

Effect of microplastics on silty loam soil properties and radish growth

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Abstract: Microplastics (particles of plastics <5 mm) affect the physical, biological and hydrological properties of agricultural soil, as well as crop growth. We investigated the effect of the addition of three microplastics (high-density polyethylene (HDPE), polyvinyl chloride (PVC), and polystyrene (PS)) at a concentration of 5% (w/w) to a silty loam soil on selected soil properties and growth of radish (*Raphanus sativus* L. var. *sativus*). Changes in the soil properties and radish growth in three microplastic treatments were compared with the control. Soil properties (bulk density, hydraulic conductivity, sorptivity, water repellency) were estimated for each treatment at the beginning and at the end of the radish growing period (GP). The bulk density was significantly lower in the HDPE and PVC treatments compared to the control within the measurement at the beginning of the GP and in all microplastic treatments compared to the control at the end of the GP. The values of hydraulic conductivity and water sorptivity did not show significant differences between any treatments within the measurement at the beginning of GP, but they were significantly higher in the HDPE treatment compared to the control at the end of the GP.

The growth of radish was characterized by the plant biomass and effective quantum yield of Photosystem II (Y (II)). We did not find a statistically significant difference in the total biomass of radish between any of the experimental treatments, maybe due to used concentration of microplastics. The mean value of Y (II) was significantly higher in all microplastic treatments compared to control only within the last measurement at the end of the GP. A statistically significant change of Y(II) in all microplastic treatments may indicate functional shift in soil properties; however, the measured values of the soil characteristics have not shown the significant changes (except for the bulk density values in all microplastic treatments and hydraulic conductivity together with sorptivity in HDPE treatment within the measurement at the end of GP).

Keywords: Microplastics; Silty loam soil; Radish; Soil properties.

INTRODUCTION

Soil is generally defined as the top layer of the earth's crust, formed by mineral particles, organic matter, water, air and living organisms. It is the interface between earth, air and water and hosts most of the biosphere (Thematic Strategy for Soil Protection, 2006). Soil is subjected to a series of degradation processes or threats, including contamination by microplastics (particles of plastic <5 mm) originating from the fragmentation of large plastic litter or from direct environmental emission. Microplastics present an important set of issues because of two main reasons. Firstly, they are small enough to be taken up by biota and thus can accumulate in the food chain, and secondly, they can adsorb pollutants, enriching them on these particle surfaces (Rillig, 2012). Senathirajah et al. (2021) estimated that, as a global average, humans may ingest 0.1–5 g of microplastics weekly through various exposure pathways.

As of 2015, approximately 6300 Mt of plastic waste had been generated, around 9% of which had been recycled, 12% was incinerated, and 79% was accumulated in landfills or the natural environment (Geyer et al., 2017). Although the annual plastic release to land is estimated at 4–23 times of that released to the oceans (Horton et al., 2017), microplastic effects in terrestrial ecosystems have only recently moved into focus after about a decade of research being limited to aquatic systems (Rillig et al., 2019).

Low-density polyethylene (LDPE) is commonly used for mulching and the resulting macro- and microscopic plastic

residues in agricultural soil have aroused concerns for years. Qi et al. (2018, 2020a) investigated the effect of 1% (w/w) LDPE on physicochemical and hydrological properties of sandy soils and wheat (*Triticum aestivum* L.) growth in the presence and absence of earthworms. Macro- and micro-plastic residues affected both above- and below-ground parts of the wheat plant during both vegetative and reproductive growth. Earthworms have mitigated wheat damage caused by the addition of plastic residues (Qi et al., 2018). The bulk density, porosity, saturated hydraulic conductivity, field capacity and soil water repellency alters significantly in the presence of plastic debris, while pH, electrical conductivity and aggregate stability does not substantially change (Qi et al., 2020a). Steinmetz et al. (2016) also found that LDPE mulching induced soil water repellency and increased erosion potential.

Zang et al. (2020) investigated the effect of polyvinyl chloride and polyethylene on wheat (*Triticum aestivum* L.) growth. They report that microplastics reduced above-ground and underground productivity (by 22.9% and 8.4%, respectively), increased soil microbial biomass (by 43.6%) and changed the structure and metabolic status of the microbial community.

Machado et al. (2019) investigated the effect of six microplastics (polyester, polyamide, polyethylene, polyester terephthalate, polypropylene and polystyrene) on the physicochemical and hydrological properties of loamy sand soil and spring onion (*Allium fistulosum* L.) growth in the 200 mL glass beakers in a greenhouse. They observed significant changes in plant biomass, tissue elemental composition, root traits, and soil micro-

bial activities, which can be explained by changes in soil structure and water dynamics.

The effects of microplastics on biomass in terrestrial ecosystems have been studied in several species, including wheat (Gong et al., 2021; Qi et al., 2018), onion (Machado et al., 2019), common bean (Meng et al., 2021), pumpkin (Colzi et al., 2022; Gonnelli, 2022), Italian lettuce, radish and corn (Gong et al., 2021). Colzi et al. (2022) indicated that the higher the microplastics concentration, the greater the negative effect on chlorophyll content and photosynthetic efficiency. The effects of microplastics on soil properties and plant growth depend on the type of soil, crop and microplastics material.

Chlorophyll fluorescence analysis is one of the most powerful and widely used techniques to study the effect of stresses on the photosynthetic process (Guidi et al., 2019). Chlorophyll fluorescence can be used as a measure of plant stress because environmental stresses (e.g., extremes of temperature, light and water availability) can reduce the ability of a plant to metabolise normally (Hazrati et al., 2016). In this study, the stress induced by the addition of microplastics to soil was assessed using the effective quantum yield of Photosystem II or Y(II). In the good condition of light, Y(II) reflects the actual photosynthetic efficiency of plants at present (Lichtenthaler and Babani, 2004). Under stress conditions, the photosynthetic capacity of plants weakens (Ashraf and Harris, 2013).

The objectives of this research were: i. to measure the soil hydrophysical characteristics and grow parameters of radish (*Raphanus sativus* L.) after addition of three microplastics (HDPE, PVC, and PS) to the silty loam soil; and ii. to compare the measured parameters with soil characteristics and radish growth in the control silty loam soil without microplastics.

MATERIALS AND METHODS

Plastic materials

Three types of plastic were used in the conducted experiment: high-density polyethylene (HDPE), polyvinyl chloride (PVC), and polystyrene (PS). The selected three plastic materials were processed following the same procedure, by milling with consequent sieving to a powder with particle size <400 µm. The detailed characteristics of plastic material are in the Table 1.

Soil preparation

The studied soil was taken from the experimental site of the Slovak University of Agriculture in Nitra located in Dolná Malanta, Slovakia (48°19'00" N; 18°09'00" E), which was used for conventional agricultural purposes. According to the Köppen-Geiger climate classification (Kottek et al., 2006), the region exhibits a warm summer subtype of the humid continental climate (Dfb) (Melo et al., 2013). The mean annual air temperature is 9.8°C, and the mean annual precipitation is 540 mm (Šimanský et al., 2018). The soil is classified as Haplic Luvisol (WRB, 2014). The topsoil (upper 20 cm horizon of soil profile) has silty loam texture (Soil Survey Division Staff, 1993) with

sand, silt, and clay contents of 15.2%, 59.9%, and 24.9%, respectively. The soil organic carbon content is 9.13 g kg⁻¹, and pH 5.71 (Šimanský et al., 2018; Toková et al., 2020).

Collected soil was homogenized using a hammer mill, oven-dried and sieved (2.5 mm mesh size). Microplastics were prepared as described before and mixed manually (with metal spoon for 5 min) at a concentration of 5% (w/w) with the soil in a large container, to provide an equal distribution throughout the soil. Consequently, the bulk density of homogenized untreated soil and microplastics treatments was measured in Kopecky cylinders as arithmetic mean of weight/volume of three evenly filled cylinders according to the methodology stated in following subchapter.

Then each cylindrical pot (with 160 mm inner diameter, 250 mm height, and 3000 mL volume) was filled with the microplastics-soil mixtures or untreated soil to reach the previously determined bulk density value for each treatment (presented in Table 2). The pot experiments with three microplastics treatments (PS, HDPE, and PVC) and control (i.e., 4 treatments in total) were done with 5 replicates for each treatment (a total of 20 pots were cultivated).

Plant material

Radish (*Raphanus sativus* L.) comes from the Asian countries, from where it spread rapidly to the European continent. At present, it is grown almost all over the world. The total radish production represents roughly 2% of the global vegetable production (Zhang et al., 2021). Radish is typically grown very fast during all seasons. Radish seeds (*Raphanus sativus* L. var. sativus) cultivar LADA was acquired from a commercial supplier (Moravoseed CZ a.s). Radish is a semi-early variety, specific for year-round cultivation, with a 33–37-days growing period (GP). Radish has been selected as a test plant due to its low tolerance of a water stress (Stagnari, 2018), while the water stress can be potentially enhanced by presence of microplastics in soil (Zhou et al., 2021).

Experiment setup

The pot experiments were done in a laboratory with a natural light, temperature of 23–25 °C and humidity of 50–60%. The pots were irrigated every other day to achieve a water content of 0.20 cm³ cm⁻³; actual soil water content was continuously monitored by moisture sensors. The measurements of photosynthetic parameters were carried out on the first right leaf in the middle. Plants were grown in a regular GP of radish, the experiment was established at the end of July and the vegetation period of radish was from August 2 to 23, 2021.

Laboratory measurements and data processing

Measurement of soil hydrophysical parameters

The volumetric water content, θ (cm³ cm⁻³), was measured with a calibrated moisture meter type ECH₂O Check and soil moisture sensor ECH₂O EC-5 (Decagon, 2012b). The bulk

Table 1. Characteristics of plastic material, product of origin, bulk density of microplastic powder and persistence of soil water repellency, determined through the WDPT test. Detailed methodologies for bulk density measurement and WDPT test are stated in following subchapter.

Material	Product of origin	Bulk density of powder (g cm ⁻³)	WDPT (s)
HDPE	pipe	0.35	10200
PVC	bottle	0.46	11100
PS	packaging material	0.54	11400

density ρ_d (g cm⁻³) was determined with the core method (ISO 11272, 2017; Soil Survey Division Staff, 1993) and calculated as the ratio of the mass of the oven-dried soil from the Kopecky cylinder and the 100 cm³ volume of the cylinder. The bulk density was measured before GP on samples, prepared by filling the cylinders with homogenized soil and microplastics–soil mixtures. Bulk density after GP was measured on undisturbed samples (four samples for each treatment), taken carefully from the pots at the same time as the radish plants were harvested.

Water infiltration measurements were performed with a minidisk infiltrometer (MDI) (Decagon, 2012a) under a negative tension $h_0 = -2$ cm (Alagna et al., 2017, 2019). For alleviating boundary effects, the diameter of pots was 3.5 times greater than the MDI base diameter (Bordoloi et al., 2019). Measurements were performed without plants before GP and with plants after GP. Measuring points were shifted to the edge of pots (about 2 cm from the edge of pots and about 1.5 cm from the plant, if present). The cumulative infiltration I was calculated based on the Philip infiltration equation:

$$I = C_1 t^{1/2} + C_2 t + C_3 t^{3/2} + \dots + C_m t^{m/2} + \dots \quad (1)$$

where C_1 , C_2 , C_3 , ..., and C_m are coefficients, and t is time.

The sorptivity of water, $S_w(-2 \text{ cm})$, was estimated from the first term of the Philip infiltration equation ($I = C_1 t^{1/2}$) during early-time infiltration of water (Clothier et al., 2000):

$$S_w(-2 \text{ cm}) = I / t^{1/2} \quad (2)$$

Duration of early-time infiltration is 60–180 s (Hallett, 2008). During this time the process is dominated by the capillarity and the other terms of the Philip infiltration equation can be neglected.

Water infiltration measurements with the MDI under a negative tension $h_0 = -2$ cm were used to estimate the hydraulic conductivity $k(-2 \text{ cm})$. Zhang (1997) proposed to use the first two terms of the Philip infiltration equation to fit the cumulative infiltration vs. time relationship and estimate the hydraulic conductivity $k(-2 \text{ cm})$ from the equation:

$$k(-2 \text{ cm}) = C_2 / A \quad (3)$$

where A is a dimensionless coefficient.

Fitting of the two terms infiltration equation to cumulative infiltration data was performed through the linearization technique suggested by the Minidisk Infiltrometer User's Manual (Decagon, 2012a). A value of $A = 1.73$, corresponding to sandy soil and suction $h_0 = -2$ cm, was used to calculate the hydraulic conductivity $k(-2 \text{ cm})$. Given capillarity can be ignored at long times, estimation of k is expected to be more accurate as t increases (Zhang, 1997). The MDI experiments were therefore conducted until apparent steady-state conditions were observed.

The persistence of soil water repellency was assessed by the WDPT test. It involves placing a 50 ± 5 μL water drop from a standard medicine dropper or pipette on the soil surface and recording the time of its complete penetration. A standard droplet release height of approximately 10 mm above the soil surface was used to minimize the cratering effect on the soil surface (Doerr, 1998; Tinebra et al., 2019). The WDPT test determines how long strong water repellency persists in the contact area of a water droplet (Dekker et al., 2009). The following classes of the persistence of soil water repellency were distinguished: wettable or non-water-repellent soil (WDPT < 5 s), slightly (WDPT = 5–60 s), strongly (WDPT =

60–600 s), severely (WDPT = 600–3600 s), and extremely (WDPT > 3600 s) water repellent soil (Bisdorf et al., 1993).

Measurement of photosynthetic parameters

The photochemical response at the Photosystem II level was analyzed by a chlorophyll fluorometer OS1p (ADC BioScientific Ltd., UK) instrument with a sensor for detecting photosynthetic active radiation (PAR). Photosystem II is a membrane protein supercomplex that executes involved in the primary reactions of photosynthesis.

Effective Quantum Yield of Photosystem II, referred to as $Y(\text{II})$, $\Delta F/F_m'$ or ΦPSII , was calculated from chlorophyll fluorescence measurements as (Genty et al., 1989):

$$Y(\text{II}) = (F_m' - F_s)/F_m' \quad (4)$$

where F_s is the chlorophyll fluorescence intensity value emitted by a plant at steady-state photosynthesis. It is also known as F' . F_m' is the maximum fluorescence measurement when a saturating light is applied to the leaf at steady-state photosynthesis.

$Y(\text{II})$ represents a normalized measurement ratio and is an indication of the amount of energy used in photochemistry by Photosystem II under steady-state photosynthetic lighting conditions. It is affected by the closure of reaction centers and heat dissipation caused by non-photochemical quenching (Gamon et al., 1997). An increase in effective quantum yield of Photosystem II reflects higher light use efficiency in plants, the typical $Y(\text{II})$ will be less than 1 (Baker, 2008). In the good condition of light, the $Y(\text{II})$ of Photosystem II could reflect the actual photosynthetic efficiency of plants at present. Under stress conditions, the photosynthetic capacity of plants is weakened. $Y(\text{II})$ has the advantage that it is more sensitive to a larger number of plant stress types than F_v/F_m (F_v - variable fluorescence and F_m - maximal fluorescence). Leaf absorption characteristics can vary by water content, age, and other factors. In this study, the $Y(\text{II})$ values were estimated from two repeated measurements on five plants for each microplastic treatment.

Measurement of plant biomass

For growth analysis, the weight of fresh biomass and the dry weight of biomass of aboveground and belowground plant parts were measured after GP. After ripening, the plants were removed from the soil, cleaned, weighed, oven-dried (leaves - 2 days, bulbs with roots - 5 days, at the temperature 60 °C), and weighed again for the dry biomass.

Statistical analysis

Differences between the parameters estimated in different treatments were evaluated using the single factor ANOVA with Tukey's Honest Significant Difference (HSD) post-hoc test. The Tukey-Kramer method (also known as Tukey's HSD method) uses the Studentized Range distribution to compute the adjustment to the critical value. The statistical significance in the analysis was defined at $P < 0.05$. The statistically significant differences in the soil characteristics between the microplastic treatments (HDPE, PVC, PS) and control were estimated separately within the measurement at the beginning and at the end of the GP. The significant differences in grow parameters between HDPE, PVC, PS and control were estimated separately within each of four measurements during the GP, resp. for one measurement after the GP in the case of the biomass weight.

RESULTS AND DISCUSSION

Effect of microplastics on hydrophysical characteristics of soil

Soil hydrophysical characteristics, measured before and after the GP of radish are presented in Table 2. During the experiment, after several wetting–drying cycles, we visually observed the formation of physical soil surface crust in pots. The tested soil consisted of a relatively large proportion of clay ($\approx 25\%$), which caused shrinking and swelling, leading to cracking of topsoil in pots. The parameters of surface soil crust were not evaluated or quantified separately within this study.

Volumetric water content, θ , of air-dried soil measured before and after GP was low ($2.2\text{--}2.8 \times 10^{-2} \text{ cm}^3 \text{ cm}^{-3}$) (Fig. 1a). The difference in θ between the experimental treatments was not statistically significant, either before or after the GP. Higher standard deviation of θ values within measurement after GP may be a due the more heterogeneous profile in pots (with mineral soil surface crust), compared to the homogenous substrate at the beginning of the experiment (Table 2; Fig. 1a).

The addition of plastics to the soil reduced the bulk density (ρ_d) of the microplastic treatments of the experiment (PS, HDPE and PVC) within both measurements, before and after the GP. This finding is in accordance with Machado et al. (2018), who found the decrease in ρ_d after contamination of loamy sand soil with polyacrylic fibers, polyamide and polyethylene. Our finding might be explained by the fact that plastics are less dense than soil minerals. Before GP, mean value of ρ_d in HDPE (1.320 g cm^{-3}) and PVC (1.334 g cm^{-3}) showed a statistically significant difference, compared with the control (1.430 g cm^{-3}). The value of ρ_d in all treatments decreased very slightly after GP compared to the measurements before GP. A statistically significant difference was found between mean values of ρ_d measured after GP in all treatments except HDPE and PVC (Fig. 1b).

We did not find a significant difference between mean values of unsaturated hydraulic conductivity, $k(-2 \text{ cm})$, in the

microplastic and control treatments, measured before GP (Fig. 2a). This is consistent with the findings of Machado et al. (2018), who did not detect significant changes in saturated hydraulic conductivity despite the microplastics in their study induced noticeable changes in soil bulk density. The minimum value of $k(-2 \text{ cm})$ was measured in the control (0.652 cm h^{-1}), while the maximum was in HDPE treatment (1.615 cm h^{-1}). The $k(-2 \text{ cm})$ after the GP showed slightly lower values compared with those measured before GP (at control by 0.079 cm h^{-1} , at PS by 0.095 cm h^{-1} , at HDPE by 0.215 cm h^{-1} and at PVC by 0.503 cm h^{-1}), similar to the soil bulk density values. This may be due the fact that the presence of surface soil crusts (which were visually observed at the time of measurement) generally lowers the hydraulic conductivity in soil. The $k(-2 \text{ cm})$ measured after GP in HDPE treatment and control showed a statistically significant difference.

The mean values of water sorptivity, $S_w(-2 \text{ cm})$, measured in all treatments before GP showed no statistically significant difference between each other, and they were higher than $S_w(-2 \text{ cm})$ after GP (Fig. 2b). A statistically significant difference was found between control and HDPE treatment (Fig. 2b) in the measurement after the GP. The estimated statistical differences in hydraulic conductivity and sorptivity between the treatments within the experiment were similar.

The mean values of the persistence of soil water repellency, assessed by the WDPT test were higher for all of the treatments with microplastic contamination as compared to the control, which is in line with the findings of Qi et al. (2020b), who observed that soil water repellency was altered in the presence of LDPE residues. A statistically significant difference was found only between the control and HDPE treatment before the GP. The HDPE treatment showed the highest persistence of soil water repellency in both measurement periods (Fig. 3). Mean values of the WDPT of all treatments were below the 5 s threshold defining wettable / non-repellent soils.

Table 2. Soil hydrophysical characteristics (arithmetic mean and standard deviation) in individual treatments (high-density polyethylene (HDPE), polyvinyl chloride (PVC), polystyrene (PS) and control) measured before and after the GP of radish. Arithmetic means (within the same measurement period) with the same letter are not significantly different from each other (Tukey's HSD test, $P < 0.05$).

Treatment	Attribute	Before growing period			After growing period		
		Arithmetic mean	Standard deviation	N	Arithmetic mean	Standard deviation	N
control	$\theta (\text{cm}^3 \text{ cm}^{-3})$	$2.8 \times 10^{-2} \text{ a}$	4.1×10^{-3}	10	$2.6 \times 10^{-2} \text{ a}$	1.1×10^{-2}	10
	$\rho_d (\text{g cm}^{-3})$	1.43 a	3.27×10^{-2}	3	1.41 a	2.36×10^{-2}	4
	$k(-2 \text{ cm}) (\text{cm h}^{-1})$	0.652 a	0.791	4	0.573 a	0.454	10
	$S_w(-2 \text{ cm}) (\text{cm s}^{-1/2})$	0.159 a	2.71×10^{-2}	4	$2.28 \times 10^{-2} \text{ a}$	9.53×10^{-3}	10
	WDPT (s)	0.6 ^a	0.89	5	0.4 ^a	0.55	5
PS	$\theta (\text{cm}^3 \text{ cm}^{-3})$	$2.5 \times 10^{-2} \text{ a}$	2.2×10^{-3}	10	$3.1 \times 10^{-2} \text{ a}$	1.0×10^{-2}	10
	$\rho_d (\text{g cm}^{-3})$	1.38 a,b	2.71×10^{-2}	3	1.37 b	1.72×10^{-2}	4
	$k(-2 \text{ cm}) (\text{cm h}^{-1})$	1.211 a	0.526	4	1.116 a,b	0.801	10
	$S_w(-2 \text{ cm}) (\text{cm s}^{-1/2})$	0.169 a	1.25×10^{-2}	4	$3.15 \times 10^{-2} \text{ a,b}$	8.35×10^{-3}	10
	WDPT (s)	2.2 a,b	2.05	5	1.8 a	0.84	5
HDPE	$\theta (\text{cm}^3 \text{ cm}^{-3})$	$2.4 \times 10^{-2} \text{ a}$	3.6×10^{-3}	10	$3.6 \times 10^{-2} \text{ a}$	1.3×10^{-2}	10
	$\rho_d (\text{g cm}^{-3})$	1.32 b	2.35×10^{-2}	3	1.31 c	2.57×10^{-2}	4
	$k(-2 \text{ cm}) (\text{cm h}^{-1})$	1.615 a	0.706	4	1.400 b	0.534	10
	$S_w(-2 \text{ cm}) (\text{cm s}^{-1/2})$	0.182 a	1.49×10^{-2}	4	$3.63 \times 10^{-2} \text{ b}$	9.53×10^{-3}	10
	WDPT (s)	4.8 b	3.03	5	3.4 a	2.70	5
PVC	$\theta (\text{cm}^3 \text{ cm}^{-3})$	$2.2 \times 10^{-2} \text{ a}$	2.7×10^{-3}	10	$2.7 \times 10^{-2} \text{ a}$	7.1×10^{-3}	10
	$\rho_d (\text{g cm}^{-3})$	1.33 b	3.69×10^{-2}	3	1.30 c	1.74×10^{-2}	4
	$k(-2 \text{ cm}) (\text{cm h}^{-1})$	1.467 a	0.389	4	0.964 a,b	0.431	10
	$S_w(-2 \text{ cm}) (\text{cm s}^{-1/2})$	0.179 a	1.53×10^{-2}	4	$3.21 \times 10^{-2} \text{ a,b}$	6.32×10^{-3}	10
	WDPT (s)	1.4 a, b	1.95	5	1.0 a	1.73	5

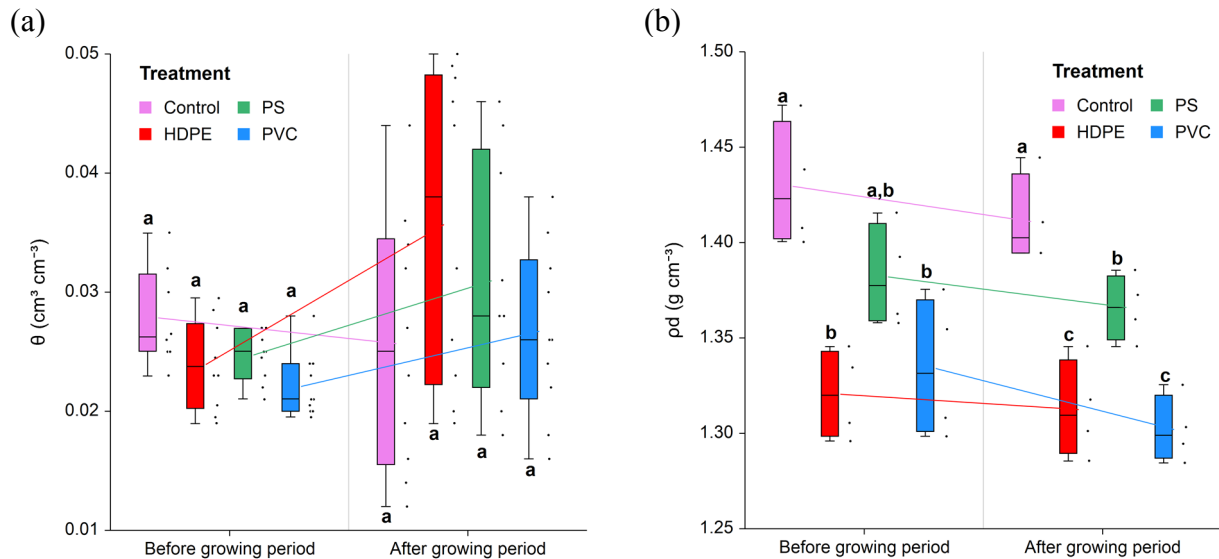


Fig. 1. Values of (a) the volumetric water content of air-dried soil, θ ($\text{cm}^3 \text{cm}^{-3}$), and (b) bulk density, ρ_d (g cm^{-3}), in all treatments measured before and after GP; lines connect the arithmetic means, the whiskers show minima and maxima and points represent individual measurements. Box plots denoted with different letters within the same measurement period are significantly different on significance level 0.05.

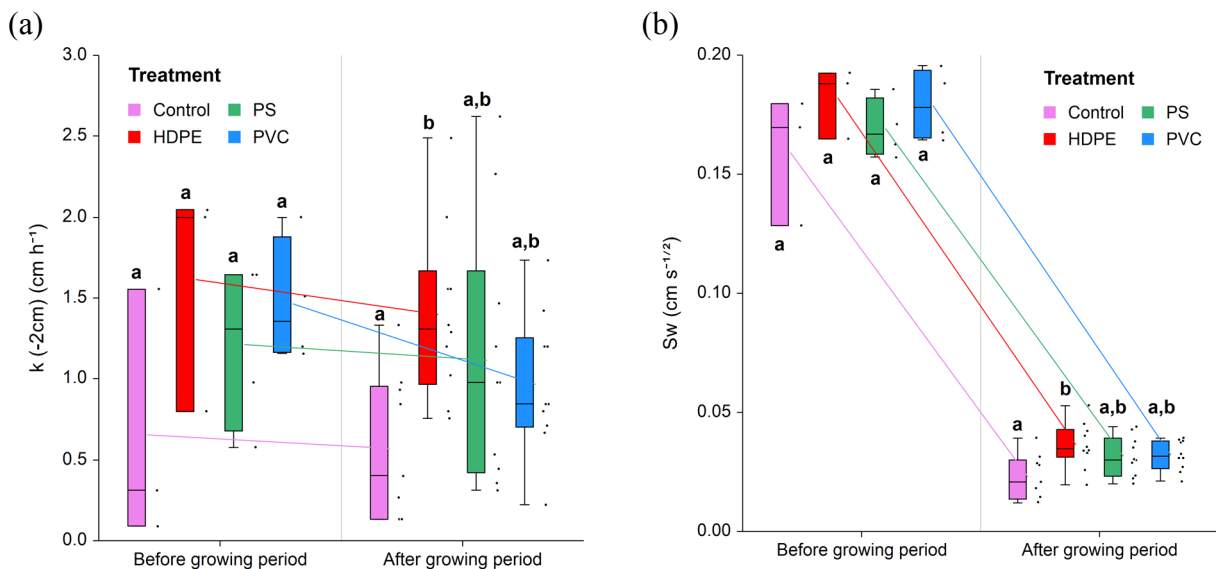


Fig. 2. Values of (a) unsaturated hydraulic conductivity, $k(-2\text{ cm})$ (cm h^{-1}), and (b) sorptivity of water, $S_w(-2\text{ cm})$ ($\text{cm s}^{-1/2}$), measured in pots before and after GP. Lines connect the arithmetic means; the whiskers show minima and maxima and points represent individual measurements. Box plots denoted with different letters within the same measurement period are significantly different on significance level 0.05.

Effect of microplastics on plant biomass

Understanding the impact of microplastics on plant growth and plant production is of paramount importance for the agriculture system and can involve human health in case contaminants enter the food chain.

Our results showed that the given concentration of the three tested microplastic materials in tested soil induced negligible effects on plant development. PS triggered increases in root biomass, compared to control, however, it was not statistically significant (Table 3). No positive or negative effect on root biomass was observed in plants grown in soil treated with HDPE and PVC, compared to control. Our result contradicts the study of Zang et al. (2020), who recorded a negative impact of microplastics on plant growth affecting both above- (−22.9%) and below-ground productivity (−8.4%).

Within our experiment, we did not find significant difference in plant root biomass between control and microplastic treat-

ments probably due to the used concentration of microplastics. Significant increase in hydraulic conductivity and sorptivity in HDPE treatment compared to control did not affect the root biomass in this treatment. This result is in contrast with study of Machado (2019) that confirmed the effect of microplastics on soil structure and following changes in the onion plant growth. It is unclear if microplastics influence root biomass development because they impact the substrate physical structure or whether they have a direct negative effect on the root system (Khalid et al., 2020). The probable explanation is that even though the microplastics could not get through the root cuticle into the tested radish plants, they could negatively affect the plant growth. This is done by creating a layer of microplastics on the root surface that blocks the uptake or utilization of essential nutrients or water (Jiang et al., 2019).

Data of the leaf biomass for individual treatments of the experiment, presented in Fig. 4, shows that HDPE and PVC treatments are not significantly different from control treatment.

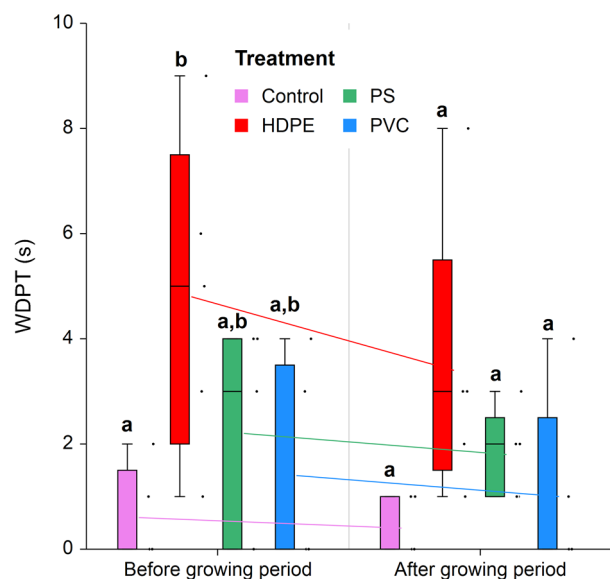


Fig. 3. Values of the persistence of soil water repellency, assessed by the WDPT test, measured in pots before and after GP. Lines connect the arithmetic means; the whiskers show minima and maxima and points represent individual measurements. Box plots denoted with different letters within the same measurement period are significantly different on significance level 0.05.

On the other hand, the difference between PS and control was statistically significant, indicating that PS has a positive effect on leaf biomass. Our results are in contrast with the work of Colzi et al. (2022), which report microplastic toxicity (reduction of leaf size, chlorophyll content, and photosynthesis efficiency) in pumpkin plants. Again, we did not find signifi-

cant shifts of soil characteristics in PS treatment which could induce the leaf biomass increase (through the improved water and nutrient accessibility for plants).

There was no significant difference in the amount of total biomass between the microplastic treatments of experiment and the control. For comparison, Gong et al. (2021) found the zero effect of PS contamination on total biomass of radish and wheat, a slight effect on maize and a significant effect on lettuce at early seedling growth.

Effect of microplastics on the effective quantum yield of Photosystem II

Measured values of the effective quantum yield $Y(II)$ were evaluated in four time periods from the beginning to the end of the GP. The measured values of $Y(II)$ were variable during the first three measurements and did not show clear trend confirming the effect of microplastics addition on $Y(II)$ (Fig. 5). Within the last measurement at the end of GP, we found significant differences in $Y(II)$ between all microplastic treatments and control. These results may indicate that the plants grown in soils treated with microplastics had higher light use efficiency. Statistical difference estimated in all microplastic treatments compared to control cannot be satisfactorily explained by the change in soil characteristics, as the significant change in hydraulic conductivity and sorptivity was confirmed only for the HDPE treatment. Change of $Y(II)$ cannot be explained neither by a reduction in bulk density, because this change did not result in a change of hydraulic conductivity and thus cannot substantially change the accessibility of soil water and nutrients for plants. This change was probably caused by other factors (e.g., physiological) that were not monitored in the conditions of our experiment.

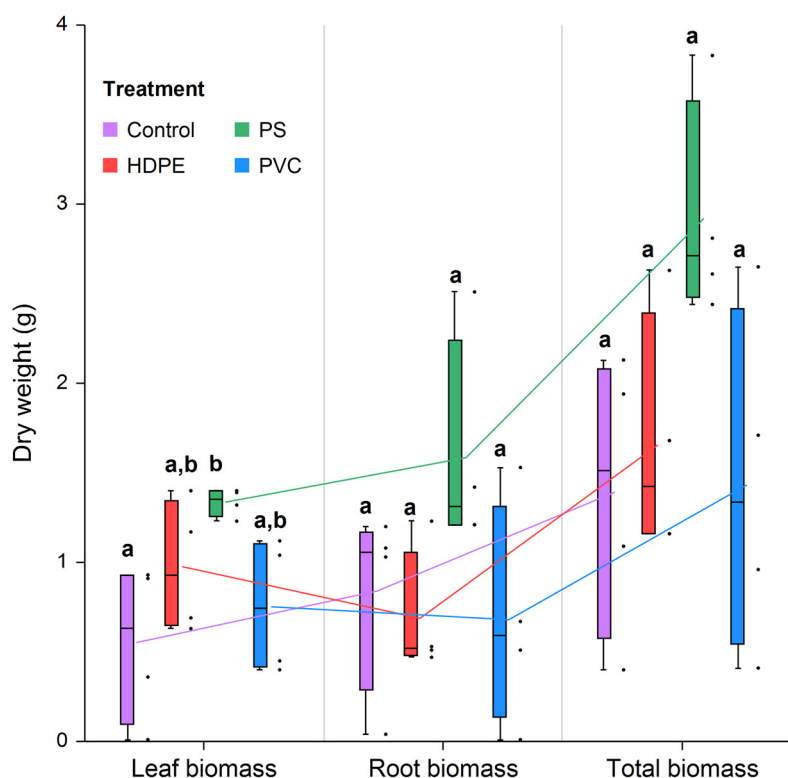


Fig. 4. Dry weight of leaf, root and total biomass of radish plants for all treatments of experiment after GP. Lines connect the arithmetic means; the whiskers show minima and maxima and points represent individual measurements. Box plots denoted with different letters are significantly different on significance level 0.05.

Table 3. Fresh weight (g) of biomass ($\pm$ standard deviation) of radish plants for all treatments of experiment after GP. Arithmetic means with the same letter are not significantly different from each other (Tukey's HSD test, $P < 0.05$).

Treatment	Leaf biomass	Root biomass	Total biomass
	Fresh weight (g)	Fresh weight (g)	Fresh weight (g)
Control	8.89 $\pm$ 2.88	12.76 $\pm$ 7.18	21.66 $\pm$ 10.04 ^a
PS	15.23 $\pm$ 1.36	28.53 $\pm$ 10.37	43.76 $\pm$ 9.29 ^a
HDPE	12.50 $\pm$ 3.20	12.18 $\pm$ 3.78	24.69 $\pm$ 5.98 ^a
PVC	11.23 $\pm$ 4.20	12.50 $\pm$ 9.64	23.72 $\pm$ 13.32 ^a

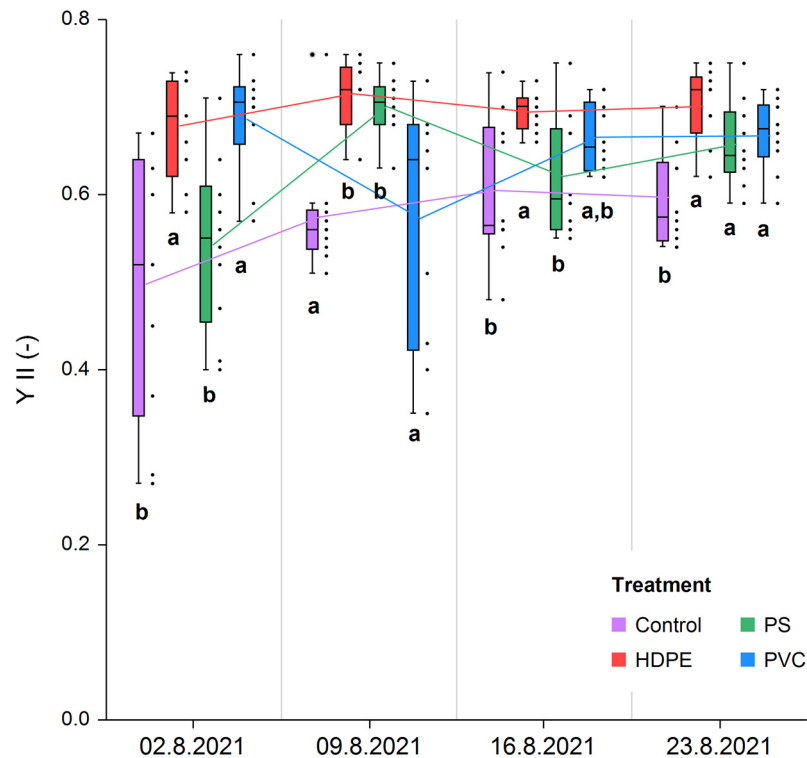


Fig. 5. The measured values of effective quantum yield of Photosystem II, $Y(II)$ (%) of radish plants during GP for the individual treatments of experiment. Lines connect the arithmetic means; the whiskers show minima and maxima and points represent individual measurements. Box plots denoted with different letters within the same measurement period are significantly different on significance level 0.05.

CONCLUSIONS

The present study evidenced that all three types of microplastic contamination (HDPE, PVC, PS) significantly decreased the bulk density of silty loam soil, measured after GP. The results also confirmed significant increase in the hydraulic conductivity and water sorptivity in HDPE treatment, measured after GP, and significant increase in the persistence of SWR in HDPE treatment, measured before GP.

Confirmed shifts of hydrophysical characteristics of soil in microplastic treatments (particularly in the HDPE treatment), which can affect the accessibility of water and nutrients for plants, did not result in the direct significant increase in total biomass of radish at microplastic treatments. Measured values of the effective quantum yield of Photosystem II ($Y(II)$) also did not show the unambiguous effect of added microplastic material. Significant increase of the $Y(II)$ in all microplastic treatments measured at the last measurement period cannot be satisfactorily explained only on the basis of the soil characteristics measured during our experiment.

To assess risks of agricultural microplastic pollution in different soil-plant systems, further research is needed on different soil types, with different crops and concentration of microplastics.

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REFERENCES

- Alagna, V., Iovino, M., Bagarello, V., Mataix-Solera, J., Lichner, L., 2017. Application of minidisk infiltrometer to estimate water repellency in Mediterranean pine forest soils. *J. Hydrol. Hydromech.*, 65, 3, 254–263.
- Alagna, V., Iovino, M., Bagarello, V., Mataix-Solera, J., Lichner, L., 2019. Alternative analysis of transient infiltration experiment to estimate soil water repellency. *Hydrological Processes*, 33, 4, 661–674.
- Ashraf, M.H.P.J.C., Harris, P.J., 2013. Photosynthesis under stressful environments: an overview. *Photosynthetica*, 51, 2, 163–190.
- Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.*, 59, 89–113.
- Bisdorf, E.B.A., Dekker, L.W., Schoute, J.F.T., 1993. Water

- repellency of sieve fractions from sandy soils and relationships with organic material and soil structure. *Geoderma*, 56, 105–118.
- Bordoloi, S., Yamsani, S.K., Garg, A., Sekharan, S., 2019. Critical assessment of infiltration measurements for soils with varying fine content using a mini disk infiltrometer. *Journal of Testing and Evaluation*, 47, 868–888.
- Clothier, B.E., Vogeler, I., Magesan, G.N., 2000. The breakdown of water repellency and solute transport through a hydrophobic soil. *Journal of Hydrology*, 231, 255–264.
- Colzi, I., Renna, L., Bianchi, E., Castellani, M. B., Coppi, A., Pignattelli, S., Loppi, S., Gonnelli, C., 2022. Impact of microplastics on growth, photosynthesis and essential elements in *Cucurbita pepo* L. *Journal of Hazardous Materials*, 423, Article Number: 127238.
- Decagon, 2012a. Mini Disk Infiltrometer – User's Manual. Version 10. Decagon Devices, Inc., Pullman.
- Decagon, 2012b. EC-5 Soil Moisture Sensor – User's Manual. Version 2. Decagon Devices, Inc., Pullman.
- Dekker, L.W., Ritsema, C.J., Oostindie, K., Moore, D., Wesseling, J.A., 2009. Methods for determining soil water repellency on field-moist samples. *Water Resources Research*, 45, Article Number: W00D33.
- Doerr, S.H., 1998. On standardizing the "Water Drop Penetration Time" and the "Molarity of an Ethanol Droplet" techniques to classify soil hydrophobicity: a case study using medium textured soils. *Earth Surface Processes and Landforms*, 23, 663–668.
- Gamon, J., Serrano, L., Surfus, J.S., 1997. The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. *Oecologia*, 112, 4, 492–501.
- Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta*, 990, 87–92.
- Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. *Science Advances*, 3, 7, Article Number: e1700782.
- Gong, W., Zhang, W., Jiang, M., Li, S., Liang, G., Bu, Q., Xua, L. Zhu, H., Lu, A., 2021. Species-dependent response of food crops to polystyrene nanoplastics and microplastics. *Science of the Total Environment*, 796, Article Number: 148750.
- Gonnelli, C., 2022. Impact of microplastics on growth, photosynthesis and essential elements in *Cucurbita pepo* L. *Journal of Hazardous Materials*, 423, Article Number: 127238.
- Guidi, L., Lo Piccolo, E., Landi, M., 2019. Chlorophyll fluorescence, photoinhibition and abiotic stress: Does it make any difference the fact to be a C3 or C4 species? *Frontiers in Plant Science*, 10, Article Number: 174.
- Hallett, P.D., 2008. A brief overview of the causes, impacts and amelioration of soil water repellency – a review. *Soil and Water Research*, 3, 1, 521–528.
- Hazrati, S., Tahmasebi-Sarvestani, Z., Modarres-Sanavy, S.A.M., Mokhtassi-Bidgoli, A., Nicola, S., 2016. Effects of water stress and light intensity on chlorophyll fluorescence parameters and pigments of *Aloe vera* L. *Plant Physiology and Biochemistry*, 106, 141–148.
- Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of the Total Environment*, 586, 127–141.
- ISO 11272, 2017. Soil Quality. Determination of dry bulk density. International Organization of Standardization, Geneva.
- Jiang, X., Chen, H., Liao, Y., Ye, Z., Li, M., Klobučar, G., 2019. Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant *Vicia faba*. *Environmental Pollution*, 250, 831–838.
- Khalid, N., Aqeel, M., Noman, A., 2020. Microplastics could be a threat to plants in terrestrial systems directly or indirectly. *Environmental Pollution*, 267, Article Number: 115653.
- Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F., 2006. World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, 15, 259–263.
- Lichtenthaler, H.K., Babani, F., 2004. Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. In: Govindjee, G., Papageorgiou, G. (Eds.): *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Springer, Dordrecht, pp. 713–736.
- Machado, A.A.d.S., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M.C., 2018. Impacts of microplastics on the soil biophysical environment. *Environmental Science & Technology*, 52, 17, 9656–9665.
- Machado, A.A.d.S., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S., Rillig, M.C., 2019. Microplastics can change soil properties and affect plant performance. *Environmental Science & Technology*, 53, 10, 6044–6052.
- Melo, M., Lapin, M., Kopolková, H., Pecho, J., Kružicová, A., 2013. Climate trends in the Slovak part of the Carpathians. In: Kozak, J., Ostapowicz, K., Bytnerowicz, A., Wyzga, B. (Eds.): *The Carpathians: Integrating Nature and Society Towards Sustainability*. Springer, Heidelberg, Germany, 717 p.
- Meng, F., Yang, X., Riksen, M., Xu, M., Geissen, V., 2021. Response of common bean (*Phaseolus vulgaris* L.) growth to soil contaminated with microplastics. *Science of the Total Environment*, 755, 142516.
- Qi, Y.L., Yang, X., Pelaez, A.M., Lwanga, E.H., Beriot, N., Gertsen, H., Geissen, V., 2018. Macro-and micro-plastics in soil-plant system: effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. *Science of the Total Environment*, 645, 1048–1056.
- Qi, R., Jones, D.L., Li, Z., Liu, Q., Yan, C., 2020a. Behavior of microplastics and plastic film residues in the soil environment: A critical review. *Science of the Total Environment*, 703, Article Number: 134722.
- Qi, Y., Beriot, N., Gort, G., Lwanga, E.H., Gooren, H., Yang, X., Geissen, V., 2020b. Impact of plastic mulch film debris on soil physicochemical and hydrological properties. *Environ. Pollut.*, 266, 115097.
- Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? *Environmental Science & Technology*, 46, 6453–6454.
- Rillig, M.C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C.A., Buchert, S., Wulf, A., Iwasaki, A., Roy, J., Yang, G., 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. *Science*, 366, 6467, 886–890.
- Senathirajah, K., Attwood, S., Bhagwat, G., Carbery, M., Wilson, S., Palanisami, T., 2021. Estimation of the mass of microplastics ingested—A pivotal first step towards human health risk assessment. *Journal of Hazardous Materials*, 404, Article Number: 124004.
- Soil Survey Division Staff, 1993. *Soil Survey Manual*. Soil Conservation Service. U.S. Department of Agriculture Handbook 18, 437 p.
- Stagnari, F., Galieni, A., D'Egidio, S., Pagnani, G., Pisante, M., 2018. Responses of radish (*Raphanus sativus*) to drought

- stress. *Annals of Applied Biology*, 172, 2, 170–186.
- Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Troeger, J., Munoz, K., Fror, O., Schaumann, G.E., 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Sci. Total Environ.*, 550, 690–705.
- Šimanský, V., Igaz, D., Horák, J., Šurda, P., Kolenčík, M., Buchkina, N.P., Uzarowicz, Ł., Juriga, M., Šrank, D., Pauková, Ž., 2018. Response of soil organic carbon and water-stable aggregates to different biochar treatments including nitrogen fertilization. *J. Hydrol. Hydromech.*, 66, 429–436.
- Thematic Strategy for Soil Protection, 2006. Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions. SEC(2006)620; SEC(2006)1165. Brussels. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A52006DC0231>
- Tinebra, I., Alagna, V., Iovino, M., Bagarello, V., 2019. Comparing different application procedures of the water drop penetration time test to assess soil water repellency in a fire affected Sicilian area. *Catena*, 177, 41–48.
- Toková, L., Igaz, D., Horák, J., Aydin, E., 2020. Effect of biochar application and re-application on soil bulk density, porosity, saturated hydraulic conductivity, water content and soil water availability in a silty loam Haplic Luvisol. *Agronomy*, 10, 7, Article Number: 1005.
- WRB, 2014. World Reference Base for Soil Resources 2014. World Soil Resources Reports No. 106. FAO, Rome.
- Zang, H., Zhou, J., Marshall, M.R., Chadwick, D.R., Wen, Y., Jones, D.L., 2020. Microplastics in the agroecosystem: are they an emerging threat to the plant-soil system? *Soil Biology and Biochemistry*, 148, Article Number: 107926.
- Zhang, R., 1997. Determination of soil sorptivity and hydraulic conductivity from the disk infiltrometer. *Soil Science Society of America Journal*, 61, 1024–1030.
- Zhang, J., He, P., Ding, W., Ullah, S., Abbas, T., Li, M., Ai, Ch., Zhou, W., 2021. Identifying the critical nitrogen fertilizer rate for optimum yield and minimum nitrate leaching in a typical field radish cropping system in China. *Environmental Pollution*, 268, Article Number: 115004.
- Zhou, J., Wen, Y., Marshall, M.R., Zhao, J., Gui, H., Yang, Y., Yanga, Y., Zenga, Z., Jonesbe, D.L., Zang, H., 2021. Microplastics as an emerging threat to plant and soil health in agroecosystems. *Science of the Total Environment*, 787, Article Number: 147444.

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