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Radish growth and soil aggregate stability affected by microplastic contamination

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Abstract: The major objective of this study is to investigate changes in radish growth parameters (fresh and dry biomass and photosynthetic efficiency) and the stability of water-stable soil aggregates under contamination by different microplastic materials (high-density polyethylene (HDPE), polyvinyl chloride (PVC), and polystyrene (PS)) in contrasting soil types (Chernozem, Luvisol and Umbrisol). The study was conducted as a pot experiment under laboratory conditions, including three soil types and three microplastic types, with five replicates per variant. The results indicate that the effects of microplastics on plant biomass were significantly dependent on the interaction between soil type and microplastic type. In Chernozem, PS contamination significantly increased both dry and fresh biomass production (by approximately 46% and 50%, respectively) compared with the control. This response was directly associated with increased photosynthetic efficiency and greater stability of water-stable microaggregates in this variant. These findings confirm the stimulatory effect of PS on plant growth in Chernozem. The presence of PS in Chernozem appears to promote the formation of stable microaggregates and decrease macroaggregates, thereby altering the pore system (distribution of micro- and macropores) and supporting plant growth and nutrient absorption. An improvement in the stability of water-stable microaggregates was also observed in Luvisol and Umbrisol under PS contamination (statistically significant in Umbrisol); however, these changes were not reflected in the analysed plant growth parameters. In contrast, HDPE and PVC microplastic pollution had no statistically significant effect on biomass production or photosynthetic efficiency in Chernozem, Luvisol, or Umbrisol. Overall, the findings demonstrate that the effects of microplastic contamination on soil properties and plant growth are strongly influenced by both the type of microplastic polymer and soil characteristics.

Keywords: environmental contamination; pollutant; micro-particle effects; crop performance

Ecological research has traditionally focused on how plants and soils respond to global change factors, particularly climate change. However, increasing attention is being given to emerging anthropogenic stressors such as environmental contamination by plastics (Krehl et al. 2022). Since the 20th century, global plastic production and consumption have risen sharply, resulting in increasing amounts of plastic waste entering the environment (Ragusa et al. 2021). If current trends continue, global plastic production could exceed 12.000 million tonnes by

2050. Due to their widespread presence in terrestrial, aquatic, and atmospheric systems, plastics are now recognised as a major environmental problem and potential risk to public health (Cottom et al. 2024). In the environment, plastics persist for long periods and gradually fragment through processes such as physical abrasion, ultraviolet radiation, and mechanical disturbance (Jin et al. 2022). These processes produce microplastics – commonly defined as plastic particles smaller than 5 mm that can further break down into nanoparticles (< 1 000 nm), which

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may enter biological tissues and cells and potentially cause toxic effects (Lehner et al. 2019). Although the < 5 mm definition is widely used, there is still no internationally standardised classification of plastic particle size classes (Hartmann et al. 2019).

Recent findings confirm that microplastics affect soil biological functions by modulating the microbial community. Changes in microbiota composition, especially the decline of actinobacteria and other beneficial microbes, can lead to weakened soil particle bonding, reduced aggregation, and disruption of soil water and nutrient regimes. These changes may ultimately impair plant growth and ecosystem functioning. Microplastics can disrupt soil ecosystems, be absorbed by plants, interfere with nutrient cycling, decrease soil quality and health, and eventually enter the food chain. Due to their resistance to degradation, they persist as pollutants, remaining in the soil for extended periods and adversely affecting its physical and chemical properties (Hanif et al. 2024).

Microplastics affect the physical properties of soil, including texture, structure, aggregate stability, porosity, bulk density, water-holding capacity (WHC), and saturated hydraulic conductivity, and reduce rainwater infiltration time (Joos and De Tender 2022, Li et al. 2022). With a rise in microplastic concentration (> 1%), cohesion among microplastics, soil particles, and aggregates could progressively lead to the encasement of soil particles by microplastics, thereby reducing interactions between soil particles (Hanif et al. 2024). Due to other previous studies, it can be concluded that the incorporation of large microplastics (MPs) like microfibers (high-density polyethylene (HDPE), polyester (PES), or polyacrylic fibres) can decline water-stable soil aggregates (Boots et al. 2019, Li et al. 2022).

The effects of microplastics on plants vary depending on their size, shape, type, and concentration (Wu et al. 2021). Microplastics can negatively influence plant development by impairing seed germination and root growth, inducing oxidative stress, reducing plant height and biomass, lowering seed production, and limiting water and nutrient uptake. In addition, they may accumulate in plant tissues, decrease photosynthetic activity, alter cell membrane integrity, and disrupt plant-symbiont interactions (Gao et al. 2019, Li et al. 2022).

Recent research suggests that microplastics can change soil properties and microbial communities, and may also influence plant physiology, although

the extent and even the direction of these effects vary significantly depending on the experimental method, polymer type, particle size, and concentration used. On one hand, a meta-analysis of controlled experiments – mostly reflecting more environmentally realistic exposure levels – found only a slight average reduction in plant biomass (~1%), alongside a more substantial decrease in chlorophyll content (~28%), with smaller particles and earlier exposure producing stronger effects (Shi et al. 2024). On the other hand, pot experiments using significantly higher, modelled concentrations report much more severe responses: in wheat, polystyrene microplastics – especially in powder form and at concentrations $\geq 3\%$ – dramatically reduced plant height (by ~58–60%), fresh biomass (by ~54–55%), and dry biomass (by ~61–62%), while also increasing photosynthesis, enzymatic activity, and oxidative stress (Riaz et al. 2025).

Microplastics can affect photosynthesis indirectly; MPs can affect leaves, show signs of inhibited growth, hinder chlorophyll fluorescence, reduce chlorophyll content, inhibit protein synthesis, modulate energy levels, affect amino acid metabolism, and interfere with the antioxidant defence system of plants (Hanif et al. 2024). The impact of microplastics on plants appears to be either direct toxicity – where microplastics clog root hairs, impair nutrient and water uptake, induce oxidative stress, and disrupt photosynthetic cycles – or adverse effects through changes in soil properties and nutrient cycling (Ren et al. 2024). De Souza Machado et al. (2019) reported the increased root length, area, and biomass in the onion (*Allium fistulosum* L.) under 2% polyethylene high density (PEHD), polystyrene (PS), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP), and polyester (PES). Under the influence of 1% low-density polyethylene (LDPE) and 1% starch-based BDM, root mass decreased significantly in wheat (*Triticum aestivum* L.), also affecting the above-ground and below-ground parts of wheat during reproductive and vegetative growth phases.

These findings highlight the growing problem of microplastic accumulation in soils, where they can substantially impair plant growth, photosynthetic activity, and crop yield, particularly under field conditions where multiple environmental stressors interact simultaneously. Current research has primarily focused on the general impacts of microplastics on soils and plants, whereas the combined effects of polymer composition and soil type on crop performance remain insufficiently understood. In particular,

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comparative studies examining the effects of HDPE, PS, and PVC microplastics in agricultural soils are still limited. Therefore, this study investigates how three widely distributed microplastic polymers (HDPE, PS and PVC) influence radish (*Raphanus sativus* L. var. *sativus*) performance and soil aggregate stability in three contrasting soil types (Chernozem, Haplic Luvisol and Umbrisol). In accordance with the two-factor experimental design (soil type $\times$ microplastic type) and the measured response variables (fresh biomass (FB), dry biomass (DB), maximum photochemical efficiency of PSII (F_v/F_m); water-stable macro- and microaggregates, WSMAA and WSMIA), the following hypotheses were formulated:

(H1) HDPE, PS and PVC microplastics will affect FB, DB and F_v/F_m of radish to a different extent.

(H2) The magnitude of the microplastic-induced response of FB, DB and F_v/F_m will differ among the three soil types.

(H3) A statistically significant interaction between soil type and microplastic type will be detected for at least one of the plant response variables.

(H4) Microplastic contamination will shift the distribution of water-stable aggregates.

MATERIAL AND METHODS

Soil sampling and analytical methods. Three topsoil samples (0–20 cm) were collected from agricultural sites located in western Slovakia: Maslovce (48°05'41"N, 17°26'34"E), Malanta-Nitra (48°19'23"N, 18°09'01"E), and Borský Mikuláš (48°37'01"N, 17°12'54"E). The selected locations represent three contrasting soil types: Chernozem (Maslovce), Haplic Luvisol (Malanta-Nitra), and Umbrisol (Borský Mikuláš). Soil classification was conducted in accordance with the World Reference Base for Soil Resources (WRB 2015). Soil samples were collected after the harvest of the preceding crop, which was maize (*Zea mays* L.) at Maslovce, spring

wheat (*Triticum aestivum* L.) at Nitra-Malanta, and maize (*Zea mays* L.) at Borský Mikuláš.

Disturbed soil samples were collected from the 0–20 cm layer at ten representative points per site, avoiding atypical areas. Subsamples (~10 kg each) were collected, mixed in jute containers, and transported under cool, dry conditions. In the laboratory, soils were air-dried, crushed and sieved through a 2.5 mm mesh before analysis.

The Baksheev method (Šimanský et al. 2023) was used to determine the contents of water-stable aggregate (WSA) size fractions. The WSA of > 0.25 mm size fraction was considered water-stable macroaggregates (WSMAA), while the WSA of < 0.25 mm size fraction was considered water-stable microaggregates (WSMIA).

Basic soil properties were determined on disturbed soil samples, with two replicates, at the Laboratory of the Soil Science and Protection Research Institute in Bratislava and at the Institute of Agrochemistry and Soil Science in Nitra. Samples for static contact angle (CA) measurements were prepared according to the procedure described by Bachmann et al. (2000) and were estimated using the dpiMAX version 1.51.90.75 (DataPhysics Instruments GmbH, Filderstadt, Germany) from image recordings obtained with the optical goniometer OCA 11 (DataPhysics Instruments GmbH, Filderstadt, Germany). The properties of the tested soils are listed in Table 1.

Microplastic materials. Three types of plastic were used in the conducted experiment: high-density polyethylene, polyvinyl chloride, and polystyrene. The three selected plastic materials were processed using the same procedure: milling followed by sieving to obtain microplastics (MPs) with particle sizes < 400 μm . The characteristics of the plastic materials are presented in Table 2.

Study design. To ensure uniformity, the collected soil was homogenised using a hammer mill, followed by sieving through a 2.5 mm mesh. Subsequently,

Table 1. Soil parameters of the topsoil horizon (0–20 cm) of the study sites

	Sand content	Silt content	Clay content	$\text{pH}_{\text{H}_2\text{O}}$	SOC	C_aCO_3	CA
		(%)				(%)	(°)
Chernozem	27.1	55.1	17.8	7.42	2.9	4.0	2.24
Luvisol	24.9	59.9	15.2	6.89	1.2	0.06	2.18
Umbrisol	84.5	11.9	3.6	6.71	1.0	0.1	7.83

SOC – soil organic carbon content; CA – contact angle, representing the severity of soil water repellency before adding microplastics

Table 2. Characteristics of plastic materials

Material	Product of origin	Bulk density (g/cm ³)	CA (°)	WDPT (s)
HDPE	crude plastic	0.35	135.17	10 200
PVC	pipe	0.46	131.40	11 100
PS	cup	0.54	123.01	11 400

HDPE – high-density polyethylene; PVC – polyvinyl; PS – polystyrene; WDPT – water drop penetration time; CA – contact angle

microplastics were incorporated into the soil at a concentration of 5% (microplastics weight/total weight) in each pot (200 mm × 150 mm × 150 mm, 3 000 mL). The microplastic concentration was defined on a mass basis of the total mixture, i.e. 5% by weight (1 000 g of mixture = 50 g of microplastics + 950 g of soil). Before establishing the experiment, we first determined the bulk density of three disturbed pure control soil samples (without microplastics). In the control variants containing only pure soil, each 3-L (3 000 cm³) pot therefore contained 3 480 g of soil for Chernozem, 3 840 g for Luvisol, and 4 410 g for Umbrisol, corresponding to the measured bulk densities of the respective soils. MPs were mixed by hand with soil in a large container for 5 minutes to ensure uniform distribution throughout the soil. Each pot was then filled with an equal volume of substrate or pure soil to maintain consistent bulk density across experimental variants. Following preparation, all pots were acclimatised under laboratory conditions for 14 days, during which three wetting-drying cycles were simulated.

Given the potential impact of MPs on soil moisture availability, radish (lat. *Raphanus sativus* L. var. *sativus*) was selected for cultivation due to its low tolerance to water stress (Stagnari et al. 2018). The chosen semi-early radish variety is known for its large, spherical shape, light red skin, and a 33–37-day growing season.

Pot experiments comprising three MP variants (PS, HDPE, and PVC) and a control were conducted in a laboratory environment with natural light, temperatures of 23–25 °C, and humidity maintained at 50–60%. Each variant had five replicates. Irrigation was performed every other day to achieve a volumetric water content of 20%. Plant growth occurred from October 19 to December 2, 2024

Plant analyses. During the growth analysis, assessments were conducted of the fresh biomass and dry biomass weights of both the above-ground parts and the bulbs. Following ripening, the plants

were extracted from the soil, meticulously cleaned, weighed, and desiccated in an oven at 60 °C for 5 days. Subsequently, the dry biomass was weighed once more.

During the experiment, the portable, battery-powered fluorometer FluorPen FP 110 (Photon Systems Instruments, Drásov, Czech Republic) was used to rapidly and precisely measure chlorophyll fluorescence parameters (OJIP) in plants.

The FluorPen device was used to implement the protocol for recording the rapid fluorescence transient known as OJIP, a phenomenon observed when photosynthetic organisms are exposed to high irradiance. The accompanying FluorPen software enabled the transfer of measured data to a computer, where OJIP curves and calculated parameters could be visualised and analysed. The OJIP protocol includes several measured and derived parameters, including the maximum quantum yield of stable charge separation in photosystem II (ϕ PSII). This parameter is calculated from chlorophyll fluorescence measurements according to the Eq. (1) (Parkhill et al. 2001):

$$F_v/F_m = (F_m - F_0)/F_m \quad (1)$$

Where: F_0 – minimum fluorescence yield in the dark-adapted state; F_m – maximum fluorescence yield in the dark-adapted state. This parameter is commonly used as an indicator of plant stress.

Chlorophyll fluorescence was measured on the first fully developed leaf, on the central part of the leaf blade. Measurements were carried out weekly during plant growth over a period of five weeks (one measurement per week). Each variant and the control were represented by five replicates (five plants); for each soil, three microplastic variants and one control were established ($4 \times 5 = 20$ plants per soil). With three soil types in total, 60 plants were measured on the same day each week throughout the five-week measurement period.

Statistical analyses. The data underwent initial analysis using the omnibus normality test, which

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combines skewness and kurtosis into a single measure. Subsequently, if the data met the criteria of the omnibus normality test, distinctions among the parameters were assessed *via* single-factor ANOVA, accompanied by Tukey's honest significant difference (*HSD*) post-hoc test. In cases where the normality assumption was not met, the nonparametric Kruskal-Wallis test with the multiple-comparison Kruskal-Wallis Z test was employed. This distribution-free method does not require normality assumptions and is suitable for testing pairs of medians using the Kruskal-Wallis test. Statistical significance was established at $P < 0.05$. All statistical analyses were conducted using NCSS statistical software (NCSS 12 Statistical Software 2018, Kaysville, USA).

RESULTS AND DISCUSSION

Effect of microplastic type on the water-stable micro- and macroaggregate stability of soils: WSMIA, WSMAA. Changes in WSA stability in Chernozem, Luvisol, and Umbrisol contaminated with different types of MPs are shown for micro- and macroaggregates in Table 3.

For Chernozem soil, the single-factor analysis of variance (ANOVA) revealed a statistically significant

effect of PS on WSA for both WSMIA (< 0.25 mm) and WSMAA (> 0.25 mm) compared to the control (Table 3). Specifically, WSMIA increased by approximately 49%, and WSMAA decreased by approximately 4%, based on the mean values (Table 3). Although other MP variants exhibited a similar pattern, these changes were not statistically significant (Table 3).

In contrast, for Luvisol, pairwise *t*-tests revealed significant differences between the control and certain MP variants (PS and PVC; Table 3). However, when all variants were analysed simultaneously using ANOVA followed by Tukey's *HSD* test, no statistically significant differences were detected between the control soil and MP variants (Table 3). Nevertheless, PVC showed significant differences compared with both the HDPE and PS variants (Table 3).

In Umbrisol, we observed a statistically significant increase in WSMIA and a statistically significant decrease in WSMIA in the PS-contaminated variant compared to the control soil and the MP variants (Table 3).

These findings indicate that PS contamination positively affects the WSMIA (Table 3) across all soil types, likely due to the formation of micropores ($0.1\text{--}5\text{ }\mu\text{m}$) (Li et al. 2025), which enhance soil water retention and thereby increase water availability for plant uptake.

Table 3. WSMIA and WSMAA for different microplastic (MP) types and soil groups

Soil type	MP type	WSMIA (%) ($n = 3$)	WSMAA (%) ($n = 3$)
Chernozem	C	6.52 (0.75) ^b	93.48 (0.76) ^a
	HDPE	6.83 (1.04) ^{a,b}	93.16 (1.03) ^{a,b}
	PS	↑ 9.67 (1.84) ^{*a}	↓ 90.33 (1.84) ^{*b}
	PVC	6.58 (0.82) ^{a,b}	93.42 (0.82) ^{a,b}
Luvisol	C	11.65 (1.75) ^{a,b}	88.35 (1.76) ^{a,b}
	HDPE	14.15 (2.32) ^a	85.84 (2.32) ^b
	PS	↑ 15.43 (0.54) ^{*a}	↓ 84.57 (0.55) ^{*b}
	PVC	↓ 8.10 (1.10) ^{*b}	↑ 91.90 (1.10) ^{*a}
Umbrisol	C	4.77 (0.87) ^b	95.23 (0.88) ^a
	HDPE	5.75 (0.52) ^b	94.25 (0.52) ^a
	PS	↑ 8.12 (0.79) ^{*a}	↓ 91.88 (0.78) ^{*b}
	PVC	4.58 (0.79) ^b	95.42 (0.79) ^a

Values are arithmetic means of replicates of variants (n); standard deviations are given in parentheses. Arithmetic means of MP variants within a soil type, marked with an asterisk (*), are significantly different from the respective control according to a *t*-test with a significance level of 0.05. A red arrow pointing downwards indicates a significant decrease in the parameter, and a green arrow pointing upwards indicates a significant increase in the parameter compared to the control. Arithmetic means of MP variants within a soil type with different letters differ significantly at the 0.05 significance level based on ANOVA with the *HSD* post hoc test. C – control soil; HDPE – high-density polyethylene; PVC – polyvinyl; PS – polystyrene; WSMIA – content of water-stable microaggregates; WSMAA – content of water-stable macroaggregates

The influence of microplastics on water-stable aggregates in our study was not clearly polymer-specific. At the same time, PS increased the proportion of WSMIA consistently across all three soils (by approximately 3–4% relative to the control), PVC (on the contrary) decreased WSMIA, but only in the Luvisol (with no change in the Chernozem and Umbrisol). HDPE produced no significant change in any soil. These contrasting responses can be explained by mechanisms jointly governed by the polymer's physicochemical characteristics and the soil's intrinsic properties.

Previous studies indicated that microplastic contamination significantly reduced macro-aggregate fractions and altered the distribution of water-stable aggregates (Boots et al. 2019). One possible explana-

tion is that microplastics may become incorporated into soil aggregates, altering the binding mechanisms and hindering macroaggregate formation (Boots et al. 2019). Additionally, soil organic carbon, or organic matter, is a key binder in the formation of water-stable aggregates. Zhao et al. (2021) found that the addition of microplastics reduced organic carbon content in aggregates, potentially affecting their mechanical formation.

The influence of microplastics on the composition of soil aggregates can vary with soil type and conditions, such as pH and organic matter levels. Future research may uncover how microplastics affect the dynamics and rates of soil aggregate formation. Since microplastic particles vary in shape and surface properties from natural soil particles, they can become

Table 4. Crop growth parameters (F_v/F_m and biomass) for different microplastic (MP) types and soil groups

Soil type	MP type	F_v/F_m (–) ($n = 90$)	FB (g) ($n = 5$)	DB (g) ($n = 5$)
Chernozem	C	0.796 (0.018) ^a	56.78 (8.40) ^a	2.93 (0.45) ^a
	HDPE	0.792 (0.026) ^a	48.04 (5.19) ^a	2.60 (0.38) ^a
	PS	↑ 0.804 (0.016) ^{*b}	↑ 79.35 (12.94) ^{*b}	↑ 4.09 (0.54) ^{*b}
	PVC	0.798 (0.021) ^a	46.78 (8.68) ^a	2.67 (0.24) ^a
Luvisol	C	0.797 (0.019) ^{a,b}	45.59 (17.06) ^a	2.67 (1.17) ^a
	HDPE	0.801 (0.014) ^{a,b}	56.10 (14.23) ^a	2.98 (0.91) ^a
	PS	0.795 (0.020) ^a	44.79 (19.01) ^a	2.73 (0.95) ^a
	PVC	0.802 (0.023) ^b	38.91 (20.11) ^a	2.19 (1.22) ^a
Umbrisol	C	0.791 (0.025) ^a	44.35 (11.67) ^a	2.51 (0.61) ^a
	HDPE	0.788 (0.029) ^a	43.59 (17.96) ^a	2.38 (0.94) ^a
	PS	0.792 (0.016) ^a	41.5 (15.01) ^a	2.35 (0.78) ^a
	PVC	0.789 (0.032) ^a	47.57 (13.05) ^a	2.86 (0.93) ^a
Means of all MP variants in the same soils				
Chernozem		0.795 (0.023) ^b	57.28 (17.44) ^a	3.08 (0.79) ^a
Luvisol		0.801 (0.019) ^b	46.59 (18.19) ^{a,b}	2.63 (1.02) ^a
Umbrisol		0.788 (0.031) ^a	44.22 (14.56) ^b	2.53 (0.86) ^a
Means of all MP variants with identical plastics				
	HDPE	0.794 (0.025) ^a	49.24 (13.65) ^a	2.65 (0.77) ^a
	PS	0.797 (0.018) ^a	53.48 (22.57) ^a	2.99 (1.04) ^a
	PVC	0.796 (0.027) ^a	44.25 (14.54) ^a	2.57 (0.91) ^a

Values are arithmetic means of replicates of variants (n); standard deviations are given in parentheses. Arithmetic means of MP variants within a soil type, marked with an asterisk (*), are significantly different from the respective control according to a t -test with a significance level of 0.05. A red arrow pointing downwards indicates a significant decrease in the parameter, and a green arrow pointing upwards indicates a significant increase in the parameter compared to the control. Arithmetic means of MP variants within a soil type with different letters differ significantly at the 0.05 significance level based on ANOVA with the *HSD* post hoc test. Arithmetic means of variants with the same soils or with the same plastics with the same letter are not significantly different from each other according to the Kruskal-Wallis or *LSD* post-hoc test (if relevant) with a significance level of 0.05. C – control soil; HDPE – high-density polyethylene; PVC – polyvinyl; PS – polystyrene; F_v/F_m – maximum photochemical efficiency of PSII; FB – fresh biomass; DB – dry biomass

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part of newly formed aggregates and act as weak points, reducing the stability of larger aggregates.

Effect of microplastic type on fresh and dry weight of biomass. Fresh and dry weights of radish biomass grown in control soils and in soils with different types of microplastic contamination are shown in Table 4.

The results for Chernozem confirmed significant differences in fresh biomass production between the experimental variants. PS resulted in a statistically significant increase in fresh biomass weight compared with the other variants. The median biomass weight in the PS variant is approximately 78 g, representing an increase of more than 50% compared to the control, which has a median biomass weight of approximately 55 g. The HDPE and PVC are not statistically significantly different from the control, with median values of around 45–50 g. This result suggests that PS may paradoxically stimulate crop growth in Chernozem (Table 4).

In Luvisol, we did not observe statistically significant differences in fresh biomass weight between the experimental variants. Nevertheless, some variability in median values can be observed, with the HDPE variant having the highest values (median approximately 55 g), followed by the PVC (median approximately 50 g), the control (median approximately 47 g), and the PS had the lowest values (median approximately 38 g). There is also high variability in values across the variants, especially in the control and HDPE, indicating inconsistent crop response to Luvisol growing conditions. Overall, however, microplastic pollution has no statistically demonstrable effect on biomass production in this soil type (Table 4).

In Umbrisol, no statistically significant differences in fresh biomass production were observed among experimental variants, as was the case in Luvisol. The median values of all variants are in a relatively narrow range of approximately 40–50 g. The control has a median of approximately 47 g, the HDPE vari-

ant approximately 50 g, the PS variant approximately 42 g, and the PVC variant approximately 45 g. This low variability between the variants suggests that the crops grown in Umbrisol are relatively resistant to the effects of microplastics (Table 4).

A two-factor analysis of variance showed no significant effect of soil type ($P = 0.065$) or microplastic type ($P = 0.275$) on fresh biomass. However, a significant interaction effect between soil type and microplastic type ($P = 0.018$) was confirmed (Table 5).

We observed high variability in Chernozem, with PS increasing biomass production while HDPE and PVC decreased it. In the Luvisol, we observed the opposite trend: a decrease in PS, with no change in HDPE and PVC. The lowest sensitivity to microplastics was observed in the Umbrisol.

Although the main effects of soil type and microplastic type were not statistically significant, the significant interaction effect suggests that the combination of a particular soil type and microplastic can have a statistically significant impact on fresh biomass production. The most significant positive effect was observed for PS microplastics in Chernozem, while Luvisol showed an adverse effect for the same microplastic type. Umbrisol appears to be the least sensitive soil type to microplastic contamination with respect to biomass production. These results highlight the importance of interactions between soil physical properties and microplastic contamination characteristics on crop growth.

Contamination of the Chernozem with PS led to a significant increase in dry biomass compared with the other variants. We observed a median DB weight of approximately 4.1 g for crops grown in the PS, an increase of approximately 46% compared to the control (2.8 g). No statistically significant differences were confirmed in the HDPE (median approximately 2.6 g) and PVC (median approximately 2.8 g) compared to the control. This result confirms the stimulatory effect of PS on crop growth in Chernozem, as

Table 5. Results of two-factor ANOVA for fresh crop biomass grown on three soil types contaminated with three types of microplastic (MP)

	Sum of squares	df	Mean sum of squares	F	P	η^2_p
Soil type	1 322.09	2	661.05	2.96	0.065	0.15
MP type	598.72	2	299.36	1.34	0.275	0.07
Soil type $\times$ MP type	3 076.26	4	769.07	3.45	0.018	0.29

A bolded P -value indicates a statistically significant difference between means at the $P < 0.05$ significance level. df – degree of freedom; F – F -statistic; η^2_p – effect size of parameter

observed for fresh biomass (Table 4). Furthermore, the presence of PS in Chernozem appears to promote the development of a more stable soil structure and a balanced pore system (micro- and macropores; Table 3), thereby supporting root penetration and aeration.

No statistically significant differences in dry biomass mass were observed in the Luvisol between the experimental variants. However, a high degree of variability is evident within the PVC variant, with dry biomass values ranging from approximately 0.9 g to 3.2 g (median approximately 2.8 g). This high variability suggests an inconsistent effect of PVC microplastics on crop growth in Luvisol (Table 4).

In Umbrisol, as in Luvisol, no statistically significant differences in dry biomass production were observed between the experimental variants. The median values are relatively balanced, with the control at approximately 2.5 g, HDPE at 2.4 g, PS at 2.2 g, and PVC at 3.0 g. Although the median of the PVC variant is approximately 20% higher than that of the control, this difference is not statistically significant. Thus, a similar trend to that observed for fresh biomass was confirmed in the Umbrisol, namely, relative resistance to microplastic pollution in terms of its impact on biomass production (Table 4).

A two-factor analysis of variance (ANOVA) did not confirm an effect of soil type on ($P = 0.212$) or an effect of microplastic type on dry biomass mass ($P = 0.389$), nor was there an interaction effect between soil type and microplastic type ($P = 0.064$) (Table 6). Although the factors and their interaction did not show a significant effect ($P = 0.064$, near the significance level), the interactions indicate trends in crop response to microplastic pollution across soil types.

We observed an increase in dry biomass production in PS, while HDPE and PVC showed decreases under the experimental conditions. Luvisol reaches similar values in HDPE and PS, with a decrease in PVC (Table 4).

Although statistical analysis did not reveal significant differences in dry biomass production by soil

type or microplastic type, the graphical representation suggests trends that may be biologically relevant.

Our measurement results for fresh and dry biomass (except Chernozem variant with PS) are consistent with the study of Gong et al. (2021), which found no significant effect of microplastics on radish biomass (e.g.). Also, other studies have reported similar results, including reduced root and shoot growth in pumpkin (Colzi et al. 2022) and negative effects on crop biomass (Zang et al. 2020). While FB provides information on the total mass production of the crop, including water, DB (obtained by drying at 60 °C to a constant weight) represents the actual organic matter and nutrient content produced by the crop. The assessment of DB is important because it eliminates variability due to differences in chaff water content, which can be affected by current harvest conditions. Consequently, DB provides a more reliable indicator of crop growth and metabolic activity, enabling a more accurate assessment of MPs' effects on crop productivity.

In conclusion, a significant change in biomass was observed only in the Chernozem-PS combination, where biomass increased relative to the control. In contrast, no significant biomass response occurred in the other soil-microplastic combinations. This indicates that a biomass increase required both a microplastic-induced change in soil properties and favourable intrinsic soil characteristics acting together: only the Chernozem, with its high SOC content, high fertility, and good aeration, provided the conditions under which the PS-induced structural change translated into enhanced biomass.

Effect of microplastic type on maximum photochemical efficiency of PSII. The values of maximum photochemical efficiency of PSII (F_v/F_m), measured on leaves of crops grown in control soil and in soil with different types of MP contamination (HDPE, PS, PVC), were evaluated over a five-week growing period as an average for each week and for the whole period of observation (Table 4).

Table 6. Results of two-factor ANOVA for dry biomass of crops grown on three soil types contaminated with three types of microplastic (MP)

	Sum of squares	<i>df</i>	Mean sum of squares	<i>F</i>	<i>P</i>	η^2_p
Soil type	2.31	2	1.16	1.62	0.212	0.09
MP type	1.38	2	0.69	0.97	0.389	0.05
Soil type × MP type	6.99	4	1.75	2.45	0.064	0.22

A bolded *P*-value indicates a statistically significant difference between means at the $P < 0.05$ significance level. *df* – degree of freedom; *F* – *F*-statistic; η^2_p – effect size of parameter

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Changes in F_v/F_m values of crops grown in Chernozem during the five-week measurement period are shown in Table 4. Significant differences in F_v/F_m between the control and MP variants (PVC and PS) were observed at the beginning and at the end of the growing season. During the fourth week of measurements, we observed a significant decrease in F_v/F_m in HDPE compared to the control Chernozem.

From a temporal perspective, some non-significant trends in F_v/F_m can be identified during the growing season. The initial and final phases of the growing season appear to be the most sensitive to microplastic impacts, especially in Chernozem and Umbrisol. The middle phase of the growing season in Chernozem and Umbrisol (except the fourth week in Chernozem and the second week in Umbrisol) shows relatively stable F_v/F_m values. In Luvisol, we observed high variability of F_v/F_m throughout the growing season (Table 4).

A two-factor analysis of variance (ANOVA) confirmed a statistically significant effect of soil type on F_v/F_m ($P < 0.001$); the effect of microplastic type was not confirmed ($P = 0.187$) (Table 7).

A statistically significant interaction between soil type and microplastic type ($P = 0.003$) was demonstrated. The maximum photochemical efficiency of PSII varies significantly across soil types, and its response to microplastics differs among them.

The graphs indicate that the highest sensitivity to MP contamination was observed for Chernozem – F_v/F_m decreases in HDPE (≈ 0.793), increases in PS (≈ 0.804), and decreases again in PVC (≈ 0.796), with the difference between PS and the other variants being statistically significant. In the Luvisol, the following trend was observed: the highest value was in PVC (≈ 0.802), a decrease in PS (≈ 0.795), and an increase again in HDPE (≈ 0.801), confirming the interaction effect. In the Umbrisol, the lowest F_v/F_m values were recorded across all soils, with slight differences between variants (HDPE ≈ 0.788 , PS ≈ 0.793 , PVC ≈ 0.789), but these differences were not statistically significant.

The increased F_v/F_m values in some MP variants suggest that microplastics may paradoxically improve photosystem II efficiency, which may be related to altered soil properties (e.g., changes in water-stable aggregates, WSA) that secondarily affect crop physiology, thereby modifying water and nutrient availability to crops and indirectly affecting photosynthesis.

The polystyrene-induced increase in WSMIA was a widespread effect, statistically confirmed compared to the control in all three soils (Chernozem, Luvisol, and Umbrisol) following PS contamination. In contrast, the increase in the maximum photochemical efficiency of PSII was confirmed only in the PS-treated Chernozem. In contrast, no significant F_v/F_m response was observed in the Luvisol or Umbrisol, or in any soil treated with HDPE or PVC. Our findings align with the study by Liu et al. (2025), who reported that PS MPs notably stimulated the growth of *S. lycopersicum* L.

The key question is why, despite a statistically significant PS-induced increase in microaggregates across all soils, F_v/F_m rose only in the Chernozem. The answer may lie in the fact that improved microaggregate stability is a necessary but not sufficient condition for a physiological benefit: structural changes can be converted into a measurable increase in photochemical efficiency only where the intrinsic soil conditions are already favourable. The Chernozem, with its high soil organic carbon content, high fertility, good aeration, and carbonate buffering, provided a soil matrix in which the additional gain in pore structure translated into improved water and nutrient supply to the roots and, therefore, into higher F_v/F_m . In the Luvisol and, especially, the coarse-textured, low-SOC Umbrisol, the same relative increase in microaggregates was superimposed on an intrinsically less fertile and less structured matrix.

From an applied perspective, this underlines that soil-physical indicators (aggregate stability, pore-size distribution, hydraulic properties) and plant-physiological indicators (F_v/F_m , biomass, root traits,

Table 7. Results of two-factor ANOVA for the maximum photochemical efficiency of PSII (F_v/F_m) grown on three soil types contaminated with three types of microplastic (MP)

	Sum of squares	df	Mean sum of squares	F	P	η^2_p
Soil type	0.01	2	0.01	13.13	< 0.001	0.03
MP type	0	2	0	1.68	0.187	0
Soil type $\times$ MP type	0.01	4	0	4.09	0.003	0.02

The bolded P -value indicates a statistically significant difference between means at the significance level $P < 0.05$. df – degree of freedom; F – F -statistic; η^2_p – effect size of parameter

nutrient uptake) should be assessed jointly. Future work should therefore extend the present pot experiment to additional concentrations, particle sizes, and crop species, and complement it with long-term studies to elucidate the mechanisms underlying the polymer-specific responses observed here, in particular the unexpected stimulatory effect of polystyrene in Chernozem.

Evaluation of the formulated hypotheses. The data obtained allow a direct evaluation of the four hypotheses outlined in the Introduction. Hypothesis H1 is partially supported: the three polymers did not behave identically, since PS induced a pronounced stimulation of FB (~+50 %), DB (~+46 %) and F_v/F_m in Chernozem, whereas HDPE and PVC produced no statistically significant change in any of the three soils. However, the main effect of polymer type in the two-way ANOVA was generally not significant, indicating that polymer identity becomes relevant only when combined with a specific soil matrix.

Hypothesis H2 is not supported in its simple main-effect form: the main effect of soil type, considered separately, was not statistically significant for FB and F_v/F_m , and the expected gradient Chernozem > Haplic Luvisol > Umbrisol did not appear as a direct main effect. The soil-related signal is real, but it operates through the interaction with microplastic type rather than as an independent factor.

Hypothesis H3 is fully confirmed and represents the central finding of the study: the two-way ANOVA revealed a statistically significant soil × microplastic interaction for fresh biomass ($P = 0.018$) and for F_v/F_m ($P = 0.003$), and the PS-induced stimulation of radish occurred only in Chernozem, while the same polymer remained neutral in Haplic Luvisol and Umbrisol – a pattern that can only be explained by an interactive, not additive, action of the two factors. Hypothesis H4 is partially supported: PS contamination significantly shifted the distribution of water-stable aggregates towards microaggregates (WSMIA +49%) in Chernozem, in parallel with increases in FB, DB and F_v/F_m , consistent with the proposed mechanism (a changed pore architecture that supports aeration and water availability). A comparable PS-induced increase in WSMIA was also recorded in Haplic Luvisol and Umbrisol (statistically significant in Umbrisol). Still, it did not translate into measurable changes in plant performance, confirming that enhanced microaggregate stability is a necessary but not sufficient condition for biomass stimulation and that additional soil properties (SOC content, texture,

carbonate buffering) co-determine whether the plant utilises the structural benefit.

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