
			1	–	–	↓	↓
		PBAT	0.01	–	–	–	–
			0.1	–	–	↓	↓
			1	–	–	↓	↓
	Lettuce	PE	0.01	–	↓	–	–
			0.1	–	–	–	–
			1	–	–	–	–
		PBAT	0.01	–	↓	–	–
			0.1	–	↓	–	–
			1	–	↓	–	–

Table 3
The effects of low, medium, and high PE and starch-PBAT blend concentrations (0.01, 0.1, 1 % *w*/w) on the seed germination (%) halfway and at the end of germination of barley (*Hordeum vulgare*) and lettuce (*Lactuca sativa*) buds. Barley seedlings were grown for 14 days, and lettuce seedlings for 21 days. All values are presented as mean ± SE. Different letters indicate significant differences (ANOVA and Tukey's post-hoc test, $p < 0.05$) between treatments.

Plant species	Concentration (% w/w)	Germination (%)	
		Halfway (day 2)	Final (day 4)
Barley	Control	90 ± 3.8 **a**	95 ± 3.3 **a**
	PE 0.01	68 ± 7.5 **ab**	95 ± 3.3 **a**
	PE 0.1	88 ± 5.3 **ab**	85 ± 6.3 **a**
	PE 1	85 ± 5.0 **ab**	90 ± 3.8 **a**
	PBAT 0.01	80 ± 5.3 **ab**	90 ± 3.8 **a**
	PBAT 0.1	65 ± 8.3 **ab**	75 ± 7.3 **a**
	PBAT 1	63 ± 5.2 **b**	74 ± 7.2 **a**
Lettuce	Control	80 ± 8.5	98 ± 2.5
	PE 0.01	83 ± 7.0	95 ± 3.3
	PE 0.1	78 ± 4.5	85 ± 3.3
	PE 1	80 ± 7.6	90 ± 3.8
	PBAT 0.01	70 ± 8.5	90 ± 5.3
	PBAT 0.1	80 ± 7.6	90 ± 6.5
	PBAT 1	80 ± 5.3	83 ± 4.5

no effects on carrot roots were observed, we did find adverse effects on shoot length and bud biomass. Similar responses were found in previous studies on soybean (*Glycine* max) exposed to PE (6.5 μm; Wang et al., 2021) and Komatsuna (*Brassica napa*) subjected to PBAT (<5 mm, Inubushi et al., 2022).

In a second experiment on the chronic exposure (14 days for barley and 21 days for lettuce), effects on growth parameters were minimal for both lettuce and barley. Interestingly, the germination rate of barley in soil was reduced by starch-PBAT blend, while during the acute experiment no impact was observed. This difference has been also noticed for soybean exposed to PE. Soybean seeds germinated in a hydroponic culture were not affected by PE (Wang et al., 2021), while seeds grown in soil contaminated with PE showed a lower germination viability (Li et al., 2021b). This difference in results highlights that the medium in which experiments are carried out might also affect the outcome of the study. Moreover, the shoot fresh weight was reduced by the starch-PBAT blend for both barley and lettuce. This is in line with other studies, such as Wang et al. (2024), in which lettuce seedlings were exposed to both PE and starch-PBAT blend at the same concentrations as in our study. Nonetheless, the shoot biomass was also decreased by the PE treatments. Here, the particle size could play a role as the particles used by Wang et al. (2024) were 40 μm, compared to our particle size that ranged from 50 to 1000+ μm. Size of particles is an important key factor, as it influences uptake and fate in plants (Li et al., 2020b).

Lettuce was the only plant which showed altered chlorophyll content after MP exposure. Chlorophyll content was only affected for lettuce, in which we noted an increase in chlorophyll content. Increases in chlorophyll levels do happen in plants exposed to plastic particles but are less common compared to the decrease (Zantis et al., 2023a). For example, compared to our latest study, in a hydroponic system, we observed that the chlorophyll content in lettuce seedlings was decreased after 7 days, while no effect was determined after 14 and 21 days (Zantis et al., 2023b). It is hypothesized that the increase of chlorophyll content could be explained through two mechanisms: a) the decreased in chlorophyll degradation or b) the increased chlorophyll synthesis (Li et al., 2015; Pignattelli et al., 2021). We also observed an increase in the leaf number and total surface of lettuce seedlings, which might be linked to the increase in chlorophyll content. From previous research, light perception by plants was determined by leaf area growth (Koester et al., 2014; Melnyk et al., 2020), which promotes the photosynthesis process within leaves.

One goal of our study was to compare the impact of PE and starch-PBAT blend MPs on plants. Our results show that both types of

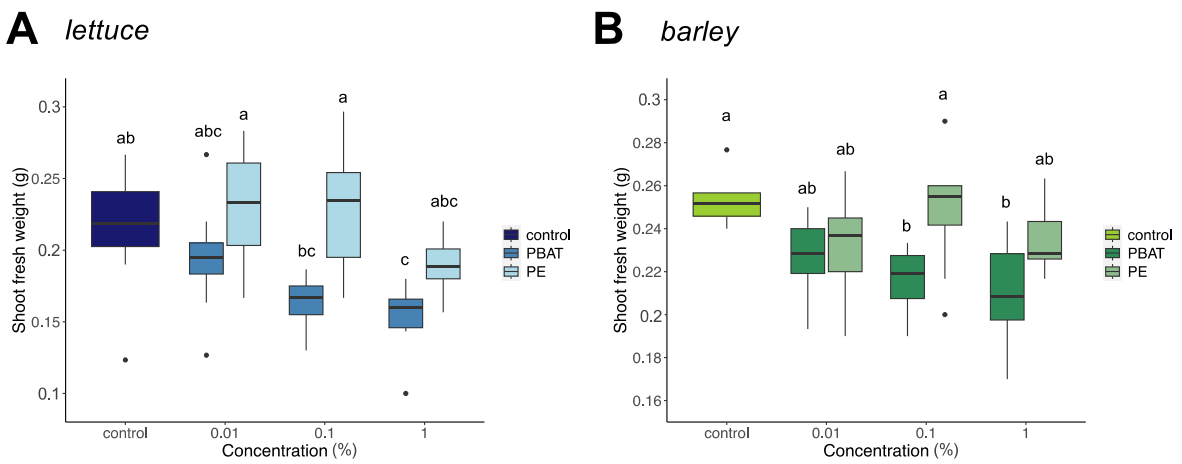

Fig. 2. The mean ± SE shoot fresh biomass of lettuce (*Lactuca sativa*) [A] and barley (*Hordeum vulgare*) [B] after exposure to low, medium and high PE and starch-PBAT blend concentrations (0.01, 0.1, 1 % w/w). Barley seedlings were grown for 14 days, and lettuce seedlings for 21 days. Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments.

Table 4
The effects of low, medium and high PE and PBAT concentrations (0.01, 0.1, 1 % *w*/w) on the root length and biomass, shoot length and biomass, and the specific leaf area (SLA) of barley (*Hordeum vulgare*) and lettuce (*Lactuca sativa* L.). Barley seedlings were grown for 14 days, and lettuce seedlings for 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 (% *w*/w)	Root length (cm)	Root fresh weight (g)	Root dry weight (mg)	Shoot length (cm)	Shoot dry weight (mg)	Number of leaves (pcs)	SLA (cm^2/mg)
Barley	Control	15 ± 0.7	0.130 ± 0.008	28 ± 2.1	20 ± 0.5	30 ± 2.2	2 ± 0	0.153 ± 0.012
	PE 0.01	14 ± 0.9	0.141 ± 0.014	26 ± 2.3	19 ± 0.9	33 ± 2.1	2 ± 0	0.150 ± 0.007
	PE 0.1	16 ± 0.6	0.179 ± 0.011	26 ± 2.4	18 ± 0.5	26 ± 2.1	2 ± 0	0.158 ± 0.021
	PE 1	16 ± 0.4	0.154 ± 0.012	26 ± 3.5	19 ± 0.5	28 ± 1.4	2 ± 0	0.136 ± 0.008
	PBAT 0.01	15 ± 0.5	0.162 ± 0.011	23 ± 1.8	18 ± 0.6	26 ± 2.0	2 ± 0	0.143 ± 0.009
	PBAT 0.1	16 ± 0.7	0.161 ± 0.014	29 ± 3.5	18 ± 1.1	33 ± 4.3	2 ± 0	0.136 ± 0.012
	PBAT 1	16 ± 0.7	0.167 ± 0.012	26 ± 3.1	18 ± 1.0	23 ± 1.8	2 ± 0	0.131 ± 0.008
Lettuce	Control	9 ± 0.9	0.026 ± 0.002	5 ± 0.9	5 ± 0.1	16 ± 1.7	6 ± 0.1 **a**	0.578 ± 0.043
	PE 0.01	9 ± 1.0	0.021 ± 0.003	4 ± 0.7	5 ± 0.2	16 ± 0.9	6 ± 0.1 **ab**	0.630 ± 0.056
	PE 0.1	8 ± 0.9	0.018 ± 0.002	3 ± 0.6	5 ± 0.1	14 ± 2.1	6 ± 0.2 **abc**	0.664 ± 0.045
	PE 1	11 ± 0.9	0.025 ± 0.003	5 ± 1.3	5 ± 0.1	14 ± 1.7	7 ± 0.1 **bc**	0.693 ± 0.040
	PBAT 0.01	8 ± 1.0	0.018 ± 0.002	4 ± 1.3	5 ± 0.2	16 ± 1.9	7 ± 0.1 **c**	0.668 ± 0.072
	PBAT 0.1	10 ± 1.0	0.022 ± 0.002	4 ± 0.7	5 ± 0.1	14 ± 1.6	7 ± 0.1 **bc**	0.798 ± 0.085
	PBAT 1	9 ± 1.1	0.017 ± 0.002	3 ± 0.6	5 ± 0.1	13 ± 1.5	6 ± 0.1 **abc**	0.694 ± 0.069

plastics impacted the growth of tested crops, but effects were more commonly observed and stronger when plants were exposed to the biodegradable MPs. For example, during acute exposure, dicots were more severely impacted by the starch-PBAT blend. All concentrations of starch-PBAT blend reduced the root length of lettuce, and the shoot length and bud biomass of carrots were affected by the two highest concentrations of the starch-PBAT blend, while this was the case only for the highest PE treatment. Moreover, in the chronic experiment, we observed that the shoot biomass of both barley and lettuce was significantly reduced by the starch-PBAT blend compared to conventional PE MPs. This decrease induced by biodegradable plastic was also reported by Meng et al. (2021), in which a decrease in shoot biomass at concentrations of 1.5 %, 2 % and 2.5 % *w*/w of bioplastic was noted. Nonetheless, several studies have also reported negative effects for both plastic types in direct comparison, such as on soybean (Li et al., 2021b), lettuce (Wang et al., 2024) or rice (Yang and Gao, 2022), or no effect at all by both plastic types, for example on wheat (Qi et al., 2018). Interestingly, the high concentration of both plastic types increased the number of lettuce leaves in our study. This is in contrast to two other studies which showed a decreasing trend. For instance, the number of leaves of lettuce was significantly reduced by both PE and PBAT at exposure concentrations of 0.1 % and 1 % w/w (Wang et al., 2024), and for wheat only for the starch-based biodegradable MPs (Qi et al., 2018).

Including ours, to date only eleven studies (with 13 experiments in total) have directly compared the impacts of conventional and biodegradable plastics on plant growth (see Table 6 for a summary of all

Table 5
Summary of effects of different concentrations (0.01 %, 0.1 % and 1 % w/w) of PE and starch-PBAT blend on seed germination, root length, root biomass, shoot length, shoot biomass, number of leaves, specific leaf area (SLA) and chlorophyll content. Barley seedlings were grown for 14 days, and lettuce seedlings for 21 days. A downward arrow (↓) and the red colour signify decrease in the endpoint, while an upward arrow (↑) and the green colour signifies increase. No effect (−) was shown as in blue.

		Plastic	Concentration (% w/w)	Germination	Root length	Root wet weight	Root dry weight	Shoot length	Shoot wet weight	Shoot dry weight	Number of leaves	SLA	Chl *a*	Chl b	Chl *a+b*
Monocot	Barley	PE	0.01	−	−	−	−	−	−	−	−	−	−	−	−
			0.1	−	−	−	−	−	−	−	−	−	−	−	−
			1	−	−	−	−	−	−	−	−	−	−	−	−
		PBAT	0.01	−	−	−	−	−	−	−	−	−	−	−	−
			0.1	−	−	−	−	−	↓	−	−	−	−	−	−
			1	↓	−	−	−	−	↓	−	−	−	−	−	−
Dicot	Lettuce	PE	0.01	−	−	−	−	−	−	−	−	−	↑	−	−
			0.1	−	−	−	−	−	−	−	−	−	↑	↑	↑
			1	−	−	−	−	−	−	−	↑	−	−	−	−
		PBAT	0.01	−	−	−	−	−	−	−	−	−	↑	−	−
			0.1	−	−	−	−	−	−	−	↑	−	↑	−	↑
			1	−	−	−	−	−	↓	−	−	−	−	−	−

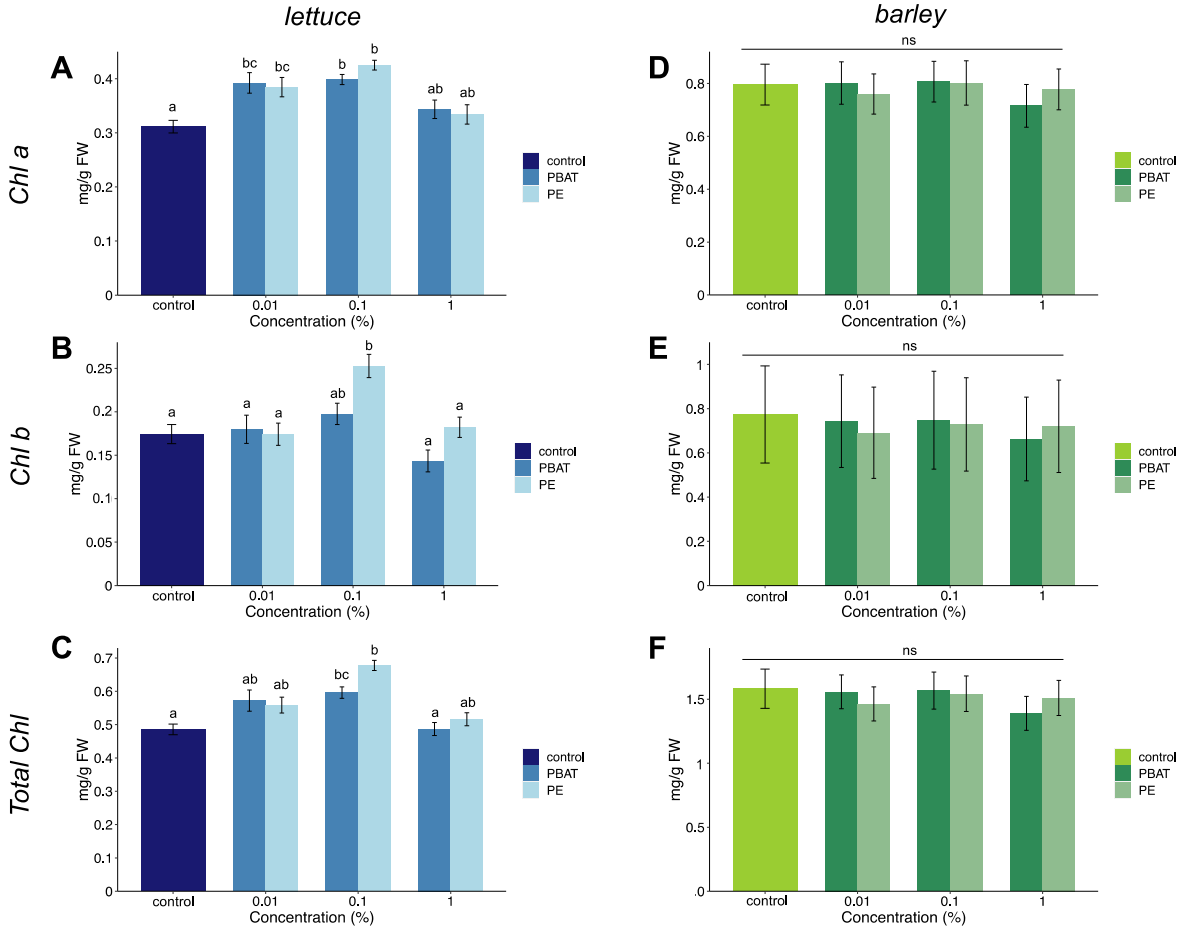

Fig. 3. The mean ± SE chlorophyll *a* [A: lettuce, D: barley], chlorophyll *b* [B: lettuce, E: barley] and total chlorophyll content [C: lettuce, F: barley] of lettuce (*Lactuca sativa*) and barley (*Hordeum vulgare*) after exposure to low, medium and high PE and starch-PBAT blend concentrations (0.01, 0.1, 1 % w/w). Barley seedlings were grown for 14 days, and lettuce seedlings for 21 days. Different letters indicate significant differences (ANOVA followed by Tukey's post-hoc test, $p < 0.05$) between treatments. ns means not significant.

Table 6
Summary of research studies ($n = 8$ studies) comparing the effects of conventional plastics and biodegradable plastics within the same study on plants. Abbreviations: LDPE: low density polyethylene; LLDPE: low linear density polyethylene; HDPE: high density polyethylene; PBAT: polybutylene adipate terephthalate; PE: polyethylene; PHB: polyhydroxybutyrate; PLA: polylactic acid; PP: polypropylene; PS: polystyrene; PVC: polyvinyl chloride.

Plant species		Plastic tested	Concentration (w/w)	Exposure time	Observation of study	Source
Monocot	Barley	LLDPE & PBAT	0.01, 0.1, 1 %	4 days	No effect during seed germination and early development (acute experiment).	Our study
		LLDPE & PBAT	0.01, 0.1, 1 %	14 days	Bio-MPs reduced germination, while no effect observed on PE. Shoot fresh biomass only reduced for medium and high bio-MPs treatments (chronic experiment).	Our study
	Maize	PE & PLA + PBAT	0.1, 1, 10 %	30 days	Leaf area and total chlorophyll content only reduced by bio-MPs. Upregulation in enzymatic antioxidant defence mechanism for both plastic types, but stronger for bio-MPs.	Sun et al. (2023)
		HDPE & PLA	0.1, 1, 10 %	1 month	Stronger negative impacts of PLA on shoot and root DW, and shoot/root ratio compared to HDPE.	Yang et al. (2021)
	Rice	PVC & PLA	10 %	1 month	Both PLA and PVC negatively impacted the growth of rice.	Song et al. (2023)
		PE & PBAT	1 %	4 months	Root biomass, shoot biomass and plant height were decreased by both plastic types.	Yang and Gao (2022)
	Sorghum	PP & PHB, PLA	0.02, 0.095, 0.48, 2.38, 11.9 %	3 days	No effect during seed germination. Bio-based plastics acted stronger than the petroleum-based plastic on the root growth.	Liwarska-Bizukojc (2022)
	Wheat	LDPE & starch-based bioplastic	1 %	4 months	Negative effects only seen for bio-MPs on number of leaves, leaf area and stem diameter.	Qi et al. (2018)
		LLDPE & PBAT	0.01, 0.1, 1 %	4 days	No effect on seed germination. Only decrease in root length observed for the low PE treatment (acute experiment)	Our study
Dicot	Carrot	LLDPE & PBAT	0.01, 0.1, 1 %	10 days	No effect on seed germination. Decrease in shoot length and bud biomass for medium and high PBAT, and high PE treatment (acute experiment).	Our study
	Common bean	LDPE & PBAT+PLA	0.5, 1, 1.5, 2, 2.5 %	105 days	Negative effects only seen for bio-MPs on shoot and root biomass, fruit biomass and shoot/root ratio. Specific root length and module increased by all bio-MPs treatment, while only for the highest PE treatments.	Meng et al. (2021)
	Cress	PP & PHB, PLA	0.02, 0.095, 0.48, 2.38, 11.9 %	3 days	No effect during seed germination. Bio-based plastics acted stronger than the petroleum-based plastic on the root growth.	Liwarska-Bizukojc (2022)
	Lettuce	PE & PBAT	0.1, 1 %	30 days	Shoot fresh and dry biomass, number of leaves, carotenoid and total chlorophyll content were decreased by both plastic types. Lipid peroxidation was up regulated for both plastic types.	Wang et al. (2024)
		LLDPE & PBAT	0.01, 0.1, 1 %	4 days	No effect on seed germination. Root length reduction for all bio-MPs treatment, while only for the low PE MPs concentration (acute experiment)	Our study
		LLDPE & PBAT	0.01, 0.1, 1 %	21 days	Shoot biomass increased only for high bio-MPs. Number of leaves and chlorophyll content increased for both plastic types (chronic experiment).	Our study
	Mustard	PP & PHB, PLA	0.02, 0.095, 0.48, 2.38, 11.9 %	3 days	No effect during seed germination. Bio-based plastics acted stronger than the petroleum-based plastic on the root growth.	Liwarska-Bizukojc (2022)
	Peanut	HDPE, PS & PLA	1, 10 %	2 months	No effect by all three MPs on biomass but increase in plant heights by PS and PLA. Chlorophyll shows mixed effects for all MPs.	Wang et al. (2023)
	Soybean	PE & PBAT+PLA	0.1, 0.5, 1 %	4 months	Plant height increased for all bio-MPs treatments, while a decrease is seen for PE MPs. Similar reduction in shoot biomass and total biomass for both plastic types.	Li et al. (2021b)
		PE & PLA	0.1, 1 %	49 days	PE had no phytotoxic effect, while root length was decreased by PLA. Antioxidant enzymes were impacted by both plastic types.	Lian et al. (2022)

papers to date). Similar to our study, effects were more commonly detected when plants were exposed to PBAT and/or PLA. For monocots, we observe that effects were more commonly observed when plants were exposed to biodegradable plastics compared to conventional plastics (four out of six experiments showed a stronger effect by biodegradable plastics). For example, leaf characteristics of wheat (Qi et al., 2018) and maize (Sun et al., 2023) were significantly reduced only by biodegradable MPs. For dicots, a similar pattern is observed with five out of seven experiments showing a major effect when plants were exposed to biodegradable plastics. For example, biomass of roots and shoots of common beans were reduced by the exposure to biodegradable plastics while no effect was observed when exposed to LDPE (Meng et al., 2021). Although this is based on a small sample size of only eleven studies, and a total of twelve different plant species, it does highlight that adverse effects of biodegradable MPs on plant health under controlled conditions are common. This highlights the urgent need to unravel the mechanisms behind the differences in impact, especially for biodegradable plastic. We require a better understanding of which components (physically or chemically) of biodegradable plastic persist compared to the conventional persistent MPs.

This raises a final, yet crucial point to consider; the environmental relevance when studying the impact of both biodegradable and conventional plastics on plants. In the current study we used soil exposure to assess the MP-induced phytotoxicity, which is one step closer to environmental relevant conditions compared to hydroponic cultures which have been commonly used (Zantis et al., 2023a). For future research, testing these materials in environmentally relevant field conditions would shed knowledge on actual degradation rates of plastic mulch and also its interaction with soil and organisms. Especially, the use of biodegradable plastic mulch in agriculture has been suggested as an environmentally friendly alternative to conventional plastic mulch. However, limited is known about its interaction and integration into agricultural soils, and its effects on soil microbiomes and organisms remain understudied (Serrano-Ruiz et al., 2021). More research is needed on the fate and effects of biodegradable plastics in terrestrial ecosystems. The degradation rate for biodegradable plastics is often

faster than conventional plastics (Chamas et al., 2020). However, these degradation rates are not always accurate as an overall discrepancy between biodegradation conditions in the laboratory and in the field is observed (Choe et al., 2021). In addition, one area of future research is the impact of additives which may leach from plastics. Leached additives might also impact plant health (Cao et al., 2023), and, importantly, biodegradable plastics also often contain additives (Savva et al., 2023). Considering the mentioned factors would result in a more environmentally realistic assessment of the potential risks posed by both conventional and biodegradable MPs to crop growth and food safety (Nelis et al., 2023).

5. Conclusion

This study compared the short and long-term effects of conventional and biodegradable MPs on crops. Effects were measured on seed germination, early development and plant growth of four commonly grown crops. No effect was recorded on the seed germination of all crops, but negative impacts were recorded on the early development of the two dicot species, lettuce and carrot. During long term exposure, effects by both plastic types were limited, except for the shoot biomass. Here a clear decreasing trend in biomass was observed for both barley and lettuce seedlings. Overall, our results show that effects induced by the starch-PBAT blend MPs on the plant development were more common compared to conventional MPs, which was confirmed by a mini-review on current studies directly comparing the impacts conventional and biodegradable microplastics on plant performance.

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CRediT authorship contribution statement

Laura J. Zantis: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Sylwia Adamczyk:** Writing – review & editing, Methodology, Investigation. **Sannakajsa M. Velmala:** Writing – review & editing. **Bartosz Adamczyk:** Writing – review & editing, Methodology, Investigation. **Martina G. Vijver:** Writing – review & editing. **Willie Peijnenburg:** Writing – review & editing. **Thijs Bosker:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Laura 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. Willie Peijnenburg reports financial support was provided by Horizon Europe. Martina G. Vijver reports financial support was provided by European Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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.scitotenv.2024.173265.

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