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Biodegradable microplastics induce profound changes in lettuce (*Lactuca sativa*) defense mechanisms and to some extent deteriorate growth traits☆

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ABSTRACT

The development of agricultural technologies has intensified the use of plastic in this sector. Products of plastic degradation, such as microplastics (MPs), potentially threaten living organisms, biodiversity and agricultural ecosystem functioning. Thus, biodegradable plastic materials have been introduced to agriculture. However, the effects of biodegradable plastic substitutes on soil ecosystems are even less known than those of traditional ones. Here, we studied the effects of environmentally relevant concentrations of MPs prepared from a biodegradable plastic (a starch-polybutylene adipate terephthalate blend, PBAT-BD-MPs) on the growth and defense mechanisms of lettuce (*Lactuca sativa*) in CLIMECS system (CLImatic Manipulation of ECosystem Samples). PBAT-BD-MPs in the highest concentrations negatively affected some traits of growth, i.e., dry weight percentage, specific leaf area, and both C and N contents. We observed more profound changes in plant physiology and biochemistry, as PBAT-BD-MPs decreased chlorophyll content and triggered a concerted response of plant defense mechanisms against oxidative stress. In conclusion, exposure to PBAT-BD-MPs induced plant oxidative stress and activated plant defense mechanisms, leading to oxidative homeostasis that sustained plant growth and functioning. Our study highlights the need for in-depth understanding of the effect of bioplastics on plants.

1. Introduction

For decades, plastics have been seen as biochemically inert to the environment and organisms due to their chemical structure and large molecular size (Teuten et al., 2009). However, under the action of environmental factors, plastic debris may break down into smaller fragments such as micro- and nanoplastics (1 μm-5 mm and <100 nm, respectively) (Helmberger et al., 2020; Hurley et al., 2020). Due to their common use in agriculture, microplastic (MP) concentrations in soils are high, reaching 40 mg kg^{-1} (0.004 %) of conventional microplastics and in excess of 300 mg kg^{-1} (0.03 %) of mesoplastics in soil treated with mulching films (Li et al., 2020). MPs found in agricultural soil originate, among other sources, from the degradation of agricultural plastics (especially mulching films) and contaminated sewage sludge and biosolid applications (Sajjad et al., 2022), however, wastewater irrigation, pesticides, and atmospheric deposition also contribute to increasing soil MP concentrations (Nizzetto et al., 2016; Zantis et al., 2023a).

The potential effects of non-biodegradable MPs on plants include impaired nutrient uptake, alterations in plant root traits (Wang et al., 2020) and seed germination (Hassan et al., 2022). MPs may also cause oxidative stress in plant cells, manifested by an overproduction of reactive oxygen species (ROS), e.g. superoxide anion radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), leading to damage in cell structures, including oxidative deterioration of lipids (lipid peroxidation) (Jia et al., 2023; Zantis et al., 2023a). Plant defense mechanisms may respond to MPs with elevated activities of ROS-scavenging enzymes (superoxide dismutase scavenging $O_2^{\bullet-}$ and catalase scavenging H_2O_2), and increased concentrations of non-enzymatic antioxidants, i.e., phenolics and the stress-related hormone salicylic acid (Jia et al., 2023).

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In recent years, biodegradable materials have been introduced as a substitute for conventional non-biodegradable materials in agricultural plastics, especially in the manufacturing of mulching films. This solution provides an economic benefit for farmers, overcoming the costs of end-of-life and waste management (Hurley et al., 2020). A biodegradable material commonly used in mulching applications is polybutylene adipate terephthalate (PBAT) blended with biobased materials (such as starch or cellulose) (Fu et al., 2020). Another example of biodegradable plastic is polylactic acid (PLA) (Huerta-Lwanga et al., 2021; Yang et al., 2021). PBAT and PLA blends are degradable in composting conditions and to some extent in soil. During degradation, biodegradable plastics break down, forming biodegradable MPs (BD-MPs) that may progressively accumulate in soils if the application rates exceed the rate of their degradation. Recent study provided first BD-MPs (PBAT and PLA-based) soil concentrations at the level of up to 500 particles per kg after 2 years of application to soil (Li et al., 2023). However, almost no data on the concentrations of BD-MPs in agricultural soils exists, and their effects on plant physiology and biochemistry are less studied than those of synthetic MPs (Fan et al., 2022).

The limited data available on the effects of BD-MPs on plant growth yields fragmentary and sometimes inconsistent knowledge. Based on available literature, plant response to BD-MPs varies, with some studies showing positive, and others showing negative or no significant effect on plant performance in concentration above 1 % (Chah et al., 2022). For example (Kanwal et al., 2022), showed no toxic effect of degradation products of PBAT on the growth of Chinese cabbage (*Brasicca rapa*), and (Palsikowski et al., 2018) did not detect any phytotoxic, cytotoxic, or genotoxic effects of PLA and PBAT on meristematic cells of onion (*Allium cepa*). However, 1 % PBAT-BD-MPs affected rice (*Oryza sativa*) and buck-horn plantain (*Plantago coronopus*) growth negatively, but not as much as conventional MPs (Courtene-Jones et al., 2024; Irshad et al., 2024; Yang and Gao, 2022). The mode of action of BD-MPs seems to be similar to that of conventional ones, which includes oxidative stress and negative effects on photosynthesis via a decrease in chlorophyll content (Sun et al., 2023; Yang and Gao, 2022). Inconsistency among the results from different studies may derive from BD-MP dosages, differences in bioplastic physicochemical properties, experimental designs and conditions, and species-specific effects on plants, as also shown for conventional MPs (Zantis et al., 2023b). Moreover, we are lacking a holistic view on the effect of MPs and especially their biodegradable substitutes on plant health and crop yield under relevant more natural conditions, as studies concentrate mainly on laboratory conditions. Studies of the effect of PBAT-BD-MPs on plants are rare (just 44 reported articles, Table S1) and often they do not include multiple plant stress endpoints (just 7 reported articles, Table S1). Thus, experiments that resemble realistic, multitrophic environment are needed to disentangle the effect of PBAT-BD-MPs on plant performance and health.

In this study we used CLIMECS (CLImatic Manipulation of ECosystem Samples) setup as a model of terrestrial ecosystems containing plants (lettuce, *Lactuca sativa*), natural soil, soil microorganisms, and soil invertebrates (including two earthworm species and three springtail species), aims to elucidate how PBAT-BD-MPs affect plants under environmentally relevant conditions. Lettuce was chosen as it is often cultivated with the use of mulch and this species is commonly used in ecotoxicity experiments. The soil of the CLIMECS system was spiked with environmentally relevant concentrations of PBAT-BD-MPs, ranging from 0.025 % to 0.8 % (w/w) of soil dry mass. This range contains the expected level of MPs originating from mulching films degrading on the soil surface and being diluted in the top few cm of the soil (Hurley et al., 2020), including conditions of repeated continuous applications (yearly or multiple times per year). At three harvesting timepoints (4, 8, and 11 weeks), lettuce growth traits and biochemical traits were measured Overall this study adds new knowledge on understudied effect of biodegradable microplastic-induced impacts on plant growth and defense mechanisms.

2. Materials and methods

2.1. CLIMECS system

Cylindrical soil columns measuring 12 cm in depth and 16.6 cm in diameter were constructed and put into the CLIMECS system (CLImatic Manipulation of ECosystem Samples, CLIMECS, Amsterdam, the Netherlands, Ligtelijn et al., 2024) (see Fig. S1 for picture of the CLIMECS). Four concentrations of PBAT-BD-MPs were mixed in with the soil, 0.025 %, 0.05 %, 0.2 %, and 0.8 % (w/w), and for each treatment, including control, we prepared eight replicates. Control did not obtain PBAT-BD-MPs. The columns (in the sum 40) were prepared by filling HDPE cylinders placed on a bottom plate with PBAT-BD-MP-spiked or control soil. The columns were tapped on the ground five times to allow soil compaction, to increase comparability to natural soil structure. After preparing the soil columns, they were inserted into the CLIMECS system and left for two weeks before the introduction of the animals and lettuce, to allow for stabilization of the soil's natural microbiome.

2.2. Test soil and PBAT-BD-MPs

Lufa 2.2 standard natural soil (Lufa Speyer, Germany) was dried for 48 h at 40 °C after arrival. Lufa 2.2 soil is a sandy loam soil (soil type USDA). According to the supplier, Lufa 2.2 soil has approximately (mean ± sd) 1.8 ± 0.6 % organic carbon, 0.19 (±0.05) % N, cation exchange capacity of 9.5 (±1.3) meq/100 g, a pH (0.01 M $CaCl_2$) of 5.6 ± 0.3, and a water holding capacity (WHC) of 43 ± 5 %. During the experiment, soil water content was maintained at 22 %, corresponding to 50 % WHC, by watering twice a week with 20 mL of demineralized water, as well as bringing the columns back to their original weight once every two weeks.

BD-MPs utilized in this study were obtained from shredding and cryogenically grinding a commercially available PBAT-starch blend based mulching film. The films were first shredded using a benchtop shredder (SHR3D IT, 3DEVO, The Netherlands). Cryogenic grinding was performed using a laboratory-scale 6875 Freezer/Mill High Capacity Cryogenic Grinder (SPEX Sample Prep, USA) at the Norwegian Institute for Water Research. The grinded particles were sieved and adjusted to provide a size distribution that corresponds with field observations of starch-PBAT blend mulching films in real agricultural soils (Hurley et al., 2024). The mean particle size was 131 μm (±161 μm s.d.) and the D50 was 67.0 μm (±225 μm m.a.d.). The size distribution of PBAT-BD-MPs used in this study can be found in Supplementary Table 2, including estimations of the corresponding mass (based on a measured film thickness of 15 μm). The particles were made using a virgin film and were not subject to weathering prior to the generation of test materials. This is due to limitations in homogenously ageing film materials and the physical dimension restrictions of ageing chambers versus producing sufficient amount of test materials (Hurley et al., 2024).

PBAT-BD-MPs were mixed in with the soil using a concrete mixer (PROMISCHER PM145L) at 24–29 rpm. PBAT-BD-MPs and the dry soil were mixed for 5 min, after which water was added in batches to bring the soil moisture content to 50 % WHC, but not exceeding this to prevent puddle formation, which would potentially cause PBAT-BD-MPs to float to the surface and coagulate.

2.3. Plants

Lettuce (*Lactuca sativa* L., Zwart Duits) and cress (*Lepidium sativum*) seeds were obtained from Dutch Garden Seeds (Volendam, the Netherlands). Four patches of 100 cress seeds were sown as shelter for the springtails, one in each quarter of the column, four days prior to the introduction of the animals (t = 0), leaving a 2 cm wide clearing between the patches for the lettuce seedlings. Lettuce was sown in pressed peat germination trays (Tuinplus, Heerenveen, the Netherlands), 4 × 4 × 4 cm wide, containing the same experimental soil as used to prepare the CLIMECS system. Shortly before the introduction of the animals, five patches of three lettuce seedlings were transferred to the soil column, to provide enough material to sample at different timepoints.

2.4. Earthworms and springtails

Earthworms, *Aporrectodea caliginosa* and *Lumbricus rubellus* were obtained from Prodigga (Caumont-sur-Durance, France) and Lasebo (Nijkerkerveen, the Netherlands), respectively. In total, four earthworms were added to each soil column: one adult and one subadult individual of each species. Three species of springtails were used in the CLIMECS experiment: *Sinella curviseta*, *Heteromurus nitidus*, and *Protaphorura fimata*. All species were taken from cultures kept at the Vrije Universiteit Amsterdam. Cultures of all species were kept on moist plaster of Paris, containing activated charcoal in a 10:1 ratio, and fed with dry baker's yeast weekly. Cultures were kept in climate rooms at 75 % relative humidity and a 16:8 light-dark cycle. *S. curviseta* was kept at 20 °C, *H. nitidus* and *P. fimata* at 16 °C. For each replicate CLIMECS, 125 adult and 125 subadult individuals of each species were collected from the cultures by hand using an aspirator. Adults were separated from subadults by sieving, using a 630 μm (*S. curviseta* and *H. nitidus*) or 450 μm mesh width (*P. fimata*).

2.5. Experimental conditions

The CLIMECS were kept at a light-dark regime of 14:10 h. The soil temperature was set at 15 °C, with a set daytime heating temperature of 18 °C, meaning the air column above the soil was heated by light, until 18 °C at a soil depth of 0.5 cm was reached. A set of four temperature sensors (DS28EA00U, Maxim Integrated, 1/16 °C resolution and 0.5 °C accuracy) monitored the temperature in the soil. Once this temperature was reached, the heating halogen light was switched off, but LEDs were used to maintain proper lighting.

Once the earthworms and springtails were introduced, all soil columns were monitored for an hour to ensure earthworms were alive and well and dug into the soil. If no burrowing activity was observed of an individual earthworm, it was replaced with one from a spare Petri-dish.

Two weeks after the introduction of the animals, all cress was cut down to allow the lettuce to develop. At week 4, 8 and 11, lettuce samples were taken for analysis of shoot length, number of leaves, dry weight, and biomarker analysis.

2.6. Seedling sampling and measuring growth traits

During each sampling (4, 8, 11 weeks), two replicate plants were taken from each column, and shoot length, shoot fresh weight, and the number of leaves were measured. During the final sampling (week 12), two seedlings were left inside the column. One plant was then used to determine the specific leaf area (SLA, equation [1]) and the necrotic tissue (equation [2]) of the leaves and then dried for 24 h at 60 °C to measure dry weight and C and N content with an elemental CN analyzer (LECO, Michigan, USA); analysis was based on ISO 13878 standard. Pictures of the leaves were taken using the camera of an iPhone 13 (Apple, iOS 15, dual 12 MP camera system with $f/1.6$ aperture) and the total leaf area for the SLA was determined using ImageJ (version 1.53t). The second plant was stored at −80 °C for biochemical analyses. Before analyses, leaves of plants were grounded with liquid N and plant material was divided into specific analyses (see below).

$$Specific\ leaf\ area\left(\frac{cm^2}{g}\right)=\frac{Total\ leaf\ area\ (cm^2)}{Total\ leaf\ dry\ weight\ (g)} \qquad [1]$$

$$Necrotic\ tissue\ (\%)=\frac{Necrotic\ changes\ area\ (cm^2)*100\%}{Total\ area\ (cm2)} \qquad [2]$$

2.7. Chlorophyll content

Chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) concentrations were measured spectrophotometrically (Warren, 2008). To 100 mg of grounded lettuce leaves 1 mL of 100 % methanol was added. Following centrifugation (5 min, 10 000 g) the supernatant was collected, and the pellet was re-extracted with 1 mL of methanol. Combined supernatants (2 mL) were used to measure the absorbance at 663 nm and 645 nm using a microplate reader (BMG Labtech, ClarioStar). Chlorophyll *a*, *b* and total chlorophyll were calculated using formulas from Warren (2008).

2.8. Activity of superoxide dismutase (SOD) and catalase (CAT)

For enzyme extraction, 300 mg of grounded plant material was homogenized in 1 mL of phosphate buffer (50 mM, pH 7.0) with 1 mM EDTA, 1 % PVP and 1 M NaCl. Extraction of SOD included 1 mM sodium ascorbate. After centrifugation (15 min, 5000 g, 4 °C), supernatants were used to measure the activity of catalase and superoxide dismutase.

Catalase (CAT, EC 1.11.1.6) activity was measured in an assay mixture with phosphate buffer (50 mM, pH 7.0) and 15 mM H_2O_2 (Dhindsa et al., 1981). Decomposition of hydrogen peroxide was measured at 240 nm, and CAT activity was expressed in μmol H_2O_2 min^{-1} mg^{-1} protein.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured in an assay mixture containing 3 mM EDTA, 73 μM nitrotetrazolium blue (NBT), 13 mM methionine in phosphate buffer (50 mM, pH 6.4) with 60 μM riboflavin (Beauchamp and Fridovich, 1971). Samples were placed under a UV lamp for 10 min for superoxide anion generation and the degree of inhibition of NBT reduction to diformazan by $O_2^{\cdot-}$ was measured at 560 nm. SOD activity was expressed in U mg^{-1} protein. Protein concentration was measured with the (Bradford, 1976) method.

2.9. Lipid peroxidation

Lipid peroxidation was measured via a lipid peroxidation marker, malondialdehyde (MDA) (Hodges et al., 1999). Grounded plant material (0.25 g) was mixed with 1 mL of 0.1 % trichloroacetic acid (TCA). Following centrifugation, 500 μL of supernatant was mixed with 500 μL 20 % TCA with 0.5 % thiobarbituric acid (TBA). Controls did not have TBA. Samples were incubated at 95 °C for 30 min and immediately cooled in an ice bath. The absorbances were measured in the microplate reader (BMG Labtech, ClarioStar) at 532 nm and at 600 nm to subtract non-specific absorption, and at 440 nm to subtract sucrose. The results were expressed as MDA equivalent (nmol g^{-1} FW).

2.10. Total phenolic content

The measurement of total phenolic content (TPC) followed the protocol of (Herald et al., 2012). Grounded plant material (300 mg) was mixed with 80 % methanol (3 mL) and incubated at room temperature for 1 h. Following centrifugation (5 min, 10000 g), 25 μL of supernatant was mixed with 75 μL of water and 25 μL of Folin-Ciocalteu reagent and incubated at room temperature for 6 min. Then, 100 μL 7.5 % Na_2CO_3 was added, mixed and samples were incubated at room temperature in the dark for 90 min. The absorbance at 765 nm was measured with a microplate reader (BMG Labtech, ClarioStar). Gallic acid was used as a standard. TPC was expressed as μg gallic acid (GA) equivalent g^{-1} FW.

2.11. Salicylic acid and salicylic acid glucosides

Free salicylic acid (SA) and SA-glucoside concentrations were measured according to (Allasia et al., 2018). Grounded plant material (0.25 g) was mixed with 1 mL 70 % ethanol with 32 μL anisic acid (15.25 ng μL^{-1}). After centrifugation, the pellet was re-extracted with 1 mL of 90 % methanol. Methanol and ethanol from combined supernatants were evaporated in a vacuum concentrator (Speed Vac, 173 2–18 Cdplus, Thermo Fisher) and the pellet was treated with 65 μL 20 % of TCA, and 650 μL of ethyl acetate: cyclohexane (1:1). After centrifugation, the upper phase was collected, and the water phase was re-extracted. The combined upper phase was evaporated in a vacuum concentrator (Speed Vac) to dryness. The pellet was dissolved in 100 μL 10 % methanol containing 0.1 % trifluoroacetic acid (TFA). This extraction provided free SA. The water phase was later mixed with 0.3 mL 12 M HCl, incubated at 80 °C for 1 h. After cooling down, 18 μL of anisic acid (15.25 ng μL^{-1}) was added and samples were extracted twice with 0.9 mL of ethyl acetate: cyclohexane (1:1). Combined upper phases were evaporated to dryness in a vacuum concentrator, dry residue was dissolved in 100 μL 10 % methanol with 0.1 % TFA. This fraction represented SA-glucosides. Free and hydrolyzed SA were measured with HPLC (Arc HPLC Waters), with C18 column (Phenomenex, 250 × 4.6 mm, 5 μm) eluted with a methanol gradient from 10 % to 82 % at a flow rate of 1 mL min^{-1} at a temperature of 30 °C. Eluent contained 0.1 % TFA. Detection of SA and hydrolyzed SA (HSA) was done with a fluorescence detector (excitation at 305 nm and emission at 407 nm). SA and HSA concentrations were expressed as μg SA g^{-1} FW.

2.12. *Statistics*

We used R4.2.2. (R Core Team, 2023) with libraries car (Fox and Weisberg, 2019), multcomp (Hothorn et al., 2008), corrplot (Wei and Simko, 2021), FactoMineR (Lê et al., 2008) and factoextra (Kassambara and Mundt, 2020). We used correlations to study pairwise relationships between quantitative variables at different timepoints at each concentration of MP and factor analysis of mixed data (FAMD) to explore the associations between both quantitative and qualitative variables. We used two-way-ANOVA with PBAT-BD-MPs treatments and sampling timepoints (4, 8, and 11 weeks) and their interactions as explanatory variables with Tukey's honest significance test (HSD) post-hoc test. If needed, the response variable was transformed to meet the assumptions of normality (based on Levene's test). For most response variables, there was a significant interaction of time and treatment, and thus treatment effect was analyzed for all timepoints separately. We adjusted the p-values for multiple comparisons with Holms's method. Figures were generated with ggplot2 (Wickham, 2016).

3. Results

3.1. *Growth traits*

Shoot height showed a constant declining pattern with increasing MP concentrations over all sampling timepoints (Fig. 1a). Lettuce dry weight after exposure to PBAT-BD-MPs was not significantly different from the control (Fig. 1b–Table 1). Dry weight percentage significantly increased at higher PBAT-BD-MPs after 4 weeks (0.2 % and 0.8 %) by 42 % and 65 % respectively, and after 11 weeks (0.8 %) by 29 % compared to the control (Fig. 1c–Table 1).

Specific leaf area (SLA) tended to decrease linearly after 4 and 11 weeks of exposure to increasing concentrations of PBAT-BD-MPs; however, this difference was statistically significant only at the highest PBAT-BD-MP concentration (0.8 %) in which 67 % decrease was observed after 4 weeks compared to the control (Fig. 1d). Necrotic tissue levels of lettuce leaves were at a similar level to the control for most PBAT-BD-MP concentrations (Fig. 1e), except for 20 % lower necrotic tissue at the higher PBAT-BD-MP concentrations after 8 and 11 weeks. Number of leaves tended to decrease with increasing concentrations of PBAT-BD-MPs and was 17–29 % lower than the control at 0.8 % PBAT-BD-MPs after 8 and 11 weeks (Fig. 1f–Table 1).

Lettuce leaf CN content was measured only at weeks 8 and 11 due to insufficient sample size at week 4. Plant leaf C content showed a constant declining pattern with increasing PBAT-BD-MP concentrations over all sampling timepoints (Table 1). It was 5 % lower at the highest PBAT-BD-MP concentration (0.8 %) compared to the control (Fig. 2a). Also, N content was decreased by 5–33 % at the higher PBAT-BD-MP concentrations compared to the control after 8 weeks (0.8 %) and 11 weeks (0.2 % and 0.8 %) (Fig. 2b–Table 1). Leaf C/N ratio tended to increase with increasing concentrations of PBAT-BD-MPs after 11 weeks of the experiment and was significantly increased by 50–76 % compared to the control at the highest PBAT-BD-MP concentrations (0.2 % and 0.8 %) (Fig. 2c–Table 1).

3.2. *Chlorophyll*

The concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total chlorophyll in plant leaves were not significantly affected by the PBAT-BD-MPs concentrations after 4 weeks except for elevated Chl *a* by 9 % at 0.025 % PBAT-BD-MPs (Fig. 3a). However, observations after 8- and 11-weeks showed significant and opposite effects: after 8-weeks Chl *a* and total chlorophyll concentrations were significantly decreased by 7 % at 0.05 % and 0.2 % PBAT-BD-MPs, and significantly increased by 26 % at 0.8 % PBAT-BD-MPs (Fig. 3a and c, Table 1) compared to the control. After 11 weeks, all chlorophyll concentrations were significantly decreased by 64% at all PBAT-BD-MP concentrations except for 0.05 % and 0.2 % for Chl *b*. In all cases, the responses did not show a monotonic pattern (Fig. 3).

3.3. *Superoxide dismutase (SOD) and catalase (CAT) activities*

SOD activity in lettuce leaves was significantly affected by PBAT-BD-MPs at all concentrations tested (Fig. 4a, Table 1). Lower PBAT-BD-MP concentrations (0.025 and 0.05 %) increased SOD activity by at least 15 % compared to the control throughout the experiment. After 8 and 11 weeks also 0.2 % PBAT-BD-MPs increased SOD activity by more than 10 %. However, the highest PBAT-BD-MPs concentration (0.8 %) decreased SOD activity by 48 % after 11 weeks.

CAT activity in lettuce leaves increased by at least 33 % at the lower PBAT-BD-MP concentrations (0.025 %, 0.05 %) (Fig. 4b), while it also increased by 30 % at 0.2 % PBAT-BD-MPs throughout the whole experimental duration. The highest PBAT-BD-MP concentration (0.8 %) increased CAT activity only after 4 weeks by less than 10 % but did not differ from the control later on.

3.4. *Lipid peroxidation (MDA concentration)*

Lettuce leaf MDA concentration tended to increase with increasing concentrations of PBAT-BD-MPs (Fig. 4c). MDA was significantly increased compared to the control at all PBAT-BD-MP concentrations throughout the whole experiment (Fig. 4c–Table 1), except for the lower PBAT-BD-MP concentrations: 0.025 % and 0.05 % after 4 weeks and 0.025 % after 8 weeks. After 11 weeks all PBAT-BD-MP concentrations significantly increased MDA concentrations in the lettuce leaves by at least 55 %. (Fig. 4c–Table 1).

3.5. *Total phenolic content (TPC)*

After 4 weeks, all PBAT-BD-MP concentrations significantly increased TPC in lettuce leaves by at least 75 % (Fig. 4d–Table 1). However, after 8 weeks only at 0.025 % and 0.2 % PBAT-BD-MP total phenolic content increased by 1000 % compared to the control (Fig. 4d). After 11 weeks, the lower PBAT-BD-MP concentrations (0.025 %, 0.05 %) did not affect TPC while the higher PBAT-BD-MP concentrations (0.2 % and 0.8 %) showed even 21 % decrease of TPC levels compared with the control.

3.6. *Salicylic acid (SA) and SA-glycosides (HSA)*

SA concentration in lettuce leaves was significantly increased by 100 % compared to the control by all PBAT-BD-MP concentrations after 4

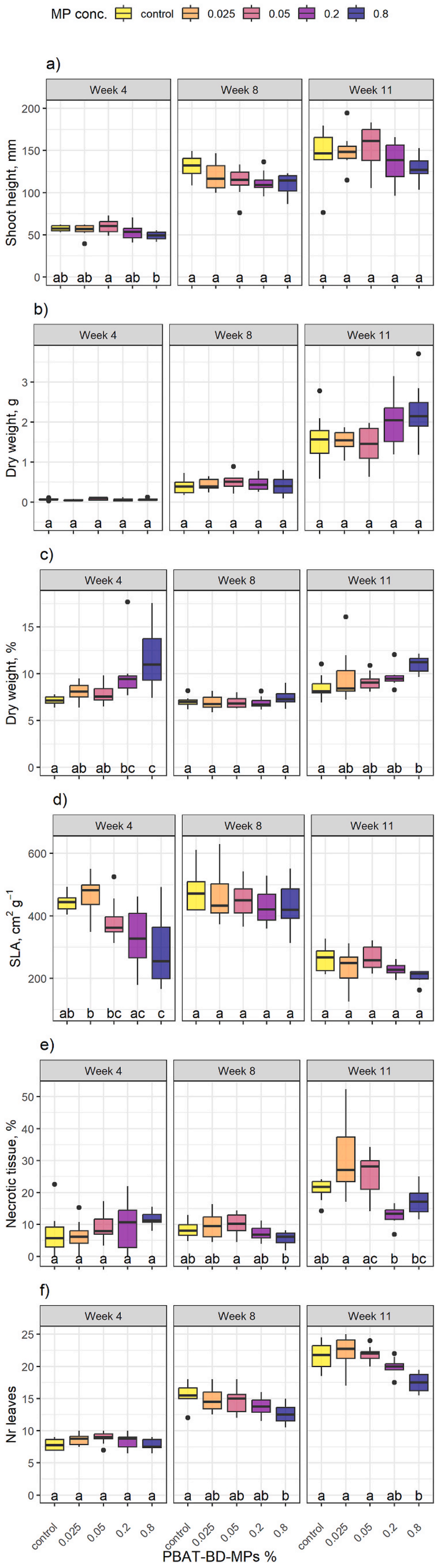

Fig. 1. Growth traits of lettuce: a) seedling shoot height, b) seedling dry weight, c) seedling dry weight percentage, d) specific leaf area (SLA, $cm^2 g^{-1}$), e) necrotic tissue percentage and f) number of leaves (pcs – pieces) following exposure to polybutylene adipate terephthalate biodegradable microplastics (PBAT-BD-MPs) in CLIMECS system. Results are presented as median, interquartile range and the standard deviation including outliers; Lowercase letters indicate significant differences between treatments ($p < 0.05$) based on Tukey's HSD test (Holms adj.p).

(caption on next column)

weeks (Fig. 4e–Table 1). After 8 weeks, the lowest PBAT-BD-MP concentration (0.025 %) still elevated SA concentrations by 24 %, but at the higher PBAT-BD-MP concentrations (0.2 % and 0.8 %) SA levels were 12 % lower than in the control. After 11 weeks, SA concentrations were increased by 16 % at the lower PBAT-BD-MP concentrations (0.025 and 0.05 %), but decreased by 90 % compared to the control at highest PBAT-BD-MPs concentration (0.8 %).

The HSA level in the lettuce leaves was significantly reduced by 12%–251 % compared with the control throughout the whole experiment (Fig. 4f–Table 1) at the highest PBAT-BD-MP concentrations (0.8 %), however, medium PBAT-BD-MPs concentrations increased HSA concentrations by more than 100 % after 11 weeks (Fig. 4f).

3.7. Correlations, principal components and correspondence analysis

Studied biomarkers showed both, positive and negative correlations (Fig. S2 in the Supplementary Material). SA positively correlated with enzymatic ROS-scavengers (with SOD after 11 weeks, with CAT after 4 and 11 weeks) for all PBAT-MPs concentrations. When PBAT-BD-MP effects were correlated separately for each concentration, SA correlated positively with SOD for 0.2 % and 0.8 % PBAT-BD-MPs throughout the whole experiment. SA positively correlated with non-enzymatic ROS-scavengers, TPC (after 4 weeks). However, SA correlated negatively with lipid peroxidation studied via MDA concentration (after 4 and 8 weeks, but not after 11 weeks) for the lower PBAT-BD-MP concentrations (0.025 % and 0.05 %). TPC correlated negatively with MDA and SOD (after 4 weeks), and with chlorophyll (after 8 weeks). TPC correlated negatively with 0.8 % PBAT-BD-MPs throughout the whole experiment.

Associations of both quantitative and qualitative variables with factor analysis of mixed data (FAMD) was conducted to look at all dependent variables together. FAMD analysis separated the control samples, that were not exposed to PBAT-BD-MPs, from samples from the PBAT-BD-MP exposures at all sampling timepoints along the first dimension. After 4 weeks, the first dimension, which separated the high MP treatments from the low MP treatments and the control, was explained by increasing SOD and decreasing TPC content (Supplementary Material Fig. S3). At later growth stages (8 and 11 weeks), the second dimension that separated the control and the lowest PBAT-BD-MP concentrations from the higher ones was explained by increasing lipid peroxidation MDA (Supplementary Material Figs. S3–S5). Moreover, the lowest PBAT-BD-MP concentrations (0.025 % and 0.05 %) were grouped together at week 4, but they separated after 8 and 11 weeks. At week 11, the lowest PBAT-BD-MP concentration was closest to the control.

4. Discussion

CLIMECS experiment aimed to elucidate how PBAT-BD-MPs added to soil affect lettuce plants under environmentally relevant conditions as due to limited number of studies the effect of these bioplastics on plant health is not-well known (Table S1). The results revealed that PBAT-BD-MPs altered severely lettuce biochemical traits and, to a lesser extent, several growth traits. The effects were dependent on the time of exposure and concentration of the PBAT-BD-MPs in soil. The impact on soil invertebrates was less pronounced, with no alterations observed in the springtail community composition and no effects on earthworm survival

Table 1
Analysis of variance (ANOVA) of data on the effects of polybutylene adipate terephthalate biodegradable microplastics (PBAT-BD-MPs) on lettuce growth and biochemical traits. Statistically significant differences were determined based on alpha level P = 0.05. ns - nonsignificant; w4 – week 4, w8 – week 8, w11 – week 11. Chl - chlorophyll, Nr leaves – number of leaves, tot Chl – total chlorophyll, SOD – superoxide dismutase, CAT – catalase, MDA – malondialdehyde, TPC – total phenolic content, SA – salicylic acid, HSA – SA glicosides.

Response variable	Two-way ANOVA			One-way ANOVA		
	explanatory variable		Interaction[a]	separate test for each timepoint		
Endpoint	concentration	week	concentration x week	w4	w8	w11
dry weight	$F_4 = 0.6$; ns	$F_2 = 537.1$; $P < 0.001$	$F_8 = 2.0$; $P < 0.05$	ns	ns	ns
shoot height	$F_4 = 3.9$; $P < 0.01$	$F_2 = 419.7$; $P < 0.001$	$F_8 = 0.8$; ns	$P < 0.05$	ns	ns
dry weight %	$F_4 = 9.6$; $P < 0.001$	$F_2 = 39.5$; $P < 0.001$	$F_8 = 2.3$; $P < 0.05$	$P < 0.001$	ns	$P < 0.05$
specific leaf area	$F_4 = 7.1$; $P < 0.001$	$F_2 = 106.2$; $P < 0.001$	$F_8 = 2.2$; P0.05	$P < 0.001$	ns	$P < 0.05$
necrotic tissue %	$F_4 = 4.8$; $P < 0.01$	$F_2 = 375.2$; $P < 0.001$	$F_8 = 1.5$; ns	ns	ns	$P < 0.05$
nr leaves	$F_4 = 11.2$; $P < 0.001$	$F_2 = 587.4$; $P < 0.001$	$F_8 = 2.6$; $P < 0.05$	ns	$P < 0.05$	$P < 0.001$
C	$F_4 = 7.9$; $P < 0.001$	$F_2 = 3.4$; ns	$F_4 = 1.3$; ns	-	$P < 0.001$	ns
N	$F_4 = 19.1$; $P < 0.001$	$F_2 = 241.7$; $P < 0.001$	$F_4 = 3.8$; $P < 0.01$	-	$P < 0.05$	$P < 0.001$
C:N ratio	$F_4 = 25.3$; $P < 0.001$	$F_2 = 196.0$; $P < 0.001$	$F_4 = 13.7$; $P < 0.001$	-	ns	$P < 0.001$
Chl a	$F_4 = 25.8$; $P < 0.001$	$F_2 = 799.4$; $P < 0.001$	$F_8 = 125.5$; $P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$
Chl b	$F_4 = 3.4$; $P < 0.05$	$F_2 = 63.2$; $P < 0.001$	$F_8 = 6.6$; $P < 0.001$	ns	$P < 0.01$	$P < 0.001$
total Chl	$F_4 = 22.2$; $P < 0.001$	$F_2 = 622.5$; $P < 0.001$	$F_8 = 81.6$; $P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$
SOD	$F_4 = 170.3$; $P < 0.001$	$F_2 = 68.9$; $P < 0.001$	$F_8 = 25.5$; $P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
CAT	$F_4 = 110.9$; $P < 0.001$	$F_2 = 1.0$; ns	$F_8 = 11.5$; $P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
MDA	$F_4 = 27.6$; $P < 0.001$	$F_2 = 143.7$; $P < 0.001$	$F_8 = 3.4$; $P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$
TPC	$F_4 = 372.8$; $P < 0.001$	$F_2 = 5.3$; $P < 0.01$	$F_8 = 257.6$; $P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
SA	$F_4 = 40.6$; $P < 0.001$	$F_2 = 546$; $P < 0.001$	$F_8 = 25.8$; $P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
HSA	$F_4 = 257.8$; $P < 0.001$	$F_2 = 643.4$; $P < 0.001$	$F_8 = 16.2$; $P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

(van Loon et al. unpublished).

Though statistically significant effects of PBAT-BD-MPs on the dry weight of the lettuce plants was not observed, some negative effects of the highest PBAT-BD-MPs (0.2, 0.8 %) were observed on the shoot height, number of leaves, specific leaf area, and C and N content. This suggests that PBAT-BD-MPs may deteriorate lettuce growth if present at relatively high concentrations in the soil. In a study using a high PBAT-BD-MP concentration (1 %), *Oryza sativa* growth was affected negatively (Yang and Gao, 2022). Similarly, 1.5 % PLA-PBAT-BD-MPs severely affected the growth of common beans (*Phaseolus vulgaris*) (Meng et al., 2021), and dandelion (*Taraxacum officinalis*) (Xingfan Li et al., 2024b). However, PLA-BD-MPs showed both, negative and positive effects on the growth of plants; for example at 0.1 % PLA-BD-MPs increased, but at 10 % PLA-BD-MPs decreased the growth of maize (*Zea mays*) (Yang et al., 2021). Thus, BD-MPs may adversely affect the growth of plants and the magnitude of the effect depends on BD-MP properties, concentration, time of exposure, and potentially also plant species.

In this study, lettuce physiology and biochemistry seemed to be more sensitive indicators of PBAT-BD-MP stress than growth *per se*, as confirmed by decreased chlorophyll concentrations, increased oxidative stress, and significant modifications in plant defense mechanisms. A similar effect on chlorophyll was observed also for maize (*Zea mays*) exposed to a high (10 %) PLA-BD-MP concentration (Sun et al., 2023; F. Wang et al., 2020), common beans exposed to PBAT-PLA-BD-MPs (0.5–5 %) (Meng et al., 2021) and rice at 1 % PBAT-BD-MPs (Yang and Gao, 2022). Decreases in chlorophyll concentrations after exposure to PBAT-BD-MPs in soil may partially emerge from decreased N uptake, and its transportation and assimilation by plants, as revealed by transcriptomic studies with rice (Yang and Gao, 2022). As chlorophyll is built up from an N-containing porphyrin ring, low plant N status caused by PBAT-BD-MP exposure may well explain the decrease in chlorophyll concentrations. In addition, the N content of plant leaves decreased at higher concentrations of PBAT-BD-MPs, supporting this hypothesis of plant N deficiency. Alternatively, a low plant N status leading to decreased chlorophyll synthesis can emerge from an increased microbial immobilization of N observed after the addition of PBAT-BD-MPs to soil (Reay et al., 2023).

Changes in the ratio of Chl *a* and Chl *b* may also suggest drought stress due to PBAT-BD-MPs. Under drought stress, the reduction of Chl *b* is greater than that of Chl *a* (Ashraf and Harris, 2013), which was observed for lettuce exposed for 11 weeks to 0.8 % PBAT-BD-MPs. Potential drought stress due to high 0.8 % PBAT-BD-MPs after 11 weeks is also confirmed by the elevated dry weight percentage of the lettuce plants in our study. It is possible that in our experiment, PBAT-BD-MPs accumulated in the vicinity of the lettuce roots, decelerating water uptake. It was shown for lettuce that conventional MPs may induce drought effects (Zhang et al., 2023).

In higher plants, synthesis and accumulation of chlorophylls may increase due to low levels of stress and decrease in conditions of high levels of stress (Agathokleous et al., 2020). This could partially explain the non-linear response of chlorophylls to PBAT-BD-MPs exposure. Stress in plants is inseparable from oxidative stress, during which reactive oxygen species production is elevated and activity and production of ROS-scavengers are modified accordingly to provide oxidative homeostasis (Ekner-Grzyb et al., 2022). In line with that, activity of SOD scavenging $O_2^{\bullet -}$ and CAT activity scavenging H_2O_2 increased after exposure to low PBAT-BD-MP concentrations (0.025–0.05 %). Also in *Brassica parachinensis*, the activity of SOD and ROS production increased after exposure to high 2 % PBAT-BD-MPs in soil (Xinyang Li et al., 2024a). PBAT-BD-MPs at a concentration as high as 1 % also elevated ROS production in rice (Yang and Gao, 2022). In line with the increases of ROS-scavenging enzymes (SOD, CAT), also lipid peroxidation, measured via concentration of MDA, increased. Similar effects have been observed in other studies with exposure to PBAT-BD-MPs (Liu et al., 2023; Yang and Gao, 2022). In our study, the high MDA concentrations after exposure to 0.8 % PBAT-BD-MPs coincided with lower activities of SOD and CAT. This could emerge from an overproduction of ROS exceeding the catalytic abilities of these enzymes, leading to excessive accumulation of ROS resulting in cell membrane damage (Hodges et al., 1999). Accordingly, another line of plant defense, total phenolic compounds were also elevated by PBAT-BD-MP exposure (4 weeks) to gradually decrease to the control level after 11 weeks. TPC was negatively correlated with SOD activity (4 weeks) underlining its role in scavenging these ROS, which were not effectively removed by the enzymatic line of plant defense. On one hand, TPC levels similar to or even below the control after 11 weeks could point to decreased oxidative stress, on the other hand, the negative correlation between TPC and MDA after 4 weeks could mean that the oxidative stress exceeded the ability of plant defense mechanisms leading to lipid peroxidation and thus to higher MDA concentrations. The decreased level of TPC after 11

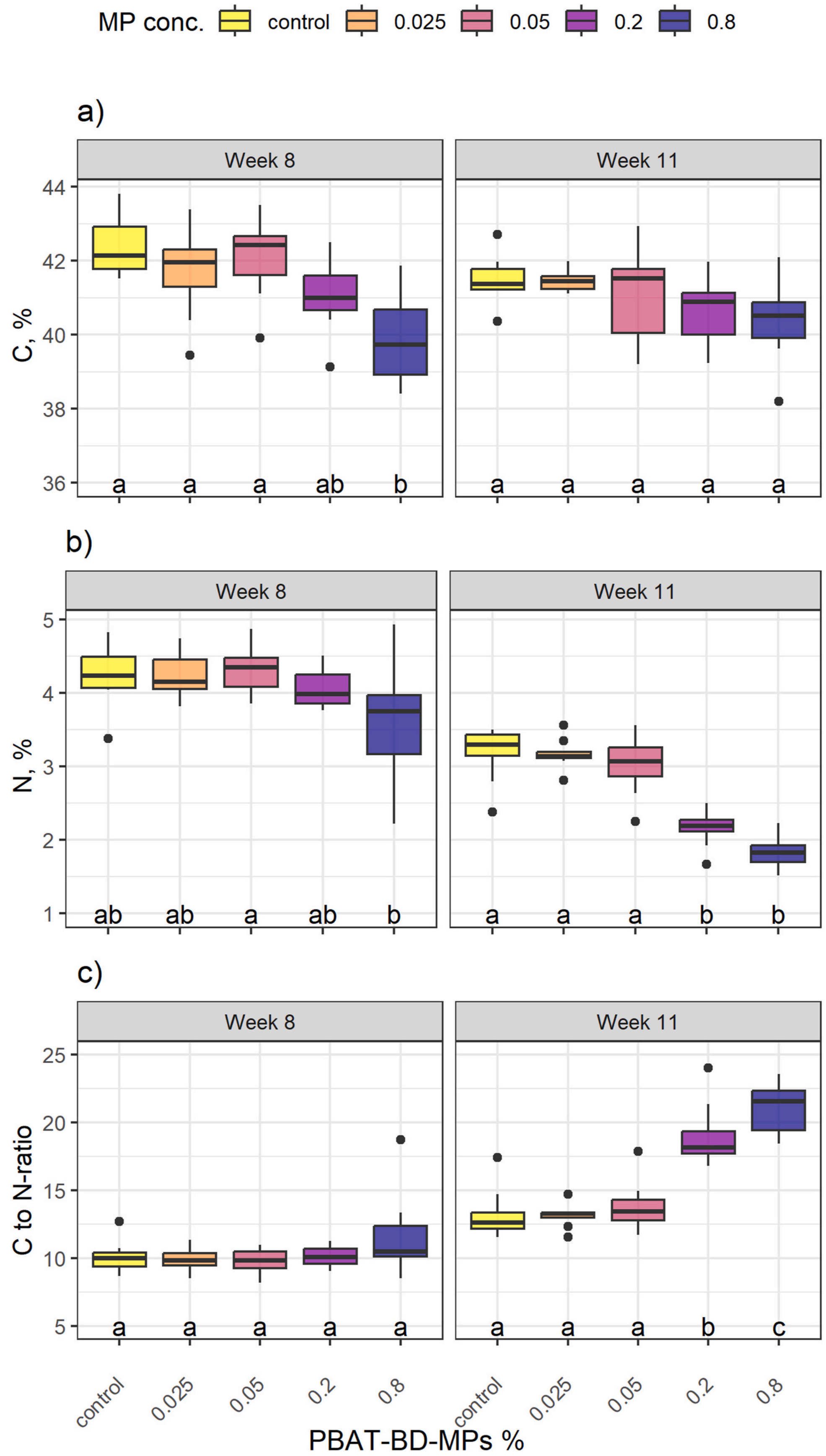

Fig. 2. Content of carbon (C) (a) and nitrogen(N) (b), percentage, and C-to-N ratio (c) in lettuce following exposure to polybutylene adipate terephthalate biodegradable microplastics (PBAT-BD-MPs) in CLIMECS system. Results are presented as median, interquartile range and the standard deviation including outliers; Lowercase letters indicate significant differences between treatments ($p < 0.05$) based on Tukey's HSD test (Holms adj.p).

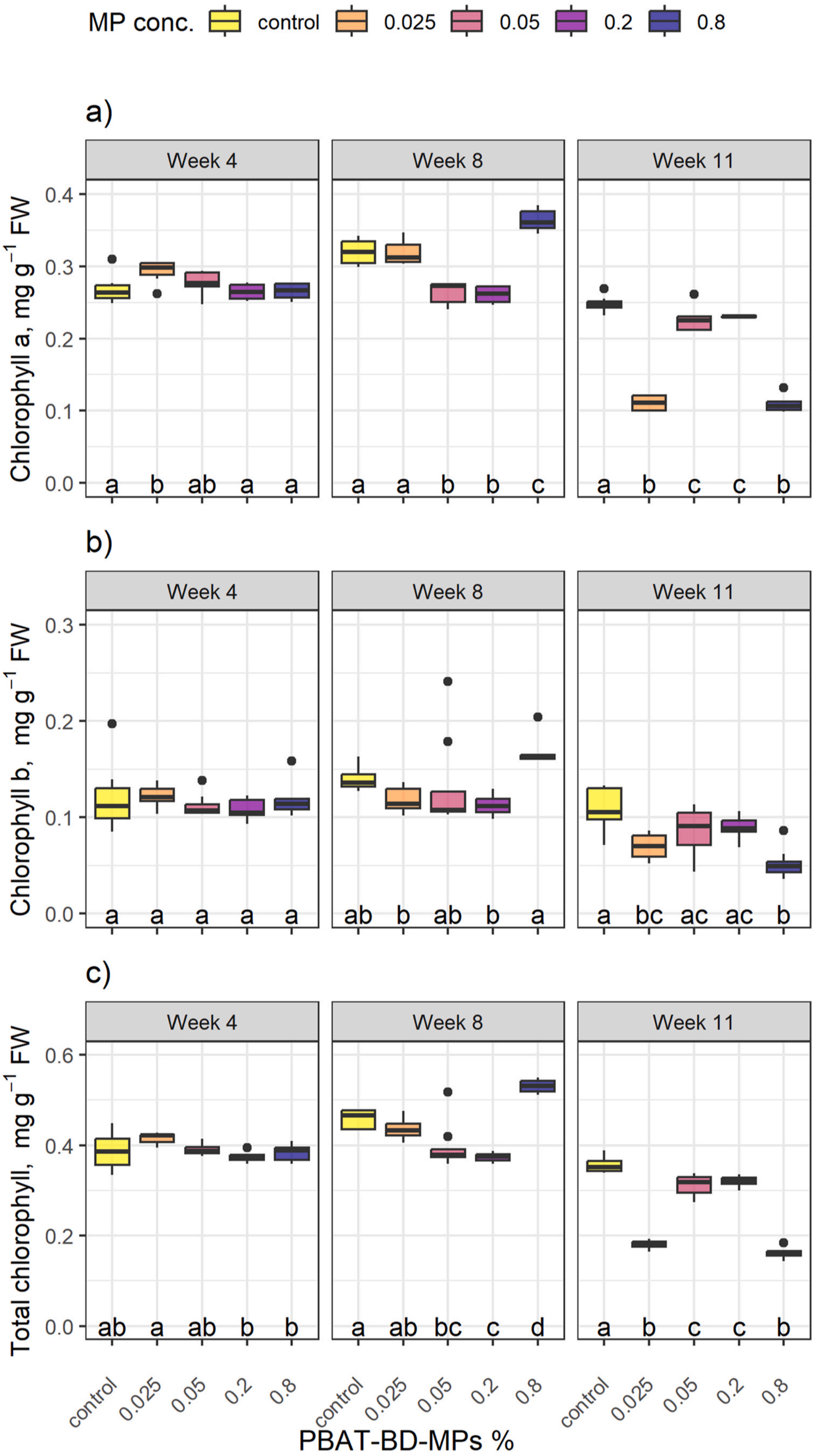

Fig. 3. Concentration of chlorophyll in lettuce following exposure to polybutylene adipate terephthalate biodegradable microplastics (PBAT-BD-MPs) in CLIMECS system: a) chlorophyll *a*, *b*) chlorophyll *b, and c*) total chlorophyll. FW – fresh weight. Results are presented as median, interquartile range and the standard deviation including outliers; Lowercase letters indicate significant differences between treatments ($p < 0.05$) based on Tukey's HSD test (Holms adj.p).

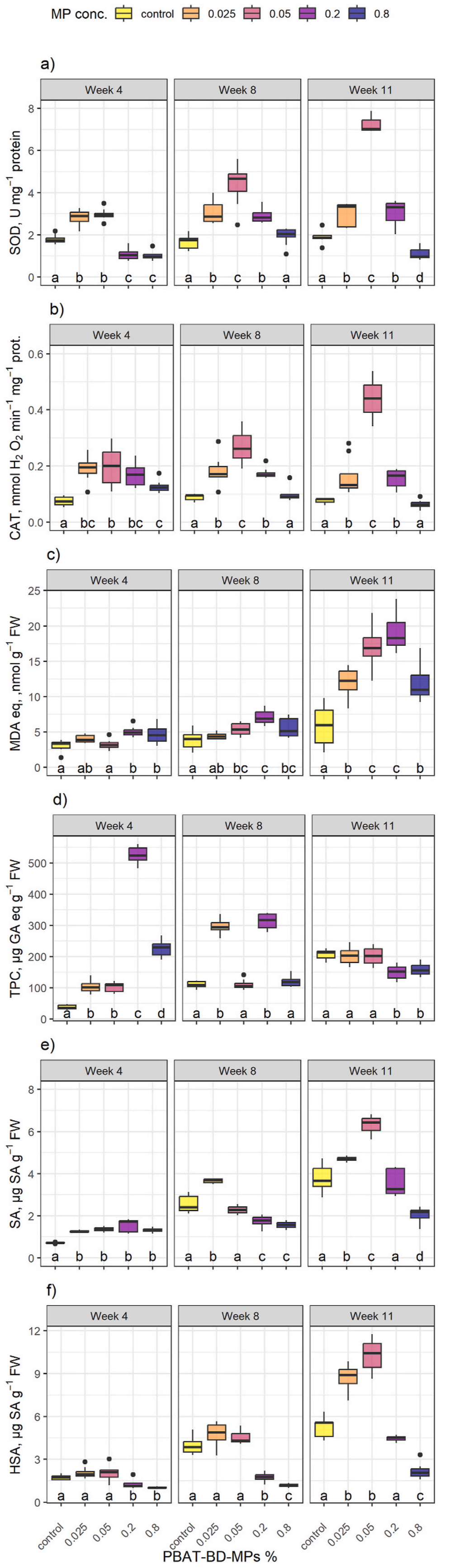

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Fig. 4. Performance of plant defense systems in lettuce following exposure to polybutylene adipate terephthalate biodegradable microplastics (PBAT-BD-MPs) in CLIMECS system: a) specific activity (U mg $^{-1}$ protein) of superoxide dismutase, SOD, b) activity of catalase, CAT, c) concentration of malondialdehyde, MDA, Eq = equivalents d) total phenolic content, TPC, e) salicylic acid, SA, and f) SA-glycosides, HSA. FW = fresh weight. Results are presented as median, interquartile range and the standard deviation including outliers; Lowercase letters indicate significant differences between treatments ($p < 0.05$) based on Tukey's HSD test (Holms adj.p).

weeks of exposure may also emerge from the downregulation of the phenylpropanoid biosynthesis pathway by the PBAT-BD-MPs (Yang and Gao, 2022). The phenylpropanoid biosynthesis pathway is activated under abiotic stress leading to the production of various phenolic compounds (Sharma et al., 2019).

Salicylic acid was also included to disentangle to effect of PBAT-BD-MPs on lettuce resilience, as it plays a central role in stress signalling (Janda et al., 2020). The SA concentration in the lettuce leaves was initially elevated after 4 weeks of exposure to PBAT-BD-MPs, correlating positively with other elements of plant defense mechanisms (enzymatic and TPC). This is in line with the available literature showing that SA enables plants to cope with stress by regulation of antioxidant enzyme activities (Souza et al., 2017), and the activity of phenylalanine ammonia-lyase, a key enzyme in the biosynthesis of phenolic compounds (Janda et al., 2020). Taken together, PBAT-BD-MPs significantly affected all studied biomarkers of plant defense clearly underlining that these MPs are not inert to plant health in near-field concentrations.

5. Conclusions

This study investigates the effects of PBAT-BD-MPs on lettuce growth, physiology and biochemistry in near-field conditions. Exposure of lettuce to PBAT-BD-MPs induced plant oxidative stress and activated plant defense mechanisms leading to oxidative homeostasis and sustaining plant growth and development, though some negative effects on plant growth were observed, mainly after the longest exposure duration to the highest PBAT-BD-MP concentration (0.8 %) under the conditions of the experiment. To conclude, PBAT-BD-MPs though does not affect lettuce growth much, do affect lettuce metabolism and resilience and these aspects should be further explored. Following steps in understanding the effect of PBAT-BD-MPs on plant health should include extrapolation to field conditions, experiments with a wide set of plant species and experiments with numerous stressors, better reflecting natural conditions.

CRediT authorship contribution statement

Sylwia Adamczyk: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laura J. Zantis:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Sam van Loon:** Writing – review & editing, Methodology, Conceptualization. **Cornelis A.M. van Gestel:** Writing – review & editing, Validation, Supervision, Conceptualization. **Thijs Bosker:** Writing – review & editing, Supervision, Conceptualization. **Rachel Hurley:** Writing – review & editing, Project administration. **Luca Nizzetto:** Writing – review & editing, Validation, Funding acquisition. **Bartosz Adamczyk:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Sannakajsa Velmala:** Writing – review & editing, Visualization, Formal analysis, Conceptualization.

Declaration of competing interest

No conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125307.

Data availability

Data will be made available on request.

References

Agathokleous, E., Feng, Z., Peñuelas, J., 2020. Chlorophyll hormesis: are chlorophylls major components of stress biology in higher plants? Sci. Total Environ. 726, 138637. https://doi.org/10.1016/j.scitotenv.2020.138637.

Allasia, V., Industri, B., Ponchet, M., Quentin, M., Favery, B., Keller, H., 2018. Quantification of salicylic acid (SA) and SA-glucosides in Arabidopsis thaliana. BIO-Protoc. 8. https://doi.org/10.21769/BioProtoc.2844.

Ashraf, M., Harris, P.J.C., 2013. Photosynthesis under stressful environments: an overview. Photosynthetica 51, 163–190. https://doi.org/10.1007/s11099-013-0021-6.

Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44, 276–287. https://doi.org/10.1016/0003-2697(71)90370-8.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Chah, C.N., Banerjee, A., Gadi, V.K., Sekharan, S., Katiyar, V., 2022. A systematic review on bioplastic-soil interaction: exploring the effects of residual bioplastics on the soil geoenvironment. Sci. Total Environ. 851, 158311. https://doi.org/10.1016/j.scitotenv.2022.158311.

Courtene-Jones, W., Cheung, S.W.H., Thompson, R.C., Hanley, M.E., 2024. Effect of biodegradable and conventional microplastic exposure in combination with seawater inundation on the coastal terrestrial plant Plantago coronopus. Environ. Pollut. 360, 124573. https://doi.org/10.1016/j.envpol.2024.124573.

Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A., 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32, 93–101. https://doi.org/10.1093/jxb/32.1.93.

Ekner-Grzyb, A., Duka, A., Grzyb, T., Lopes, I., Chmielowska-Bąk, J., 2022. Plants oxidative response to nanoplastic. Front. Plant Sci. 13, 1027608. https://doi.org/10.3389/fpls.2022.1027608.

Fan, P., Yu, H., Xi, B., Tan, W., 2022. A review on the occurrence and influence of biodegradable microplastics in soil ecosystems: are biodegradable plastics substitute or threat? Environ. Int. 163, 107244. https://doi.org/10.1016/j.envint.2022.107244.

Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, third ed. Sage, Thousand Oaks, CA.

Fu, Y., Wu, G., Bian, X., Zeng, J., Weng, Y., 2020. Biodegradation behavior of poly (butylene adipate-Co-terephthalate) (PBAT), poly(lactic acid) (PLA), and their blend in freshwater with sediment. Molecules 25, 3946. https://doi.org/10.3390/molecules25173946.

Hassan, I.F., Ajaj, R., Gaballah, M.S., Ogbaga, C.C., Kalaji, H.M., Hatterman-Valenti, H. M., Alam-Eldein, S.M., 2022. Foliar application of nano-silicon improves the physiological and biochemical characteristics of 'kalamata' olive subjected to deficit irrigation in a semi-arid climate. Plants 11, 1561. https://doi.org/10.3390/plants11121561.

Helmberger, M.S., Tiemann, L.K., Grieshop, M.J., 2020. Towards an ecology of soil microplastics. Funct. Ecol. 34, 550–560. https://doi.org/10.1111/1365-2435.13495.

Herald, T.J., Gadgil, P., Tilley, M., 2012. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour: high-throughput microplate assays for screening sorghum. J. Sci. Food Agric. 92, 2326–2331. https://doi.org/10.1002/jsfa.5633.

Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611. https://doi.org/10.1007/s004250050524.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363. https://doi.org/10.1002/bimj.200810425.

Huerta-Lwanga, E., Mendoza-Vega, J., Ribeiro, O., Gertsen, H., Peters, P., Geissen, V., 2021. Is the polylactic acid fiber in green compost a risk for Lumbricus terrestris and Triticum aestivum? Polymers 13, 703. https://doi.org/10.3390/polym13050703.

Hurley, R., Binda, G., Briassoulis, D., Carroccio, S.C., Cerruti, P., Convertino, F., Dvorakova, D., Kernchen, S., Laforsch, C., Löder, M.G.L., Pulkrabova, J., Schettini, E., Spanu, D., Tsagkaris, A.S., Vox, G., Nizzetto, L., 2024. Production and characterisation of environmentally relevant microplastic test materials derived from agricultural plastics. Sci. Total Environ. 174325. https://doi.org/10.1016/j.scitotenv.2024.174325.

Hurley, R., Horton, A., Lusher, A., Nizzetto, L., 2020. Plastic waste in the terrestrial environment. In: Plastic Waste and Recycling. Elsevier, pp. 163–193. https://doi.org/10.1016/B978-0-12-817880-5.00007-4.

Irshad, M.K., Aqeel, M., Saleem, S., Javed, W., Noman, A., Kang, M.W., Khalid, N., Lee, S. S., 2024. Mechanistic insight into interactive effect of microplastics and arsenic on growth of rice (Oryza sativa L.) and soil health indicators. Sci. Total Environ. 955, 176875. https://doi.org/10.1016/j.scitotenv.2024.176875.

Janda, T., Szalai, G., Pál, M., 2020. Salicylic acid signalling in plants. Int. J. Mol. Sci. 21, 2655. https://doi.org/10.3390/ijms21072655.

Jia, L., Liu, L., Zhang, Y., Fu, W., Liu, X., Wang, Q., Tanveer, M., Huang, L., 2023. Microplastic stress in plants: effects on plant growth and their remediations. Front. Plant Sci. 14, 1226484. https://doi.org/10.3389/fpls.2023.1226484.

Kanwal, A., Zhang, M., Sharaf, F., Li, C., 2022. Enzymatic degradation of poly (butylene adipate co-terephthalate) (PBAT) copolymer using lipase B from Candida Antarctica (CALB) and effect of PBAT on plant growth. Polym. Bull. 79, 9059–9073. https://doi.org/10.1007/s00289-021-03946-w.

Kassambara, A., Mundt, F., 2020. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR : an *R* package for multivariate analysis. J. Stat. Softw. 25. https://doi.org/10.18637/jss.v025.i01.

Li, S., Ding, F., Flury, M., Wang, J., 2023. Dynamics of macroplastics and microplastics formed by biodegradable mulch film in an agricultural field. Sci. Total Environ. 894, 164674. https://doi.org/10.1016/j.scitotenv.2023.164674.

Li, W., Wufuer, R., Duo, J., Wang, S., Luo, Y., Zhang, D., Pan, X., 2020. Microplastics in agricultural soils: extraction and characterization after different periods of polythene film mulching in an arid region. Sci. Total Environ. 749, 141420. https://doi.org/10.1016/j.scitotenv.2020.141420.

Li, Xinyang, Cheng, X., Wu, J., Cai, Z., Wang, Z., Zhou, J., 2024a. Multi-omics reveals different impact patterns of conventional and biodegradable microplastics on the crop rhizosphere in a biofertilizer environment. J. Hazard Mater. 467, 133709. https://doi.org/10.1016/j.jhazmat.2024.133709.

Li, Xingfan, Zeng, G., Du, X., Zhou, R., Lian, J., Liu, J., Guo, X., Tang, Z., 2024b. Effects of polyethylene and biodegradable microplastics on the physiology and metabolic profiles of dandelion. Environ. Pollut. 352, 124116. https://doi.org/10.1016/j.envpol.2024.124116.

Ligtelijn, M., Henrik Barmentlo, S., Van Gestel, C.A.M., 2024. Field-realistic doses of the neonicotinoid acetamiprid impact natural soil arthropod community diversity and structure. Environ. Pollut. 359, 124568. https://doi.org/10.1016/j.envpol.2024.124568.

Meng, F., Yang, X., Riksen, M., Xu, M., Geissen, V., 2021. Response of common bean (Phaseolus vulgaris L.) growth to soil contaminated with microplastics. Sci. Total Environ. 755, 142516. https://doi.org/10.1016/j.scitotenv.2020.142516.

Nizzetto, L., Futter, M., Langaas, S., 2016. Are agricultural soils dumps for microplastics of urban origin? Environ. Sci. Technol. 50, 10777–10779. https://doi.org/10.1021/acs.est.6b04140.

Palsikowski, P.A., Roberto, M.M., Sommaggio, L.R.D., Souza, P.M.S., Morales, A.R., Marin-Morales, M.A., 2018. Ecotoxicity evaluation of the biodegradable polymers PLA, PBAT and its blends using Allium cepa as test organism. J. Polym. Environ. 26, 938–945. https://doi.org/10.1007/s10924-017-0990-9.

Reay, M.K., Greenfield, L.M., Graf, M., Lloyd, C.E.M., Evershed, R.P., Chadwick, D.R., Jones, D.L., 2023. LDPE and biodegradable PLA-PBAT plastics differentially affect plant-soil nitrogen partitioning and dynamics in a Hordeum vulgare mesocosm. J. Hazard Mater. 447, 130825. https://doi.org/10.1016/j.jhazmat.2023.130825.

Sajjad, M., Huang, Q., Khan, S., Khan, M.A., Liu, Y., Wang, J., Lian, F., Wang, Q., Guo, G., 2022. Microplastics in the soil environment: a critical review. Environ. Technol. Innov. 27, 102408. https://doi.org/10.1016/j.eti.2022.102408.

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., Zheng, B., 2019. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. Molecules 24, 2452. https://doi.org/10.3390/molecules24132452.

Souza, L.A., Monteiro, C.C., Carvalho, R.F., Gratão, P.L., Azevedo, R.A., 2017. Dealing with abiotic stresses: an integrative view of how phytohormones control abiotic stress-induced oxidative stress. Theor. Exp. Plant Physiol. 29, 109–127. https://doi.org/10.1007/s40626-017-0088-8.

Sun, H., Shi, Y., Zhao, P., Long, G., Li, C., Wang, J., Qiu, D., Lu, C., Ding, Y., Liu, L., He, S., 2023. Effects of polyethylene and biodegradable microplastics on photosynthesis, antioxidant defense systems, and arsenic accumulation in maize (Zea mays L.) seedlings grown in arsenic-contaminated soils. Sci. Total Environ. 868, 161557. https://doi.org/10.1016/j.scitotenv.2023.161557.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284.

Wang, F., Zhang, X., Zhang, Shuqi, Zhang, Shuwu, Sun, Y., 2020. Interactions of microplastics and cadmium on plant growth and arbuscular mycorrhizal fungal communities in an agricultural soil. Chemosphere 254, 126791. https://doi.org/10.1016/j.chemosphere.2020.126791.

Warren, C.R., 2008. Rapid measurement of chlorophylls with a microplate reader. J. Plant Nutr. 31, 1321–1332. https://doi.org/10.1080/01904160802135092.

Wei, T., Simko, V., 2021. R package "corrplot": visualization of a correlation matrix, Version 0.92. https://github.com/taiyun/corrplot.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Yang, C., Gao, X., 2022. Impact of microplastics from polyethylene and biodegradable mulch films on rice (Oryza sativa L.). Sci. Total Environ. 828, 154579. https://doi.org/10.1016/j.scitotenv.2022.154579.

Yang, W., Cheng, P., Adams, C.A., Zhang, S., Sun, Y., Yu, H., Wang, F., 2021. Effects of microplastics on plant growth and arbuscular mycorrhizal fungal communities in a soil spiked with ZnO nanoparticles. Soil Biol. Biochem. 155, 108179. https://doi.org/10.1016/j.soilbio.2021.108179.

Zantis, L.J., Borchi, C., Vijver, M.G., Peijnenburg, W., Di Lonardo, S., Bosker, T., 2023a. Nano- and microplastics commonly cause adverse impacts on plants at environmentally relevant levels: a systematic review. Sci. Total Environ. 867, 161211. https://doi.org/10.1016/j.scitotenv.2022.161211.

Zantis, L.J., Rombach, A., Adamczyk, S., Velmala, S.M., Adamczyk, B., Vijver, M.G., Peijnenburg, W., Bosker, T., 2023b. Species-dependent responses of crop plants to polystyrene microplastics. Environ. Pollut. 122243. https://doi.org/10.1016/j.envpol.2023.122243.

Zhang, T., Luo, X.-S., Xu, J., Yao, X., Fan, J., Mao, Y., Song, Y., Yang, J., Pan, J., Khattak, W.A., 2023. Dry–wet cycle changes the influence of microplastics (MPs) on the antioxidant activity of lettuce and the rhizospheric bacterial community. J. Soils Sediments 23, 2189–2201. https://doi.org/10.1007/s11368-023-03479-x.