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Legacy herbicide bound to PLA-PBAT and LDPE plastic mulch film fragments in soil can induce phytotoxicity to subsequent crops

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ABSTRACT

Agricultural herbicides are essential for maximizing yields through effective weed control; however, their interaction with plastic residues in soil, particularly from mulch films, remains poorly understood. This study investigated the absorption, persistence and phytotoxic effects of the herbicide trifluralin (2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzenamine) when combined with macro- (2 × 2 cm) and microplastics (63–500 µm) of either conventional low-density polyethylene (LDPE) or biodegradable poly(lactic acid) – poly (butylene adipate-*co*-terephthalate) (PLA-PBAT) plastic film. Plastics were incorporated into soil at realistic field application rates (400 kg ha^{-1}) and their effects on maize (*Zea mays* L.) growth, soil nutrient dynamics, and plant elemental composition were assessed over 45 days. PLA-PBAT plastic absorbed approximately four times more ^{14}C-trifluralin than LDPE, while the ^{14}C-trifluralin mineralisation rate remained low for both PLA-PBAT (ca. 4%) and LDPE (<1%). In the absence of trifluralin, the soil addition of micro- or macro-plastics (orientated vertically or horizontally) had minimal effect on plant growth. However, leaching of trifluralin from contaminated LDPE and PLA-PBAT reduced maize biomass by up to 87%, particularly in the microplastic treatments. The inhibition of root growth from the trifluralin-contaminated plastics led to major changes in soil chemistry (increased NO_3^-) due to reduced nutrient uptake. These findings suggest that plastic particle size can influence environmental impact, with microplastics posing higher risks as herbicide vectors than larger fragments. Biodegradable plastics may also act as more effective vectors for herbicide absorption than conventional plastics, leading to enhanced phytotoxicity when plastic residues contaminated with herbicides are incorporated into agricultural soils.

1. Introduction

Weeds threaten global food production by competing with crops for essential resources (e.g., nutrients, light, water; [1,2]), with diminished yields resulting in significant annual losses of approximately USD$33 billion in the US [3,4], $11 billion in India [5], and AUD$3.3 billion in the Australian grain industry [4,6]. Herbicides effectively manage both existing weed populations and the soil seedbank, enhancing on-farm efficiency when utilised correctly [7,8]. However, the persistence of herbicides in soil can jeopardise environmental and human health [9], especially when they persist longer than intended persistence [10,11], depending on chemical (e.g., photolysis, hydrolysis, oxidation and reduction) and biological (e.g., non-enzymatic and enzymatic transformation) processes, soil temperature and moisture content [12]. This persistence may result in soil and groundwater contamination [11], reductions in above- and below-ground soil and plant biodiversity [13], and modified microbial community functioning [14,15].

Trifluralin (2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine) is a widely utilised pre-emergence herbicide employed to manage annual weeds in crops including cotton, soybean, wheat, and oilseed [16,17], with an annual consumption of ca. 4400 tons worldwide each year [18]. In soil, it is degraded at the surface by UV light and deeper in the soil by microbial mineralisation [19], with an average half-life of 45 days (varying from 14 days to 128 days; [20]), typically resulting in less than 10% persisting one-year post-application [20,21]. Trifluralin can modify soil microbial and mesofaunal community abundance, diversity, and activity [20], especially in anaerobic or low organic matter soils, where its breakdown occurs at a slower rate [22–24]. Consequently, understanding the behaviour and nutrient cycling dynamics of trifluralin in conventionally managed agricultural soil is essential for assessing its environmental impact.

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The use of plastic mulch films in agriculture (often referred to as plasticulture) can enhance crop yield, reduce soil degradation [25], reduce agrichemical usage, suppress weeds [26], and minimise water usage [27]. However, although vital for maintaining global food security [28], their use has contributed to the buildup of legacy plastic in soil [29], highlighting concerns regarding their unintended environmental consequences [30]. Macro- (> 5 mm) and microplastic (> 1 µm to ≤ 5 mm) fragments of conventional plastic mulch film polymers such as polyethylene (PE), polypropylene (PP), and polyvinyl chloride (PVC) are environmentally persistent [31] and may interact with agrichemicals (e.g., herbicides) in the soil [32,33] to provide a pollutant vector effect [34]. In addition, remaining plastic particles may be oriented in any directions in the soil. However, despite the growing popularity of biodegradable alternatives, such as poly(lactic acid)/poly(butylene adipate-*co*-terephthalate) (PLA-PBAT), their interaction with herbicides remains largely unknown.

Biodegradable mulch films are designed to degrade *in-situ*, with this process largely controlled by a range of biotic (e.g., microbial activity; soil fauna) and abiotic (e.g., pH, moisture, temperature) factors [35]. However, due to their physicochemical nature (e.g., porous surface, easily hydrolysable bonds; [36]) and different additive composition, biodegradable mulch films may have a much greater sorption capacity for agrochemicals (e.g., herbicides) than conventional plastics [34,37], leading to repercussions for non-target organisms (e.g., soil biota) by enhancing the persistence of agrichemicals in the environment [38,39]. In some cases, biodegradable mulches are also mixed with trifluralin to improve their weed suppressiveness [40]. However, the adsorption of herbicide onto plastic may be influenced by factors such as size, shape, surface area, and the presence of surface functional groups [8,41].

Although plastic mulch residues can sorb pesticides and herbicides in soil, differences in herbicide sorption and release between conventional plastics (e.g., LDPE) and biodegradable films (e.g., PLA-PBAT) remain poorly understood. The effects of plastic particle size and orientation on herbicide availability and plant exposure in soil–plant systems are also largely unknown. To better understand the interactions of herbicides with legacy plastic in soil, this study investigated the absorption and persistence of trifluralin in macro- and microplastic of low-density polyethylene (LDPE) and biodegradable (PLA-PBAT) plastic and their subsequent impact on plant-soil interactions. We hypothesised that (1) the physiochemical properties of the PLA-PBAT mulch films enhance the molecular interactions with trifluralin, resulting in greater sorption compared to LDPE; (2) when incorporated into the soil, the weaker binding affinity and faster degradation of PLA-PBAT is expected to promote greater desorption of trifluralin into the soil solution, improving microbial access and mineralisation; (3) horizontally oriented macroplastic will result in greater phytotoxicity than vertically oriented plastic due to increased probability of root-particle contact; and (4) these changes in herbicide bioavailability are expected to alter microbial nutrient cycling, while the phytotoxic effects of increased trifluralin exposure will suppress maize growth (as model crop species) and alter forage quality.

2. Materials and methods

2.1. Soil chemical characteristics

In December 2023, soil samples (0–20 cm depth, 40 kg; $n = 4$) were randomly collected from a lowland arable area at Bangor University's Henfaes Research Centre in Abergwyngregyn, North Wales, UK (53°14′ N, 4°01′ W). The region has an oceanic climate with an average annual temperature and rainfall of 10 °C and 1060 mm, respectively. The soil is classified as a freely draining Eutric Cambisol with a sandy clay loam texture with no previous history of plastic mulch film use. After collection, the soil was sieved to ≤ 5 mm to remove plant material and stones prior to determining the chemical characteristics (Table S1).

Briefly, pH and electrical conductivity were determined on 1:5 (*w/v*) soil:DI H_2O suspensions using standard electrodes. Soil pH was measured using a Jenway 3510 pH meter (Bibby Scientific Ltd, UK), and electrical conductivity was determined with a Jenway 4520 Conductivity meter (Bibby Scientific Ltd, UK). Gravimetric soil moisture was determined by oven drying (105 °C, >24 h) and organic matter was quantified by loss-on-ignition in a muffle furnace (450 °C, 16 h; [42]). Bioavailable N and P levels in soil were determined following a 1:5 (*w/v*) soil:1 M KCl and 1:5 (*w/v*) soil:0.5 M CH_3COOH (acetic acid) extraction, respectively. Soil nitrate (NO_3^-) and ammonium (NH_4^+) in the KCl extracts were measured by the colorimetric methods of Miranda et al. [43] and Mulvaney [44], respectively. Phosphate (PO_4^{3-}) was measured in the acetic acid extracts using the colorimetric molybdate blue method of Murphy and Riley [45].

2.2. Plastic composition

The experiment utilised two types of macro- and microplastic, a conventional low-density polyethylene (LDPE, 23 µm thickness; GroMax Industries Ltd, Hadleigh, UK) and biodegradable poly(lactic acid) - poly (butylene adipate-*co*-terephthalate) (PLA-PBAT; 15:85 *w/w*; 10 µm thickness; GroMax Industries Ltd, Hadleigh, UK) mulch film. Both black-coloured LDPE and PLA-PBAT plastics were either cut into 2 × 2 cm macro-sized plastic pieces or ground up and sieved to a size range of 63–500 µm using a basic batch mill (A10, IKA Ltd, Oxford, UK) to represent the microplastic fraction. The plastic films were new and had not been previously exposed to soil or agrochemicals, and all cutting and grinding procedures were carried out in a clean, separate workspace to minimise potential contamination prior to herbicide exposure. The mechanical and chemical properties of each plastic are reported in Table 1 and Fig. S6.

Briefly, film property tests were conducted to assess the physical and functional performance of plastic films used in the study based on Graf et al. [46]. Film thickness was measured using a digital gauge. Surface roughness was analysed according to ISO 4287:1997 using a Surftest SJ-210 (Mitutoyo Corp., Huntersville, NC). Water vapor transmission rate (WVTR) was determined using a Permatran-W 1/50 analyser (Mocon Inc., Minneapolis, MN) at 38 °C and 90% relative humidity following GB/T 1037–2021, using one replicate per film type. Tensile strength was evaluated via an Instron 3345 (Instron, Norwood, MA), using standard film specimens cut with a directional stencil, and tested under controlled load and speed as per GB/T 35795–2017. Abrasion resistance was assessed with a standardised test involving sandpaper abrasion under fixed pressure and movement until visible damage appeared or 100 cycles were completed. Light transmittance was measured at 350, 650, and 1000 nm using a UV spectrophotometer to evaluate UV, visible, and near-infrared transparency.

2.3. Exposure of plastic to trifluralin

A commonly used, commercially available, pre-emergence herbicide

Table 1
Mechanical properties of the conventional LDPE and biodegradable PLA-PBAT plastic mulch film used in the experiment. Values represent mean ± SEM ($n = 3$), except for water vapor transmission rate (WVTR) ($n = 1$).

Variable	Unit	LDPE	PLA-PBAT
Thickness	µm	23.0 ± 0.3	10.5 ± 0.2
Surface roughness	µm	0.81 ± 0.02	1.53 ± 0.04
Specific surface area	$m^2\ g^{-1}$	0.09	0.15
WVTR	$g\ m^{-2}\ d^{-1}$	12	1069
Nominal strain fracture	%	806 ± 1	625 ± 7
Maximum force	N	5.64 ± 0.38	3.72 ± 0.06
Tensile strength	MPa	24.5 ± 1.7	35.5 ± 0.6
Abrasion resistance	Cycles	> 100	2.7 ± 0.7
Light transmittance at 350 nm	%	0.0 ± 0.0	0.03 ± 0.03
Light transmittance at 650 nm	%	0.0 ± 0.0	0.03 ± 0.03
Light transmittance at 1000 nm	%	0.4 ± 0.0	0.50 ± 0.00

trifluralin (IPAUC: a,a,a-trifluoro-2,6-dinitro-N,N-dipropylp-toluidine; CAS 1582–09–8; Weed Stopper II; Monterey Lawn and Garden Products Inc., Fresno, CA) was used at a recommended field concentration (9.14 ml of trifluralin concentrate per litre of water, 5.1 g active ingredient l^{-1}). Prior to conducting the soil and plant assay, the LDPE and PLA-PBAT macro- and microplastic pieces were placed in 50 ml of the trifluralin solution for 24-hours. The plastics were then removed and rinsed with deionised water, prior to placing in 50 ml of DI H_2O for 1-hour to remove any trifluralin on the surface of the plastics. This rinsing step was subsequently repeated three times after which the plastic particles were air dried for use in the experiment.

2.4. *Experimental design*

A 45-day mesocosm experiment in a temperature-controlled greenhouse investigated the effects of plastic composition (LDPE vs. PLA-PBAT), particle size (macro- vs. micro-plastic), spatial placement in soil (vertical and horizontal orientation of the macroplastic), and trifluralin addition (Fig. S1). As macroplastics may be oriented in any directions (whether vertical or horizontal) in the soil, the orientation was considered as an experimental factor. The LDPE and PLA-PBAT growth trials were undertaken at different times. The LDPE experiment was carried out first, followed by the PLA-PBAT experiment; however, all protocols were the same for both. The experimental treatments included: control (no plastic, no trifluralin - Control); macro-plastic placed vertically (Vertical); macro-plastic placed horizontally (Horizontal); microplastic mixed throughout the soil (Micro); trifluralin without plastic (Trifluralin); macro-plastic exposed to trifluralin placed vertically (Vertical-Trifluralin); macro-plastic exposed to trifluralin placed horizontally (Horizontal-Trifluralin); microplastic exposed to trifluralin mixed with the soil (Micro-Trifluralin); both for LDPE and PLA-PBAT plastic mulch films (Table 2). For the positive controls, where trifluralin was added to the soil in the absence of plastics, the amount added was equivalent to that present in each of the plastics (1.51 mg trifluralin in LDPE *vs.* 7.08 mg trifluralin in PLA-PBAT per pot, ca. ~1.9–9.0 kg a.i ha^{-1}; see Section 2.4.2 for more details). This was added directly to the soil matrix as a solution (50 ml) prior to starting the experiment.

Table 2
Summary of the 14 treatments used in the experiment. The experiment used two types of plastics (LDPE vs. PLA-PBAT) each in different size ranges (macro- vs. micro-plastics) with or without trifluralin herbicide contamination. The macroplastics were also orientated either vertically or horizontally in the soil. *NA* indicates not applicable for that treatment.

Treatment	Plastic	Size of plastic	Orientation in soil	Trifluralin present	Symbol
1a	No	*NA*	*NA*	No	Control (LDPE)
1b				No	Control (PLA-PBAT)
2a				Yes[a]	Trifluralin (LDPE)
2b				Yes[a]	Trifluralin (PLA-PBAT)
3	LDPE	Macro	Vertical	No	Macro-Vert
4			Horizontal		Macro-Horiz
5			Vertical	Yes	Macro-Vert
6			Horizontal		Macro-Horiz
7		Micro	*NA*[b]	No	Micro
8				Yes	Micro
9	PLA-PBAT	Macro	Vertical	No	Macro-Vert
10			Horizontal		Macro-Horiz
11			Vertical	Yes	Macro-Vert
12			Horizontal		Macro-Horiz
13		Micro	*NA*[§]	No	Micro
14				Yes	Micro

[a] Added directly to the soil at 2 different concentrations corresponding to trifluralin absorption of LDPE and PLA-PBAT.

[b] No orientation for the microplastics, as these were mixed in the soil matrix.

Soil (< 5 mm, 0–20 cm, 800 g) for each mesocosm ($n = 5$ per treatment) was repacked into a 1 litre (10 cm diameter) plastic pot and maintained under controlled environmental conditions with continuous light (200–500 µmol m^{-2} s^{-1}), temperature (16/22 °C, night/day), humidity (60%), and moisture (70% WHC) in a greenhouse. All treatments were planted with two pre-germinated maize (*Zea mays* L.) seedlings with ca. 2 cm long roots. Macro- and micro-plastics were applied at a rate of equal to pot surface coverage (400 kg ha^{-1} per pot) and placed either horizontally, vertically, or mixed within the soil to represent an annual application of plastic through mulch film use (Fig. S1).

2.4.1. *Assessing agronomic impacts*

Plant height and chlorophyll content were measured weekly after establishment. Leaf chlorophyll content was measured by taking three random measurements on the youngest fully emerged leaf using a non-destructive portable chlorophyll meter (SPAD-502 Plus, Konica Minolta, Tokyo, Japan) and expressed as SPAD units. At the end of the experiment (after 45-days), the fresh biomass of maize shoots and roots (after rinsing the soil off with tap water) was determined. The pots were destructively sampled and the soil biogeochemistry determined as described previously.

Root morphology was analysed using a root scanner and WinRHIZO image analysis system (Regent Instrument Inc., Quebec, Canada) to determine root length, volume, surface area, and average diameter. Plant dry weight and composition was determined by oven-drying (80 °C, 48 h) and grinding to a fine powder using a MM200 ball mill (Retsch GmbH, Haan, Germany) prior to analysis of phosphorous (P). sulfur (S). chlorine (Cl), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) on a S2 Picofox TXRF spectrometer (Bruker Inc., Billerica, MA), using Ga as an internal standard and validated using a range of certified plant standards (WEPAL-QUASIMEME, Wageningen, the Netherlands). The C:N ratio of plant tissue was analysed using a Vario EL Cube organic elemental analyser (Elementar UK Ltd, UK).

2.4.2. *^{14}C-trifluralin absorption to the plastic and degradation in the soil*

To quantify the absorption of trifluralin by each plastic, macro-plastic pieces (1 cm^2 of LDPE or PLA-PBAT plastic, $n = 5$ per plastic type) were placed in sterile 20 ml glass vials. Subsequently, 383 µl of ^{14}C-trifluralin (3.34 kBq per sample; American Radiolabeled Chemicals Inc., St Louis, USA) was mixed with the commercial formulation at field rates (9.14 ml^{-1} Weed Stopper II, purity 43%, ca. 5.10 g trifluralin l^{-1}) and added to the plastic surface. The plastic pieces were soaked in the ^{14}C-trifluralin solution for 24 h, after which they were removed and transferred to new vials.

To remove any residual ^{14}C-trifluralin from the film surface, 5 ml of DI H_2O was added to each plastic piece and shaken at 170 rev min^{-1} for 45 min. This washing process was repeated twice, resulting in a total of three washes per sample. The wash solutions were collected, and 1 ml from each was mixed with 4 ml of Optiphase HiSafe 3 scintillation cocktail (Perkin Elmer Corp., MA, USA) before determination of the ^{14}C content using a Hidex 600 SLE liquid scintillation counter (Hidex Oy, Turku, Finland). Following the washing steps, the plastics were placed in a new sterile 20 ml glass scintillation vial and extracted with 5 ml of methanol for 24 h to recover any ^{14}C-trifluralin sorbed by the plastic. A 1 ml aliquot of the methanol extract was combined with 4 ml of scintillation cocktail and the ^{14}C content determined as described above.

In a 20 ml glass scintillation vial, 440 µl of ^{14}C-trifluralin (12 kBq ml^{-1}) was applied onto the surface of a 1 cm^2 plastic square and allowed to absorb over 24 h. Subsequently, the plastic square was removed, and any excess liquid was removed by shaking. To assess the persistence of trifluralin absorbed by plastic in soil, the ^{14}C-labelled plastic was placed in 5 g of field-moist soil in a sterile 50 ml polypropylene tube. The residual ^{14}C-trifluralin solution was also recovered and applied directly to the soil surface in a separate tube containing 5 g of field-moist soil. This treatment was chosen to reflect herbicide run-off from the plastic directly into the soil. After addition of the ^{14}C label to the soil, a 6 ml scintillation vial containing 1 ml of 1 M NaOH was placed above the soil surface to capture emitted $^{14}CO_2$ and the tubes sealed and incubated in the dark at 20 °C. The NaOH traps were replaced after 3 h and then at regular intervals on days 1, 2, 6, 10, 14, 21, 28, 35 and 45.

Due to slow rates of microbial activity and degradation of the ^{14}C-trifluralin, on day 34, 0.5 ml of 50 mM glucose was evenly applied to the soil surface of all treatments to stimulate microbial activity and respiration. On day 45, the final NaOH trap was removed, and 25 ml of n-hexane was added to each sample (trifluralin solubility in hexane, 59 g l^{-1}). The samples were then shaken at 200 rev min^{-1} for 30 min, and a 1 ml aliquot of hexane was removed, centrifuged at 18,000 rev min^{-1}, then the ^{14}C content determined as described previously.

2.5. Statistical analysis

Unlike the sorption experiments, for logistical reasons, the LDPE and PLA-PBAT growth trials were undertaken at different times preventing direct comparison. The LDPE experiment was carried out first, followed by the PLA-PBAT experiment; however, all protocols were the same for both. Graphical and statistical analysis of data was performed in R (v 4.2.3; [47]) using the '*agricolea*' [48], '*multcompview*' [49], '*corrplot*' [50], and '*tidyverse*' and '*ggplot2*' [51] packages. Normal distribution of data was assessed by Shapiro-Wilk test ($p \leq 0.05$) using the '*dplyr*' package [52]. An analysis of variance (one-way ANOVA) was used to assess the effects of treatments and plastic type on the plant and soil

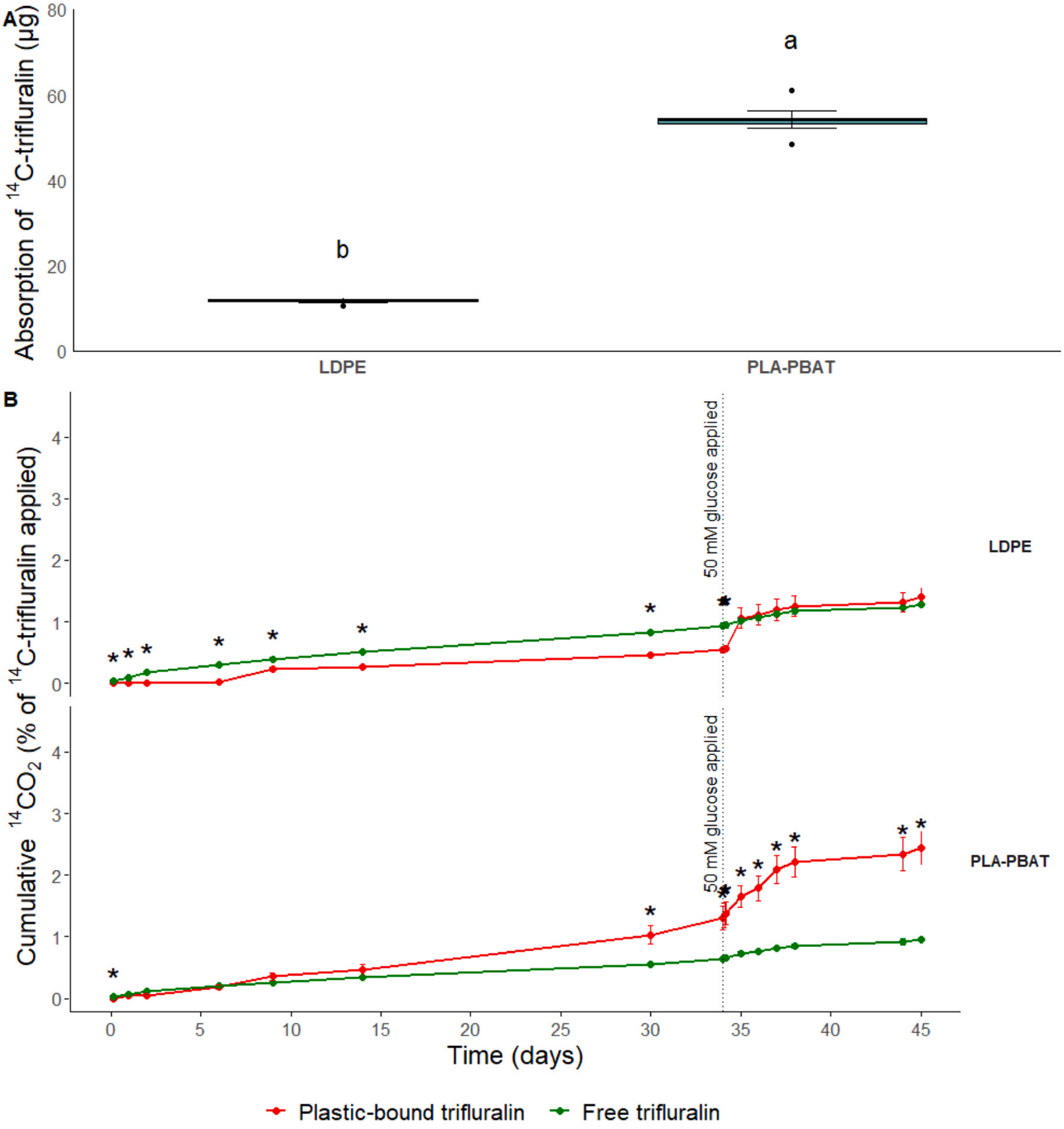

Fig. 1. ^{14}C-trifluralin absorption by LDPE and PLA-PBAT (A) and ^{14}C-trifluralin mineralisation in the soil (B). In Panel A, boxplots display the median and interquartile range, with whiskers showing minimum and maximum values in the data, and dots indicate potential outliers while different letters indicate a significant difference between treatments ($p \leq 0.05$). Panel B shows the mineralisation of ^{14}C trifluralin when bound to the plastic or when added directly to the soil. The dashed line in panel B represents the addition of 50 mM glucose to stimulate microbial activity while asterisks indicate a significant difference between pairs ($p \leq 0.05$). Values represent mean ± SEM, $n = 5$ per treatment.

parameters. To identify significant differences between treatment means, post hoc comparisons were performed using Tukey's test. A Student's *t*-test ($p \leq 0.05$) was also run to check for statistically significant differences in the absorption of trifluralin by plastics. Differences in forage chemical composition were evaluated with principal component analysis, as well as 3-way and 4-way ANOVA with factors including treatment, plastic type (LDPE *vs.* PLA-PBAT), trifluralin exposure, and tissue (shoot *vs.* root) using the '*stats*' package [53]. The significance threshold was set at $p \leq 0.05$ for all analyses. Values presented in the text represent mean ± SEM unless stated otherwise.

3. Results

3.1. ^{14}C-trifluralin absorption in plastic and microbial degradation in soil

The absorption of ^{14}C-trifluralin in macroplastic was determined after 24-hours (Fig. 1A) and its subsequent persistence in the soil assessed in a 45-day incubation experiment (Fig. 1B). Of the 383 µl of ^{14}C-trifluralin solution applied to each plastic piece to assess absorption (Fig. 1A), the PLA-PBAT absorbed significantly ($T_{(8)} = 20.84$, $p < 0.001$) more (54.2 ± 2.0 µg of active ingredient; ca. 2.77% of the total added) ^{14}C-trifluralin than LDPE (11.6 ± 0.3 µg of active ingredient; ca. 0.59%).

In an incubation experiment, each plastic was soaked in trifluralin solution for 24-hours prior to burial in soil (Fig. 1B). Greater microbial mineralisation of the ^{14}C-trifluralin from the PLA-PBAT (2.44 ± 0.27%) than the LDPE (1.40 ± 0.14%) occurred after 45-days ($F_{(8)} = 11.296$, $p = 0.001$), with a sharp increase in mineralisation rates observed from both plastics after soils were amended with 50 mM of glucose on day 34 to promote microbial activity. In comparison, when the ^{14}C-trifluralin solution was directly applied to the soil surface, microbial mineralisation was more rapid during the first 30 d, however, after 45 d it was similar to that present in LDPE (1.28 ± 0.04%; $T_{(4.75)} = 0.822$,

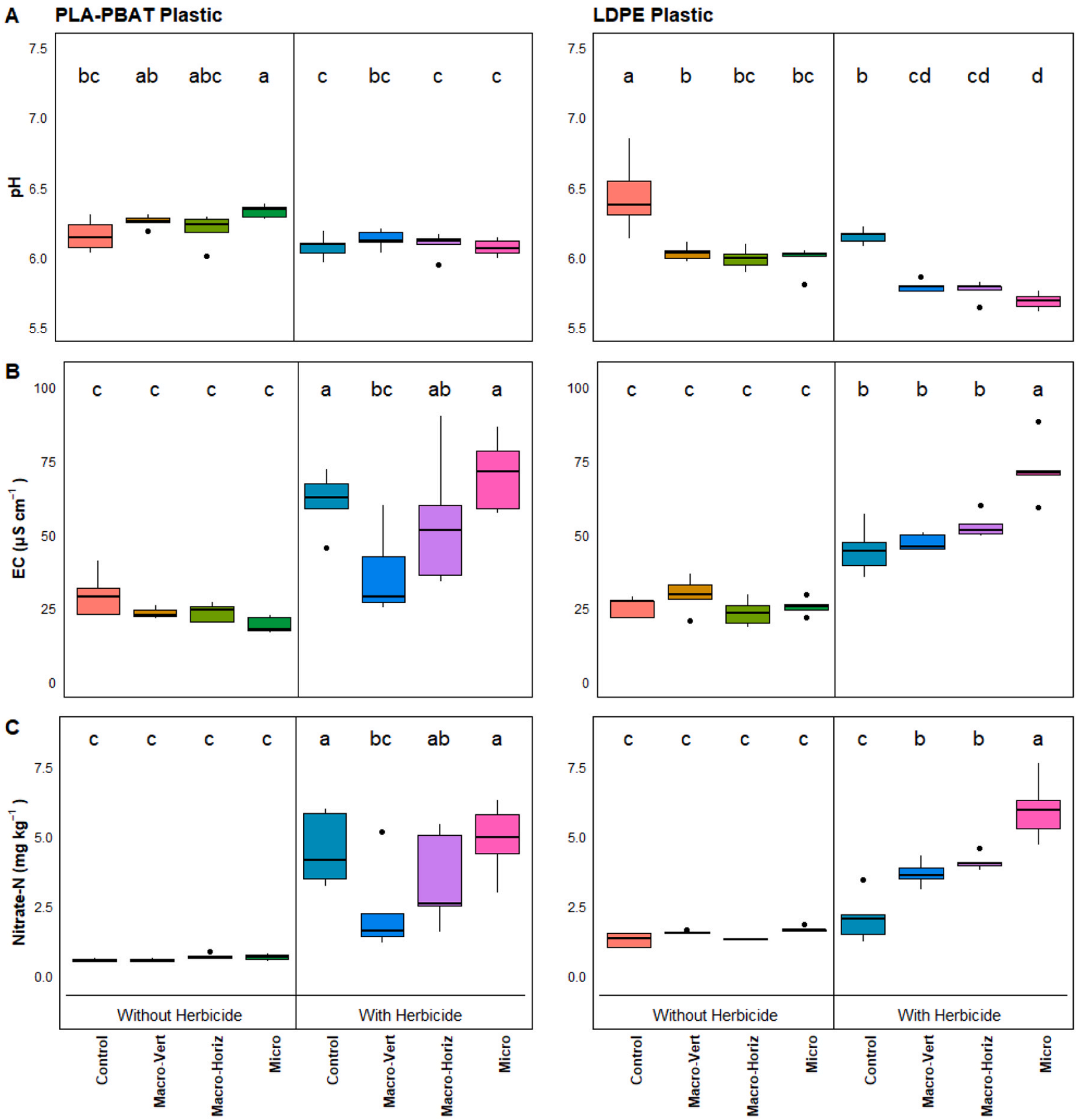

Fig. 2. Soil pH (A), electrical conductivity (B) and extractable nitrate (C) determined from each mesocosm after 45-days. The treatments included two plastic types (LDPE *vs.* PLA-PBAT), two size classes (macro- *vs.* micro-plastic), and the orientation of macro-plastic in soil (vertical *vs.* horizontal) either with or without trifluralin contamination (Table 2). Each treatment had 5 replicates. Different letters indicate a significant difference between treatments ($p \leq 0.05$). Boxplots display the median and interquartile range, with whiskers showing minimum and maximum values in the data, dots indicate potential outliers.

$p = 0.450$). In contrast, significantly lower mineralisation was observed from the ^{14}C-trifluralin added directly to the soil in comparison to that in the PLA-PBAT plastic ($0.96 \pm 0.05\%$; $T_{(4.32)} = 5.326$, $p = 0.005$).

3.2. Agronomic impacts of buried plastic mulch films contaminated with trifluralin

3.2.1. Soil nutrient cycling

The mesocosm experiment was destructively sampled and changes in soil biogeochemistry assessed after 45 days (Fig. 2 & S2, Tables S4 & S6). Overall, soil pH was significantly lower in the treatments exposed to trifluralin in comparison with the same treatments without trifluralin presence (Fig. 2A; $p < 0.001$), and organic matter content was not significantly affected by either herbicide presence, plastic type, orientation, or size (Fig. S2C; $p > 0.05$). Trifluralin significantly increased soil electrical conductivity compared to the control with LDPE (25.4 ± 1.6 µS cm^{-1}; $F_{(7, 32)} = 42.86$, $p < 0.001$) or PLA-PBAT (29.4 ± 3.4 µS cm^{-1}; $F_{(7, 32)} = 14.48$, $p < 0.001$), with the greatest difference in soil electrical conductivity observed in soils amended with LDPE (72.08 ± 4.67 µS cm^{-1}) or PLA-PBAT (70.5 ± 5.6 µS cm^{-1}) microplastic particles (Fig. 2B).

Soil ammonium concentrations were extremely low (< 1.5 mg NH_4-N kg^{-1}) and did not differ from the control (Fig. S2A). However, in comparison, soil nitrate concentration was greater in soil amended with either pure trifluralin or trifluralin-contaminated plastics (Fig. 2C), with plastic particle size driving significant differences between groups ($F_{(7, 32)} = 49.61$, $p < 0.001$ for LDPE and $F_{(7, 32)} = 15.44$, $p < 0.001$ for PLA-PBAT). Soil nitrate concentrations peaked at 5.97 ± 0.49 mg NO_3-N kg^{-1} in LDPE and 4.88 ± 0.58 mg NO_3-N kg^{-1} in PLA-PBAT when amended with trifluralin-exposed microplastic particles, representing increases of 362 and 775% respectively, compared to the control (1.29 ± 0.11 and 0.56 ± 0.02 mg NO_3-N kg^{-1}). Soil phosphate concentrations were low (ca. 1.5 mg PO_4-P kg^{-1}) and did not differ between the LDPE treatments (Fig S2B; $F_{(7, 32)} = 0.821$, $p = 0.577$), with only slight differences observed within the mesocosms amended with PLA-PBAT film ($F_{(7, 32)} = 7.182$, $p < 0.001$).

3.2.2. Maize aboveground biomass and root morphology

Plastic type and contamination with trifluralin had different effects on maize aboveground biomass (Figs. 3 & 4) and root morphology (Fig. S4) at harvest. Overall, non-herbicide contaminated macro- or micro-plastic addition to the soil had no significant effect on shoot height compared to the control ($F_{(7, 32)} = 0.679$, $p = 0.577$; Fig. 3). Similarly, plastic type in treatments without trifluralin had no significant effect on root biomass compared to the control ($F_{(7, 32)} = 2.466$, $p = 0.100$ for LDPE *vs.* $F_{(7, 32)} = 2.088$, $p = 0.142$ for PLA-PBAT); Fig. 4B).

The presence of trifluralin (whether in contaminated plastics or direct trifluralin application in the soil) reduced the above- and belowground biomass in shoots ($F_{(7, 32)} = 244.35$, $p < 0.001$) and roots ($F_{(7, 32)} = 331.03$, $p < 0.001$), regardless of plastic composition, particle size, or orientation within the soil (vertical *vs.* horizontal placement) (Fig. 4). The greatest reduction in aboveground biomass was observed in soils amended with microplastic particles exposed to trifluralin in LDPE or PLA-PBAT, equivalent to a 93% and 87% reduction, respectively, compared to the control (Tables S4 & S6). In addition, direct application of trifluralin in the soil showed higher reduction in PLA-PBAT treatment than LDPE treatment. Similar trends were observed in the belowground

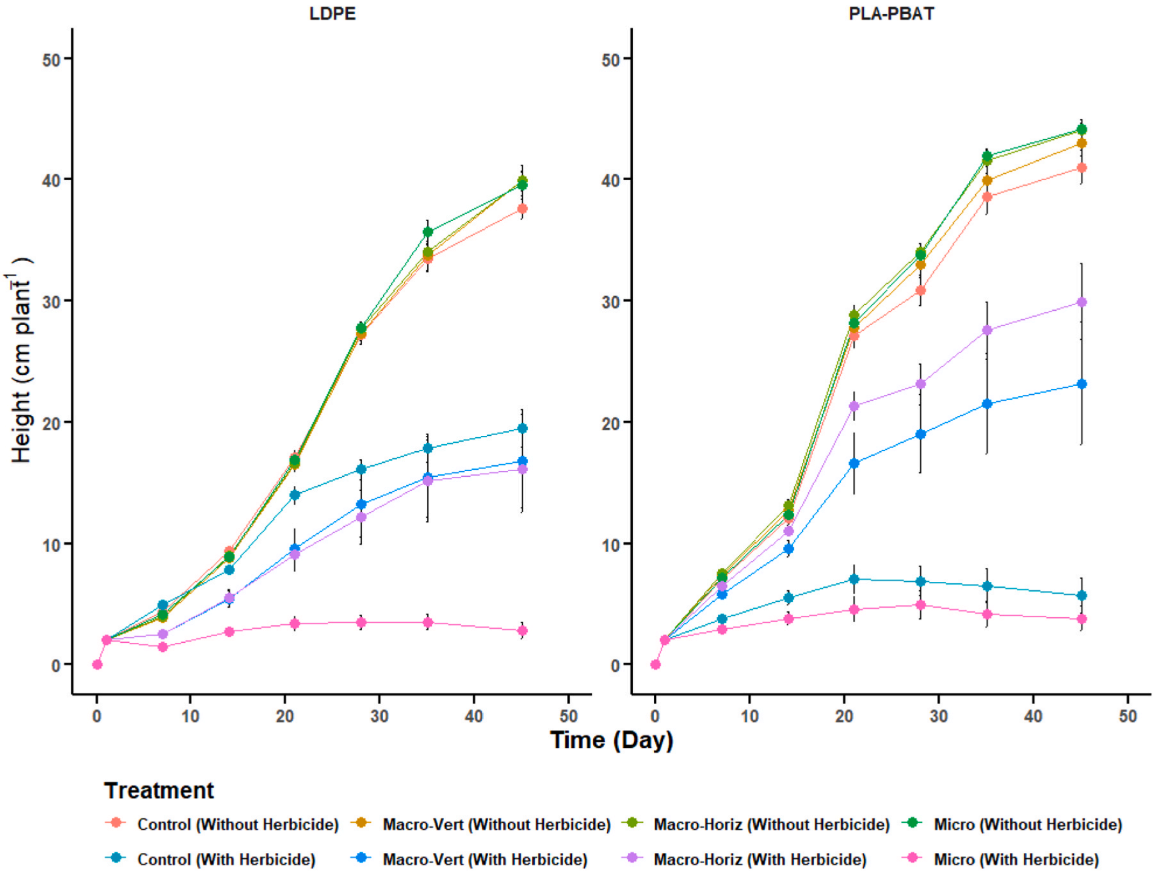

Fig. 3. Maize plant height measured from each mesocosm during the 45-day experiment. The treatments included two plastic types (LDPE *vs.* PLA-PBAT), two size classes (macro- *vs.* microplastic), and the orientation of macro-plastic in soil (vertical *vs.* horizontal) either with or without trifluralin contamination (Table 2). Values represent mean ± SEM, $n = 5$ per treatment.

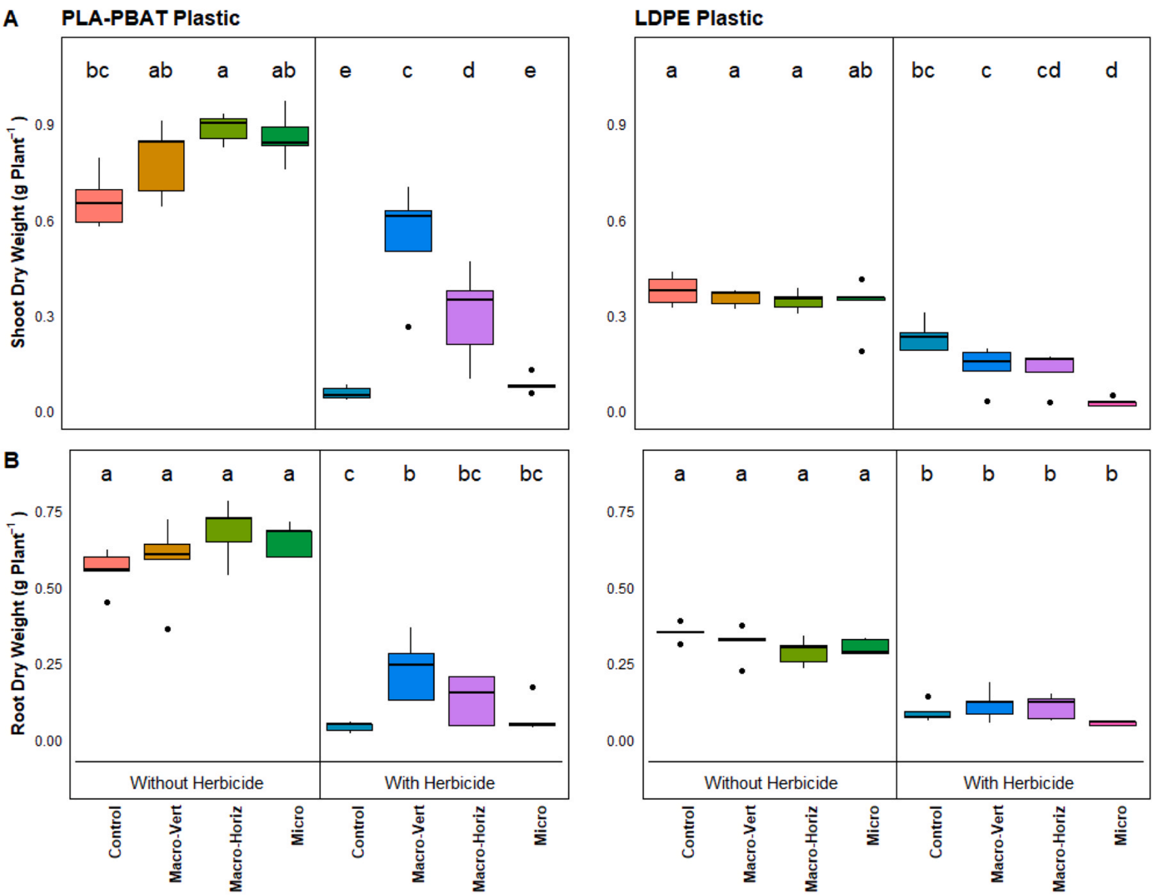

Fig. 4. Maize shoot (A) and root (B) biomass determined 45 days after exposure to plastic mulch film and trifluralin. The treatments included two plastic types (LDPE *vs.* PLA-PBAT), two size classes (macro- *vs.* microplastic), and the orientation of macro-plastic in soil (vertical *vs.* horizontal) either with or without trifluralin contamination (Table 2). Each treatment had 5 replicates. Different letters indicate a significant difference between treatments ($p \leq 0.05$). Boxplots display the median and interquartile range, with whiskers showing minimum and maximum values in the data, dots indicate potential outliers.

biomass in soils amended with microplastic particles exposed to trifluralin in LDPE or PLA-PBAT, equivalent to an 84% and 87% reduction respectively compared to the control (Fig. 4B).

Maize root morphology assessed at the end of the 45-day mesocosm experiment revealed that plastic type and trifluralin presence drove significant differences in root length ($F_{(7,\ 32)} = 55.056$, $p < 0.001$), diameter ($F_{(7,\ 32)} = 23.882$, $p < 0.001$), and volume ($F_{(7,\ 32)} = 61.693$, $p = < 0.001$) (Fig. S4, Tables S4 & S6), with no observed effect of plastic particle size (macro- *vs.* microplastic) or burial orientation (vertical *vs.* horizontal) in the soil ($p > 0.05$) (Fig. S4). Root length and volume significantly ($p < 0.001$; Table S4) decreased compared to the control when trifluralin was present in the soil or the plastic particles (Fig. S4)

In comparison to the unamended control, exposure to non-herbicide contaminated LDPE or PLA-PBAT showed no distinct responses in plant height and foliar chlorophyll content (Fig. S3). However, similar to biomass production, the introduction of trifluralin herbicide (whether through contaminated-plastics or direct trifluralin application) negatively impacted plant height ($F_{(7,\ 32)} = 48.877$, $p < 0.001$) and chlorophyll content ($F_{(7,\ 32)} = 16.930$, $p < 0.001$), with notable reductions observed under herbicide-exposed microplastic conditions and direct application of trifluralin in PLA-PBAT treatments. The reduction was more apparent in the micro-sized plastic particles exposed to herbicide, showing > 84% and > 100% reduction in LDPE plastic and > 87% and > 100% reduction in PLA-PBAT plastic in comparison with the control, for plant height and chlorophyll content, respectively.

3.2.3. *Forage quality*

After 45 days, maize shoot and root C:N ratio was negatively affected

Table 3

C:N ratio of *Zea mays* shoot and roots determined 45-days following exposure to plastic mulch film and trifluralin. The treatments included two plastic types (LDPE *vs.* PLA-PBAT plastic), two size classes (macro- *vs.* microplastic), and the orientation of macro-plastic in soil (vertical *vs.* horizontal) either with or without trifluralin contamination (Table 2). Data represents mean ± SEM, $n = 5$ per treatment. Superscripted letters indicate significant differences ($p \leq 0.05$) between treatments in each group using Tukey HSD test.

Plastic	Treatment	Shoot C:N ratio	Root C:N ratio
LDPE	Control	31.3 ± 1.24^{a}	42.6 ± 0.77^{a}
	Vertical	29.5 ± 1.27^{a}	41.9 ± 1.31^{a}
	Horizontal	28.3 ± 1.31^{ab}	40.2 ± 1.89^{a}
	Micro	28.0 ± 1.14^{ab}	40.5 ± 1.35^{a}
	Trifluralin	27.1 ± 1.60^{abc}	24.8 ± 1.46^{bc}
	Vertical-Trifluralin	21.3 ± 1.47^{c}	24.7 ± 0.37^{bc}
	Horizontal-Trifluralin	22.8 ± 1.69^{bc}	26.3 ± 0.37^{b}
	Micro-Trifluralin	9.0 ± 0.2^{d}	18.8 ± 0.58^{c}
	F-value	19.784	60.530
	p-value	< 0.001	< 0.001
PLA-PBAT	Control	47.0 ± 0.99^{a}	53.5 ± 0.75^{a}
	Vertical	48.5 ± 1.87^{a}	53.0 ± 2.31^{a}
	Horizontal	48.6 ± 0.70^{a}	57.1 ± 2.47^{a}
	Micro	49.5 ± 2.76^{a}	55.5 ± 2.50^{a}
	Trifluralin	10.8 ± 1.68^{b}	20.2 ± 0.10^{c}
	Vertical-Trifluralin	34.5 ± 3.26^{b}	35.4 ± 3.05^{b}
	Horizontal-Trifluralin	22.0 ± 3.03^{b}	23.6 ± 1.37^{c}
	Micro-Trifluralin	9.6 ± 0.8^{b}	18.9 ± 1.75^{c}
	F-value	19.859	54.353
	p-value	< 0.001	< 0.001

by trifluralin presence in soils amended with LDPE and PLA-PBAT (Table 3 & S3), regardless of plastic particle size or orientation in the soil. In the shoots, significantly lower ($F_{(7, 32)} = 19.784$, $p < 0.001$) C:N ratios were identified in treatments amended with trifluralin in solution (ca. 10–27:1) or trifluralin-contaminated LDPE and PLA-PBAT (ca. 9:1) microplastic fractions compared to the control (ca. 31–47:1). A similar trend was observed for roots, where soils amended with trifluralin in solution (ca. 20–24:1) or contaminated LDPE and PLA-PBAT microplastic (ca. 19:1) had a much lower C:N ratio than the control (ca. 43–54:1) ($F_{(7, 32)} = 60.530$, $p < 0.001$).

The elemental accumulation analysis of maize roots and shoots revealed that Zn in the roots with LDPE plastic and Fe in the shoots with PLA-PBAT plastic did not show any significant difference between treatments ($p > 0.05$; Tables S2 & S5). In contrast, Fe accumulation ($p = 0.013$) and Zn accumulation ($p = 0.025$) in roots with PLA-PBAT plastic showed significant higher values in treatments without trifluralin presence regardless of plastic type. Additionally, the accumulation of other elements including P, S, Cl, K, Ca, Mn and Cu in both shoot and root, were significantly lower in the treatments with herbicide presence ($p < 0.001$), especially in treatments with microplastics exposed to trifluralin regardless of their plastic type (PLA-PBAT *vs*. LDPE).

Principal Component Analysis (PCA) provided further insights into treatment-driven nutrient patterns (Fig. S5). In the shoots of maize (PC1 = 77.9%, PC2 = 8.4%), trifluralin and trifluralin-exposed microplastic treatments separated from the control and treatments without trifluralin presence, driven mainly by higher Fe, Mn, and Zn. In roots of maize (PC1 = 72.6%, PC2 = 12.8%), trifluralin-exposed treatments clustered away from other treatments, associated with elevated P, S, Ca, K, Zn, Fe, and Mn. The control and plastic treatments without trifluralin presence remained near the origin, indicating minimal nutrient disruption.

4. Discussion

4.1. ^{14}C-trifluralin absorption in plastic and microbial degradation in soil

This study demonstrated a significant difference in the absorption potential of trifluralin between the biodegradable (PLA-PBAT) and conventional (LDPE) plastic mulch films. Overall, PLA-PBAT absorbed four times more trifluralin than LDPE. We attribute this enhanced sorption to the greater chemical compatibility between lipophilic trifluralin ($K_{OW} = 5.34$) and the polar ester functional groups present in the PLA-PBAT blend, combined with the more amorphous polymer structure [54,55]. Together these provide increased free volume and accessibility for herbicide penetration. This contrasts with the densely packed, non-polar LDPE matrix lacking specific chemical functionalities (e.g., oxygen-bearing group and benzene rings; [34,56]). Additionally, the diffusion kinetics of trifluralin through these polymer matrices are likely to differ significantly, with the more rigid LDPE structure impeding molecular transport compared to the more flexible PLA-PBAT network. It should be noted that the exposure time used here (24 h) may not represent equilibrium conditions, as polymer absorption may require days to weeks to reach steady state, potentially altering the absorption ratios observed [57]. Overall, our findings are also supported by Torres et al. [37] and Beriot et al. [58] who reported that the sorption capacity for a wide range of other chemical contaminants to degradable plastics (e.g., PBAT) were generally higher than their non-degradable LDPE counterparts.

Surface morphology may also contribute to higher trifluralin absorption in the PLA-PBAT films, which often exhibit a more porous structure compared to the relatively smooth surface of LDPE (Table 2), providing greater surface area for trifluralin sorption. The composition of the PLA-PBAT blend, which varies markedly depending on the manufacturer, may also influence absorption [59,60]. We hypothesised that trifluralin would likely show preferential partitioning into the PBAT phase due to favourable aromatic-aromatic interactions between trifluralin's aniline structure and PBAT's terephthalate units, together with PBAT's greater molecular flexibility and lipophilic character compared to the more rigid, polar PLA component. Further, both plastics contain a range of additives (e.g., antioxidants, UV protectants, mineral fillers) that may also influence trifluralin sorption [61]. Further work is required to better identify what properties of the plastic composition regulate pesticide sorption and subsequent desorption.

Only small amounts of trifluralin were absorbed from the liquid phase by either plastic after 24 h. We attribute this to the use of a commercial formulation which also included a range of surfactants. These surfactants are known to encapsulate hydrophobic herbicides within micelles, increasing their aqueous solubility and reducing solid phase sorption [62,63]. Pilot trials showed much higher rates of pure trifluralin sorption to both plastics in the absence of surfactants (data not shown), demonstrating the importance of using realistic commercial formulations to accurately assess environmental behaviour under field conditions [64].

The rate of trifluralin mineralisation was low for both plastic types, similar to that added directly to the soil. In the case of free trifluralin, it is known that trifluralin binds strongly to soil organic matter, reducing its bioavailability [65]. Based on the evidence that additives bound to plastics (e.g., dibutyl phthalate) are much less bioavailable than those added directly to soil [66], we attribute the slow rate of trifluralin mineralisation in plastics to both physical protection by the polymer matrix and the poor solubility of trifluralin in water (1.18 μM). Despite this, the breakdown of trifluralin bound to PLA-PBAT was higher than that for LDPE, suggesting either greater trifluralin desorption from the PLA-PBAT matrix into solution and/or microbial co-metabolism stimulated by the presence of the biodegradable polymer matrix. It should be noted, however, that the enzymes and metabolic pathways involved in trifluralin breakdown are different from those associated with PLA and PBAT degradation [67]. Previous research has shown that biodegradable plastics can influence soil microbial communities and enhance the degradation of associated contaminants [36,68,69]. The addition of glucose on day 34 further stimulated microbial activity and trifluralin degradation, particularly in the PLA-PBAT treatment. This observation suggests that co-metabolism enhances trifluralin breakdown, as observed for other organic contaminants [70,71]. These findings strongly support our first hypothesis regarding enhanced trifluralin sorption by PLA-PBAT, but provide only partial support for our second hypothesis, as mineralisation rates remained low despite the biodegradability of the PLA-PBAT matrix.

These findings have important implications for understanding the environmental impact of different types of plastics. The increased absorption and subsequent release of trifluralin from PLA-PBAT plastics could affect the fate and transport of herbicides in agricultural soils, potentially impacting soil health and non-target organisms [36]. While the enhanced microbial degradation associated with biodegradable plastics may mitigate some risks by promoting the breakdown of absorbed herbicides, there is also a possibility of increased bioavailability leading to unintended ecological effects [72,73]. It should be noted that key physicochemical properties influencing sorption behaviour, such as porosity, and water contact angle, were not measured in this study. These properties may influence the interaction between plastics and hydrophobic organic compounds such as trifluralin and should be considered in future studies examining plastic–pesticide interactions.

4.2. Soil agronomic impacts of buried plastic mulch films contaminated with trifluralin

The observed changes in soil properties across various treatments indicate the complex interactions between plastic pollution, herbicide presence, and soil chemistry. In treatments without herbicide, the presence of both LDPE and PLA-PBAT plastics caused changes in soil pH, electrical conductivity (EC), and nitrate consistent with reports by Boots et al. [74], [75,76], Yang et al. [77], and Sintim et al. [78]. A decrease in soil pH in the presence of LDPE plastic particles has previously been attributed to relatively high and possibly reactive surface area along with alterations in the soil cation exchange capacity [74,79]. However, due to the recalcitrance of LDPE in soil [80,81], we ascribe the pH response observed here to alterations in plant nutrient demand caused by herbicide contaminated plastics. Specifically, reduced plant growth and corresponding decreases in N uptake provide greater amounts of NH_4^+ available for nitrification which is an inherently acidifying process [16]. This is also consistent with the reduction in plant growth, increase in EC and NO_3^- concentration in all the herbicide treatments, particularly those with micro-sized plastic particles. These shifts in soil pH and EC, combined with elevated NO_3^- concentrations, suggest disrupted nutrient cycling that could reduce soil fertility and increase the risk of nutrient leaching, potentially compromising both crop productivity and environmental quality in plastic contaminated soils.

The plant growth parameters measured in this study reveal significant impacts from both plastic particles and trifluralin herbicide on the development of shoots and roots. In treatments without herbicide, the presence of LDPE and PLA-PBAT plastics at field-relevant concentrations resulted in no negative impacts on plant growth. This contrasts with many previous studies that have shown that both LDPE and biodegradable plastics can repress shoot and root development when added at unrealistically high concentrations [82–84], but aligns with findings of studies using realistic plastic concentrations [85–88].

A significant decrease was observed in the treatments with herbicide present. Trifluralin, both alone and in combination with plastics, drastically reduced all measured plant growth parameters. Plastic treatments with herbicide (both sizes and directions), showed substantial reductions in plant growth parameters, though to a slightly lesser extent in PLA-PBAT than LDPE treatments. Root length and volume were also severely reduced. This suggests that trifluralin, when absorbed by LDPE particles, becomes more bioavailable and toxic to plants, exacerbating the herbicide's phytotoxic effects. On the other hand, this might indicate that PLA-PBAT plastics, while still harmful and absorb more trifluralin (~4 times more), do not enhance the bioavailability of trifluralin to the same extent as LDPE. The adverse effects of trifluralin presence on the plant growth are reported by Bijanzadeh et al. [89]; 1.2–1.4 kg a.i ha^{-1}), Alshallash [90]; up to 1.0 kg trifluralin a.i ha^{-1}), Chowdhury et al. [91]; 0.075–2.40 mg trifluralin a.i kg^{-1} dry soil) and Li et al. [18]; 3.19–5.98 μg trifluralin kg^{-1} dry soil). Compared with this study, the concentration of trifluralin in the LDPE experiment was similar to those applied in previous studies. In contrast, the concentration in the PLA-PBAT experiment was much higher, where stronger negative effects were expected.

The more severe phytotoxic effects seen in the microplastic treatments exposed to herbicide are probably due to the fact that smaller plastic particles have a larger surface area and higher sorption capacity [34,39]. Microplastics have a greater reactive surface area per unit mass than macroplastic fragments [92,93], facilitating the absorption and desorption of hydrophobic organic compounds like trifluralin [34]. Therefore, microplastics could be better at transporting and releasing herbicides in soil, making them more available to plants in the rhizosphere and increasing their exposure [94]. In addition, finer particles are easier to spread throughout the soil matrix and can interact more directly with plant roots [95], which could make herbicides move more easily into plant tissues.

The herbicide treatments resulted in irregular root growth with some treatments (e.g., macro sizes in both directions) showing increased root diameters. This is consistent with the mode of action of trifluralin, which binds to root tubulin proteins disrupting microtubule polymerisation and preventing normal cell elongation [96–98]. Our experimental design cannot distinguish whether the observed phytotoxic effects result from trifluralin desorption into the soil solution or from direct physical contact between plant tissues and herbicide-contaminated plastic particles. We hypothesised that horizontally oriented plastic would exhibit greater phytotoxicity than vertically oriented plastic due to increased probability of root-particle contact, however, our results did not support this. One possible explanation is that root systems develop in three-dimensional soil environments, making differences in root–plastic contact probability between vertical and horizontal plastic orientations difficult to control or quantify under experimental conditions. The greatest inhibition of shoot growth was observed in the herbicide-contaminated microplastics treatment, suggesting that the increased surface area and more uniform distribution of smaller particles throughout the soil matrix enhanced trifluralin bioavailability. Our results for strong inhibition of shoot growth are also consistent with the upward translocation of trifluralin from the roots to the aerial parts [99,100]. In addition, although the plants were well watered, the reduced root surface area and altered root morphology caused by trifluralin exposure would have severely limited nutrient acquisition, contributing to the observed shoot growth reduction. However, our results suggest that this was not the primary mechanism as the plants exposed to trifluralin-contaminated plastics contained higher levels of N than in those with non-contaminated plastics. Further, no visual symptoms of nutrient deficiency were observed in herbicide-treated plants or in the measurements, suggesting that trifluralin's primary mode of action was direct disruption of cellular processes rather than secondary nutrient stress. This is also supported by measurements of plant-available nutrients in the soil which generally showed an adequate supply. This contrasts with previous research has shown that certain herbicides can impact soil microbial communities and N cycling [16,23,101], noting that some of the concentrations used in previous studies are greater than used here for LDPE treatments and lower than used for PLA-PBAT treatments. Overall, contrary to our third hypothesis, we conclude that plastic orientation (horizontal vs. vertical placement) had no major effect on phytotoxicity, indicating that herbicide bioavailability is primarily controlled by desorption into soil solution rather than direct root-plastic contact probability. However, our fourth hypothesis was strongly supported, with trifluralin-contaminated plastics greatly altering nutrient cycling and severely suppressing plant growth, particularly when present as microplastic particles.

The results of plant elemental composition showed significant decreases in nutrient accumulation in the treatments exposed to trifluralin. Hartzler et al. [102] reported a significant decrease in P, K and Zn content in corn tissues following a low application dose of trifluralin. These decreases were attributed to the growth inhibition properties of trifluralin which disrupts cell division in meristematic tissues, thereby inhibiting root cell growth and nutrient uptake [103]. The strong inhibition of radicle development by trifluralin observed here, affected both growth of the main root alongside the emergence of secondary roots. Thickening of the hypocotyls [91] and swelling of the root tips [104] were also observed here, further indicating uncoordinated cell expansion in the growing zone following dysregulation of the cytoskeleton.

5. Conclusions

This study showed that biodegradable plastic mulch films pose a previously underappreciated risk as herbicide vectors in agricultural soils. PLA-PBAT plastic absorbed four times more trifluralin than conventional LDPE, yet herbicide mineralisation remained extremely low (<4%) regardless of plastic type over 45 days, indicating persistent soil contamination. Critically, microplastic particles caused disproportionately severe impacts on plant and soil health compared to macroplastic suggesting that plastic fragmentation in soil amplifies environmental risks.

In terms of soil management, the enhanced herbicide absorption by biodegradable plastics challenges the assumption that these materials are inherently safer alternatives to conventional mulch films. Therefore, farmers using biodegradable mulch films should consider the timing and persistence of herbicide applications, as interactions between herbicides and biodegradable plastic residues may influence herbicide availability and legacy in soil. The accumulation of microplastic fragments poses particular concern, as these particles demonstrate higher surface area-to-volume ratios that facilitate greater contaminant sorption and bioavailability and potentially transport deeper into the root zone.

We acknowledge that our findings are based on a greenhouse trial with a single lipophilic herbicide. Further long-term field studies (>5 years) are essential to assess the persistence and cumulative effects of herbicide-contaminated plastic residues under realistic agricultural conditions. Research should be expanded beyond trifluralin to evaluate interactions with other commonly used herbicides, fungicides, and insecticides. In addition, our mechanistic understanding of the factors regulating herbicide sorption and desorption from different plastic and pesticide formulations remains poor necessitating the need for further experimentation. Development of standardised protocols for plastic residue detection and contaminant quantification are also needed. Although these exist for microplastics [105], many protocols use oxidants to remove organic matter (e.g., H_2O_2) that may also remove contaminants from the plastic surface. Finally, ecosystem-scale studies examining impacts on soil microbial communities, non-target organisms, and broader food web effects are necessary to fully understand the environmental implications of plastic-pesticide interactions in agricultural landscapes.

CRediT authorship contribution statement

Davey L. Jones: Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **David R. Chadwick:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Stefano Loppi:** Writing – review & editing, Supervision. **Martine Graf:** Writing – review & editing, Methodology, Investigation, Data curation. **Emily C. Cooledge:** Writing – review & editing, Visualization, Methodology, Data curation. **Majid Ghorbani:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Nazanin Azarnejad:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.hazmp.2026.100055.

Data availability

Data will be made available on request.

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