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Research Paper

Breaking ground: The effect of leachates from conventional and biodegradable mulching films on plants

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ABSTRACT

The increasing use of plastics in agriculture, for instance mulching film, contributes to plastic residues in soils. Biodegradable plastics have been considered as an alternative to conventional plastic due to their assumed faster degradation rate. Both plastic types undergo weathering processes and leach breakdown products into the environment. Until now, little is understood about the effects of these leached chemicals on plant health. This study investigated the impact of artificially weathered plastic leachates on four widely cultivated crops: the monocots barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), and the dicots carrot (*Daucus carota*) and lettuce (*Lactuca sativa*), by comparing conventional low linear density polyethylene with a biodegradable starch-polybutylene adipate-terephthalate blend. Effects on plant growth were tested on germination and early development (acute) and in a chronic pot-plant setup (only lettuce and barley), using environmentally relevant concentrations. Limited effects were found, with the root length of both monocots and dicots being the most affected in both experiments. Interestingly, an increase in root length was observed in barley for both exposures, while the root system of lettuce increased in the acute experiment. In addition, lettuce seedlings showed a decrease in shoot fresh weight. During chronic exposure the plant defence mechanisms were upregulated in both plants for both plastic types, highlighting that exposure to leachates causes stress to plants. Our work is one of the first to compare directly the effects of leachates from conventional and biodegradable plastics on plants, thereby providing important new data in this rapidly evolving field of research.

1. Environmental implication

There is currently significant focus on the impact of microplastics on plants and agroecosystems, yet limited research is conducted on the effects of plastic chemicals leaching into the soil. As plastics degrade, they release chemicals, including additives, and byproducts that can alter soil chemistry, impair plant growth, and disrupt microbial communities. This is likely true for conventional plastics as well as biodegradable alternatives. These changes may reduce crop yields, affect food quality, and compromise soil health over time. Most studies to date examine only single species or individual plastic types, and as a result the effects of leachates from plastic pollution are still largely unknown. Understanding these interactions is essential to safeguard crop productivity and soil health in the face of rising plastic pollution in agricultural landscapes.

2. Introduction

In recent years, the emphasis on plastic pollution is shifting from aquatic towards terrestrial ecosystems [1,2], as terrestrial ecosystems also act as sinks for both primary and secondary plastics [3–5]. Nano- and microplastics (NMPs) accumulation in agro-ecosystems are caused by a range of sources, including nano-pesticides [6–8] and larger plastics (e.g., plastic mulch, trays) undergoing weathering and resulting in

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Table 1
The effects of low, medium, and high concentrations (0.01 %, 0.1 %, 1 % *w*/w) of low linear density polyethylene (LLDPE) and starch-polybutylene adipate terephthalate (PBAT) blend leachates on seed germination (percentage and germination rate index), shoot length, root length, and bud fresh weight ($n = 8$) of lettuce (*Lactuca sativa*), carrot (*Daucus carota*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*) following acute exposure. Measurements for lettuce, wheat, and barley buds were taken after four days, while carrot buds were assessed after eight days. Data are presented as mean ± SE. Significant differences determined by one-way ANOVA are indicated with lowercase letters, whereas uppercase letters denote significant interactions between plastic type and concentration identified by two-way ANOVA; both analyses were followed by Tukey's post-hoc test ($p < 0.05$).

Plant type	Plant species	Treatment (% w/w)	Shoot length (mm)	Wet weight (mg)	Germination	
					Complete germination (%)	Germination rate index
Dicot	Carrot	Control	130 ± 9.8 **ab**	5 ± 0.5	89 ± 3.5	7.4 ± 0.3
		LLDPE 0.01	118 ± 12.7 **ab**	5 ± 0.4	75 ± 5.7	6.4 ± 0.5
		LLDPE 0.1	152 ± 8.6 **a**	5 ± 0.3	84 ± 6.5	7.4 ± 0.5
		LLDPE 1	121 ± 13.3 **ab**	4 ± 0.3	86 ± 4.2	6.9 ± 0.4
		PBAT 0.01	96 ± 6.5 **b**	4 ± 0.8	80 ± 6.0	6.6 ± 0.4
		PBAT 0.1	115 ± 9.9 **ab**	5 ± 1.5	83 ± 3.4	7.3 ± 0.3
		PBAT 1	120 ± 9.2 **ab**	6 ± 1.7	92 ± 2.6	7.6 ± 0.3
	Lettuce	Control	51 ± 4.4	4 ± 0.4 **AB**	75 ± 4.6	7.9 ± 0.7
		LLDPE 0.01	46 ± 2.1	4 ± 0.3 **AB**	69 ± 3.5	7.5 ± 0.6
		LLDPE 0.1	53 ± 1.8	5 ± 0.2 **A**	68 ± 1.6	9.6 ± 0.6
		LLDPE 1	44 ± 5.3	4 ± 0.3 **AB**	71 ± 4.3	8.8 ± 0.8
		PBAT 0.01	41 ± 5.1	4 ± 0.3 **B**	68 ± 3.7	7.2 ± 0.5
		PBAT 0.1	55 ± 4.1	4 ± 0.3 **AB**	60 ± 6.5	7.9 ± 1.1
		PBAT 1	48 ± 5.3	5 ± 0.2 **AB**	68 ± 3.7	8.7 ± 0.9
	Barley	Control	34 ± 2.2 **ab**	166 ± 8.6	93 ± 4.1	9.0 ± 0.6 **ab**
		LLDPE 0.01	37 ± 1.2 **a**	172 ± 3.0	94 ± 3.8	9.2 ± 0.5 **ab**
		LLDPE 0.1	40 ± 2.0 **a**	185 ± 10.8	94 ± 3.2	9.5 ± 0.5 **a**
		LLDPE 1	36 ± 1.4 **ab**	184 ± 12.7	79 ± 5.2	7.5 ± 0.5 **b**
		PBAT 0.01	37 ± 1.1 **a**	180 ± 3.8	93 ± 4.1	9.1 ± 0.4 **ab**
		PBAT 0.1	38 ± 1.0 **a**	181 ± 6.4	94 ± 2.6	9.3 ± 0.4 **ab**
		PBAT 1	30 ± 1.6 **b**	174 ± 14.7	89 ± 3.0	8.3 ± 0.3 **ab**
Monocot						
	Wheat	Control	98 ± 17.2	102 ± 2.6	80 ± 4.6	5.5 ± 0.5
		LLDPE 0.01	107 ± 4.6	94 ± 1.4	76 ± 3.2	5.2 ± 0.3
		LLDPE 0.1	109 ± 14.4	94 ± 2.7	79 ± 5.5	5.0 ± 0.3
		LLDPE 1	89 ± 7.2	101 ± 12.9	70 ± 4.6	4.5 ± 0.5
		PBAT 0.01	84 ± 10.1	103 ± 12.1	74 ± 5.0	4.5 ± 0.4
		PBAT 0.1	74 ± 8.0	108 ± 14.5	84 ± 2.6	5.7 ± 0.4
		PBAT 1	80 ± 10.7	98 ± 2.4	75 ± 3.3	4.8 ± 0.4

secondary NMPs [9,10]. Rising rates of the usage of mulching films in the agricultural sector, which, for example, in China increased from 625,746 tons in 1997 to 1,436,607 tons in 2017 [11], underline the need to also closely examine their environmental degradation processes, plastic pollution, and potential risk for the environment.

NMPs can affect the physiological, chemical, and biological characteristics of the soil as well as soil biota [12,13]. For instance, [14] showed that the application of low linear density polyethylene (LDPE) films and polyacrylnitril (PAN) fibres altered the physiology and respiration of the soil. Plastic pollution in terrestrial environments poses a potential risk to soil organisms [6,15], and recent reviews have highlighted that effects of NMPs on terrestrial plants are commonly observed [16,17]. At environmentally relevant NMP levels, plant defence mechanisms are commonly upregulated, and deviations in the biomass, root and shoot parameters are frequently observed [17]. Although the exact mechanisms remain unclear, proposed modes of action for plastic particles include the blockage of root pores in terrestrial plants and/or the uptake of NMP into the plants [18–20].

As concerns about plastic mulching films are rising, biodegradable plastics have been promoted as an environmentally friendly alternative due to their enhanced degradation rates [21,22]. However, several studies have found that biodegradable plastics can also have adverse effects on organisms, with some studies indicating even higher impacts than conventional plastics [19,23,24]. Moreover, it seems that the effects of biodegradable plastics might be species-dependent [24,25]. For instance, [24] observed a significant reduction in the root length of lettuce (*Lactuca sativa* L.) at all tested concentrations (0.01 %, 0.1 %, 1 % *w*/w) of the biodegradable starch-polybutylene adipate terephthalate (PBAT) blend, while no clear patterns of response were observed for other species (wheat, carrot and barley) tested.

One possible cause of the adverse effects of both biodegradable and conventional plastics on terrestrial plants is the leaching of chemicals from the plastic particles [26,27]. Plastic chemicals are (un)intentionally added chemicals that modify the physiochemical properties of plastics to enhance their functions [28,29]. The degradation of plastics in the environment results in the release of chemicals into the ecosystem [30]. For example, plastic chemicals are ubiquitous in agricultural soils across China, with an average value of 988 μg/kg for certain additives, such as phthalates or bisphenols [31]. Moreover, [31] found a correlation between the occurrence of additives and the utilization of plastic mulching. Studies on the effects of leached chemicals on plants are limited to date but show that this is an important gap in our knowledge. For example, [32] compared the physiochemical toxicity mechanisms of photoaged polyvinyl chloride (PVC)-MPs compounds by comparing the effects of leachate, leached particles and unleached particles on wheat (*Triticum aestivum L.*) seedlings. Results suggested a synergy of altered physical adsorption in the roots caused by the plastic particles and enhanced toxicity of leached particles due to the photoaging process. In addition, [33] tested the phytotoxic effects of non-biodegradable and biodegradable leachates on cotton, showing slight deviations of biochemical parameters and early development compared to the control. Findings of these studies in this novel research field on plastic chemicals suggest further testing is needed [32,33]. Given that plant health and crop yield are essential to global food security, the rising accumulation of plastics and their potential release of chemicals to the environment are of significant concern [34,35].

To provide new insights in this emergent topic, we investigate the effect of leachates from conventional (low linear density polyethylene)

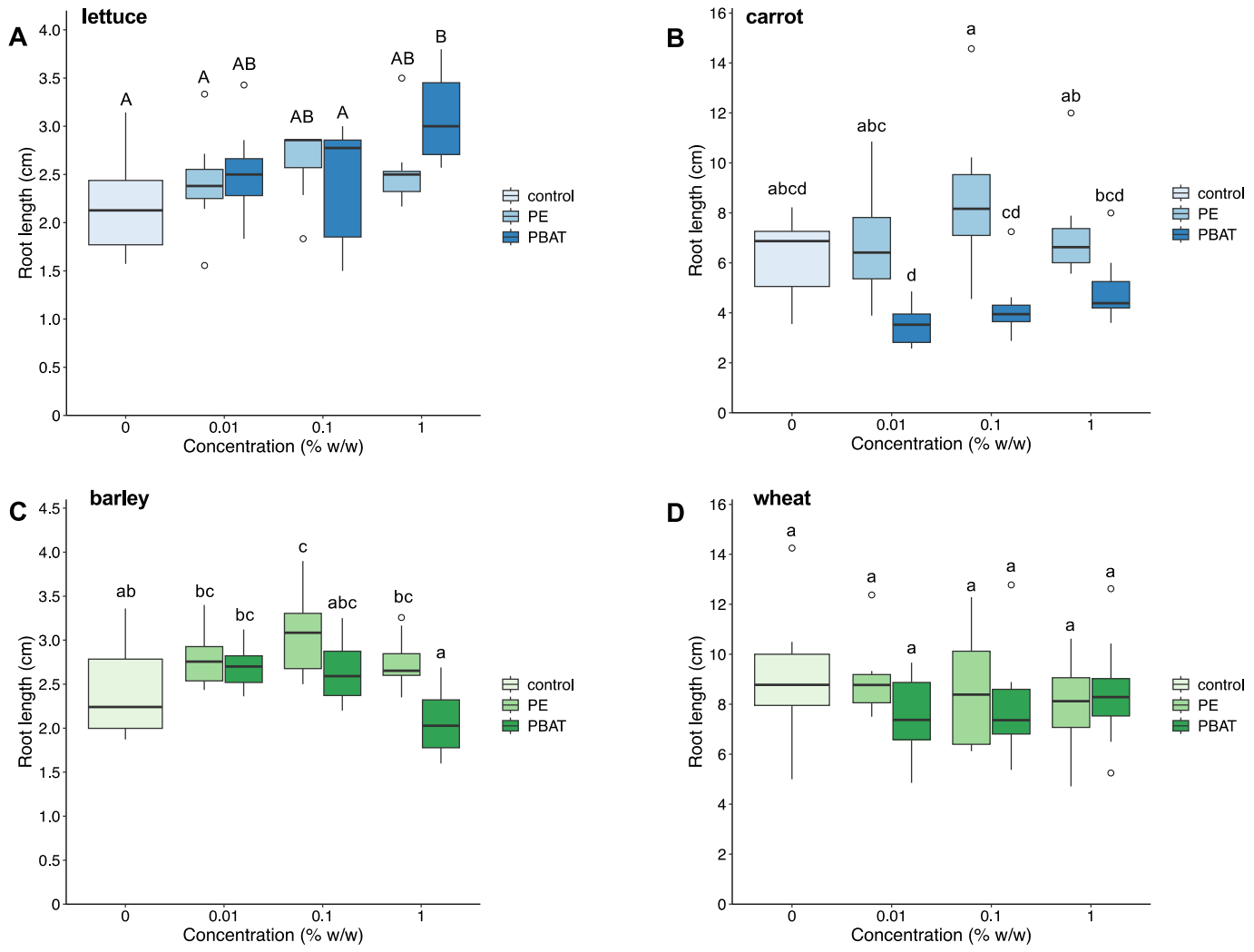

Fig. 1. The average ***root length*** ($n = 8$) of lettuce (*Lactuca sativa* [A]), carrot (*Daucus carota* [B], barley (*Hordeum vulgare* [C]), and wheat (*Triticum aestivum* [D]) seedlings following exposure to low, medium and high conventional low linear density polyethylene (LLDPE) and biodegradable starch polybutylene adipate terephthalate (PBAT) blend leachate concentrations (0.01 %, 0.1 %, 1 % *w*/w). Measurements for lettuce, wheat, and barley buds were taken after four days, while carrot buds were assessed after eight days. Significant differences determined by one-way ANOVA are indicated with lowercase letters, whereas uppercase letters denote significant interactions between plastic type and concentration identified by two-way ANOVA; both analyses were followed by Tukey's post-hoc test ($p < 0.05$).

and biodegradable (starch-polybutylene adipate-terephthalate blend) on germination, plant development and biochemistry of four common crop species: the monocots barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), and the dicots carrot (*Daucus carota*) and lettuce (*Lactuca sativa*). Non-target/suspect screening (NTS/SS) of the leachates was conducted to assess the chemicals leaching from the starch-PBAT blend and LLDPE samples and enable their tentative identification.

3. Materials and methods

3.1. Plant species and cultivation

Wheat (*Triticum aestivum* L.; obtained from Cruydt-Hoeck, Nijeberkoop, The Netherlands) and barley (*Hordeum vulgare* L., Fennica; obtained from Boreal, Finland) were chosen as the representative monocot species. For the dicot group, lettuce (*Lactuca sativa* L., Zwart Duits) and carrot (*Daucus carota* L., Summer Carrot Amsterdam) were chosen. The selected species are agriculturally important and well-studied crop plants. All experiments were conducted in a climate-controlled room at 21 ± 1 °C, under a 16:8-h light-dark cycle, with a light intensity set to 100 lx and relative humidity maintained at 75 %.

3.2. Polymer characteristics and leachate preparation

Two plastic types, one conventional low linear density polyethylene (LLDPE), and one biodegradable starch-polybutylene adipate terephthalate (PBAT) blend, were used to obtain the leachates. The plastics used were sourced and cryomilled from agricultural mulching films of low-density polyethylene (M-PEDE-45-black-A0) and starch-PBAT blend (M-BIOEL-15-black-A0). Information about the plastic properties can be found in [36].

The leachate stock solutions were prepared according to [27,37] with some modifications. In short, 100 g of conventional or biodegradable MPs were added to 1 L of ¼ Hoagland solution (L/S ratio 10:1; details of the Hoagland solution composition are listed in Table S1) to achieve a 10 % *w*/w concentration. The flasks were placed on a stirring plate with the settings of 53 ± 1 °C and 300 rpm for seven days to stimulate environmental conditions [38]. Every day, the leachates were stirred manually to ensure the exposure of the plastic particles to the solution. After the degradation treatment, the leachate stock solution was filtered twice. Big particles were filtered through a mesh of 32 µm to remove them from the leachate. Subsequently, the liquid and the solids in the remaining leachate were separated through vacuum filtration on

Table 2
The effects of low, medium, and high concentrations (0.01 %, 0.1 %, 1 % *w*/w) of low linear density polyethylene (LLDPE) and starch-polybutylene adipate terephthalate (PBAT) blend leachates on the shoot length, root fresh weight, number of leaves, leaf area, total dry weight, and the specific leaf area (SLA) ($n = 8$) of barley (*Hordeum vulgare*) and lettuce (*Lactuca sativa* L.). Measurements for barley seedlings were taken after 14 days, while lettuce seedlings were assessed after 21 days. Data are presented as mean ± SE. Significant differences determined by one-way ANOVA are indicated with lowercase letters, whereas uppercase letters denote significant interactions between plastic type and concentration identified by two-way ANOVA; both analyses were followed by Tukey's post-hoc test ($p < 0.05$).

Plant type	Plant species	Treatment (% w/w)	Root fresh weight (mg)	Shoot length (cm)	Number of leaves (pcs)	Total dry weight (mg)	SLA (cm^2/mg)	Area (cm^2)
Dicot	Lettuce	Control	13 ± 0.9	6.8 ± 0.5	5 ± 0.2 **AB**	12 ± 1.9	510 ± 60.5	6.5 ± 1.3
		LLDPE 0.01	11 ± 0.6	5.7 ± 0.6	6 ± 0.1 **A**	10 ± 0.6	419 ± 25.5	4.1 ± 0.5
		LLDPE 0.1	14 ± 1.2	6.0 ± 0.4	5 ± 0.1 **A**	7 ± 0.8	643 ± 94.2	4.2 ± 0.4
		LLDPE 1	15 ± 1.5	6.4 ± 0.5	5 ± 0.1 **A**	11 ± 1.4	644 ± 81.8	7.3 ± 0.4
		PBAT 0.01	13 ± 1.3	6.4 ± 0.4	5 ± 0.1 **AB**	9 ± 2.6	488 ± 96.5	5.2 ± 2.2
		PBAT 0.1	13 ± 1.2	6.5 ± 0.4	5 ± 0.2 **A**	13 ± 4.1	429 ± 36.1	5.7 ± 1.9
		PBAT 1	11 ± 1.0	6.1 ± 0.4	4 ± 0.2 **B**	11 ± 3.5	531 ± 56.8	5.7 ± 1.5
Monocot	Barley	Control	126 ± 13.8	24.1 ± 0.7	2 ± 0.10	50 ± 3.7 **A**	255 ± 24.2	12.6 ± 1.0 **AB**
		LLDPE 0.01	134 ± 21.5	23.0 ± 0.8	2 ± 0.04	52 ± 6.2 **AB**	187 ± 19.2	9.4 ± 1.2 **B**
		LLDPE 0.1	156 ± 18.5	25.3 ± 0.5	2 ± 0.00	48 ± 2.7 **AB**	266 ± 22.8	12.6 ± 1.1 **AB**
		LLDPE 1	91 ± 6.9	25.8 ± 0.6	2 ± 0.06	46 ± 4.7 **A**	238 ± 21.6	10.8 ± 1.3 **AB**
		PBAT 0.01	141 ± 26.0	25.4 ± 0.9	2 ± 0.04	54 ± 3.9 **AB**	295 ± 32.9	15.1 ± 0.7 **A**
		PBAT 0.1	117 ± 8.6	24.2 ± 1.1	2 ± 0.04	45 ± 5.2 **A**	263 ± 26.7	11.2 ± 0.9 **AB**
		PBAT 1	127 ± 19.1	26.3 ± 0.7	2 ± 0.17	80 ± 16.1 **AB**	202 ± 27.9	15.6 ± 2.0 **AB**

Whatman borosilicate glass microfiber filters (grade GF/F) at a pore size of 0.7 μm according to EPA method TCLP 1311 [39].

Further for the acute and chronic exposure, the leachate stock solution was diluted with ¼ Hoagland solution to obtain dilutions of 1 % (high), 0.1 % (medium) and 0.01 % (low) concentrations to reach environmental relevant conditions. The concentrations selected for this study were informed by [40], who reported that biodegradable plastic residues in soils typically range from 0.004 % to 0.4 % *w*/w under environmentally plausible conditions. Accordingly, our low and medium concentrations align with this range, while the high concentration represents a projected future soil level [40]. The control contains only ¼ Hoagland solution and was treated the same as the leachate dilutions.

3.3. Chemical analyses of leachates

3.3.1. Sample extraction and cleanup

Chemical analysis was conducted on three leachates: ¼ Hoagland PBAT (10 % *w*/w), ¼ Hoagland LLDPE (10 % w/w), and ¼ Hoagland control (0 % w/w). Sample extraction and cleanup were performed following the method described by [41], with minor modifications. Briefly, 20 mL of each leachate was extracted by solid phase extraction (SPE) with hydrophilic lipophilic balance (HLB) cartridges (Oasis® HLB, 150 mg, 6 mL, Waters Corporation). Elution of plastic additives was performed with 6 mL of methanol and 6 mL of ethyl acetate. Using a nitrogen evaporator, the combined eluates were concentrated to near dryness (TurboVap®, Biotage) at 30 °C and reconstituted in 500 μL of 20 % methanol in Milli-Q water. Based on a test injection, the extracts were diluted 100-fold due to high intensities of some features. Finally, the diluted extract was spiked with 50 μL of an injection standard (IS) solution in methanol and stored at 4 °C until analysis by liquid chromatography tandem to high resolution mass spectrometry (LC-HRMS).

3.3.2. Suspect screening of plastic additives using LC-HRMS

NTS/SS of chemical additives in the PBAT and LLDPE leachates was performed following the workflow developed by [42] and further detailed by [41]. Sample extracts were injected using a 1290 Infinity HPLC system (Agilent Technologies), achieving separation of the analytes on a BEH C18 column (XBridge, 100 × 2.1 mm, 2.5 μm, Waters) Corporation). HRMS data were analysed on a Bruker Daltonics Compact II QTOF mass Spectrometer. Electrospray ionization (ESI) was employed in both positive and negative modes. Precursor ions were chosen for fragmentation based on data-dependent acquisition. Full details on chromatographic conditions, MS instrument settings, and MS/MS data acquisition can be found in the paper of [41].

3.4. Experiment 1: acute toxicity

The acute experiment on seed germination and the bud's early development was based on [18,24]. Ten seeds were arranged on three filter papers (Whatman Grade 201) within a Petri dish and each treatment was replicated eight times. We added 10 mL of the leachate solution to the corresponding treatment. The Petri dish was sealed by parafilm with three holes for aeration and placed in the climate chamber in a random order. The germination rate was recorded every 24 h. According to [43], seeds were classified as germinated when the radicle extended to two millimetres or more. Germination periods were four days for lettuce, wheat, and barley, and eight days for carrot. The root and shoot length (mm) and seedling fresh biomass (mg) were measured after the end of the experiment. Root and shoot length were measured using a ruler and the biomass was measured using a fine scale. The germination total percentage (GTP) [1] was calculated according to [24,27] as followed:

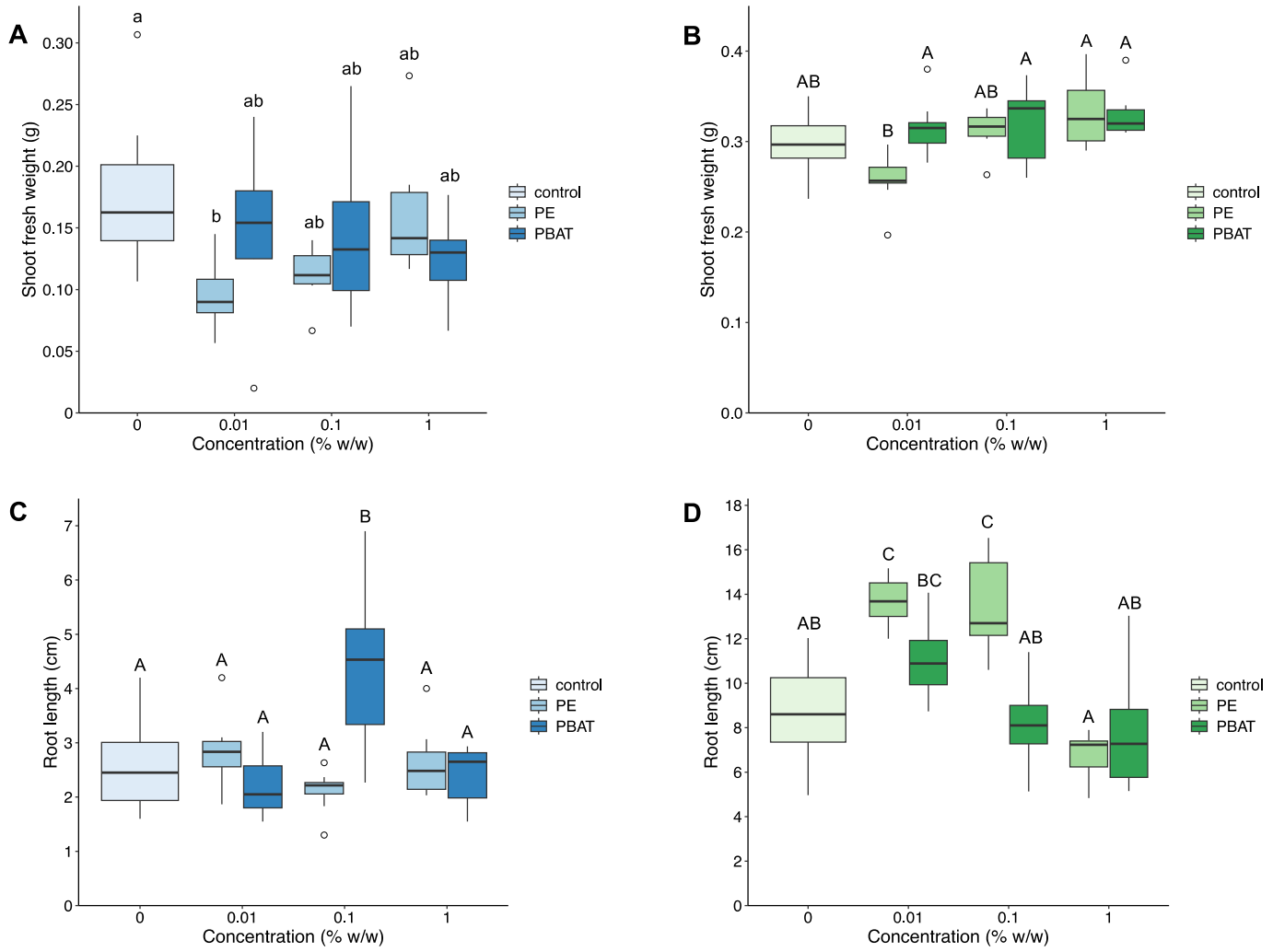

Fig. 2. The average ***shoot fresh biomass*** ($n = 8$) of lettuce (*Lactuca sativa* [A]) and barley (*Hordeum vulgare* [B]) and the average ***root length*** ($n = 8$) of lettuce [C] and barley [D] seedlings following exposure to low, medium and high linear density polyethylene (LLDPE) and biodegradable starch polybutylene adipate terephthalate (PBAT) blend leachate concentrations (0.01 %, 0.1 %, 1 % *w*/w). Measurements for barley seedlings were taken after 14 days, while lettuce seedlings were assessed after 21 days. Significant differences determined by one-way ANOVA are indicated with lowercase letters, whereas uppercase letters denote significant interactions between plastic type and concentration identified by two-way ANOVA; both analyses were followed by Tukey's post-hoc test ($p < 0.05$).

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

The germination rate index (GMI) [2 and 3] includes the speed of germination and was calculated according to [27] as followed:

$$\textit{Germination rate index}(\textit{lettuce}, \textit{barley}, \textit{wheat}) = \frac{N1}{T1} + \frac{N2}{T2} + \frac{N3}{T3} + \frac{N4}{T4} \quad (2)$$

$$\textit{Germination rate index}(\textit{carrot}) = \frac{N1}{T1} + \frac{N2}{T2} + \ldots + \frac{N8}{T8} \quad (3)$$

where N1, N2, …, N8 are the number of seeds that germinated at day T1, T2, …, T8.

3.5. Experiment 2: chronic toxicity

Lufa 2.2 standard natural soil (bought from Lufa Speyer, Germany) was dried on 40 °C for 24 h (see Table S2 for soil characteristics provided by the supplier). The leachate stock solution of high, medium or low (1 %, 0.1 %, 0.01 % *w*/w) was added to the soil to reach a water holding capacity of 70 % by mixing it uniformly with a spoon for two minutes. For the control group, a ¼ Hoagland solution subjected to the same leaching procedure as the treatment dilutions was added to the soil, bringing it to 70 % of its water holding capacity. The dilutions applied to the soil provide three treatments (high, medium and low) in addition to the control group. One monocot (barley) and one dicot (lettuce) were studied to directly compare their responses, as these species showed the highest sensitivity in the acute experiment. Beakers of the size of 100 mL were filled with the treated Lufa soil and five seedlings per beaker were sown. Each treatment contains of eight replicates. A randomized design for the beakers was chosen to account for fluctuating conditions in the climate chamber [44].

3.5.1. Growth parameters

The five seedlings were trimmed down randomly to three seedlings after at least three seeds per replicate have been germinated. For replicates with a lower germination rate trimming down the seeds was done no later than on day seven. Seedlings were watered every two to three days with a 1/4 Hoagland solution, adjusting the weight of each replicate to match its initial weight at the start of the experiment. Barley was exposed and grown for 14 days, while lettuce was grown for 21 days. At the conclusion of the growth period, the seedlings were carefully removed from the soil. Measurements were taken for shoot and root length (cm), shoot and root fresh biomass (mg), and the number of

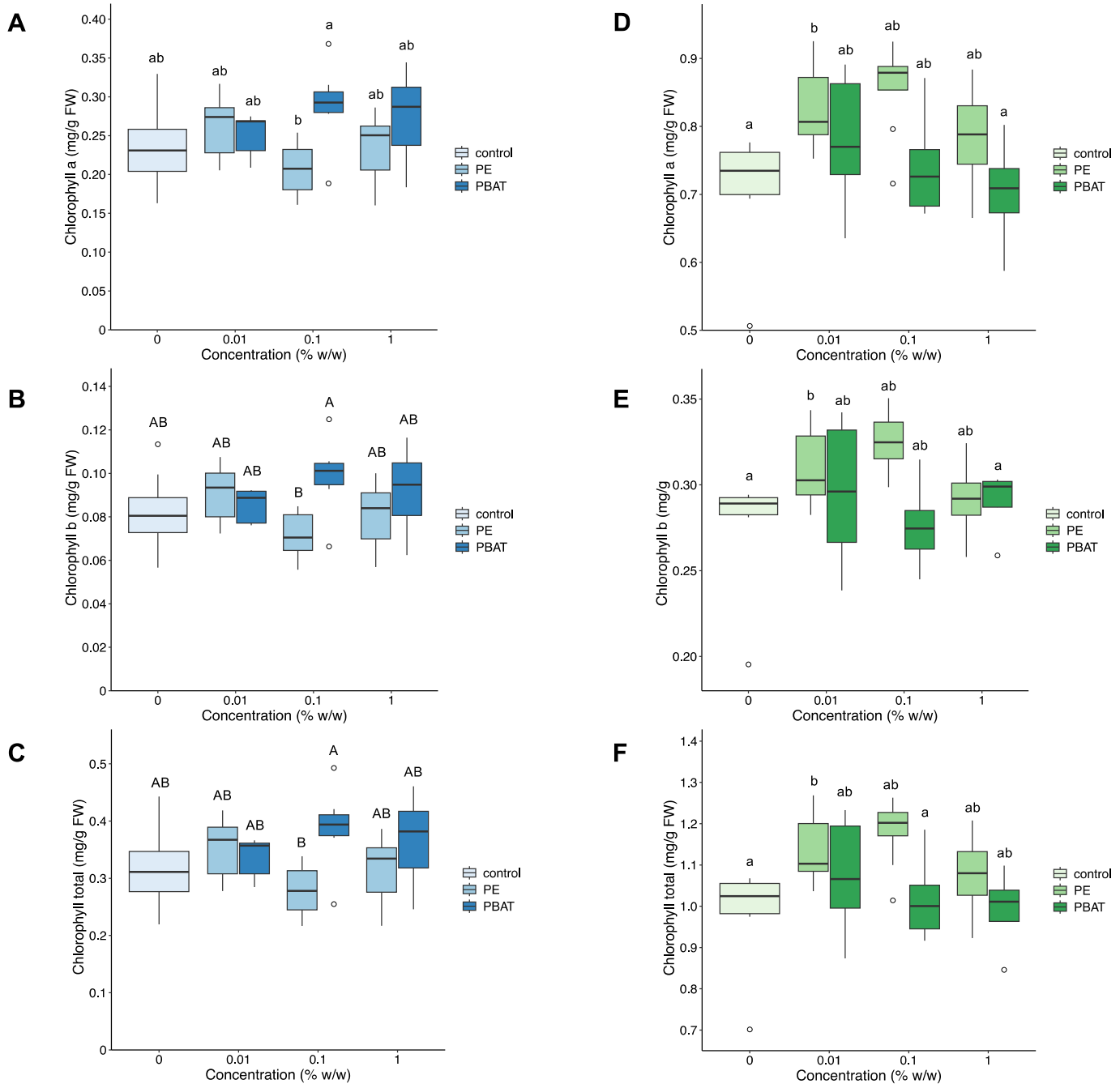

Fig. 3. The ***chlorophyll a*** [A: lettuce, D: barley], ***chlorophyll b*** [B: lettuce, E: barley] and ***total chlorophyll*** contents ($n = 8$) [C: lettuce, F: barley] in lettuce (*Lactuca sativa*) and barley (*Hordeum vulgare*) seedlings following exposure to low, medium and high conventional low linear density polyethylene (LLDPE) and biodegradable starch polybutylene adipate terephthalate (PBAT) blend leachate concentrations (0.01 %, 0.1 %, 1 % *w*/w). Measurements for barley seedlings were taken after 14 days, while lettuce seedlings were assessed after 21 days. Significant differences determined by one-way ANOVA are indicated with lowercase letters, whereas uppercase letters denote significant interactions between plastic type and concentration identified by two-way ANOVA; both analyses were followed by Tukey's post-hoc test ($p < 0.05$).

leaves. One seedling from each replicate was selected to determine the specific leaf area (SLA) before being dried at 60 °C for 24 h to obtain dry weight. The SLA was calculated based on the total leaf dry weight and total leaf area, with the leaf area measured using ImageJ (Version 1.53):

$$\textit{Specific leaf area}\left(\frac{cm^2}{mg}\right) = \frac{\textit{Total leaf area}\ (cm^2)}{\textit{Total leaf dry weight}\ (mg)} \quad (4)$$

If there was only one seed germinated in a replicate beaker, the specific leaf area measurement was chosen, and the analysis of the chlorophyll content was not taken.

3.5.2. Biochemical analyses

The remaining two seedlings were frozen and stored at −80 °C for biochemical analysis. Before starting the biochemical analysis, the lettuce shoots were ground with liquid nitrogen and plant material was split into portions for specific analysis (see below).

3.5.2.1. Chlorophyll analysis. Chlorophyll *a* and *b* concentrations were measured spectrophotometrically following the method described by [45]. For this, 100 mg of ground plant material was extracted with 1 mL of 100 % methanol. After centrifuging the mixture at 10,000 g for 5 min,

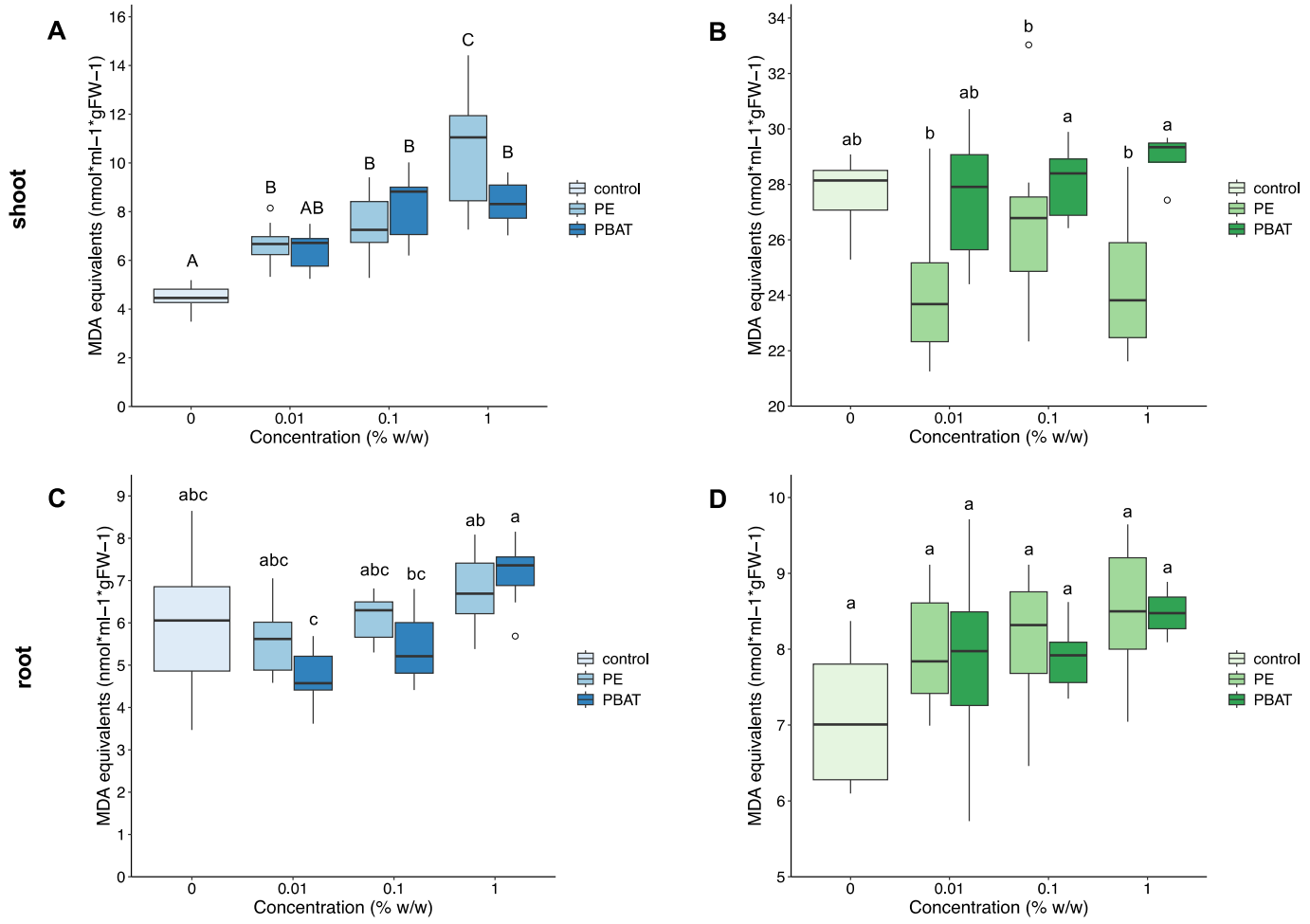

Fig. 4. The **lipid peroxidation as MDA** (malondialdehyde) **content** ($n = 8$) in the shoots [A: lettuce, B: barley], and roots [C: lettuce, D: barley] of lettuce (*Lactuca sativa*) and barley (*Hordeum vulgare*) seedlings following exposure to low, medium and high conventional low linear density polyethylene (LLDPE) and biodegradable starch polybutylene adipate terephthalate (PBAT) blend leachate concentrations (0.01 %, 0.1 %, 1 % *w*/w). Measurements for barley seedlings were taken after 14 days, while lettuce seedlings were assessed after 21 days. Significant differences determined by one-way ANOVA are indicated with lowercase letters, whereas uppercase letters denote significant interactions between plastic type and concentration identified by two-way ANOVA; both analyses were followed by Tukey's post-hoc test ($p < 0.05$).

the supernatant was collected, and the pellet underwent a second extraction. The absorbance of the combined supernatants was then read at 663 nm and 645 nm using a microplate reader (BMG Labtech, ClarioStar). Finally, the concentrations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll were calculated using the formulas provided by [45].

3.5.2.2. Lipid peroxidation. Lipid peroxidation was assessed using malondialdehyde (MDA) as a marker, following [46]. Approximately 0.25 g of ground plant material was mixed with 1 mL of 0.1 % trichloroacetic acid (TCA). After centrifugation, 0.5 mL of the supernatant was combined with 0.5 mL of 20 % TCA containing 0.5 % thiobarbituric acid (TBA). Control samples lacked TBA. The sample was heated at 95 °C for 30 min before being rapidly cooled in an ice bath. Absorbance readings were taken with a microplate reader (BMG Labtech, ClarioStar) at 532 nm, with adjustments made at 600 nm to account for non-specific absorption and at 440 nm to correct for sucrose interference. The results were reported as malondialdehyde (MDA) equivalents, expressed in nmol per gram of fresh weight (FW).

3.5.2.3. Total phenolic content. The total phenolic content (TPC) was measured according to the method outlined by [47]. To 0.3 g of ground plant material, 3 mL of 80 % methanol was added and incubated at room temperature for 1 h. After centrifugation (5 min, 10,000 g), 25 µL of the supernatant was mixed with 75 µL of distilled deionized water, followed by 25 µL of Folin-Ciocalteu reagent and incubation at room temperature for 6 min. Then, 0.1 mL of 7.5 % Na_2CO_3 was added, the mixture was mixed thoroughly, and samples were incubated at room temperature in the dark for 1.5 h. Absorbance was measured at 765 nm using a microplate reader (BMG Labtech, ClarioStar). Gallic acid served as the standard, and total phenolic content (TPC) was expressed as micrograms of gallic acid (GA) equivalents per gram of fresh weight (FW).

3.5.2.4. Salicylic acid and salicylic acid glucosides. Free salicylic acid (SA) and SA-glucosides (HSA) were quantified following the method of [48]. This measurement was only done for barley seedlings as there was not enough material available to perform this analysis on lettuce seedlings. Approximately 0.25 g of ground plant material was extracted with 1 mL of 70 % ethanol containing 32 µL of anisic acid (15.25 ng μL^{-1}). After centrifugation, the pellet was re-extracted with 1 mL of 90 % methanol. The combined supernatants were concentrated using a vacuum concentrator (Speed Vac, 173 2–18 Cdplus, Thermo Fisher), while the pellet was treated with 65 µL of 20 % trichloroacetic acid (TCA) and 650 µL of a 1:1 mixture of ethyl acetate and cyclohexane. Following centrifugation, the upper phase was collected, and the water phase was re-extracted. The upper phases were combined and evaporated to dryness in a vacuum concentrator. The residue was dissolved in 0.1 mL of

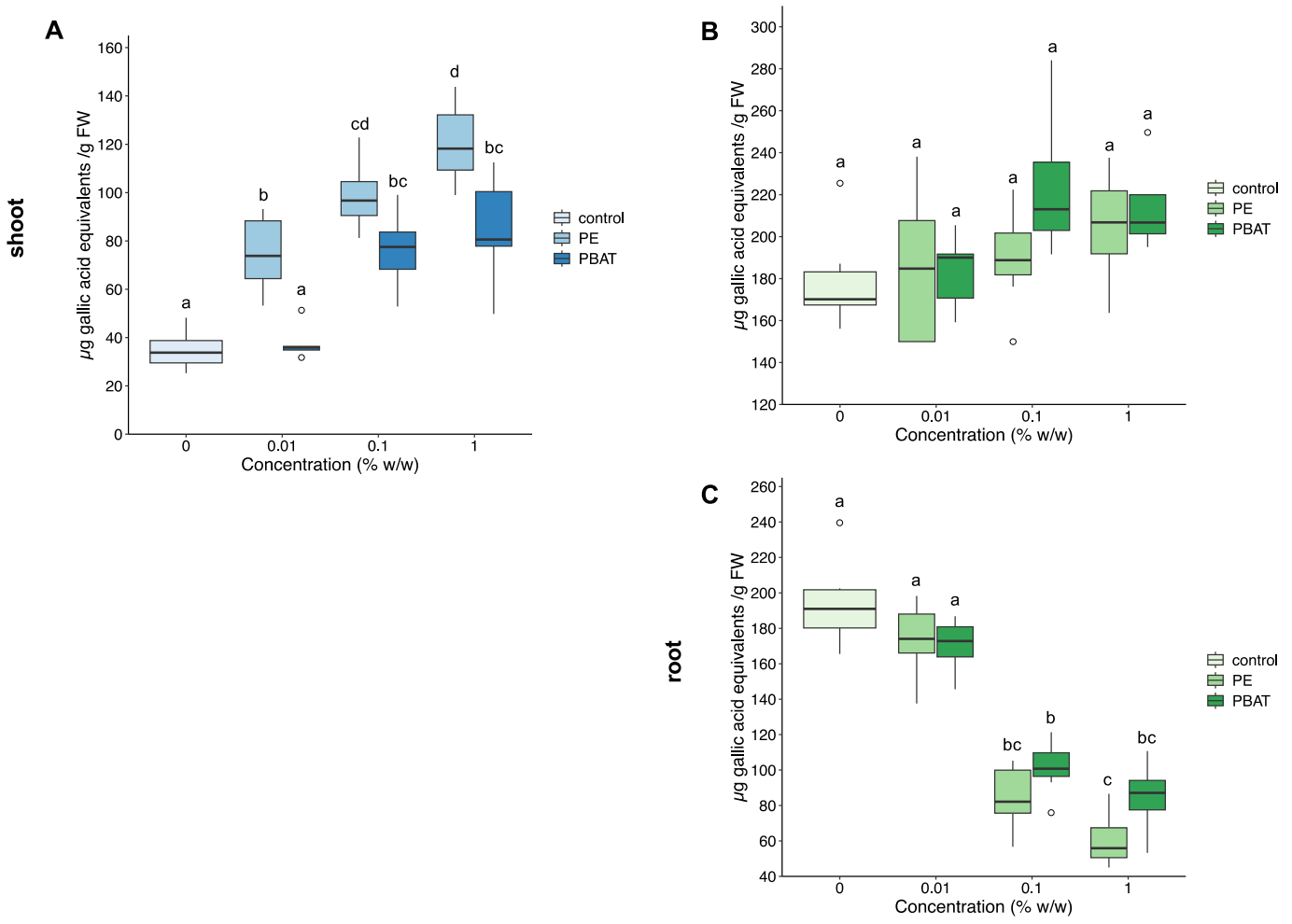

Fig. 5. The **total phenolic content** (TPC; $n = 8$) in the shoots [A: lettuce, B: barley] and roots [C: barley] of lettuce (*Lactuca sativa*) and barley (*Hordeum vulgare*) seedlings following exposure to low, medium and high conventional low linear density polyethylene (LLDPE) and biodegradable starch polybutylene adipate terephthalate (PBAT) blend leachate concentrations (0.01 %, 0.1 %, 1 % *w*/w). Measurements for barley seedlings were taken after 14 days, while lettuce seedlings were assessed after 21 days. Not enough root material was available from lettuce seedlings to assess the TPC content in lettuce roots. Significant differences determined by one-way ANOVA are indicated with lowercase letters, whereas uppercase letters denote significant interactions between plastic type and concentration identified by two-way ANOVA; both analyses were followed by Tukey's post-hoc test ($p < 0.05$).

10 % methanol with 0.1 % trifluoroacetic acid (TFA) to provide free SA for measurement. The aqueous phase was combined with 0.3 mL of 12 M HCl and incubated at 80 °C for one hour. Once cooled, 18 μL of anisic acid (15.25 ng μL^{-1}) was added, followed by two extractions with 0.9 mL of a 1:1 ethyl acetate and cyclohexane mixture. The combined organic layers were evaporated to dryness, and the resulting residue was reconstituted in 100 μL of 10 % methanol containing 0.1 % TFA to prepare the salicylic acid glucosides for analysis.

Concentrations of free SA and SA-glucosides were determined using HPLC (Arc HPLC Waters), with a C18 column (Phenomenex, 250 × 4.6 mm, 5 μm) eluted with methanol containing 0.1 % TFA, using a gradient from 10 %–82 % at a flow rate of 1 mL min^{-1} and a temperature of 30 °C. Fluorescence detection was performed with excitation at 305 nm and emission at 407 nm. SA and SA-glucoside concentrations were expressed as μg SA per gram of fresh weight (FW).

3.6. Statistical analysis

Statistical analysis was conducted using R Studio (Version 2023.03.0 + 386; R Core Team, 2023). Results are presented as means ± standard error (SE). Due to small sample size normality was assessed visually with Q-Q plot to ensure an ANOVA could be used. Homogeneity of variance were assessed with Levene's test and from residual plots. In the case for the lettuce shoot lipid peroxidation and barley shoot salicylic acid glucosides in the chronic experiment (which we indicated in Table S3 and in the text as appropriate) Levene's test showed heteroscedasticity, that might bias model estimates e.g. underestimate standard errors leading to incorrect *p*-values. Parametric tests were still used due to their greater statistical power compared to non-parametric tests, and their robustness [49], but when p-values were close to alpha, results should be interpreted with caution.

A two-way ANOVA using the *aov* function was initially performed to evaluate the interaction between plastic type and concentration. When a significant interaction was identified, a Tukey post hoc test was conducted to pinpoint differences between treatments. In cases where no significant interaction was detected, a one-way ANOVA ($\alpha = 0.05$) was carried out using a combined explanatory variable encompassing both plastic type and concentration. If this analysis revealed significant effects, a Tukey post hoc test (*TukeyHSD* function) was applied to determine specific treatment differences.

Further details on statistical analyses are provided in Table S3. All datasets will be made available on Zenodo (DOI: https://doi.org/10.5281/zenodo.15373391.). For boxplots, the lower and upper edges of the box correspond to the 25th and 75th percentiles respectively, defining the interquartile range. The line within the box indicates the median (50th percentile) of the data. The solid lines extending from the

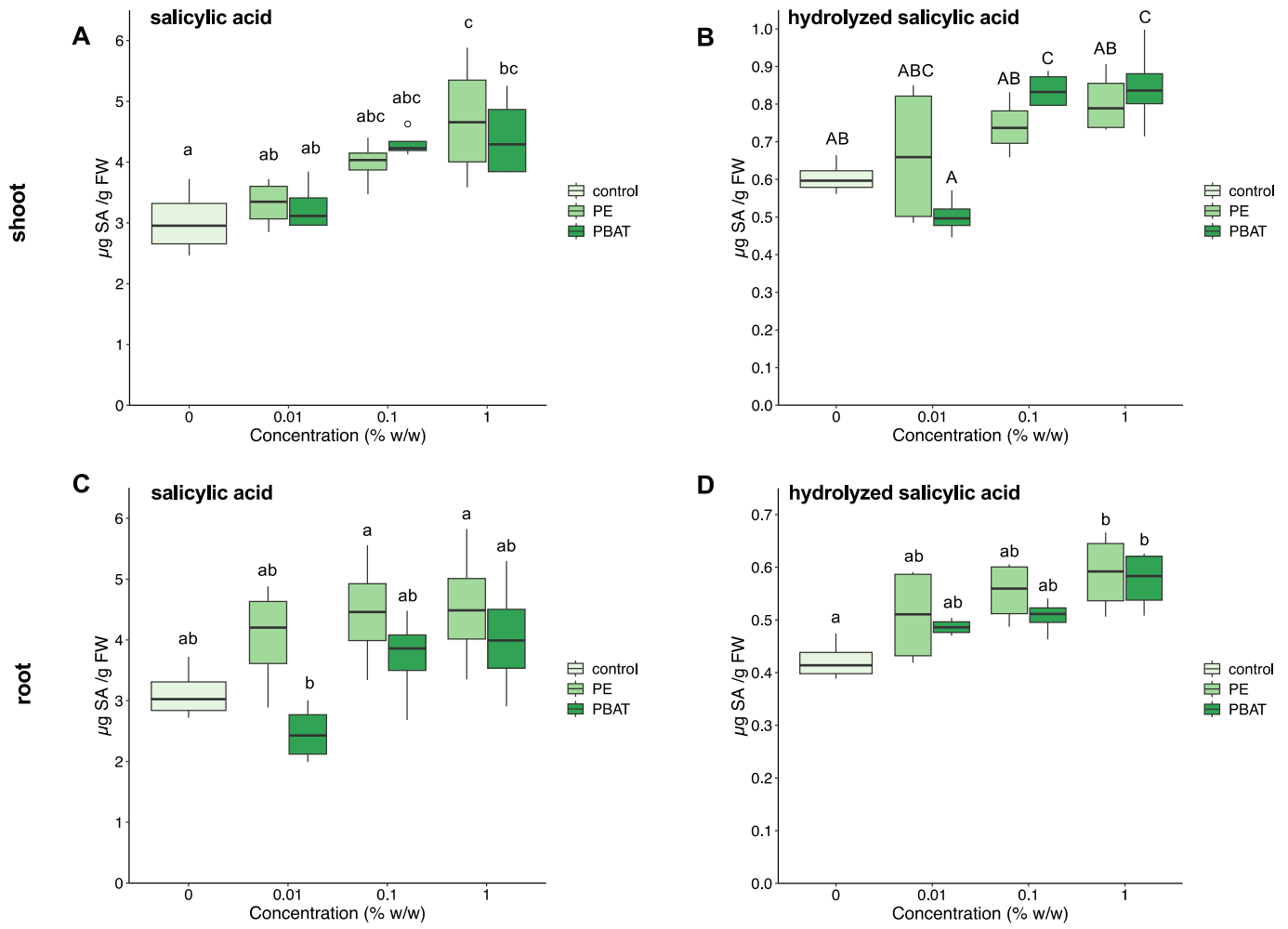

Fig. 6. The **content of salicylic acid** (SA; $n = 4$) in the shoots [A] and roots [B] and the salicylic acid glucosides (HSA; $n = 4$) in the shoots [B] and roots [D] of barley (*Hordeum vulgare*) seedlings after exposure to after exposure to low, medium and high conventional low linear density polyethylene (LLDPE) and biodegradable starch polybutylene adipate terephthalate (PBAT) blend leachate concentrations (0.01 %, 0.1 %, 1 % *w*/w). Measurements for barley seedlings were taken after 14 days. Not enough material was available to perform this analysis on lettuce seedlings. Significant differences determined by one-way ANOVA are indicated with lowercase letters, whereas uppercase letters denote significant interactions between plastic type and concentration identified by two-way ANOVA; both analyses were followed by Tukey's post-hoc test ($p < 0.05$).

box show the minimum and maximum values, excluding outliers, while points beyond these limits are identified as outliers.

4. Results

4.1. Chemical analyses

To assess the leachability of the chemicals from the starch-PBAT blend and LLDPE mulching films, NTS/SS using LC-HRMS was conducted. In total, 542 features were extracted in positive mode and 138 in negative mode from the starch-PBAT blend leachate, and 284 in positive mode and 36 in negative from the LLDPE leachate. This analysis considered a minimum intensity threshold of 3000 and the features were blank filtered (intensity 5× < control sample). Of the 680 features detected in the starch-PBAT blend leachate, 150 contained MS^2 spectra in positive mode and 81 in negative mode. In comparison, of the 320 features detected in the LLDPE leachates, 59 and 26 contained MS^2 spectra in positive and negative modes respectively. The resulting features were annotated with a spectral library (MassBank EU) and a suspect list (plastChem). In the ¼ Hoagland leachates, six compounds were tentatively identified in starch-PBAT blend and two in LLDPE, both in positive mode. Additionally, one compound was identified in negative mode in the starch-PBAT blend (Level 2a-3; Table S4-S5). The quality of the feature identification was based on the guidelines described by [50].

4.2. Experiment 1: acute exposure

For all four species, we found limited effects of the treatments on the early physical traits of the plants (Table 1 and Fig. 1). A significant interaction was observed between *plastic type* and *concentration* for the root length and bud wet weight of lettuce (Two-way ANOVA; $p = 0.045$ and $p = 0.036$, respectively). For the other endpoints and plant species, no interactive effect was noted.

No significant impact was recorded on the germination percentage or germination index for all four species (Table 1). The only endpoint affected was root length, which increased for barley ($p < 0.001$; Fig. 1) and lettuce ($p = 0.045$). The root length of barley seedlings exposed to the LLDPE leachate at the medium concentration was 15 % higher than the control (Fig. 1). No effects were found on the shoot and root length, and wet weight of wheat (Fig. 1). For the dicots, the root length of lettuce exposed to the high concentration of starch-PBAT blend leachate increased by 48 % ($p = 0.003$; Fig. 1). Although a significant interaction between concentration and plastic type was recorded for the wet weight of lettuce, no significant difference compared to control were observed (Table 1). For carrot no significant impact was found by the treatments compared to the control (Table 1; Fig. 1).

Table 3
Overview of research studies examining the effects of plastic leachates on plants; * leachate concentrations as understood from the cited papers. HDPE: high-density polyethylene; PP: polypropylene; PVC: polyvinyl chloride; PE: polyethylene; PO: polyolefin; PLA + PBAT: polylactic acid + polybutylene adipate terephthalate; PC: polycarbonate PBAT, polybutylene adipate terephthalate; LLDPE, low linear density polyethylene.

Plant species		Plastic tested	Experimental design	Leachate concentration (*w/w*) *	Leachate concentration (L/S ratio) *	Exposure time	Research observations	Source
Monocot	*Hordeum vulgare*	LLDPE, PBAT	Petri dish + filter paper	0.01 % 0.1 % 1 %	1000:1 100:1 10:1	4 days	Exposure to low LLDPE (0.01 % w/w) leachate increased root length of *Hordeum vulgare*. All other germination and growth parameters remained unaffected.	This study
			Soil substrate	0.01 % 0.1 % 1 %	1000:1 100:1 10:1	14 days	Exposure to increasing concentrations (low, medium, high) LLDPE and PBAT leachates did not affect growth parameters of *Hordeum vulgare*.	This study
	Triticum aestivum L.	LLDPE, PBAT	Petri dish + filter paper	0.01 % 0.1 % 1 %	1000:1 100:1 10:1	4 days	Exposure to increasing concentrations (low, medium, high) LLDPE and PBAT leachates did not affect germination or growth parameters of *Triticum aestivum* L.	This study
	Lolium multiflorum	HDPE (virgin)	Soil substrate	3 %	10:1	7 days	Exposure to new HDPE leachate significantly delayed and reduced germination, growth parameter (root and shoot length and fresh weight) of Italian raygrass.	[37]
	Allium cepa	HDPE, PP and HDPE + PP (virgin and aged)	Petri dish + filter paper	0.125 % 0.25 % 0.5 % 1 %	80:1 40:1 20:1 10:1	3 days	Exposure to naturally aged HDPE and PP leachates and the leachate of both plastics combined led to a reduction of the germination percentage, whereas the leachate from the virgin MPs showed no difference.	[26]
	Thinopyrum junceum	HDPE, PP and HDPE + PP (virgin)	Sand substrate	0.125 % 0.25 % 0.5 % 1 %	80:1 40:1 20:1 10:1	5 days	Exposure to leachate of virgin PP (0.1 %) slightly affected the radicle length (reduction).	[26]
	Triticum aestivum L.	PVC	Hydroponic conditions	0.1 %	100:1	2, 4.5 days	Exposure to photoaged PVC leachate decreased growth parameters (root dry weight, root length and root diameter), plant nutrients and root activity and increases oxidative stress responses significantly.	[32]
Dicot	*Daucus carota*	LLDPE, PBAT	Petri dish + filter paper	0.01 % 0.1 % 1 %	1000:1 100:1 10:1	8 days	Exposure to increasing concentrations (low, medium, high) LLDPE and PBAT leachates did not affect germination and growth parameters of *Daucus carota*.	This study
	Lactuca sativa L.	LLDPE, PBAT	Petri dish + filter paper	0.01 % 0.1 % 1 %	1000:1 100:1 10:1	4 days	Exposure to high PBAT (1 % *w/w*) leachate increased root length of *Lactuca sativa* L. All other germination and growth parameters remained unaffected.	This study
			Soil substrate	0.01 % 0.1 % 1 %	1000:1 100:1 10:1	21 days	Exposure to medium PBAT (0.1 % w/w) leachate increased root length of *Lactuca sativa* L. Exposure to low LLDPE (0.01 % w/w) leachate decreased shoot fresh weight. All other growth parameters remained unaffected.	This study
	Gossypium (XJ001 and XJ003)	PE, PO, PLA + PBAT	Sand substate	1 %	100:1	7 days	Exposure to leachate of PLA + PBAT (Bio-M) affected (increase) mean germination time (MGT) and affected (increase) the shoot height for XJ001. Besides that, most indicators were unaffected.	[33]
	L. sativum	HDPE, PP (aged and virgin)	Petri dish + filter paper	0.125 % 0.25 % 0.5 % 1 %	80:1 40:1 20:1 10:1	3 days	Exposure to leachate of virgin and naturally aged HDPE and PP slightly affected germination percentage.	[26]
		PC (virgin and artificially aged)	Petri dish + filter paper	1 % 0.5 % 0.1 %	100:1 50:1 10:1	7 days	Exposure to virgin and aged PC leachate inhibited germination. Aged PC leachate decreased the germination less than new PC leachate.	[27]
			Soil substrate	1 % 0.5 % 0.1 %	100:1 50:1 10:1	7 days	Exposure to virgin and aged PC leachate inhibited germination. Aged PC leachate decreased the germination less than new PC leachate. The effects in the substrate system exhibit in general a lower decrease of the germination compared to substrate free exposures.	[27]

4.3. Experiment 2: chronic exposure

4.3.1. Growth parameters

We found an interaction between *plastic type* and *concentration* for shoot fresh weight ($p = 0.041$), root length ($p < 0.001$), leaf area ($p = 0.004$), total dry weight ($p = 0.007$) and SLA ($p = 0.027$) for barley and number of leaves ($p = 0.019$) and root length ($p < 0.001$) for lettuce. Similar to the acute exposure test, limited statistically significant differences to control were observed (Table 2, Fig. 2). In lettuce, both shoot fresh weight and root length were affected. A significant reduction in shoot fresh weight was observed ($p = 0.037$) when treated with the LLDPE low concentration 0.01 % w/w (Fig. 2A), with a difference in mean of −45 % compared to control. For the shoot fresh weight, the mean differs from the control between −10 % (LLDPE 1 %) to −45 % (LLDPE 0.01 %), which also showed the significant difference. As already observed in the acute exposure, the root length of lettuce plants increased with the medium starch-PBAT blend concentration ($p = 0.008$; Fig. 2C). The mean root length increased compared to the control by 66 % for the medium starch-PBAT blend concentration (Fig. 2C).

The shoot fresh weight of barley seedlings was not significantly affected (Fig. 2B). No significant effects in the treatments were observed compared to the control for barley seedlings, except for root length. For both low ($p < 0.001$) and medium ($p < 0.001$) LLDPE concentrations, an increase in root length of barley seedlings were observed (Fig. 2D). Differences in mean are ranging for the conventional plastic between 31 % decrease (LLDPE 1 %) to 60 % increase (LLDPE 0.01 %).

Comparing the acute and the chronic exposure patterns, the root length was consistently affected. Lettuce root length showed an increase in mean for most treatments in the acute exposure and a decrease for some treatments, especially starch-PBAT blend, in the chronic exposure. For barley, an increase in mean was found for nearly all treatments during both acute and chronic exposures.

4.3.2. Biochemical analyses

We found a clear interactive effect between plastic type and exposure concentration (two-way ANOVA, $p < 0.001$) for four endpoints: salicylic acid in barley shoots, chlorophyll *b* and total chlorophyll content in lettuce shoots as well as lipid peroxidation. For lettuce, we observe no significant effect after exposure to all concentrations of LLDPE and starch-PBAT blend leachate concentrations for chlorophyll *a* (Fig. 3A), chlorophyll *b* (Fig. 3B) and total chlorophyll content (Fig. 3C). For barley, we also observed no significant effect on the chlorophyll content, except the low concentration of LLDPE. Chlorophyll *a* (Fig. 3D, $p = 0.01$), chlorophyll *b* (Fig. 3E, $p = 0.02$) and total chlorophyll content (Fig. 3F, $p = 0.01$) was increased by 18 %, 13 % and 16 % respectively after exposure to 0.01 % w/w LLDPE leachate.

Within lettuce shoots, a clear increasing trend is observed in the level of lipid peroxidation ($p = 0.018$, variance assumption violated; Fig. 4A), while in the roots no significant effect was found after exposure to both leachates (Fig. 4C). Low, medium and high LLDPE leachate concentrations increased the MDA content by 48 % ($p = 0.035$), 66 % ($p < 0.001$) and 137 % ($p < 0.001$) respectively. For the starch-PBAT blend leachate, only the medium ($p < 0.001$) and high ($p < 0.001$) concentrations increase the MDA content. For both barley shoots (Fig. 4B) and roots (Fig. 4D) no significant effect was determined on the lipid peroxidation by both leachate types.

The total phenolic content (TPC) in lettuce shoots was significantly increased by both leachate types ($p < 0.001$, Fig. 5A). LLDPE increased the TPC by 111 % ($p < 0.001$), 179 % ($p < 0.001$) and 241 % ($p < 0.001$) at low, medium and high concentrations respectively. For starch-PBAT blend, only the medium ($p < 0.001$) and high ($p < 0.001$) concentrations increased the TPC in lettuce shoots by 116 % and 143 % respectively. On the other hand, we observed no significant effects of both leachate types on the TPC of barley seedlings (Fig. 5B). Nevertheless, the roots of barley seemed to be affected in terms of the TPC after exposure to both leachates (Fig. 5C). For LLDPE, we observed a decrease for the medium and high of 57 % ($p < 0.001$) and 69 % ($p < 0.001$), as well as for the medium and high concentrations of starch-PBAT blend leachate reduced by 48 % ($p < 0.001$) and 57 % ($p < 0.001$) respectively.

The salicylic acid (SA) was only increased by the highest concentrations of LLDPE ($p = 0.009$) and starch-PBAT blend ($p = 0.04$, Fig. 6A) in the shoots of barley. We also observed that the medium and high concentration of starch-PBAT blend leachate increased the hydrolyzed form of SA (HSA) by 38 % ($p = 0.037$) and 40 % ($p = 0.028$) respectively (Fig. 6B) in the shoots of barley. Within barley roots, the SA was not affected by the leachates (Fig. 6C), while the HSA was increased for both leachate types at 1 % w/w ($p = 0.009$, Fig. 6D). LLDPE increased the HSA by 40 % ($p = 0.009$) and starch-PBAT blend by 36 % ($p = 0.019$).

5. Discussion

In this study, the effect of chemical leachates of conventional (LLDPE) and biodegradable (starch-PBAT blend) plastics on four crop species were investigated. Our results indicate that chemicals from both plastic types are leaching and inducing effects on growth parameters and cause a stress response in plants. Root length appeared to be a sensitive endpoint, as significant increases were detected for lettuce and barley under both acute and chronic exposure at environmentally relevant concentrations; however, these effects were not consistent across all treatments. Consistent and clear signs of stress were also observed in both lettuce and barley when exposed to both types of leachates, with dose-dependent changes in lipid peroxidation, total phenolic content, salicylic acid (SA) and SA-glucosides.

We found chemicals in the leachates associated with both conventional as well as biodegradable plastics, providing evidence that organic chemicals migrate from the starch-PBAT blend and LLDPE polymers into the leachates. Surprisingly, more chemical features and tentatively identified compounds were detected in the starch-PBAT blend leachates than in the LLDPE leachates. One possible explanation for this difference is that the starch-PBAT blend has a more complex formulation, containing a broader range or higher amounts of organic additives [51,52]. Additionally, PBAT's biodegradable nature may facilitate a faster or more extensive release of chemicals during leaching. For example, PBAT degrades more rapidly than PE; however, during this process it can release specific compounds that may be toxic to plants [53].

We did find clear effects of the leachates on plants. We found an increase in root length of both lettuce and barley seedlings, which differs from our earlier work, in which we tested the effects of starch-PBAT blend and LLDPE *particles* on root growth using an identical setup [24]. There we found that these plastic particles (the same material as tested in this study) caused a decrease in the root length for LLDPE 0.01 %, starch-PBAT blend 0.01 % and 0.1 % w/w. Interestingly, [32] also observed different responses when comparing the toxicity of *particles* and *leachates*, with a higher toxicity of PVC leachate compared to the particles, especially showing adverse effects on root characteristics. While particles may physically interfere with root development, leachates primarily introduce dissolved organic compounds that can act as chemical stressors or even growth stimulants at low concentrations (a phenomenon known as hormesis) [54,55]. Some of these organic compounds might transiently stimulate root elongation as part of a stress adaptation response. During chronic exposure, we saw some effects on the root length and shoot fresh weight of lettuce (although not a very consistent response pattern), while barley remained unaffected. This species-specific response may be attributed to physiological and structural differences between lettuce and barley, which was also observed by [56] or [57]. For instance, their root systems differ. Monocots usually develop a fibrous root system, which consists of many thin roots spreading out from the base of the plant [58], while dicots, on the other hand, form a taproot system, characterized by a main root that grows deep into the soil with smaller lateral roots branching off [59]. Although limited effects on growth parameters were found, we did observe consistent changes in the plant's defence mechanisms. Plants exhibit

clear and consistent signs of stress when exposed to plastic particles and their leachates, and this occurs at environmentally relevant exposure levels [17,60,61].

As the effects of leachates on plant health are poorly understood, we conducted a comparative analysis and summarized current research on their impact on plant growth (Table 3). Across the literature, leachates are consistently associated with adverse effects on plant development, most often reflected in reduced germination and root length in species such as *Lolium multiflorum*, *Thinopyrum junceum*, and *Triticum aestivum* [26,32,37]. However, the extent of these impacts varies with plastic type. For instance, while [33] reported limited effects of conventional plastic leachates, the biodegradable PLA + PBAT blend led to significant reductions in cotton germination and shoot height (Table 3). This contrast highlights that leachates from biodegradable plastics may, in some cases, pose equal or even greater risks than those from conventional plastics. Yet, the combined effects of the diverse suite of chemicals released from plastics remain largely unexplored, as does the role of polymer composition and degradation pathways. Considering that more than 16,000 chemicals are potentially used in plastics, including over 4200 substances of concern [62], advancing comparative research between conventional and biodegradable plastic leachates is essential to better understand and predict their impacts on plant health.

The clear stress response observed in plants when exposed to *leachates* or *plastic particles* (Table 3, [54] does not always translate into higher level responses, such as impaired growth, as was seen in our study. However, plants are often exposed to multiple stressors at once, for example those related to climate change (e.g., drought, flooding, extreme temperatures) or environmental pollution (e.g., pesticides, metals). It is known that stressors interact in ways that can amplify or mitigate their individual effects, creating unique responses that differ from those seen under single-stressor conditions [63]. The combined impact of these stressors could therefore result in reduced crop resilience and ultimately lower yields [64–66]. So far, only a limited number of studies have explored the combined effects of MPs and other anthropogenic stressors. For example, the co-exposure of MPs and drought on rice resulted in more severe effects compared to single stressors [67], while the co-occurrence of heat waves and MPs reduced rice yield and disturbed the N-cycle [68]. Given that plants show a clear stress-response, there is an urgent need to investigate these cumulative stress-scenarios in more detail.

This study addresses an important gap of our understanding of the effects of plastic leachates on plants, yet we encourage future studies to focus on the following aspects to better understand impacts under field conditions. First, although artificially aged plastic samples were used to generate leachates under controlled conditions, this approach does not fully replicate the natural degradation of mulch films in farmland, where prolonged UV exposure, microbial activity, and variable weather influence breakdown. Future studies can use more realistic aging protocols including different degradation mechanisms or interactions with microorganisms. Second, residual NPs could persist in the leachates, potentially biasing experimental outcomes and confounding the interpretation of chemical-specific effects. However, the particles used in this study were in the size range of 50 and 1000+ μm [36], making formation of NPs unlikely, but future studies should verify this. Third, while non-target screening identified a variety of leachate components, the specific compounds responsible for inducing plant stress responses remain unresolved, limiting the mechanistic understanding of observed effects. This gap could be addressed through more targeted studies on the identified compounds. For example, a Toxicity Identification Evaluation (TIE) approach, which combines chemical fractionation with bioassays to link effects to causative agents, may help resolve this [69]. Finally, the study focuses mainly on acute or chronic exposures, whereas agricultural plastic pollution is inherently cumulative. Longer term experiments are needed to understand long-term exposure to these particles, for example mesocosm or field studies [70,71].

To conclude, this study investigates the effect of plastic chemicals and break down products from conventional and biodegradable plastic mulch commonly used in agriculture on the early development, plant growth and biochemistry responses of crops. We found chemicals in the leachates from conventional as well as biodegradable plastics, and a clear and consistent stress response in both lettuce and barley due to these leachates. This highlights the urgent need to better understand the impacts of chemicals leaching of conventional and biodegradable plastics in the environment, and to investigate stress-on-stress responses in plants.

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CRediT authorship contribution statement

Laura J. Zantis: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Sophie Straetemans:** Writing – original draft, Methodology, Investigation, Conceptualization. **Sylwia Adamczyk:** Writing – review & editing, Methodology, Investigation. **Bartosz Adamczyk:** Writing – review & editing, Methodology, Investigation. **Sannakajsa Velmala:** Writing – review & editing, Resources, Project administration, Funding acquisition, Formal analysis. **Sicco Brandsma:** Writing – review & editing, Methodology, Investigation. **Maria Margalef:** Writing – review & editing, Methodology, Investigation. **Thijs Bosker:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Laura Julia Zantis reports financial support was provided by Horizon Europe. Thijs Bosker reports financial support was provided by Horizon Europe. Sylwia Adamczyk reports financial support was provided by Horizon Europe. Bartosz Adamczyk reports financial support was provided by Horizon Europe. Sannakajsa Velmala reports financial support was provided by Horizon Europe. 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.enceco.2025.10.015.

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