Macro- and microplastics leachates: characterization and impact on seed germination

Giovana P. F. Macan, Davi R. Munhoz, Leo A. J. Willems, Charlie Monkley, Charlotte E. M. Lloyd, Jos Hageman, Violette Geissen, Blanca B. Landa, Paula Harkes

Angaben zur Veröffentlichung / Publication details:

Macan, Giovana P. F., Davi R. Munhoz, Leo A. J. Willems, Charlie Monkley, Charlotte E. M. Lloyd, Jos Hageman, Violette Geissen, Blanca B. Landa, and Paula Harkes. 2024. "Macro- and microplastics leachates: characterization and impact on seed germination." *Journal of Hazardous Materials* 480: 136013. https://doi.org/10.1016/j.jhazmat.2024.136013.

Journal of Hazardous Materials 480 (2024) 136013

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Macro- and microplastics leachates: Characterization and impact on seed germination

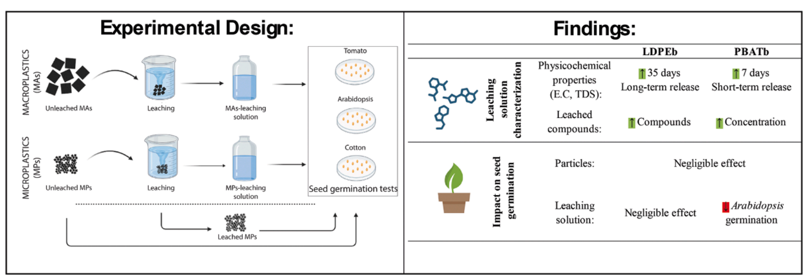
Giovana P.F. Macan[a,c,*,1], Davi R. Munhoz[b,**,1], Leo A.J. Willems[d], Charlie Monkley[e], Charlotte E.M. Lloyd[e,f], Jos Hageman[g], Violette Geissen[b], Blanca B. Landa[a], Paula Harkes[b]

[a] Institute for Sustainable Agriculture, Spanish National Research Council (IAS-CSIC), 14004 Córdoba, Spain
[b] Soil Physics and Land Management Group, Wageningen University & Research, Droevendaalsesteeg 3, 6708PB Wageningen, the Netherlands
[c] Programa de Doctorado de Ingeniería Agraria, Alimentaria, Forestal y de Desarrollo Rural Sostenible, Universidad de Córdoba, Córdoba, Spain
[d] Wageningen Seed Science Centre, Laboratory of Plant Physiology, Wageningen University, 6708PB Wageningen, the Netherlands
[e] Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol, UK
[f] School of Geographical Sciences, University of Bristol, Bristol, UK
[g] Biometris, Applied Statistics, Wageningen University & Research, Wageningen, the Netherlands

HIGHLIGHTS

- Chemical compounds are leached from conventional and biodegradable plastic mulch.
- PBATb mulch released a higher concentration of leachates than LDPEb after one week.
- High concentration of PBATb leachates reduced Arabidopsis seed germination.
- Negligible microplastic particle effects imply seed harm is mainly from leachates.
- Most leached compounds are unregulated worldwide and lack hazard information.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Plastic mulch
Environmental toxicology
Plant health
Contaminants
Toxicity
Plastic additives

ABSTRACT

Although plastic mulch enhances crop yield, its removal and disposal present significant challenges, contributing to macro- and microplastic pollution in agricultural soils. The adverse effects of this pollution on soil and plant health are not fully understood but may stem from the plastic particles or the toxicity of leached chemical additives. This study assessed the impact of macro- and microplastics from nondegradable LDPE-based (LDPEb) and biodegradable PBAT-based (PBATb) mulch films, along with their leachates, on the germination of three plant species. After seven days of incubation, PBAT mulch leached compounds that significantly inhibited Arabidopsis germination, while cotton and tomato exhibited notable tolerance. Notably, PBATb mulch released a higher concentration of compounds, whereas LDPEb mulch exhibited a greater diversity of leached chemicals. Microplastic particles alone did not hinder seed germination, indicating that plastic toxicity primarily arises from the leachates. Many of these leached compounds lack global regulation and hazard information, underscoring the urgent need for further investigation into their environmental impacts and the development of appropriate

* Correspondence to: B021 Gaia, Droevendaalsesteeg 3, 6708PB Wageningen, the Netherlands.
** Corresponding author.
E-mail addresses: gprado@ias.csic.es (G.P.F. Macan), davi.munhoz@wur.nl (D.R. Munhoz).
[1] The authors contributed equally.

https://doi.org/10.1016/j.jhazmat.2024.136013
Received 15 July 2024; Received in revised form 2 September 2024; Accepted 28 September 2024
Available online 10 October 2024

regulatory frameworks to mitigate the potential toxicity of chemicals from conventional and biodegradable mulches.

1. Introduction

Plastic pollution in agricultural soils is a growing global concern [1, 2]. The vulnerability of agricultural fields to plastic pollutants is mainly related to the use and mismanagement of various plastic products in agriculture, such as greenhouse covers, irrigation pipes, and mulch films [3]. While plastic mulch presents undeniable benefits, such as earlier harvests and higher yields, the limited recovery of their residues from the field after harvest undermines its advantages. LDPE-based plastic mulch (LDPEb) must be collected after use from fields, while biodegradable mulch films, such as PBAT-based mulch (PBATb), are supposed to degrade after 2 years in the soils, a premise that led to an increase of biodegradable plastics in agricultural soils in the past decade [4,5]. Field studies have shown that biodegradation often does not occur as predicted in laboratory experiments [6,7]. Hence, the mismanaged plastic debris undergoing fragmentation and degradation represents an additional source of particles smaller than 5 mm, commonly classified as microplastics [8,2,9,10].

In this context, several studies have assessed the impact of mulch-derived microplastics and their associated chemical compounds on soil-plant systems [11-13]. Low-density polyethylene (LDPE) is the main polymer used in mulch films, and biodegradable films composed mainly of poly(butylene adipate-co-terephthalate) (PBAT) and e polylactic acid (PLA) have also been introduced in the market as a potential alternative to conventional plastic aiming to reduce the long-term environmental impact of plastic waste [14]. Several studies have compared the environmental impact of both conventional (low-density polyethylene (LDPE-based)) and biodegradable (poly(butylene adipate-co-tere phthalate) (PBAT-based)) microplastics on soil and have observed significant impacts on the soil microbiome and in plant development [14-16,12,17-19].

While most studies on the environmental impacts of microplastics report detrimental effects on plant and soil health, such assessments often fail to elucidate the underlying causes. Unlike most soil contaminants, microplastics left behind in agricultural soils after mulch application represent an anthropogenic physical particle associated with a range of chemical additives, such as plasticizers, flame retardants, fillers, UV stabilizers, pigments, and antioxidants [20-22]. During plastic manufacturing, additives are not intrinsically bound to the polymers and thus can leach once applied onto the agricultural soil. Furthermore, unreacted monomers and chemical degradation products have also been found in the leachate of commercial and biodegradable plastic materials [23,14,24,25].

Thus, it is still unclear whether the risk posed by microplastics to plant species arises from the particles or plastic-derived compounds that leach from the plastic over time [21]. Understanding these risks from conventional and biodegradable plastics will enhance the understanding of their relative environmental impacts, assist in implementing better practices when applying plastics in agriculture, and scaffold further comprehension of chemical additives.

Menicagli et al. [26] observed that leachates from plastic bags affected the germination and early growth of two dune plants. Similarly, Balestri et al. [27] found abnormalities and reduced seedling growth in cress plants after exposure to leachates from conventional and biodegradable plastic bags. However, Barbale et al. [23] reported no adverse effects of biodegradable plastic leachates on germination and root development of cress seeds. Although these studies assessed the impact of plastic-leaching compounds on seed germination, to the best of our knowledge, none addressed the predominant sources of plastic pollution in agricultural soils, such as plastic mulch, nor jointly examined the leaching processes over time, while identifying the leached compounds.

According to the UN technical report on chemicals in plastics, additives usually comprise 4 % of plastic weight, but in some cases, they can reach up to 50 % of plastic weight for specific polymers, such as plasticizers for PVC [28]. Furthermore, a recent review paper reveals the presence of organic plastic additives in agricultural soils, particularly plasticizers, stabilizers, and antioxidants [14]. The presence of these chemicals in soil prompts questions about the role played by chemical compounds released from microplastics. Further research is needed to identify the leached compounds from various plastic materials commonly used in agriculture and assess their impact on soil and plant health.

In this study, we evaluated the 1) impact of macro- and microplastic leachates released up to 7 days from both conventional and biodegradable mulch films on the germination of three plant species: Arabidopsis (*Arabidopsis thaliana*), cotton (*Gossypium hirsutum*), and tomato (*Solanum lycopersicum*); 2) the effects of unleached microplastic particles compared to leached microplastics on seed germination and 3) plastic mulch leaching dynamics (i.e., changes in bulk parameters) for 49 days. The findings could provide valuable insights into the plastic industry and inform policies tackling certain harmful compounds while exploring environmentally friendly alternatives.

2. Materials & methods

2.1. Macro- and microplastics

Macro- and microplastics were prepared from commercially available biodegradable and conventional mulch films widely used in agriculture and considered major sources of microplastic pollution in agricultural soils, thus representing environmentally relevant test materials [2,9,29]. We selected Bionov-B®, a mulch film mainly composed of poly(butylene adipate terephthalate) (hereinafter PBATb), and BSK & Lakufol Kunstroffe GmBH® mulch film mainly composed of low-density polyethylene (hereinafter LDPEb). Both plastic films were black since it is the most commonly used type of mulch in agriculture [30,9,31], and macro- and microplastics were obtained from 1.2 m width with 15 μm thickness mulch films. Bionov-B is sold as a biodegradable mulch film, while the LDPE film is a non-degradable mulch. Macro-plastics were cut from the mulch films into 1 × 1 cm pieces with scissors, while microplastics were procured from Lavaris sro (https://cs.lavaris.eu/) by cryo-milling and subsequent size fractionation to obtain microplastics mostly ranging from 1 to 750 μm diameter (Table S2, Fig. S1A).

Macroplastics polymeric composition was characterized using Fourier transform infrared (FTIR) spectroscopy equipped with an attenuated total reflectance (FTIR-ATR) accessory (Cary 630 FTIR) within the range of 650–4000 cm^{-1}, 32 background scans and resolution of 4 cm^{-1}. Samples were measured in triplicate. Fig. S1C-D shows the detailed profiles. Microplastics' polymer chemical composition and size distribution were acquired by the Agilent 8700 LDIR using the Clarity software with a customized library (Text S1). Microplastic size distributions were calculated based on the percent (%) of microplastics within different diameters. We defined 20 μm diameter as the smallest detectable particle size to avoid over-quantification of particles < 20 μm due to LDIR technical limitations.

Macro- and microplastic polymer compositions were confirmed by matching their spectra with updated libraries used for ATR-FTIR and LDIR, respectively. LDIR analysis also provided information regarding the size distribution of the microplastic particles (Fig. S1B, Table S2). Regarding macroplastics, the ATR-FTIR spectra showed no significant differences in the absorbance spectra of the leached LDPE- and PBATb mulch films compared to the unleached materials (Fig. S1C-D).

2.2. Leachates and leached microplastic preparation

After thoroughly reviewing studies on the impact of plastic leachates on seed germination, we defined the experimental conditions of the leaching experiment (Table S1). A pilot experiment showed a negligible effect of leachates on seed germination at a concentration of 0.02 % (w/w). Thus, concentrations of 0.2 % (w/w), named Low Concentration 1 (C1), and 2 % (w/w), named High Concentration 2 (C2), were selected. These concentrations were selected based on previous toxicological studies of plastic leachates (Table S1), allowing comparison and covering environmentally relevant concentrations reported for agricultural soils [32-34,31].

Fig. 1 depicts the experimental design overview. The plastics were added to 1 L of autoclaved distilled water and shaken (Gerhardt, Laboshake, Germany) in the dark at 95 rpm at 22 ± 2 °C for 7 days. A second batch was kept leaching for 49 days. Leachates were then filtered through a 0.7 µm paper filter (Whatman International Ltd, Kent, the UK), and the filtered solution was stored in the dark at 4 °C until further analysis. Leached plastics were exposed for 7 days, followed the same filtration step, and the retained macro- and microplastics were stored under the same conditions. Three replicates were prepared for each treatment in addition to the negative controls (water without plastics), blanks (neither water nor plastics), positive control (polymer-derived organic compounds, PDC), and concentrated positive control (polymer-derived organic compounds, PDC1000x). PDC and PDC1000x were prepared with lactic acid, adipic acid, 1–4-butanediol, and terephthalic acid following previously reported concentrations (0.001 mg·L-1, 0.04 mg·L-1, 35.64 mg·L-1, 0.003 mg·L-1, respectively) [35]. We tested three different methods to expose the seeds to microplastics: 1) coating the wet seed with dry microplastics, 2) immersing seeds into a solution with microplastics, and 3) wet dispersing the solution with microplastics onto the seeds. The solution was agitated with a magnetic stirrer to ensure the microplastics even distribution and uniform suspension. The wet dispersion stood out due to their more homogeneous distribution on the seeds without microplastic clustering and was, therefore, selected (Fig. S2).

2.3. Leachates bulk characterization

2.3.1. General leachate properties

Leachates were characterized every three days by a DIST ®5 EC/TDS/Temperature tester (Hanna Instruments®, USA) to determine electrical conductivity (mS·cm^{-1}) and total dissolved solids (ppm). pH was measured with a stationary pH meter (Fisher Scientific®, Fisherbrand accumet FE150®, Netherlands). We collected the remaining filtered leachate of each treatment and further tested them for the seed germination assays and other physicochemical tests.

2.3.2. Weight loss

The gravimetric weight loss calculation of unleached and leached macro- and microplastics was calculated following Eq. 1:

$$Weight\ Loss(WL(\%)) = \left(\frac{m_{bl} - m}{m_{bl}}\right) \times 100 \quad (g) \tag{1}$$

where: m_{bl} (g) represents the dry weight of macro and microplastics before the leaching experiment, and m (g) is the dry weight of sampled macro and microplastics after the leaching experiment.

However, minimal weight loss occurs regardless of the leaching experiment due to methodological limitations. To disregard such loss, we validated our weight loss recovery rate by applying Eq. 2 (Corrected Weight Loss), which considers the specific methodological loss of each tested polymer (Fig. S3) and their respective concentrations during each filtration and weighing effort.

$$Corrected\ Weight\ Loss(cWL(\%)) = \mathrm{WL}(\%) - (m_{loss} \times f_w) \tag{2}$$

where: m_{loss} is the micro/macroplastic loss per treatment and their respective concentration when measuring weight loss during method validation (Fig. S3), and f_w is the number of times we measured weight loss for each experiment.

2.4. Leachates chemical characterization

2.4.1. Ultraviolet-visible (UV-Vis) Spectrophotometer

A full scan of the leachates using wavelengths from 190 nm to 700 nm was carried out using a UV-Vis spectrophotometer (NanoDrop™ One (Thermo Scientific™) to acquire the following indexes and characterize the humus parameters, humification, and aromaticity of the leaching solutions.

$$SR = \left(\frac{S_{275-295}}{S_{350-400}}\right) \tag{3}$$

$$HI \rightarrow \left(\frac{E_{250}}{E_{365}}\right) \tag{4}$$

$$AR \rightarrow \left(\frac{E_{253}}{E_{203}}\right) \tag{5}$$

Eq. 3 shows a proxy for humus parameters called slope ratio (SR). SR is the difference between the absorbances at 275 nm and 295 nm

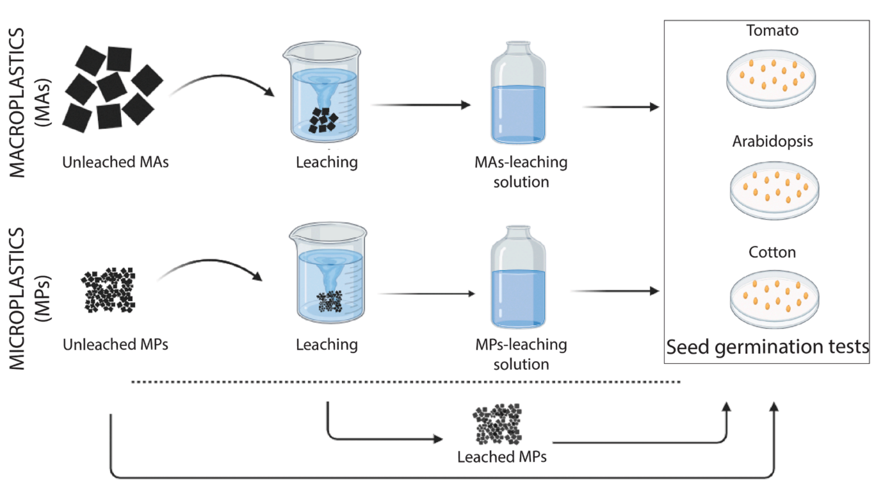


Fig. 1. Unleached macro- and microplastics undergoing the leaching process. Unleached macro- and microplastics, leached microplastics, and leachates from macro- and microplastics were then applied to seed germination tests to verify their toxicity. The image was partially created with BioRender.com®.

divided by the difference between the absorbances at 350 nm and 400 nm (X. [36]. Eq. 4 displays the absorbance ratio at 250 nm to 365 nm, described as a humification indicator (HI) that characterizes organic solute aromaticity and molecular weight [37]. Eq. 5 shows the division of the absorbance at 253 nm by the one at 203 nm and demonstrates the increasing aromatic ring substitution, i.e., working as an aromaticity indicator (AR) [38].

2.4.2. GC-MS: Leachate Preparation and Analyses

The macro- and micro C2, 7-day leachate samples were selected for qualitative compound screening due to observed effects and to aid in analyte detection. Control samples containing autoclaved distilled water only (n = 3) and procedural blanks (n = 3) were processed concomitantly to leachate samples. A subsample (150 ml) of the filtered leachate was freeze-dried (−52 °C for 72 h) before solvent transfer with 3 × 1 ml 2:1 v/v dichloromethane (DCM) / methanol (MeOH) (Rathburn Chemicals; HPLC grade, >99 % purity). The DCM/MeOH was removed under N2 at room temperature. An internal standard of butyl benzyl phthalate (BBP) (Sigma-Aldrich, Merck Life Sciences UK Ltd.) (20 µl of 1.98 mg ml-1) was added to the dried LDPE extracts, blanks, and the control samples (water only), before redissolving in a final volume of ethyl acetate (400 µl) (Rathburn Chemicals; HPLC grade, >99 % purity). The leachate from the biodegradable samples contained a yellow, oily substance, which was insoluble in the required solvent for instrument analysis (ethyl acetate). As such, the soluble fraction of the leachate was dissolved in 1 ml of ethyl acetate. The supernatant was subsampled (10 µl) and diluted into 250 µl ethyl acetate. The addition of BBP to the subsample (10 µl of 1.98 mg ml-1) enabled the normalization of the peak area and the application of a threshold for compound detection. Therefore, that process made it feasible to target the leachates' most abundant low molecular weight organic components for characterization.

Trimethylsilyl derivatization was selected to aid compound detection of alcohol and carboxylic acid functionalized components of the mulch film through GC-MS [35]. A subsample of the leachate samples (50 µl) was dried and reacted with 30 µl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) at 70 °C (1 h). Following cooling to room temperature, excess MSTFA was removed under N2, and the extract was redissolved in 50 µl ethyl acetate.

The GC-MS (Thermo Fisher Scientific™ Trace™ 1300 GC and an ISQ™ LT single quadrupole MS) was fitted with a non-polar Agilent HP-1 column (50 m x 0.32 mm × 0.17 µm). Sample (1 µl) was injected into the programmable temperature vaporizer (PTV) inlet, which operated under spitless mode at 50 °C, with constant purge flow at 5 ml min-1 and a spitless time of 2 min. The carrier gas was helium at a constant flow rate of 2 ml min-1. The GC oven temperature was initially held at 40 °C (1 min) and ramped at 5 °C min-1 to a final temperature of 300 °C (15 min). The mass range of the MS was *m/z* 40 – 700, and electron ionization was set to 70 eV.

Data interpretation was carried out in Xcalibur Version 4.1.31.9 (Thermo Fisher Scientific™ Ltd). A peak detection threshold of > 2.5 % in all triplicate repeats relative to the IS was applied to ensure that only the most abundant leachate compounds were screened for identification while reducing the potential of contaminant interference. The MS data for each compound in the extract was compared to spectral libraries (NIST 14 Main Library, NIST 2019 Additives Library, NIST 2019 Flame Retardants Library, NIST Stabilisers-Antioxidants Library, and the NIST 2019 Plasticisers Library), with a structural match requirement of ≥ 85 % required for tentative identification. Text S2 elaborates on quality assurance considerations.

2.4.3. Identification of chemical compounds extracted from LDPEb- and PBAT-b microplastic mulches with microwave-assisted solvent extraction

To compare the analytes detected in the leachate to the additive components of the mulch film, pristine mulch microplastic particles were extracted into an organic solvent through microwave extraction. Text S3 and Table S3 provide a detailed description of the methods used and all identified chemical compounds extracted from LDPEb and PBATb microplastic mulches using microwave-assisted solvent extraction, respectively.

2.5. Seed germination assays

The impact of macro- and microplastic leachates (0.2 % and 2 %), as well as unleached and leached microplastics, was tested on *Arabidopsis thaliana* wild accession Col-0, a model plant in genetic and plant biology studies and the standard species for the semi-automated germination pipeline used, and tomato (*Solanum lycopersicum,* variety money maker), and cotton (*Gassypium herbaceum* variety juncal) which are economically important crops widely cultivated with plastic mulch and are included in the list of recommended test species for seed germination and plant toxicity evaluations by various standards [39-42].

Germination experiments followed the germinator pipeline developed at Wageningen University [43] with minor adjustments for tomato and cotton seeds. Two layers of blue germination paper were placed inside germination boxes (14 ×21 cm) and wettened with 48 ml of demineralized water. Cotton seeds required an additional wettened white germination paper to maintain additional moisture. About 50–200 seeds of *Arabidopsis thaliana,* 21 tomato seeds, and 20 cotton seeds were spread on wetted papers with a frame to ensure appropriate spacing for further image analysis. Seed clustering was prevented by meticulous sorting with metal tweezers. Six replicates were prepared for *Arabidopsis thaliana* and tomato and four for cotton due to incubation feasibility. Seed stratification was not needed (Fig. S4). Stacked trays were wrapped in a closed transparent plastic bag for further incubation. The *Arabidopsis thaliana* seeds were incubated (type 5042; Seed Processing Holland, http://www.seedprocessing.nl) at 22 °C under continuous light (143 µmol m^{-2} s^{-1}) [44] for 7 days while tomato and cotton seeds were incubated at 24 °C in the dark for the same period.

Pictures were taken twice a day for 7 days with a Nikon D80 camera, fixed to a repro stand with a 60 mm macro-objective, and connected to a computer with Nikon Camera Control Pro software version 2.0, and were then processed by the Wageningen University germinator pipeline. That provides a curve fitting script based on the four-parameter Hill function that enables the interpretation of the following indexes: Maximum percentage of germination (G_{max}), time to reach 50 % germination (t_{50}), and Area Under the Curve (AUC), an integration combining information on maximum germination, t_{50}, and seed uniformity. The germination score is published in previous research [43], and the values are based on the double color thresholding of images and the cumulative germination data utilized for the curve fitting module from where G_{max}, t_{50}, and AUC are derived.

2.6. Leachate monitoring dynamics over 49 days

Since plastic mulch leaching is a continuous process lasting more than 7 days, we monitored an additional batch of microplastics leaching for 49 days and analyzed how such a process would affect the assessed bulk parameters (E.C., TDS, Weight Loss, and UV-Vis). The measurements were taken after 3, 7, 14, 21, 28, 35, 42, and 49 days, following the methodology described in Section 2.3.1.

2.7. Statistical analysis

Data analysis employed general linear models to explore the effects of plastic-type, size, and exposure on the response variables, encompassing all main effects and their interactions. ANOVA tests were conducted, and post hoc analyses were performed when observing significance. Tukey's method was used to assess the physicochemical properties of the plastic leaching solution, identifying specific differences between treatment groups, while Dunnett's multiple comparison test was applied to evaluate differences between each treatment and the water control in seed germination data.

All analyses were carried out using R version 4.4.0, graphical representations were created with GraphPad Prism® 8 software, and schematic diagrams were rendered using Adobe Illustrator 2023 (Adobe, Inc.). A significance level of $\alpha = 0.05$ was applied, and results are presented as mean ± standard deviation of the mean (SD).

3. Results

3.1. Leachates bulk characterization

A more distinguished weight loss of the PBATb mulch (>1.5 %) was observed compared to the conventional LDPEb mulch (<1 %) after the 7-day leaching period in water, regardless of material size (macro vs micro) and concentration (C1 vs C2) (Fig. 2).

The physicochemical properties of the leaching solutions were monitored over time (Fig. 3). Increased electrical conductivity (E.C.) and total dissolved solids (TDS) indicated the release of chemical compounds by macro and microplastics (Fig. 3A-B). These changes stood out in the treatment with PBATb mulch. After three days of leaching, a significantly higher electrical conductivity of PBATb microplastic (MP PBATb C2) was observed compared to the control ($p < 0.05$). After 7 days, the difference was even more pronounced, with MP PBATb C2 reaching values of 0.1 ± 0 mS/cm and 50 ± 0 ppm versus 0.01 ± 0 mS/cm and 3.3 ± 6 ppm of the control.

Fluctuations in pH values were observed in all treatments (Fig. 3C). The macro PBATb leached solutions at both concentrations exhibited a slight decrease in pH after 3 days of incubation compared to the control ($p < 0.05$), followed by its stabilization up to day 7. The solutions with microplastics exhibited a distinct behavior, with a pH peak occurring after three days of leaching followed by a decrease after seven days of leaching, regardless of plastic-type and concentration.

3.2. Leachates chemical characterization

UV–vis characterization of leachates brought to light a significant increase ($p < 0.05$) in S_R, E_{250}/E_{365}, and E_{253}/E_{203} of leachates from PBATb microplastics (C2) treatment after the 7-day leaching experiment: 4.66 ± 1.52, 19.83 ± 4.19, and 0.46 ± 0.01, respectively (Table 1).

Leachates of LDPEb macro- and microplastics showed no significant ($p > 0.05$) changes in S_R, E_{250}/E_{365}, and E_{253}/E_{203} after the leaching experiment, regardless of the concentration tested. In short, chemical compounds released from LDPE mulch after 7 days did not significantly ($p > 0.05$) alter the aromaticity, molecular weight, or aromatic ring substitution of the leachates.

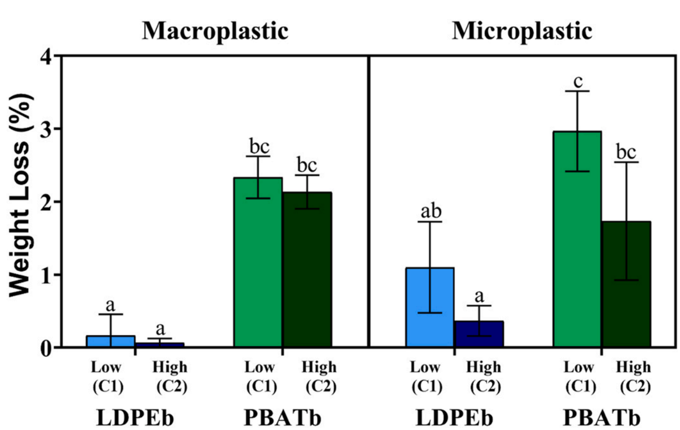


Fig. 2. Corrected plastic weight loss after 7 days of incubation in water. Means ± S.D (n = 3). Different letters indicate significant differences among treatments ($p < 0.05$; Tukey's test).

GC-MS results uncovered that PBATb mulch films leached substantially higher concentrations of compounds than the LDPEb mulch films after a 7-day leaching experiment. The concentration factor from the 150 ml H_2O LDPE leachate to the final extract volume (400 μl) was 375, whereas the effective concentration factor for the PBATb leachate was only 60. That aligns with the measured mass loss from both plastics and was similarly apparent from the visible yellow oil in the final PBATb extract, suspected to be due to polyglycerol, based on the detection of glycerol and glycerol dimers in the leachate. Fig. S5 shows the GC-MS chromatograms from leachate solutions of macro- and microplastics from LDPEb and PBATb plastic mulch films, and all detected compounds are listed in Table S4. Compound-specific quantification was not deemed viable in this case, given the partial solubility of leached content from the PBATb into final extracts and the unknown identity of many leached chemical components, for which reference material was unavailable.

Although PBATb leachates were higher in concentration than LDPEb, the diversity of compounds identified by GC-MS was higher for the latter. Table 2 summarizes all the compounds identified by the GC-MS and the possible sources and functionalities aimed for such chemical compounds in plastics. Many LDPE leachate components could not be assigned an identity through MS library comparison, making their source or function in the mulch unclear.

The identified chemical compounds leached from LDPEb and PBATb were also classified according to Plastchem lists and hazardous scores for persistence, bioaccumulation, mobility, and toxicity [50] in an attempt to corroborate observed toxic effects of specific chemicals. Although none of the identified organics present in the leachate are categorized as environmentally harmful, it can be noted that both conventional and biodegradable plastic mulch leachates contain some substances not yet regulated internationally, belonging to the grey list (Table S4), or indeed unidentified chemical constituents, for which hazard classifications cannot be made.

3.3. Impact of leachates solutions on seed germination

The assessed effects of leaching solutions from two types of plastic mulch on germination parameters of three distinct plant species (Arabidopsis, cotton, and tomato) brought to light that leachates from PBATb mulch at a higher concentration (C2) significantly decreased ($p < 0.05$) the maximum germination and area under the germination curve of Arabidopsis seeds, regardless of the size of the plastic particles (Figs. 4A and 4B, S6, and S7). Additionally, a notable increase in the time to reach 50 % germination (t50) was observed for treatments with leachate from the PBATb mulch, indicating a delay in seed germination (Table S5). Fig. S6 and S7 provide a visual insight of the Arabidopsis reduced germination No significant changes in germination uniformity (U8416) were observed in any of the assessed treatments.

For tomato and cotton, all the germination variables were not significantly affected by the leaching solutions ($p > 0.05$), revealing no adverse effect on the germination of these species compared to the water control (Fig. 4 and Table S5).

The polymer-derived organic compounds (terephthalic acid + lactic acid + adipic acid + 1,4-butanediol) at higher concentrations dramatically reduced the maximum germination of Arabidopsis and tomato seeds, consequently decreasing the area under the germination curve (Fig. 4A-D). Furthermore, a significant reduction in other germination parameters, such as uniformity, mean germination time, and t_{50} germination, was observed across all plant species (Table S5). Cotton seeds were comparatively less affected by these compounds. Although a delay in germination was observed (reduced values of t50 and increased values of mean germination time), the final number of germinated seeds remained unaffected, as shown by a non-significant effect ($p > 0.05$) on G_{max} when compared with the water control.

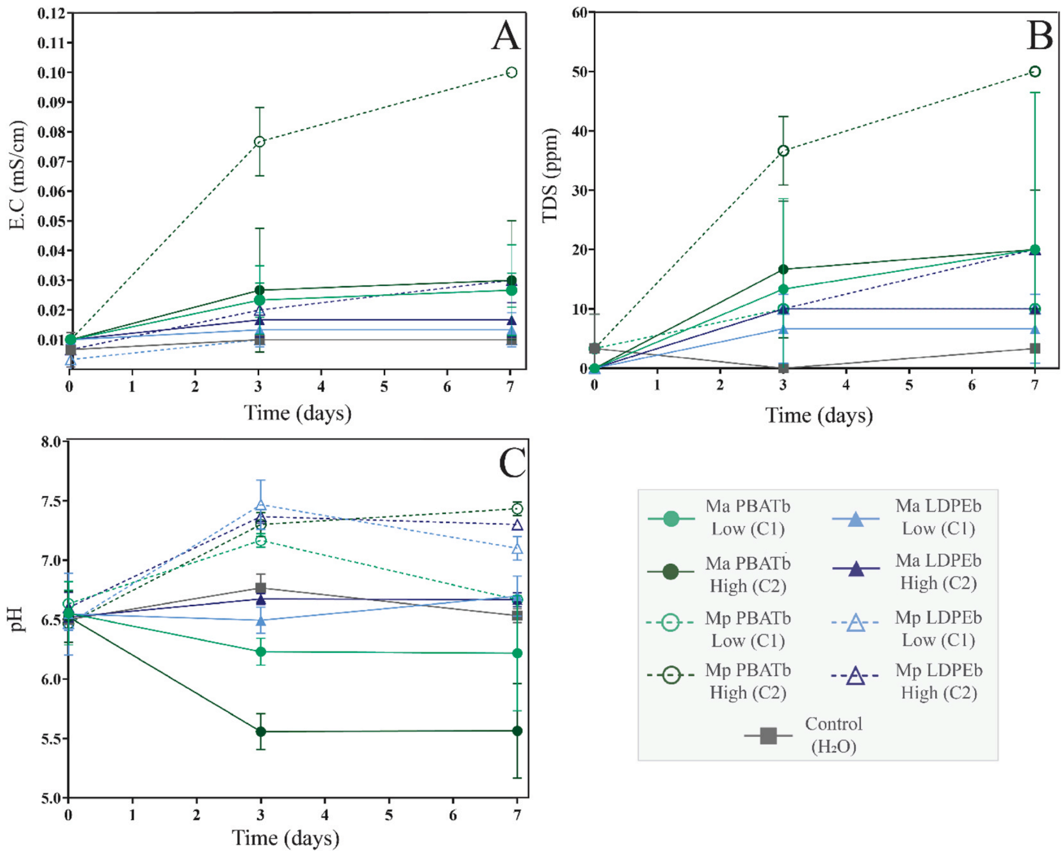


Fig. 3. Variations in the chemical properties of the leaching solution over time. A. Electrical conductivity (E.C) B. Total dissolved solids (TDS) C. pH. Means ± S.D (n = 3).Ma = macroplastics; Mp = microplastics.

Table 1
UV-Vis characterization of leachates from macro- and microplastics of both LDPEb and PBATb mulch films.

Treatment	S_R	E_{250}/E_{365}	E_{253}/E_{203}
Ma LDPEb (C1)	0.00 ± 0.00^a	0.33 ± 0.57^a	0.50 ± 0.50^{abc}
Ma LDPEb (C2)	0.67 ± 1.15^a	0.66 ± 0.57^a	0.03 ± 0.05^a
Ma PBATb (C1)	1.50 ± 0.86^a	3.38 ± 0.67^a	0.71 ± 0.04^c
Ma PBATb (C2)	2.00 ± 0.50^a	4.90 ± 1.01^a	0.58 ± 0.01^{bc}
Mp LDPEb (C1)	0.33 ± 0.57^a	1.16 ± 1.04^a	0.08 ± 0.07^{ab}
Mp LDPEb (C2)	0.33 ± 0.57^a	1.16 ± 1.04^a	0.12 ± 0.11^{ab}
Mp PBATb (C1)	0.66 ± 1.15^a	1.33 ± 2.31^a	0.52 ± 0.09^{abc}
Mp PBATb (C2)	4.66 ± 1.52^b	19.83 ± 4.19^b	0.46 ± 0.01^{abc}

Different letters indicate significant differences among treatments ($p < 0.05$; Tukey's test)

3.4. Impact of microplastic particles on seed germination

Both conventional and biodegradable microplastics directly applied to seeds, regardless of whether they were unleached or leached, exhibited a negligible effect ($p > 0.05$) compared to the water control on all assessed germination variables and plant species (Fig. S8 and Table S6).

3.5. Plastic mulch leaching dynamics

Monitoring plastic mulch leaching dynamics revealed different short- and long-term behaviors of LDPE-b and PBAT-b plastic mulches. After 7 days of incubation, there was a significant ($p < 0.05$) increase in the EC of the leachates for both types of plastics compared to the water control (Fig. 5A). The higher concentration of leachates from PBAT-b mulch microplastics showed a more pronounced peak in EC and TDS values after 7 days and stabilized afterward (Figs. 5A and 5B). In contrast, the LDPE-b leachates at both concentrations (C1 and C2) only peaked in these parameters after 35 days. Slight fluctuations in pH values were observed in the leachates of all assessed treatments and the water control (Fig. 5C). Additionally, a higher weight loss ($p < 0.05$) of PBATb at both concentrations compared to LDPEb microplastics was observed after 49 days of leaching (Fig. 5D).

4. Discussion

Leachates from conventional and biodegradable plastics can interfere with the status quo of soil health (Z. [53]. We discuss here that high concentrations of PBATb leachates (2 % w/w) hamper Arabidopsis germination after 7 days. We also address leachates' more detrimental impact on seed germination than microplastics and the relevance of long-lasting leachate monitoring due to the diverse leaching dynamics of different polymers.

4.1. Leachate characterization

The observed weight loss of the plastic mulch films provides an initial indication that compounds were released into the solution after 7 days of incubation in water. The more significant gravimetric loss of the biodegradable PBATb mulch film compared to the LDPEb has also been reported by Serrano-Ruíz et al. [35], with variations depending on the commercial brand of the mulch film.

Furthermore, the increasing values of EC and TDS in all C2 samples, more pronounced in the PBATb samples, indicate higher concentrations of dissolved organic and inorganic substances, including potential contaminant metals. Similar results of increased salinity were observed by Balestri et al. [27] when assessing leachates from conventional and

Table 2
Main compounds found in leachates from the mulch films used in the study identified by GC-MS and their possible functionalities.

Compound	Mulch film				Possible source / Functionality
	LDPEb		**PBATb**		
	Macro	Micro	Macro	Micro	
Unknown	✓ (15)	✓ (6)			Likely non-intentionally added substances (degradation/transformation products of additives)
2,2′-Oxydipropane−1,3-diol* ** *			✓	✓	Plasticizer with coating and adhesive properties [45]. Also known as dipropylene glycol and can be used as a PBAT solvent.
3-((1,3-dihydroxypropan−2-yl)oxy) propane−1,2-diol* ** *		✓	✓	✓	Compound also known as glycerol monomethacrylate. Hydrophilic monomer [46] is often used to prepare biocompatible amphiphilic networks [47] and antifouling agents [48]. Crosslinking agent; enhancer of flexibility, moisture resistance, and adhesion; coating additive; biodegradability enhancer.
3-((5-(hydroxymethyl)−1,4-dioxan−2-yl) methoxy)propane−1,2-diol* **		✓	✓	✓	Plasticizer, since compounds with dioxane rings and diol properties can act as plasticizers in polymer matrices; Crosslinking agent and PBAT solvent [49]; Compatibilizer – the hydroxyl and dioxane rings might improve the compatibility between PBAT and other polymers/additives; Surface modifier; Processing aid; Additive for UV-resistance, antistatic properties and/or antimicrobial activity [50].
Glycerol* **	✓	✓	✓	✓	Antistatic agents (United Nations, 2023), Plasticizer [51] and increase compatibility [52];
Diglycerol* ** *		✓	✓	✓	Biocide; Catalyst; Colorant; Crosslinking agent; Filler; Initiator; Intermediate; Light stabilizer; Lubricant; Monomer; Odor Agent [50].

** = 2 TMS derivatives; *** = 3 TMS derivatives; **** = 4 TMS derivatives; ***** = 5 TMS derivatives.
✓Confirms the presence of the compound.
() Shows the number of Unknown compounds found.

biodegradable bags. However, contrasting results were reported by Serrano-Ruiz et al. [54], where the conductivity of the leached solution remained unaffected after the same incubation period in water. Although we did not specifically assess trace metals in this research, previous studies have shown the presence of zinc (Zn), copper (Cu), and nickel (Ni) in the leachates of compostable bags [55].

Our results monitoring plastic leaching dynamics over an extended period of 49 days show distinct patterns of increased electrical conductivity (EC) and total dissolved solids (TDS) over time, particularly for conventional plastic mulch compared to biodegradable mulch. A first peak in the compound release is observed after 7 days, being more pronounced for PBATb C2, which tends to stabilize. In contrast, the conventional LDPEb material shows a second peak after 35 days. That indicates that conventional LDPEb materials also release significant amounts of compounds, but the process takes longer. Our findings underscore the importance of testing plastic leaching dynamics over extensive periods. Most published experiments on plastic leaching have been conducted in the short-term (3 to 7 days) (Table S1), eclipsing the long-term effects. Hence, incubation time should be carefully considered as it can lead to significantly different results.

Previous studies have also reported increased pH values in leachates of conventional polyethylene bags and acidification in solutions containing leachates from biodegradable ones [27,54]. In this context, P. Zhang et al. [56] have highlighted the impact of macro- and microplastics on pH levels in agricultural soils. The observed soil acidification in the presence of PBATb plastics has been attributed to various factors related to the material's biodegradation, such as mineralization, the production of organic acids, and the release of protons (H^+) from the breakdown of ester bonds [57].

Nevertheless, the results presented here suggest that immediate changes in the chemical properties of the surrounding environment may be somehow attributed to the initial release of compounds easily leached from PBATb plastics. Biodegradable plastic mulch begins interacting with the environment even before polymer degradation processes, particularly upon initial contact with water [35,58]. Residual monomers, solvents, and additives (such as plasticizers, lubricants, antistatic agents, antifogging agents, blowing agents, stabilizers, colorants, and fillers) that are not chemically bound to the polymer matrix can be readily leached from these plastics [21].

Most previous studies have provided limited characterization of leachates, typically using general, non-specific methods discussed beforehand that offer an initial overview or indication of released substances once these materials come into contact with water. In contrast, complementary chemical analyses, including UV-Vis spectroscopy and non-targeted GC-MS, offer more comprehensive screening and identification of organic compounds based on their wavelengths and mass spectra [21]. Due to their higher sensitivity and specificity, these advanced techniques provide crucial insights for identifying potentially toxic materials and highlighting the need for their replacement or stricter regulation.

UV-Vis spectroscopy further supported the finding that PBATb microplastics may leach more than LDPEb ones, showing a significant increase in SR, E250/E365, and E253/E203 after the 7-day leaching experiment with PBATb microplastics (C2). These values allowed us to infer that the compounds released triggered changes in aromaticity, molecular weight, and aromatic ring substitution of the leachates [38]. The increased E_{253}/E_{203} measured for PBATb macroplastics (C1) and (C2) and PBATb microplastics (C1) and (C2) may also indicate the increased quantity of carboxyl and carbonyl functional groups on aromatic rings in the solution [38].

4.1.1. Molecular composition of plastics and leachates

Most identified chemical compounds solvent extracted from the original LDPEb plastic mulch included fatty acid lubricants, amide slip agents, UV absorbers, antioxidants, and *n*-alkane oligomers (Table S3). Both tris(2,4-di-tert-butylphenyl)phosphite (Irgafos 168) and octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Irganox 1076) have been identified elsewhere as additives components in LDPE based mulch films and are typically incorporated as a synergetic antioxidant pair in plastics [59,60]. Similarly, 2-hydroxy-4-(octyloxy)benzophenone (octabenzone) is also a known UV absorber component of both LDPE and biodegradable mulches [61]. Extracts from the biodegradable mulches also contained the slip agent erucamide along with a number of cyclic oligoesters, which are usual components of polyester materials [62,63]. A number of glycerol components were additionally detected in the biodegradable mulch solvent extracts, indicating a polyglycerol component in the material.

After a 7-day leaching process, most of the chemical compounds leached from LDPEb macro- and microplastics are unknown, while for PBATb, many are glycerol derivatives (Table 2). The identification of chemical compounds extracted from the original LDPEb and PBATb plastic mulches (Table S3) reveals that some major chemical compounds are less prone to leaching than others. Discrepancies with other leaching studies are noted here, given the release of Irganox 1076 [60], Irgafos

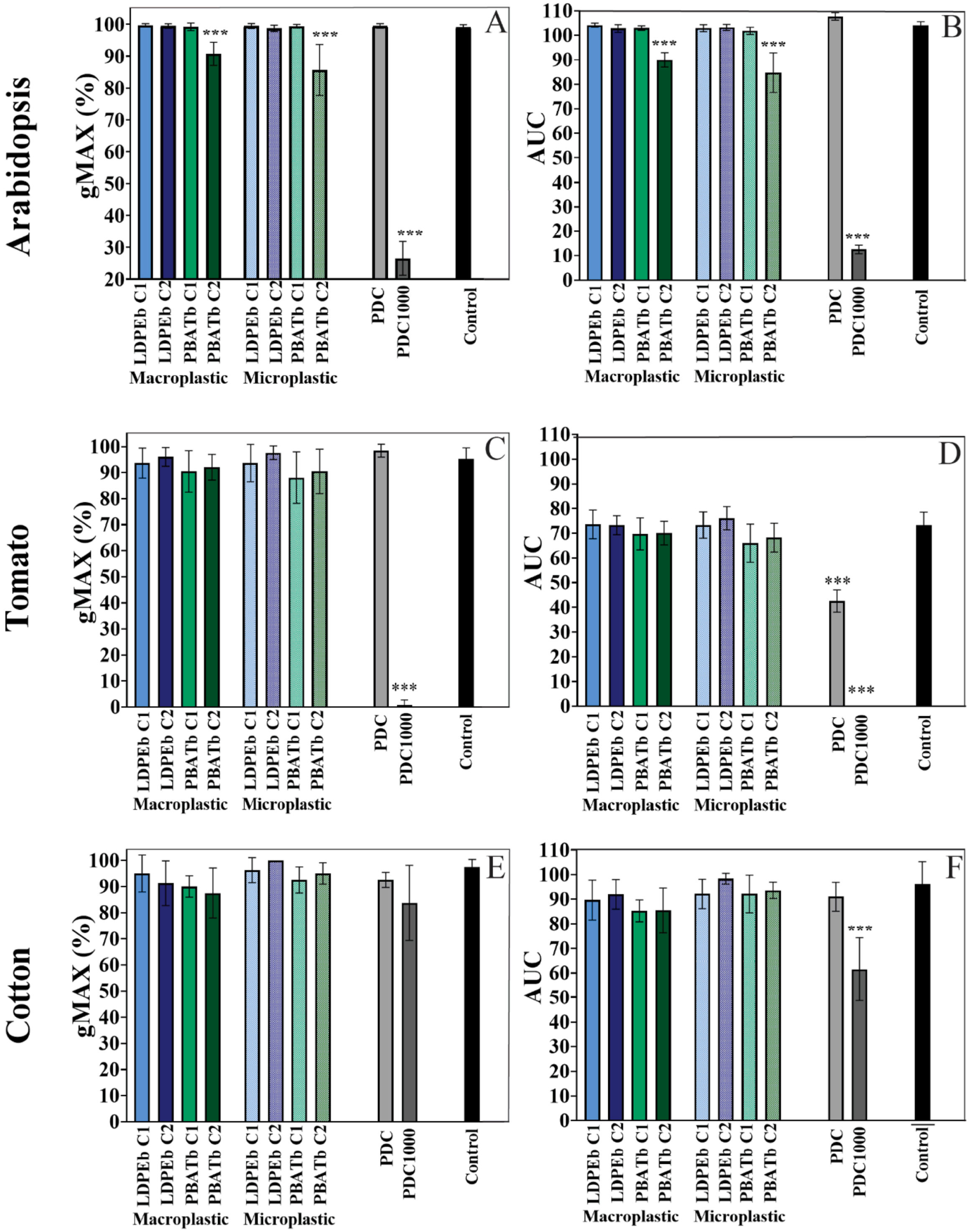


Fig. 4. Impact of leachates from macro- and microplastic on maximum germination rate and area under the curve of Arabidopsis *sp.* (A-B), tomato (C-D), and cotton (E-F) seeds. Where: PDC and PDC1000 are the polymer-derived organic compounds (terephthalic acid + lactic acid + adipic acid + 1,4-butanediol) at reported concentrations [35] and 1000x concentrations. C1, and C2 are the 0.2 %(C1) and 2 % concentration (C2). Asterisks represent significant differences compared to the control (H_2O) according to the Dunnet test (*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$).

168 [59], and octabenzone [61] from LDPE mulch over short term leaching (28 d) has been reported but not detected in this case. This is likely a result of detection limits between the targeted analyses of these studies and the untargeted characterization undertaken here. Even so, the results suggest that certain additives require more attention in an environmental context.

4.2. Impact of leachates on seed germination

This study is a pioneer in assessing the impact of plastic-derived leachates on seed germination with a well-established automated pipeline [43]. The leachate effect varied depending on the type of plastic, concentration, and the assessed plant species.

Most of the evaluated parameters regarding Arabidopsis germination

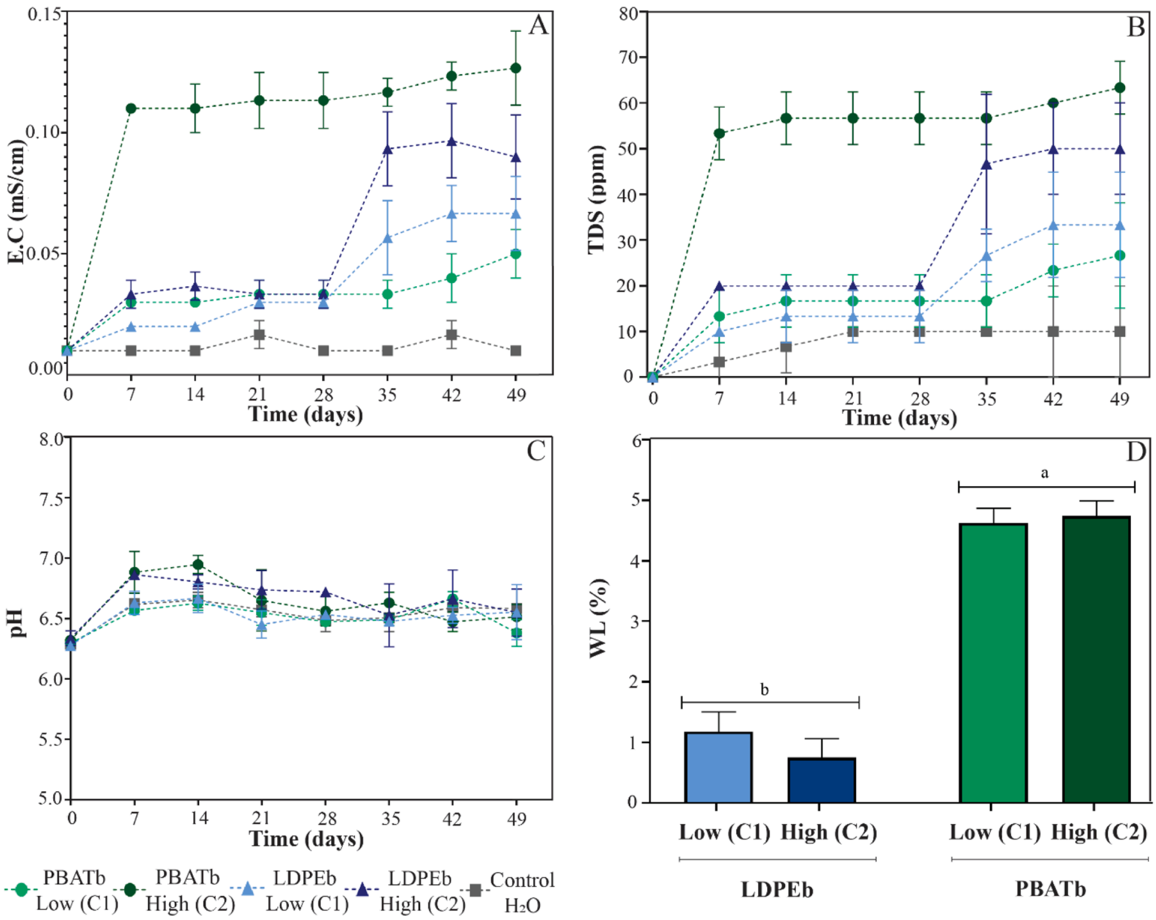


Fig. 5. Variations in the physicochemical properties of the leaching solution over 49 days to assess the plastic mulch leaching dynamics. A. Electrical conductivity (E.C) B. Total dissolved solids (TDS) C. pH. Means ± S.D (n = 3); D. Corrected weight loss (WL) after 49 days of leaching. Different letters indicate significant differences among treatments ($p < 0.05$; Tukey's test).

were impaired by higher concentrations (C2) of compounds released by PBATb plastic mulch. That impairment may be explained by induced osmotic stress and decreased water uptake, triggered by excessive dissolved solids, undermining germination and ultimately establishing poor seedlings [26]; S. [64]. Furthermore, research indicates that the sensitivity of seeds to stress varies among plant species [65].

Arabidopsis is a glycophytic plant sensitive to moderate levels of salt stress [66], particularly evident during the seed germination and seedling stages. Therefore, Arabidopsis' reduced germination may be attributed to their susceptibility to osmotic stress [67], an understanding aligned with the possibility of increased E.C values due to higher ion content in the leachates.

A recent study showed higher inhibitory effects of PBATb microplastics on the growth of Arabidopsis than LDPEb ones (B. [12]. Interestingly, the degradation products from PBAT, such as adipic acid, butanediol, and terephthalic acid, were more toxic than the PBAT microplastics themselves. B. Liu et al. [68] also reported hampered seed germination when treating those with a highly concentrated solution of these compounds, a pattern similar to what we reported when exposing the seeds to a high concentration of PDC (Fig. 4).

In this regard, Hu et al. [69] raise awareness of the increased use of biodegradable plastic packaging and agricultural films, which can lead to increased levels of potentially toxic oligomers and monomers. For instance, terephthalic acid, a monomer PBAT, is an endocrine disruptor that can harm the endocrine system of organisms. Hence, the reduced germination observed in this study may be due to either the indirect impact of the solution's salinity or the high concentration of oily compounds likely derived from polyglycerol.

The leached oily substances, likely due to the polyglycerol or lipid-based compounds in the plastics, could have hampered hydrophilic interactions and the contact between water and seeds.Furthermore, without assigned structures, it remains unclear if individual unknown constituents of the leachates are likely to have associated environmental hazards. Plastic chemicals are a worrisome black box highlighted by a recent report categorizing tens of thousands of chemical compounds found in plastics [50].

In contrast, cotton and tomato seeds showed a different pattern than Arabidopsis since none of the leaching solutions impaired their germination significantly. In a recent study evaluating the effects of macro and microplastic leachates on cotton seed germination, He et al. [70] reported a nonsignificant impact on cotton seed germination in most assessed germination parameters with minor fluctuations in oxidative stress. Interestingly, the mean germination time increased significantly in only one of the cotton varieties. That highlights the potential variation in sensitivity among different cotton varieties. In contrast to Arabidopsis, Cotton is renowned for its high-stress tolerance crop with moderate salt tolerance [71]; [72].

A study by Martin-Closas et al. [73] showed no significant impact on tomato germination and growth in response to bioplastic constituents, such as adipic and lactic acid. However, some effects manifested later in plant development, where high amounts of adipic acid in the culture medium restricted plant growth. That emphasizes the need for further studies on seed germination and on later stages of plant development, such as seedling growth, root length, and root growth [73].

Seed size and the anatomical structure of seed coats can also help explain their varying tolerance to abiotic stresses and resistance when exposed to leachate solutions. The seed coat, which varies by plant species, is considered the primary defense against adverse conditions, acting as a physical barrier [74,75]. According to studies on the effects of metals on seed germination, seed coat morphology directly influences permeability and determines whether metals are confined to the seed coat cells or translocated into the endosperm [76]. Similarly, this concept can be applied to the interaction between leachate compounds and the different plant species tested with distinct morphological traits.

Moreover, smaller seeds, such as those of Arabidopsis, can be more sensitive to contaminants and stressors than larger seeds. This increased sensitivity is due to their higher surface area-to-volume ratio, resulting in greater exposure to harmful substances and environmental stressors [77,78].

Even though the current study was carried out under controlled conditions to provide first insights into how the leachate would interact with the seeds, understanding how leachates would impact seed germination on soils is not covered in this study. Many other variables will influence how leachates affect seed germination in more complex matrices with varied texture, pH, porosity, etc. The pipeline used in this study is unfeasible for checking seed germination in soils, and other techniques are needed to achieve such outcomes.

4.3. Impact of microplastic particles on seed germination

The negligible effects of LDPEb and PBATb microplastics on all assessed variables and plant species suggest that the environmental impact of microplastics is primarily attributed to their associated chemical compounds, which can leach both acutely and chronically rather than the particles themselves.

Previous studies on the impact of microplastics on seed germination have produced divergent findings, likely due to the influence of various factors on the materials' toxicity in the environment. For instance, Shi et al. [79] observed that polystyrene (PS), polyethylene (PE), and polypropylene (PP) inhibited the germination of tomato seeds. The differences in the type and shape of the materials tested, which consisted of polymer powders rather than the commercial films examined here, suggest that factors such as polymer type and shape can influence their toxicity.

Microplastic concentration is another critical factor in determining potential environmental toxicity. At the concentrations of 0.2 % and 2 % (w/w) tested in this study, the particles did not significantly impact seed germination in the assessed species. However, higher concentrations could result in more particles adhering to the seed surface, potentially blocking water uptake and thus interfering with germination.

Although previous studies have demonstrated microplastic attachment to seed pores and germination impairment of certain species [11], we observed a different outcome here. Particle size may help us explain this discrepancy since the particles used in our study ranged mainly from 1 to 750 μm (Table S2), and their size range was in a nanoscale of 0.05, 0.5, and 4.8 μm. Research suggests that smaller particles can be more toxic since nanoparticles are more likely to adhere to seed pore surfaces, potentially clogging the pores or even penetrating cell walls (Roy et al., 2023; [80]. That could result in a greater impact seed germination and plant growth compared to larger microscale particles.

4.4. Study novelty and practical significance

In light of the above, the innovation of this study relies on the following aspects: 1) We compared the impact of leachates from both macro- and microplastics, a comparison often disregarded by other studies; 2) We analyzed the bulk and molecular characteristics of leachates from LDPE- and PBAT-based mulch films, which are environmentally relevant materials and main source of plastic pollution in agricultural soils, highlighting the differences among them and the current knowledge gaps; 3) We assessed the impact of leached and unleached plastics and leachates on Tomato, Cotton, and Arabidopsis; 4) Through such lenses, we uncovered that leachates hampered more Arabidopsis seed germination than particles themselves, echoing that the effect of chemicals leaching from plastics may be more detrimental than the microplastics; 5) Studies focusing on both the chemical leachates from the polymers and the impact of unidentified compounds on seed germination are scarce. Therefore, we decided to provide this holistic picture combining fundamental (proxies) and refined chemical techniques and the seed germination assays, enabling further reflections on strategies to control such contaminants; 6) We characterized with advanced spectral techniques (ATR-FTIR and LDIR) the macro and microplastics used in the experiments; 7) This work pioneered the use of WUR germinator pipeline to test the impact of plastic leachates on seed germination; 8) This study also highlights that most leached compounds are unregulated worldwide and lack hazard information; 9) Last but not least, by monitoring leachates release after 49 days, we established that long-term monitoring is pivotal to enhance the understanding of the contrasting short- and long-term leaching behavior of different polymers.

4.5. Environmental implications

Plastic pollution is widely reported in the agricultural environment, with over 13,000 chemicals associated with distinct plastic applications. Understanding whether macro- or microplastics from mulch films or their leachates hamper seed germination is pivotal for developing strategies to tackle such environmental issues. Leachates from macro- and microplastics at different concentrations were characterized. Here, we found that the leachates can impact seed germination and that most compounds leached from the tested mulch films are still unregulated worldwide, lacking hazard information. Therefore, more attention should be paid to the environmental hazards of macro- and microplastics and their respective leachates.

5. Conclusions

Addressing compounds leaching out from microplastics is a multilayered challenge that requires further research and action in different fields. In this study, we characterized compounds leaching from micro- and macroplastics of both PBATb and LDPEb mulch films and explored how such leachates impact seed germination. We found that the effect of PBATb on seed germination is crop-specific (i.e., undermined germination of Arabidopsis seeds but no effect on cotton and tomato germination). GC-MS and weight loss data show that the PBATb mulch film leached a higher concentration of compounds, inducing higher EC, TDS, and the release of plasticizer-derived compounds/oily substances that could be the key players hampering Arabidopsis germination. Moreover, the LDPEb and PBATb leached and unleached particles per se did not hamper tomato, cotton, and Arabidopsis germination. Detailed dynamics of the leaching procedure after 49 days show that PBATb mulch releases a higher concentration of chemical compounds after 7 days, while LDPEb release peaked around 35 days. This highlights the need for further research considering long-term leaching processes, as different materials can behave differently. This study is pioneering the use of the automated WUR pipeline to assess the impact of leachates on seed germination. Further studies aiming to complete the puzzle could target a more comprehensive range of plastic mulch films (both macro- and microplastics) and the impacts on different soil textures and/or plant species for extensive periods.

CRediT authorship contribution statement

Giovana P. F. Macan: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Davi R. Munhoz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Leo Willems:** Writing – review & editing, Methodology. **Charlie Monkley:** Writing – review & editing, Methodology. **Charlotte E. M. Lloyd:** Writing – review & editing, Supervision, Methodology. **Jos Hageman:** Writing – review & editing, Supervision, Formal analysis, Data curation. **Violette Geissen:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Blanca B. Landa:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Paula Harkes:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge the financial support from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No 955334. We also thank Wendy Beekman-Lukassen and Patrick Hendrickx for their prompt support for our urgent freeze-drying step.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.136013.

Data availability

Data will be made available on request.

References

[1] Chae, Y., An, Y.-J., 2018. Current research trends on plastic pollution and ecological impacts on the soil ecosystem: a review. Environ Pollut 240, 387–395. https://doi.org/10.1016/j.envpol.2018.05.008.
[2] Huang, Y., Liu, Q., Jia, W., Yan, C., Wang, J., 2020. Agricultural plastic mulching as a source of microplastics in the terrestrial environment. Environ Pollut 260, 114096. https://doi.org/10.1016/j.envpol.2020.114096.
[3] Hofmann, T., Ghoshal, S., Tufenkji, N., Adamowski, J.F., Bayen, S., Chen, Q., et al., 2023. Plastics can be used more sustainably in agriculture. Commun Earth Environ 4 (1), 332. https://doi.org/10.1038/s43247-023-00982-4.
[4] EN 17033, 2018. Plastics - Biodegradable mulch films for use in agriculture and horticulture - Requirements and test methods. (2018), European Standard EN 17033. European Committee for Standardization (CEN), Brussels.
[5] Meng, K., Lwanga, E.H., van der Zee, M., Munhoz, D.R., Geissen, V., 2023. Fragmentation and depolymerization of microplastics in the earthworm gut: A potential for microplastic bioremediation? J Hazard Mater 447, 130765. https://doi.org/10.1016/j.jhazmat.2023.130765.
[6] Griffin-LaHue, D., Ghimire, S., Yu, Y., Scheenstra, E.J., Miles, C.A., Flury, M., 2022. In-field degradation of soil-biodegradable plastic mulch films in a Mediterranean climate. Sci Total Environ 806, 150238. https://doi.org/10.1016/j.scitotenv.2021.150238.
[7] Qi, R., Jones, D.L., Liu, Q., Liu, Q., Li, Z., Yan, C., 2021. Field test on the biodegradation of poly (butylene adipate-co-terephthalate) based mulch films in soil. Polym Test 93, 107009. https://doi.org/10.1016/j.polymertesting.2020.107009.
[8] 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.
[9] Khalid, N., Aqeel, M., Noman, A., Fatima Rizvi, Z., 2023. Impact of plastic mulching as a major source of microplastics in agroecosystems. J Hazard Mater 445, 130455. https://doi.org/10.1016/j.jhazmat.2022.130455.
[10] Li, S., Ding, F., Flury, M., Wang, Z., Xu, L., Li, S., et al., 2022. Macro- and microplastic accumulation in soil after 32 years of plastic film mulching. Environ Pollut 300, 118945. https://doi.org/10.1016/j.envpol.2022.118945.
[11] Bosker, T., Bouwman, L.J., Brun, N.R., Behrens, P., Vijver, M.G., 2019. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*. Chemosphere 226, 774–781. https://doi.org/10.1016/j.chemosphere.2019.03.163.
[12] Liu, B., Li, W., Pan, X., Zhang, D., 2022. The persistently breaking trade-offs of three-decade plastic film mulching: microplastic pollution, soil degradation and reduced cotton yield. J Hazard Mater 439, 129586. https://doi.org/10.1016/j.jhazmat.2022.129586.
[13] Roy, T., Dey, T.K., Jamal, M., 2023. Microplastic/nanoplastic toxicity in plants: an imminent concern. Environ Monit Assess 195 (1), 27. https://doi.org/10.1007/s10661-022-10654-z.
[14] Cao, X., Liang, Y., Jiang, J., Mo, A., He, D., 2023. Organic additives in agricultural plastics and their impacts on soil ecosystems: compared with conventional and biodegradable plastics. TrAC Trends Anal Chem 166, 117212. https://doi.org/10.1016/j.trac.2023.117212.
[15] Iqbal, B., Zhao, T., Yin, W., Zhao, X., Xie, Q., Khan, K.Y., et al., 2023. Impacts of soil microplastics on crops: a review. Appl Soil Ecol 181. https://doi.org/10.1016/j.apsoil.2022.104680.
[16] Lian, Y., Liu, W., Shi, R., Zeb, A., Wang, Q., Li, J., et al., 2022. Effects of polyethylene and polylactic acid microplastics on plant growth and bacterial community in the soil. J Hazard Mater 435, 129057. https://doi.org/10.1016/j.jhazmat.2022.129057.
[17] Liu, R., Liang, J., Yang, Y., Jiang, H., Tian, X., 2023. Effect of polylactic acid microplastics on soil properties, soil microbials and plant growth. Chemosphere 329, 138504. https://doi.org/10.1016/j.chemosphere.2023.138504.
[18] 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.
[19] Yang, C., Gao, X., 2022. Impact of microplastics from polyethylene and biodegradable mulch films on rice (*Oryza sativa* L.). Sci Total Environ 828. https://doi.org/10.1016/j.scitotenv.2022.154579.
[20] Ambrogi, V., Carfagna, C., Cerruti, P., Marturano, V., 2017. 4 - additives in polymers. In: Jasso-Gastinel, C.F., Kenny, J.M. (Eds.), Modification of polymer properties. William Andrew Publishing, pp. 87–108. https://doi.org/10.1016/B978-0-323-44353-1.00004-X.
[21] Bridson, J.H., Gaugler, E.C., Smith, D.A., Northcott, G.L., Gaw, S., 2021. Leaching and extraction of additives from plastic pollution to inform environmental risk: A multidisciplinary review of analytical approaches. J Hazard Mater 414, 125571. https://doi.org/10.1016/j.jhazmat.2021.125571.
[22] Munhoz, D.R., Harkes, P., Beriot, N., Larreta, J., Basurko, O.C., 2023. Microplastics: a review of policies and responses. Microplastics 2 (1), 1–26. https://doi.org/10.3390/microplastics2010001.
[23] Barbale, M., Chinaglia, S., Gazzilli, A., Pischedda, A., Pognani, M., Tosin, M., et al., 2021. Hazard profiling of compostable shopping bags. Towards an ecological risk assessment of littering. Polym Degrad Stab 188, 109592. https://doi.org/10.1016/j.polymdegradstab.2021.109592.
[24] Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard Mater 344, 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014.
[25] Rillig, M.C., Kim, S.W., Zhu, Y.-G., 2024. The soil plastisphere. Nat Rev Microbiol 22 (2), 64–74. https://doi.org/10.1038/s41579-023-00967-2.
[26] Menicagli, V., Balestri, E., Lardicci, C., 2019. Exposure of coastal dune vegetation to plastic bag leachates: a neglected impact of plastic litter. Sci Total Environ 683, 737–748. https://doi.org/10.1016/j.scitotenv.2019.05.245.
[27] Balestri, E., Menicagli, V., Ligorini, V., Fulignati, S., Raspolli Galletti, A.M., Lardicci, C., 2019. Phytotoxicity assessment of conventional and biodegradable plastic bags using seed germination test. Ecol Indic 102, 569–580. https://doi.org/10.1016/j.ecolind.2019.03.005.
[28] United Nations Environment Programme and Secretariat of the Basel, Rotterdam and Stockholm conventions; 2023. Chemicals in plastics: a technical report. Geneva.
[29] Qi, R., Tang, Y., Jones, D.L., He, W., Yan, C., 2023. Occurrence and characteristics of microplastics in soils from greenhouse and open-field cultivation using plastic mulch film. Sci Total Environ 905, 166935. https://doi.org/10.1016/j.scitotenv.2023.166935.
[30] Agarwal, A., Prakash, O., Sahay, D., Bala, M., 2022. Effect of organic and inorganic mulching on weed density and productivity of tomato (*Solanum lycopersicum* L.). J Agric Food Res 7, 100274. https://doi.org/10.1016/J.JAFR.2022.100274.
[31] Zhang, H., Huang, Y., An, S., Wang, P., Xie, C., Jia, P., et al., 2024. Mulch-derived microplastic aging promotes phthalate esters and alters organic carbon fraction content in grassland and farmland soils. J Hazard Mater 461, 132619. https://doi.org/10.1016/j.jhazmat.2023.132619.
[32] Haixin, Z., Yimei, H., Shaoshan, A., Haohao, L., Xiaoqian, D., Pan, W., et al., 2022. Land-use patterns determine the distribution of soil microplastics in typical agricultural areas on the eastern Qinghai-Tibetan Plateau. J Hazard Mater 426, 127806. https://doi.org/10.1016/j.jhazmat.2021.127806.
[33] Liang, Y., Liu, X., Zhai, W., Guo, Q., Guo, H., Lv, S., et al., 2024. Agricultural film-derived microplastics elevate the potential risk of pesticides in soil ecosystem: The inhibited leaching by altering soil pore. J Hazard Mater 477, 135210. https://doi.org/10.1016/j.jhazmat.2024.135210.
[34] Ren, S.Y., Kong, S.F., Ni, H.G., 2021. Contribution of mulch film to microplastics in agricultural soil and surface water in China. Environ Pollut 291, 118227. https://doi.org/10.1016/j.envpol.2021.118227.
[35] Serrano-Ruíz, H., Eras, J., Martín-Closas, L., Pelacho, A.M., 2020. Compounds released from unused biodegradable mulch materials after contact with water. Polym Degrad Stab 178, 109202. https://doi.org/10.1016/j.polymdegradstab.2020.109202.
[36] Zhao, X., Wei, Y., Fan, Y., Zhang, F., Tan, W., He, X., et al., 2018. Roles of bacterial community in the transformation of dissolved organic matter for the stability and safety of material during sludge composting. Bioresour Technol 267, 378–385. https://doi.org/10.1016/j.biortech.2018.07.060.

[37] Hautala, K., Peuravuori, J., Pihlaja, K., 2000. Measurement of aquatic humus content by spectroscopic analyses. Water Res 34 (1), 246–258. https://doi.org/10.1016/S0043-1354(99)00137-2.
[38] Fang, C., Yuan, X., Liao, K., Qu, H., Han, L., He, X., et al., 2022. Micro-aerobic conditions based on membrane-covered improves the quality of compost products: Insights into fungal community evolution and dissolved organic matter characteristics. Bioresour Technol 362, 127849. https://doi.org/10.1016/j.biortech.2022.127849.
[39] ASTM, 2014. Standard guide for conducting terrestrial plant toxicity tests ASTM E1963–09. https://doi.org/10.1520/E1963-09R14.
[40] EPA. 1996. Ecological effects test guidelines. OPPTS 850.4100 terrestrial plant toxicity, tier i (seedling emergence). Available from: ⟨https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines⟩.
[41] ISO, 2012. ISO Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early growth of higher plants. ⟨https://www.iso.org/standard/51382.html⟩.
[42] OECD, 2006. Terrestrial plant test: seedling emergence and seedling growth test. OECD guidelines for the testing of chemicals, section 2. OECD,. https://doi.org/10.1787/9789264070066-en.
[43] Joosen, R.V.L., Kodde, J., Willems, L.A.J., Ligterink, W., Van Der Plas, L.H.W., Hilhorst, H.W.M., 2010. GERMINATOR: a software package for high-throughput scoring and curve fitting of Arabidopsis seed germination. Plant J 62 (1), 148–159. https://doi.org/10.1111/j.1365-313X.2009.04116.x.
[44] Yazdanpanah, F., Maurino, V.G., Mettler-Altmann, T., Buijs, G., Bailly, M., Karimi Jashni, M., et al., 2019. NADP-MALIC ENZYME 1 affects germination after seed storage in *Arabidopsis thaliana*. Plant Cell Physiol 60 (2), 318–328.
[45] Wan, T., Lin, Y., Tu, Y., 2016. Plasticizing effect of glyceryl tribenzoate, dipropylene glycol dibenzoate, and glyceryl triacetate on poly(lactic acid). Polym Eng Sci 56, 1399–1406. https://doi.org/10.1002/pen.24375.
[46] Sahoo, N.G., Rana, S., Cho, J.W., Li, L., Chan, S.H., 2010. Polymer nanocomposites based on functionalized carbon nanotubes. Prog Polym Sci 35 (7), 837–867. https://doi.org/10.1016/j.progpolymsci.2010.03.002.
[47] Gao, C., Vo, C.D., Jin, Y.Z., Li, W., Armes, S.P., 2005. Multihydroxy polymer-functionalized carbon nanotubes: synthesis, derivatization, and metal loading. Macromolecules 38 (21), 8634–8648. https://doi.org/10.1021/ma050823e.
[48] Patrucco, E., Ouasti, S., Vo, C.D., De Leonardis, P., Pollicino, A., Armes, S.P., et al., 2009. Surface-initiated ATRP modification of tissue culture substrates: poly (glycerol monomethacrylate) as an antifouling surface. Biomacromolecules 10 (11), 3130–3140. https://doi.org/10.1021/bm900856r.
[49] Xing, Q., Ruch, D., Dubois, P., Wu, L., Wang, W.J., 2017. Biodegradable and high-performance poly (butylene adipate-co-terephthalate)–lignin UV-blocking films. ACS Sustain Chem Eng 5 (11), 10342–10351. https://doi.org/10.1021/acssuschemeng.7b02370.
[50] Wagner, M., Monclús, L., Arp, H.P.H., Groh, K.J., Løseth, M.E., Muncke, J., et al., 2024. State of the science on plastic chemicals - Identifying and addressing chemicals and polymers of concern. https://doi.org/10.5281/zenodo.10701706.
[51] Rodriguez-Gonzalez, F.J., Ramsay, B.A., Favis, B.D., 2003. High performance LDPE/thermoplastic starch blends: a sustainable alternative to pure polyethylene. Polymer 44 (5), 1517–1526. https://doi.org/10.1016/S0032-3861(02)00907-2.
[52] Rodriguez-Gonzalez, F.J., Ramsay, B.A., Favis, B.D., 2004. Rheological and thermal properties of thermoplastic starch with high glycerol content. Carbohydr Polym 58 (2), 139–147. https://doi.org/10.1016/j.carbpol.2004.06.002.
[53] Liu, Z., Wen, J., Liu, Z., Wei, H., Zhang, J., 2024. Polyethylene microplastics alter soil microbial community assembly and ecosystem multifunctionality. Environ Int 183, 108360. https://doi.org/10.1016/j.envint.2023.108360.
[54] Serrano-Ruiz, H., Martin-Closas, L., Pelacho, A.M., 2023. Impact of buried debris from agricultural biodegradable plastic mulches on two horticultural crop plants: Tomato and lettuce. Sci Total Environ 856, 159167. https://doi.org/10.1016/j.scitotenv.2022.159167.
[55] Capolupo, M., Rafiq, A., Coralli, I., Alessandro, T., Valbonesi, P., Fabbri, D., et al., 2023. Bioplastic leachates characterization and impacts on early larval stages and adult mussel cellular, biochemical and physiological responses. Environ Pollut 319, 120951. https://doi.org/10.1016/j.envpol.2022.120951.
[56] Zhang, P., Yuan, Y., Zhang, J., Wen, T., Wang, H., Qu, C., et al., 2023. Specific response of soil properties to microplastics pollution: a review. Environ Res 232, 116427. https://doi.org/10.1016/j.envres.2023.116427.
[57] Zhou, J., Jia, R., Brown, R.W., Yang, Y., Zeng, Z., Jones, D.L., et al., 2023. The long-term uncertainty of biodegradable mulch film residues and associated microplastics pollution on plant-soil health. J Hazard Mater 442, 130055. https://doi.org/10.1016/j.jhazmat.2022.130055.
[58] Munhoz, D.R., Meng, K., Wang, L., Lwanga, E.H., Geissen, V., Harkes, P., 2024. Exploring the potential of earthworm gut bacteria for plastic degradation. Sci Total Environ 927, 172175. https://doi.org/10.1016/j.scitotenv.2024.172175.
[59] Gong, X.Y., Zhang, W.J., Zhang, S.Y., Wang, Y., Zhang, X.Y., Lu, Y., et al., 2021. Organophosphite antioxidants in mulch films are important sources of organophosphate pollutants in farmlands. Environ Sci Technol 55 (11), 7398–7406. https://doi.org/10.1021/acs.est.0c08741.
[60] Xu, Y., Zeng, L., Tao, Y., Xu, J., He, Y., Lu, Z., 2023. Release oF Additives from Agricultural Plastic Films in Water: Experiment and Modeling. Environ Sci Technol 57 (27), 10053–10061. https://doi.org/10.1021/acs.est.2c09738.
[61] Li, B., Liu, Q., Yao, Z., Ma, Z., Li, C., 2023. Mulch film: An overlooked diffuse source of organic ultraviolet absorbers in agricultural soil. Environ Pollut 318, 120935. https://doi.org/10.1016/j.envpol.2022.120935.
[62] Cui, H., Gao, W.C., Lin, Y.C., Zhang, J., Yin, R.S., Xiang, Z.M., et al., 2021. Development of microwave-assisted extraction and dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry for the determination of organic additives in biodegradable mulch films. Microchem J 160. https://doi.org/10.1016/j.microc.2020.105722.
[63] Monkley, C., Reay, M.K., Evershed, R.P., Lloyd, C.E.M., 2024. Mass spectral characterisation of cyclic oligoesters in a biodegradable mulch film. Rapid Commun Mass Spectrom 38 (10), e9726. https://doi.org/10.1002/rcm.9726.
[64] Zhao, S., Zhang, Q., Liu, M., Zhou, H., Ma, C., Wang, P., 2021. Regulation of plant responses to salt stress. Int J Mol Sci 22 (9). https://doi.org/10.3390/ijms22094609.
[65] Fahad, S., Hussain, S., Matloob, A., Khan, F.A., Khaliq, A., Saud, S., et al., 2015. Phytohormones and plant responses to salinity stress: a review. Plant Growth Regul 75 (2), 391–404. https://doi.org/10.1007/s10725-014-0013-y.
[66] Møller, I.S., Tester, M., 2007. Salinity tolerance of Arabidopsis: a good model for cereals? Trends Plant Sci 12 (12), 534–540. https://doi.org/10.1016/j.tplants.2007.09.009.
[67] Xiong, L., Zhu, J.K., 2002. Salt tolerance. Arab Book 1, e0048. https://doi.org/10.1199/table0048.
[68] Liu, J., Wang, P., Wang, Y., Zhang, Y., Xu, T., Zhang, Y., et al., 2022. Negative effects of poly(butylene adipate-co-terephthalate) microplastics on Arabidopsis and its root-associated microbiome. J Hazard Mater 437, 129294. https://doi.org/10.1016/j.jhazmat.2022.129294.
[69] Hu, L., Zhou, Y., Chen, Z., Zhang, D., Pan, X., 2023. Oligomers and monomers from biodegradable plastics: an important but neglected threat to ecosystems. Environ Sci Technol 57 (27), 9895–9897. https://doi.org/10.1021/acs.est.3c04423.
[70] He, M., Feng, Z., Xu, Y., Ding, H., Ying, C., Cai, Y., et al., 2023. Macro- and microplastic leachates show a slightly toxic effect on seed germination of cotton. Chemosphere 335, 139081. https://doi.org/10.1016/j.chemosphere.2023.139081.
[71] Ashraf, M., 2002. Salt tolerance of cotton: some new advances. Crit Rev Plant Sci 21 (1), 1–30. https://doi.org/10.1080/0735-260291044160.
[72] Zafar, S., Afzal, H., Ijaz, A., Mahmood, A., Ayub, A., Nayab, A., et al., 2023. Cotton and drought stress: an updated overview for improving stress tolerance. South Afr J Bot 161, 258–268. https://doi.org/10.1016/j.sajb.2023.08.029.
[73] Martin-Closas, L., Botet, R., Pelacho, A.M., 2014. An in vitro crop plant ecotoxicity test for agricultural bioplastic constituents. Polym Degrad Stab 108, 250–256. https://doi.org/10.1016/j.polymdegradstab.2014.03.037.
[74] Mohamed-Yasseen, Y., Barringer, S.A., Splittstoesser, W.E., Costanza, S., 1994. The role of seed coats in seed viability. Bot Rev 60 (4), 426–439. https://doi.org/10.1007/bf02857926.
[75] Radchuk, V., Borisjuk, L., 2014. Physical, metabolic and developmental functions of the seed coat. Front Plant Sci 5 (OCT). https://doi.org/10.3389/fpls.2014.00510.
[76] Kranner, I., Colville, L., 2011. Metals and seeds: biochemical and molecular implications and their significance for seed germination. Environ Exp Bot 72 (1), 93–105. https://doi.org/10.1016/j.envexpbot.2010.05.005.
[77] Lukina, A.O., Boutin, C., Rowland, O., Carpenter, D.J., 2016. Evaluating trivalent chromium toxicity on wild terrestrial and wetland plants. Chemosphere 162, 355–364. https://doi.org/10.1016/j.chemosphere.2016.07.055.
[78] Margenot, A.J., Rippner, D.A., Dumlao, M.R., Nezami, S., Green, P.G., Parikh, S.J., et al., 2018. Copper oxide nanoparticle effects on root growth and hydraulic conductivity of two vegetable crops. Plant Soil 431 (1–2), 333–345. https://doi.org/10.1007/s11104-018-3741-3.
[79] Shi, R., Liu, W., Lian, Y., Wang, Q., Zeb, A., Tang, J., 2022. Phytotoxicity of polystyrene, polyethylene and polypropylene microplastics on tomato (*Lycopersicon esculentum* L.). J Environ Manag 317, 115441. https://doi.org/10.1016/j.jenvman.2022.115441.
[80] Yu, Z., Xu, X., Guo, L., Jin, R., Lu, Y., 2024. Uptake and transport of micro/nanoplastics in terrestrial plants: detection, mechanisms, and influencing factors. Sci Total Environ 907, 168155. https://doi.org/10.1016/j.scitotenv.2023.168155.