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Response of common bean (*Phaseolus vulgaris L.*) growth to soil contaminated with microplastics

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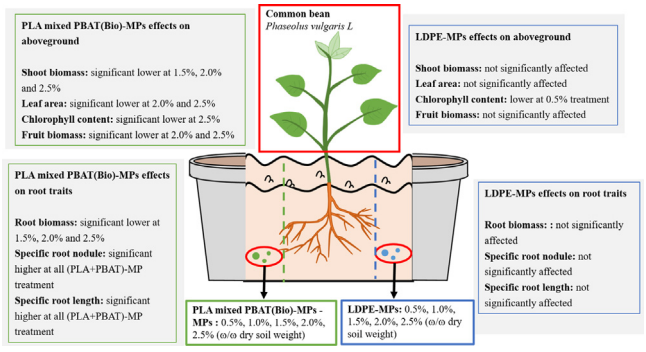
HIGHLIGHTS

- We studied effects of LDPE-MPs and PLA + PBAT(Bio)-MPs (0.5% ~ 2.5% ω/ω) on *P. vulgaris*.
- LDPE-MPs did not affect shoot, root and fruit biomass.
- Bio-MPs strongly reduced shoot, root biomass (≥1.5%) and fruit biomass (≥2%).
- Specific root length/nodules strongly increased (all Bio-MPs; LDPE: ≥1.0%/2.5%).
- We conclude that Bio-MPs negatively influenced the growth of *P.vulgaris*.

GRAPHICAL ABSTRACT



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ABSTRACT

Although concerns surrounding microplastics (MPs) in terrestrial ecosystems have been growing in recent years, little is known about the responses of plant growth to MPs pollution. Here, we conducted a pot experiment in a net house under natural condition by adding two types of MPs, low-density polyethylene (LDPE-MPs) and polylactic acid (PLA) mixed with poly-butylene-adipate-*co*-terephthalate (PBAT, Bio-MPs), to sandy soil at 5 doses (0.5%, 1.0%, 1.5%, 2.0%, 2.5% ω/ω dry soil weight). The effects of LDPE-MPs and Bio-MPs on common bean *(Phaseolus vulgaris L)* were tested. Compared to control (no MPs addition), LDPE-MPs showed no significant effects on shoot, root and fruit biomass while ≥1.0% LDPE-MPs showed significant higher specific root nodules ($n \cdot g^{-1}$ dry root biomass) and only 2.5% LDPE-MPs showed significant higher specific root length ($cm \cdot g^{-1}$ dry root biomass). 1.0% LDPE-MPs caused significant higher leaf area and 0.5% LDPE-MPs caused significant lower leaf relative chlorophyll content. For Bio-MPs treatment, compared to control, ≥1.5% Bio-MPs showed significant lower shoot and root biomass. ≥2.0% Bio-MPs showed significant lower leaf area and fruit biomass. All Bio-MPs treatments showed significant higher specific root length and specific root nodules as compared to control. The results of the current research show that both MPs induced the responses of common bean growth, and ≥1.5% Bio-MPs exerted stronger effects. Further studies of their ecological impacts on soil-plant systems are urgently needed.

1. Introduction

In arid and semi-arid regions, plastic mulching is widely used in farming to control weeds, conserve water and improve soil temperatures (Kader et al., 2017; Ma et al., 2018; Qin et al., 2015). Unfortunately,

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the plastic mulches are not always removed from the soil after harvest. As a result of machinery tillage and natural degradation, the mulches left on the fields were fragmented into microplastics (MPs, <5 mm) (Andrady, 2017; Briassoulis, 2004; Palsikowski et al., 2017a; Sintim et al., 2019; Steinmetz et al., 2016). These plastic mulches derived MPs have been identified as one of the main sources of agricultural MPs pollution (Ng et al., 2018; Wierckx et al., 2018). After entering the soil, MPs could pose serious potential threats to soil health and ultimately damage the environment (Ng et al., 2018; Piehl et al., 2018).

MPs have been reported that could be ingested by soil organisms, i.e. snails (*Achatina fulica*) and earthworm *Lumbricus terrestris* (Oligochaeta, Lumbricidae), thus affecting their growth, activities, gut microbiota and immune systems (Huerta Lwanga et al., 2016; Song et al., 2019; Zhu et al., 2018). Considering the important role of soil organisms in soil organic matter decomposition and nutrient cycling, the occurrence of MPs will pose threats to soil ecosystem (Chae and An, 2018; Rillig et al., 2017b). In addition, due to the chemical inertia and structural characteristics, MPs have been proven to have the capacity to adsorb toxic chemicals onto the surface (Ivar do Sul and Costa, 2014; Koelmans et al., 2013; Wright et al., 2013). After entering the soil, these MPs can be considered as vectors for agrichemicals and heavy metals, thus posing threats to soil health (Chae and An, 2018; Huerta Lwanga et al., 2017; Li et al., 2020a).

The increasing concerns surrounding plastic pollution in agriculture have led to the development of biodegradable materials (Bandopadhyay et al., 2018; Sintim et al., 2019). Biodegradable plastic films (BDFs) have been developed as an alternative for conventional low-density polyethylene (LDPE) films. BDFs could be left in agricultural fields after use and then degraded into CO_2 and H_2O by soil microorganisms (Bandopadhyay et al., 2018; Bettas Ardisson et al., 2014). However, the total degradation of BDFs in farmland conditions is rarely observed (Li et al., 2014; Palsikowski et al., 2017b). In addition, Sintim and Flury (2017) expressed their concerns about the toxicity of biodegradable material and indicated that "out-of-sight does not mean they are safe". Qi et al. (2020) found starch based biodegradable MPs could shift the soil bacterial communities and volatiles emitted in the rhizosphere. Research by Wang et al. (2020) also indicated that polylactic acid (PLA) MPs exhibited a noticeable phytotoxicity to maize growth. BDMs have been suggested as the most promising solution for agricultural plastic pollution. Unfortunately, the knowledge about its ecological impacts on soil-plant systems are still insufficient and require further study.

The growing body of literature have indicated that MPs could affect the soil biophysical environments, i.e. decreased soil bulk density and soil microbial activities, increased soil evaporation and desiccation cracking (de Souza Machado et al., 2018; Wan et al., 2019). However, little information is available on the effects of MPs on plant growth (Rillig et al., 2019). It has been reported that changes in soil properties by the occurrence of MPs could enhance plant performance. For example, de Souza Machado et al. (2019) found onion growth affected as a result. Qi et al. (2018) found starch based MPs led to the reduction of wheat biomass. A recent study by Li et al. (2020b) observed an effective uptake of micrometre-sized (2.0 μm) and submicrometre-sized (0.2 μm) polystyrene (PS) by wheat and lettuce root via a crack-entry mode and the translocation of 0.2 μm PS within roots, shoots and leaves of wheat and lettuce. Considering the important role of plant in terrestrial ecosystems and increasing accumulation of MPs in agricultural soils. Understanding the effects of MPs on plant thus is crucial. LDPE is the most commonly applied plastic mulching material, PLA blended with PBAT has been suggested as one of the most promising materials as an alternative for agricultural plastic film due to its durability and environment friendliness (Palsikowski et al., 2017a; Zhang et al., 2019). Therefore, a better understanding of the effects of LDPE microplastics (LDPE-MPs) and biodegradable microplastics (Bio-MPs) on plant growth will provide deeper insight into the impacts of these particles on the soil-plant systems.

In our present study, according to the previous research, we hypothesized that both LDPE-MPs and Bio-MPs affect plant growth, and that Bio-MPs have stronger impacts than LDPE-MPs. To test our hypothesis, we conducted a pot experiment by using common bean (*Phaseolus vulgaris L.*), a Leguminosae crop, as a model plant due to it often being cultivated with plastic mulching and sensitive to changes in soil conditions, such as water deficiency and soil nitrogen (Abd El-Wahed et al., 2017; Chekanai et al., 2018; Fenta et al., 2019). Common bean was exposed to two types of MPs, LDPE-MPs and biodegradable bioplastics derived from PLA/PBAT (Bio-MPs), at gradient doses (0.5%, 1.0%, 1.5%, 2.0% and 2.5% ω/ω dry soil weight). Several commonly applied growth parameters were used to assess the impacts of the MPs on the growth of common bean, i.e. shoot and root biomass, shoot to root ratio, specific root length, specific root nodules etc.

2. Materials and methods

2.1. Experimental setup

We conducted a two-factorial pot experiment from the 28th of June 2019 until the 18th of October 2019 in an outdoor net house (diameter 0.25 mm) at Unifarm, Wagenigen University & Research (WUR), the Netherlands (Fig. S1A). Fig. S2 shows the monthly temperatures in Wageningen during the experiment.

In the experiment we applied two types of microplastics: low-density polyethylene (LDPE-MPs) and biodegradable plastic (Bio-MPs). The industrial pellets of biodegradable (Bio) plastic consisted of 85% PBAT, 10% PLA and 5% calcium carbonate. The pellets of LDPE and Bio materials were first frozen with liquid nitrogen and then ground using a grinding machine into smaller particles, the particles were sieved manually using steel sieves with pore sizes of 53 μm, 125 μm, 250 μm, 500 μm and 1000 μm to ensure the particle size ranging from <53 μm to 1000 μm. The MPs used in this experiment were comprised of 250– 500 μm (60% of total MPs weight) and 500– 1000 μm (40% of total MPs weight). These two size categories were chosen based on Scheurer and Bigalke (2018) and Zhang and Liu (2018). The ratio was chosen to simulate the heterogeneity of sizes of MPs in terrestrial ecosystems. The MPs used in our research were arbitrarily shaped particles (scanned by Laser Direct Infrared system, Agilent, US), the shape and flourier transform infrared spectroscopy (FTIR) are shown in supplementary files (Fig. S3).

LDPE-MPs and Bio-MPs were applied in 5 different doses: 0.5%, 1.0%, 1.5%, 2.0% and 2.5% dry soil weight. In addition, a control treatment (CON) without MPs was prepared. The doses of MPs were chosen based on the current knowledge of MPs concentrations in soil (Corradini et al., 2019; de Souza Machado et al., 2019; Ng et al., 2018). The gradient and high doses could amplify the potential side effects that might otherwise be overlooked and also determine a potential threshold (van Weert et al., 2019). Totally, 11 treatments with 8 replicates were included (Fig. 1), so that a total of 88 pots were cultivated (Fig. S1).

The substrate used in this study was a sandy soil (87% sand, 12% silt and 1% clay with an organic matter content of 4%, and pH 6.0. More details can be found in the Supporting Information Fig. S4). The soil was collected from an agricultural field near Wageningen, the Netherlands on June 14th, 2019. The soil was immediately sieved to 4 mm to remove large roots and gravel, air-dried and homogenized. MPs were manually mixed into homogenized air dried soil using a wooden stick for 10 min in an iron tank until achieving target doses. Then, a 7 L polypropylene (PP) pot (21 cm height, 16 cm bottom diameter and 21 cm top diameter) was filled with 6 kg of homogenized soil-MPs until 5 cm below the top of the pot, resulting in a bulk density of approximately 1.16 $g \cdot cm^{-3}$. The bottom of the PP pots was covered with a piece of geotextile to prevent soil loss. After all the pots were filled, the soil moisture was unified to 10% (gravimetric water content). Pots were then placed in the outdoor net house for one week to allow interactions between the soil microbiome, soil and microplastics (the 28th of June to the 5th of July 2019).

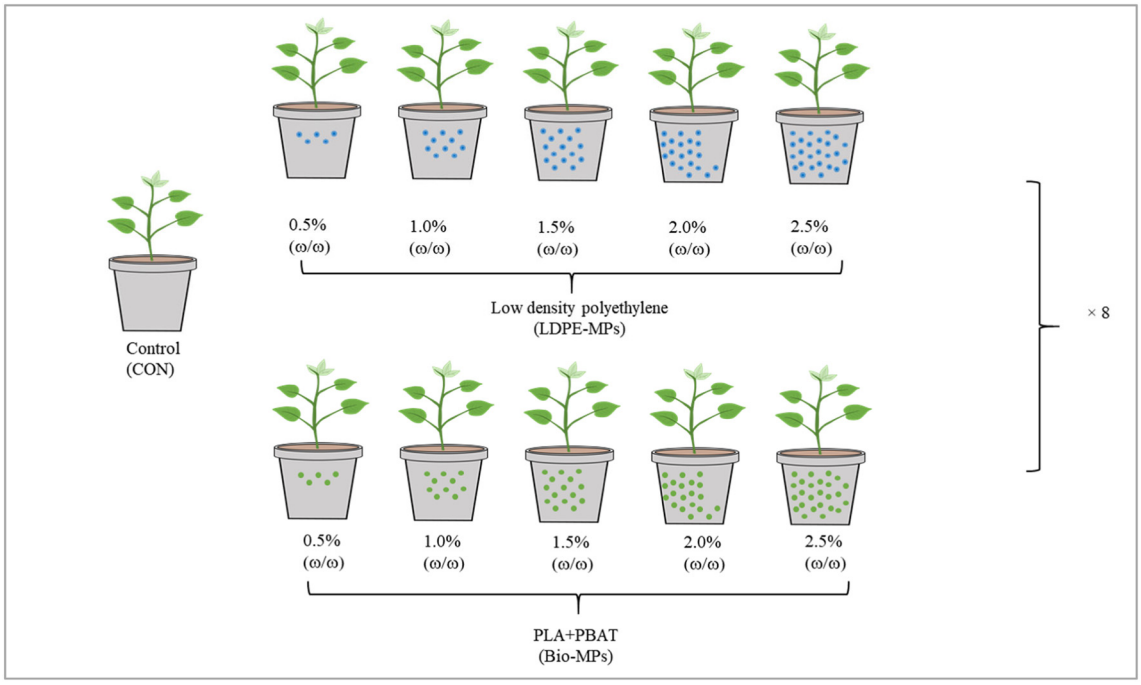

Fig. 1. Pot experimental design. All 11 treatments were repeated 8 times (4 replicates per harvesting moment).

Common bean (*Phaseolus vulgaris L.; Cultivar: Bruine Noordhollandse*) seeds were obtained from Unifarm, Wagenigen University & Research. The seeds were surface sterilized for 5 min using 10% sodium hypochlorite and then washed several times with deionized water. Five seeds were sown in each pot. Germination occurred within 14 days. 2 seedlings per pot were kept for the experiment and the rest were removed from the pots. During the growing period, 100 mL of a diluted nutrient solution (Fig. S5) was added to each pot in the 4th (26th of July) and 5th (2nd of August) week. The diluted nutrient solution contained 1/3 of the nitrogen of the original nutrient solution and served as a starter nitrogen to stimulate early growth (Chekanai et al., 2018). From the 6th to the 12th week, 100 mL of the nutrient solution was added to each pot once a week to ensure the fully development of common bean. Pots were randomly placed within the net house and their positions were shifted once a month. The water content of the pots during the whole growth period was maintained at 10(±1)% by watering twice a week.

2.2. Measurements of Phaseolus vulgaris L. growth parameters

During the growing period, the height and stem diameter of the common bean were measured once a week from the 14th to the 105th day. Plant height was measured using a steel ruler and stem diameter was measured using a Vernier caliper (Data recorded in Fig. S6).

The plants were harvested twice based on the common bean development stage (Table S1). The first harvest was performed on the 15th of August 2019, 46 days after seeding, near the end of the vegetative stage (VS) when plant root and leaves finished the early development stage. During the first harvest, plant shoot biomass (SB_VS), root biomass (RB_VS), relative leaf chlorophyll content (Chlor_VS), leaf area (LA_VS), and root traits were measured. Root samples were only collected once at vegetative stage due to most of the roots having decayed after full maturation. A second harvest was performed on the 18th of October 2019, 105 days after seeding, after full maturation (FM). During the second harvest, fruit biomass (FruitB), number of fruits (FruitNb), number of pods (PodNb) were recorded. At each time point, 4 replicates were harvested. All the measured parameters and their abbreviations are shown in Table 1.

2.2.1. Shoot measurements

At the end of the vegetative stage (15th of August 2019), Chlor_Vs was measured using a hand-held automated chlorophyll meter (SPAD-502plus, Minolta, USA) before the first harvest. Then, plants were cut 10 mm above the soil and separated into shoot and roots. Plant shoots were transported to the laboratory, fresh shoot biomass was weighed using a digital balance (DK-6200-C-M), then the leaves were cut off and measured using a Leaf Area Meter (LI-3100C Laboratory, LICOR Biosciences, USA). Thereafter shoots and leaves were dried in an oven (TYPE A 1500-145, KEMA KEUR) at 60 °C to a constant weight to determine the SB_Vs. After plant shoots had been removed, the pots were stored in a 4 °C cooling room before the root samples were collected. After full maturation (18th of October 2019), FruitB, FruitNb and PodNb were recorded.

2.2.2. Root traits

To collect the root samples, each pot was carefully rinsed with tap water to remove any traces of soil. Then, the roots were carefully placed in a steel sieve (410 μm) and gently rinsed again to remove any fine

Table 1
Measured growth parameters and their abbreviation.

Sampling time	Growth parameters	Abbreviation
Vegetative stage	Shoot biomass (g)	SB_VS
	Leaf area (cm2)	LA_VS
	Relative leaf chlorophyll content	Chlor_VS
	Root biomass (g)	RB_VS
	Root average diameter (mm)	RAD
	Specific root length ($cm \cdot g^{-1}$)	SRL
	Specific root surface area ($cm^2 \cdot g^{-1}$)	SRSA
	Specific root volume ($cm^3 \cdot g^{-1}$)	SRV
	Specific root nodules ($n \cdot g^{-1}$)	SRN
	Fine root length proportion (%)	FRL
	Fine root surface area proportion (%)	FRS
	Fine root volume proportion (%)	FRV
	Shoot to root ratio	S:R_VS
Fully mature	Fruit biomass (g)	FruitB
	Pod number (n)	PodNb

sand. The recovered roots from each pot was placed in a steel container (20 cm wide, 30 cm long and 5 cm deep) and immersed in tap water. Floating organic debris (Fig. S7A) was picked out using tweezers. After that, the roots from each pot was homogenized in the new steel container and three subsamples were randomly selected to examine the root traits. Each subsample consisted of the roots in a sample area of 8 cm length and 5 cm width (Fig. S7B). The roots were cut off using a pair of scissors. The retrieved subsample was then stored in 100 mL centrifuge tubes (polypropylene, PP) and soaked with 25% ethanol. The rest of the root sample was oven dried at 60 °C to a constant weight and recorded as root biomass$_1$ (RB_1).

To obtain the root traits, each root subsample from each pot was placed on a transparent tray (19 cm wide, 25 cm long and 2 cm deep, Fig. S7C) and evenly spread out by hand with distilled water. The Imagery Scan Screen (EPSON Expression V700XL) was used to scan the root samples to create a black and white image (600 dpi, tagged image file format [TIF], white background) (Fig. S7D). The scanned image was then analyzed using "WinRHIZO" software (Regent Instruments Inc., Quebec), which was specially designed for root architecture measurements: root length, root surface area, root volume, average diameter and proportion of fine root (roots with diameter < 0.4 mm) length (FRL,%), fine root surface area (FRA,%), and fine root volume (FRV,%) (Fenta et al., 2019; Sofi et al., 2018). After scanning, the number of nodules per subsample was manually counted. Each subsample was then oven dried at 60 °C to a constant weight and recorded. The total weight of 3 subsamples then recorded as root biomass$_2$ (RB_2). Total dry root biomass (RB) was calculated as $RB_1 + RB_2$.

Specific root length (SRL, $cm \cdot g^{-1}$), specific root surface area (SRSA, $cm^2 \cdot g^{-1}$), specific root volume density (SRV, $cm^3 \cdot g^{-1}$) and specific root nodules were calculated as root length (cm), surface area (cm^2), root volume (cm^3) and root nodule number (n) divided by biomass of each scanned root subsample, respectively (Araújo et al., 2004; Pérez-Jaramillo et al., 2017). The biomass of shoot to root ratios (S:R_VS) were calculated by dividing the dry weight of the shoot by the dry root biomass.

2.3. Data analysis

All the measured growth parameters were normalized using arcsine square root transformation to avoid violating the underlying assumptions of normality. For each type of microplastic material (LDPE-MPs and Bio-MPs treatments), comparisons of each growth parameter in different MP concentrations in contaminated soil were performed using one-way ANOVAs, growth parameters that were significant affected ($p < 0.05$) by the occurrence of MPs then tested by the LSD test (Table S2). Comparisons between LDPE-MPs and Bio-MPs were performed using the Independent-Samples *t*-Test (Table S3). In all the analyses, the significance levels were considered at $p < 0.05$ and all the plant growth parameters were presented as "Means ± Standard deviations" (Table S4).

2.4. Correlation analysis

To identify the relationships between the microplastics (types and concentrations) and the plant growth parameters, three multivariate statistical methods including correlation analysis (CA), factor analysis (FA) and redundancy analysis (RDA) were employed in this study. Firstly, correlation analysis (Table S5) was performed to explore the collinearity among measured growth parameters. The growth parameters whose correlation coefficient values with other growth parameters were larger than 0.9 or smaller than 0.35 were screened out. According to the CA (Table S5), SRL had a high collinearity with SRSA and SRV. Since SRL correlated strongly with other growth parameters, SRSA and SRV were removed from the CA while SRL was retained. FRL was retained and FRS and FRV were removed for the same reason. Growth parameter of relative Chlor_Vs, and SRN were excluded because of the low correlation (Pearson correlation $r < 0.35$) with other parameters (Table S5). Growth parameters of S:R and RAD were excluded because no significant effects were observed in the microplastic materials and microplastic concentrations and their interactions (Table S6).

In order to recognize the comprehensive effects of microplastics on common bean growth, FA was applied to classify the latent factors. All meaningful loadings (i.e. loadings >0.70) were included in the interpretation of factor analysis results. The statistical data analyses were performed using IBM SPSS Statistics 23. The factor analysis results of bean growth parameters are shown in Table 2. Finally, we used RDA to identify the relationships among microplastics and plant growth parameters. The three extracted factors by FA and the growth parameters of Chlor_Vs and SRN, which showed low correlation with other parameters, were included in the RDA (Fig. 4). The arrows represent the different plant growth parameters, and the direction of the arrows represents the correlations between each parameter and the axes as well as the relationships among the parameters. The length of the arrows represents the relative contribution of the parameters to the axes and the parameter factor relationships. RDA was performed using CANOCO 5.

3. Results

3.1. Effects of LDPE-MPs on common bean growth and root traits

In our study, LDPE-MPs showed no significant (one-way ANOVA, $p > 0.05$) impact on shoot biomass, root biomass, fruit biomass (Fig. 2A, B and D) or pod number as compared to control treatment (Fig. S8A). However, leaf area (Fig. 2F and Table S4) in 1.0% LDPE-MPs (724 ± 56.0 cm^2) was significantly higher (one-way ANOVA, $p = 0.034$) than control (626 ± 80.0 cm^2). Leaf relative chlorophyll content (Fig. 2E and Table S4) in 0.5% LDPE-MPs (27.2 ± 2.34) was significantly lower (one-way ANOVA, $p = 0.004$) than control (33.1 ± 1.16).

For root traits, the significant impacts were mainly observed from 2.5% LDPE-MPs treatment. For example, specific root length in 2.5% LDPE-MPs treatment ($20{,}047 \pm 989$ $cm \cdot g^{-1}$) was significantly higher (one-way ANOVA, $p < 0.05$) than control treatment ($16{,}604 \pm 1082$ $cm \cdot g^{-1}$, Fig. 3A and Table S4). Besides, 2.5% LDPE-MPs also showed highest fine root surface area proportion (64.5 ± 2.36%), which is significant higher than control (57.1 ± 2.03%, Fig. S8F and Table S4). In addition, except 0.5% LDPE-MPs, all LDPE-MPs led to higher specific root nodules as compared to control treatment (510 ± 58.4 $n \cdot g^{-1}$), while only 2.0% showed no significant difference (Fig. 3B).

Table 2
Variable loading coefficients (eigenvectors) of the first four factors extracted using 7 common bean growth parameters, their eigenvalues, and individual and cumulative percentage of total variance explained by each factor.

Growth indicator	Factor 1	Factor 2	Factor 3	Cumulative
RB_VS	**0.851**	0.039	−0.018	0.871
LA_VS	**0.843**	0.165	−0.218	0.791
SB_VS	**0.823**	0.204	−0.346	0.680
PodNb	0.083	**0.927**	−0.088	0.922
FruitB	0.191	**0.924**	−0.044	1.072
FRL	−0.112	−0.013	**0.919**	0.794
SRL	−0.252	−0.122	**0.843**	0.470
Eigenvalue	3.194	1.462	1.107	11.4
Variance	45.6	20.9	15.8	82.3
Cumulative variance (%)	39.1	66.5	82.3	

Note. Bold face values loadings (>0.70) are considered highly weighted.
SB_VS: Shoot biomass at the end of vegetative stage.
RB_VS: Root biomass at the end of vegetative stage.
LA_VS: Leaf area at the end of vegetative stage.
SRL: Specific root length at the end of vegetative stage.
FRL: Proportion of fine root (diameter < 0.4 mm) length at the end of vegetative stage.
FruitB: Fruit biomass after fully mature.
PodNb: Pod number after fully mature.
Factor 1: Plant shoot and root biomass;
Factor 2: Plant production;
Factor 3: Root characteristics.

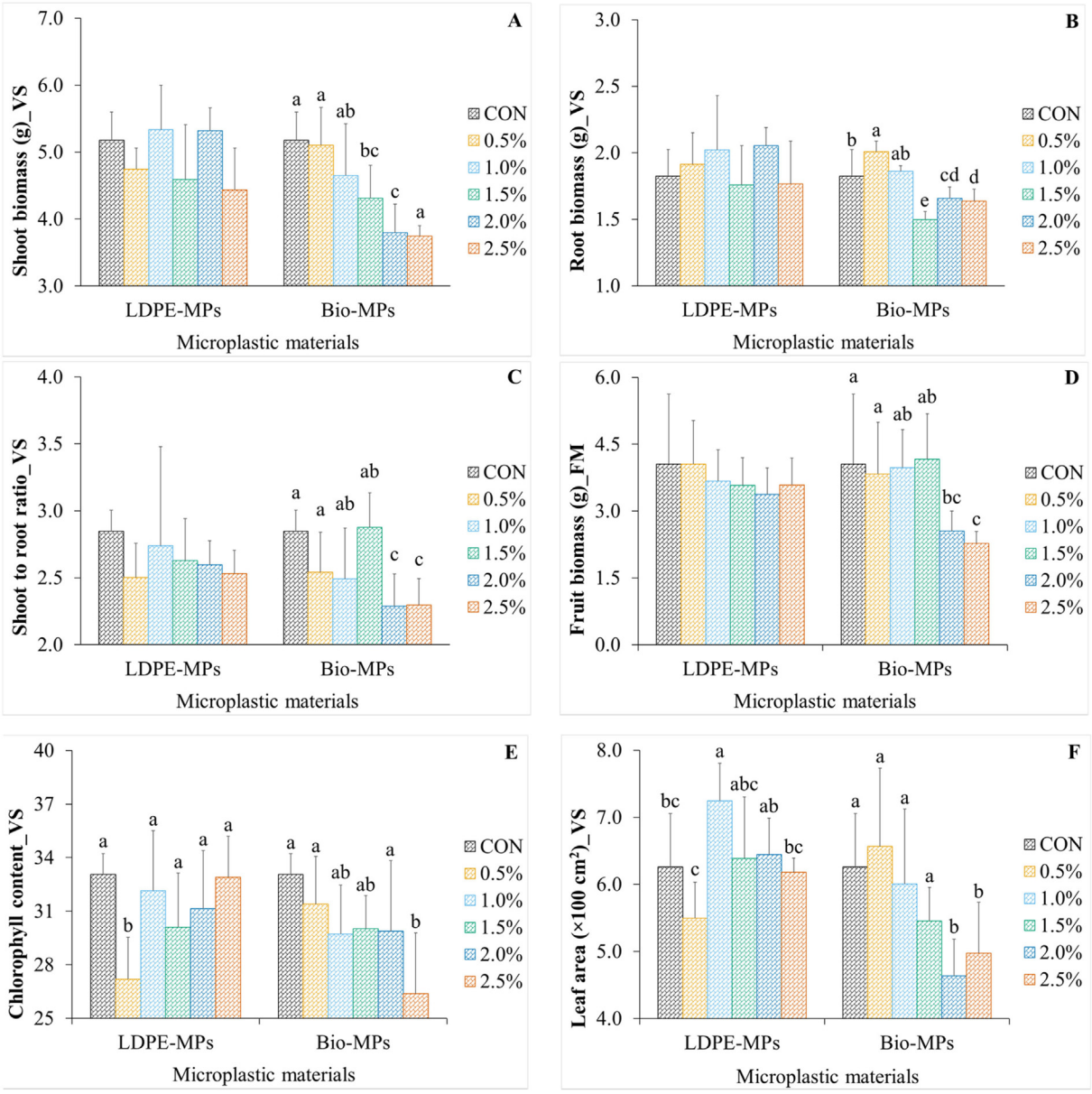

Fig. 2. The effects of LDPE microplastic (LDPE-MPs) and biodegradable microplastic (Bio-MPs) contaminated soil (0.5–2.5% ω/ω soil, uncontaminated control CON) on (A). shoot biomass at the end of vegetative stage (SB_VS); (B). root biomass at the end of vegetative stage (RB_VS); (C). shoot to root ratio of biomass at the end of vegetative stage (S:R_VS); (D). fruit biomass after fully mature (FruitB); (E). relative leaf chlorophyll content at the end of vegetative stage (Chlor_VS); (F). leaf area at the end of vegetative stage (LA_VS). Legend indicates the microplastic contamination level, including control (dark), 0.5% (orange), 1.0% (light blue), 1.5% (green), 2.0% (blue) and 2.5% (Vermillion). Error bars represent standard deviation; and the lowercase letters (a and b) indicate significant differences between control treatment and microplastic contamination treatment within each microplastic material. Post-hoc test was only performed when growth parameters were significantly affected by the occurrence of MPs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Other doses of LDPE-MPs showed no significant effects on root traits. Specific root volume (Fig. S8D and Table S4) and root average diameter (Fig. S8E and Table S4) were not significantly affected by LDPE-MPs.

3.2. Effects of bio-MPs on common bean growth and root traits

For Bio-MPs addition, shoot and root biomass were significantly affected by 1.5%, 2.0% and 2.5% Bio-MPs compared to control (Fig. 2A and B). For example, shoot biomass in 1.5%, 2.0% and 2.5% Bio-MPs treatments were 4.31 ± 0.49 g, 3.80 ± 0.43 g, and 3.75 ± 0.16 g, respectively (Table S4), which were significantly lower (one-way ANOVAs, $p < 0.05$) than in control treatment (5.18 ± 0.42 g). Root biomass in 1.5%, 2.0% and 2.5% Bio-MPs treatments were 1.50 ± 0.06 g, 1.66 ± 0.08 g and 1.64 ± 0.09 g, respectively (Table S4), which were significantly lower than control treatment (1.82 ± 0.20 g). Correspondingly, shoot to root ratio (Fig. 2C and Table S4) in 2.0% and 2.5% Bio-MPs treatments were 2.29 ± 0.24 and 2.30 ± 0.20, respectively, which were significantly lower than control treatment (2.85 ± 0.16). Fruit biomass (Fig. 2C) and leaf area (Fig. 2F) were also observed significantly lower in 2.0% and 2.5% Bio-MPs treatments, e.g. fruit biomass in 2.0% and 2.5% Bio-MPs were 2.55 ± 0.45 g and 2.28 ± 0.27 g, respectively, which were significantly lower than control treatment (4.06 ± 1.57 g). Leaf area in 2.0% and 2.5% Bio-MPs were 463 ± 54.8 cm^2 and 497 ± 75.9 cm^2, respectively, which values were significantly lower than control treatment (625 ± 80.0 cm^2, Table S4). In addition, in 2.5% Bio-MPs treatment, leaf relative chlorophyll content (Fig. 2E) and pod number (Fig. S8A) were also significantly higher than the control treatment.

Contrary to the negative effects observed on root biomass and shoot biomass, compared to the control treatment, Bio-MPs treatments showed significantly higher values on specific root length (Fig. 3A),

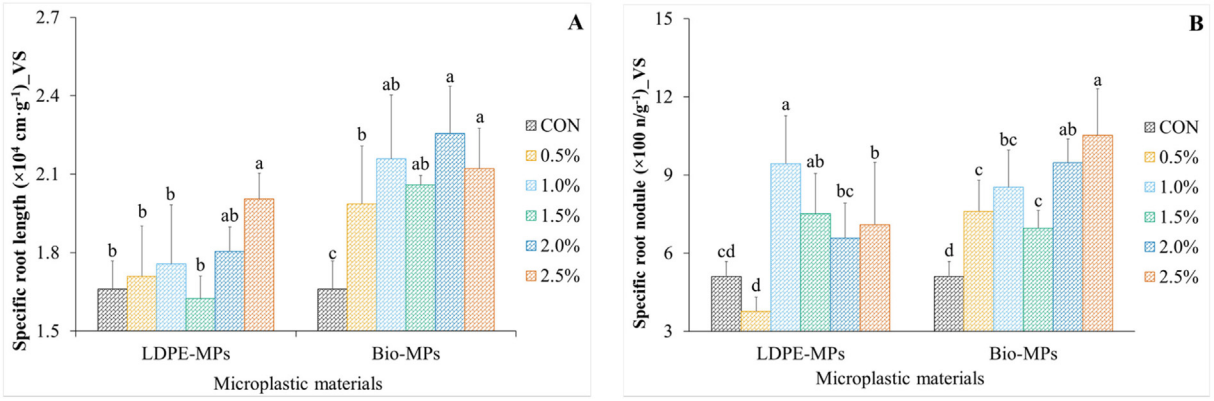

Fig. 3. The effects of LDPE microplastic (LDPE-MPs) and biodegradable microplastic (Bio-MPs) contaminated soil (0.5–2.5% ω/ω soil, uncontaminated control CON) on (A). specific root length at the end of vegetative stage; (B). specific root nodules at the end of vegetative stage (VS). Legend indicates the microplastic contamination level, including control (dark), 0.5% (orange), 1.0% (light blue), 1.5% (green), 2.0% (blue) and 2.5% (Vermillion). Error bars represent standard deviation; and the lowercase letters (a and b) indicate significant differences between control treatment and microplastic contamination treatment within each microplastic material. Post-hoc test was only performed when growth parameters were significantly affected by the occurrence of MPs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

specific root nodules (Fig. 3B) at all concentrations. The highest specific root length (22,550 ± 1816 $cm \cdot g^{-1}$, Fig. 3A and Table S4) was observed in the 2.0% Bio-MPs treatment, significantly higher than control treatment (16,604 ± 1082 $cm \cdot g^{-1}$). The highest specific root nodules was observed at 2.5% Bio-MPs treatment (1053 ± 178 $n \cdot g^{-1}$), which is significantly higher than control treatment (510 ± 58.4 $n \cdot g^{-1}$). Specific root volume (Fig. S8D) and root average diameter (Fig. S8E) were not significantly affected by the Bio-MPs addition (more data showed in Table S4).

3.3. Comparison of the effects between LDPE-MPs and bio-MPs

The impacts on growth parameters from LDPE-MPs and Bio-MPs were compared using the Independent-Samples *t*-Test (Table S3). In general, for shoot and root biomass, leaf area and relative chlorophyll content, growth parameters showed lower values in Bio-MPs treatments compared to LDPE-MPs treatments, while for root traits parameters, specific root length and specific root nodules showed higher value in Bio-MPs treatment compared to LDPE-MPs. However, the differences between the two types of materials were not always significant. For shoot and root biomass, significant differences between LDPE-MPs and Bio-MPs were only observed at 2.0% contamination level (Table S3). For specific root nodules, significant differences between LDPE-MPs and Bio-MPs were observed at 0.5% and 2.5% contamination level.

3.4. Factor analysis results and RDA analysis

Factor analysis results showed three axes (factors) with eigenvalues >1 and collectively explained about 82.3% of the variance in the original data (Table 2). This means the corresponding 7 measured growth parameters were related and that three factors effectively expressed the overall changes in the common bean growth: Factor 1 explained the highest variance (45.6%) in the results, while Factor 2 accounted for 20.9% and Factor 3 accounted for 15.8%. Factor 1 (F_1) included SB_ VS, RB_ VS and LA_VS. This group of parameters implied that Factor 1 was mainly associated with total plant biomass, thus F_1 was defined as shoot and root biomass. Factor 2 (F_2) included PodNb and FruitB, for this reason, F_2 was defined as plant production. Factor 3 included FRL and SRL. This group of parameters implied that Factor 3 was mainly associated with the root development, for this reason, F_3 was defined as root characteristics.

The relationships among the measured parameters of the common bean growth and treatment factors are illustrated in a redundancy analysis diagram (Fig. 4). The first axis explains 62.6% of the variation in the parameter-factor relationships according to the Monte Carlo permutation tests (Table S7). The diagram indicates that Pure soil and LDPE_0.5 were positively correlated to common bean production (F2). LDPE_2.0 and Bio_0.5 were positively correlated to plant biomass (F1) and Chlor_VS. While Bio_1.0, Bio_2.0 and Bio_2.5 are positively related with SRN and root characteristics (F3).

4. Discussion

In our experiment we looked at the effects of LDPE-MPs and Bio-MPs in the soil on the growth of common bean. We will first discuss the effects of both types of MPs on common bean growth separately and then followed by the limitations and implications of current research.

4.1. Effects of LDPE-MPs on common bean growth

Our experiment showed that LDPE-MPs had limited effects on common bean growth. We found no significant effect on plant shoot and root biomass. This was also observed in a study by van Weert et al. (2019), in which they exposed *Myriophyllum spicatum* and *Elodea* sp. to sediments amended with polystyrene (PS) nanoplastic (nano-PS, 50–190 nm, up to 3% sediment dry weight) and PS microplastic (micro-PS, 20–500 μm, up to 10% dry weight) under laboratory conditions. They found that micro-PS did not significantly affect shoot and root biomass while nano-PS did. They suggested the observed difference between nano-PS and micro-PS might be related to the difference in surface area, in which nano-PS could efficiently bind the nutrient, activate competition for nutrients between roots and microbial communities, thus reducing the nutrient status. Consequently, enhanced competition or reduced nutrient status triggered the root biomass growth. Our result is also in line with a study conducted by Wang et al. (2020), who reported that 1% ω/ω polyethylene high density (PEHD, 100-154 μm) had no significant effect on maize growth. A possible explanation for this is given by de Souza Machado et al. (2019), who found that up to 2% PEHD (ω/ω, 2000–3000 μm) in the soil had limited effects on soil structure and onion growth. They assumed the less pronounced effects of PEHD on the changes of soil properties due to the PEHD chemical structure: $(C2H4)_n$, which is structurally stable and contained no nutritional elements that could have elicited soil nutrient dynamics. In our research, we used LDPE, which has also a $(C_2H_4)_n$ structure but has a lower molecular weight. As for the observed variability in the leaf area and relative chlorophyll content, we have no conclusive explanations, the effects might be attributed to the common biological variability in the LDPE-MPs treatments (van Weert et al., 2019).

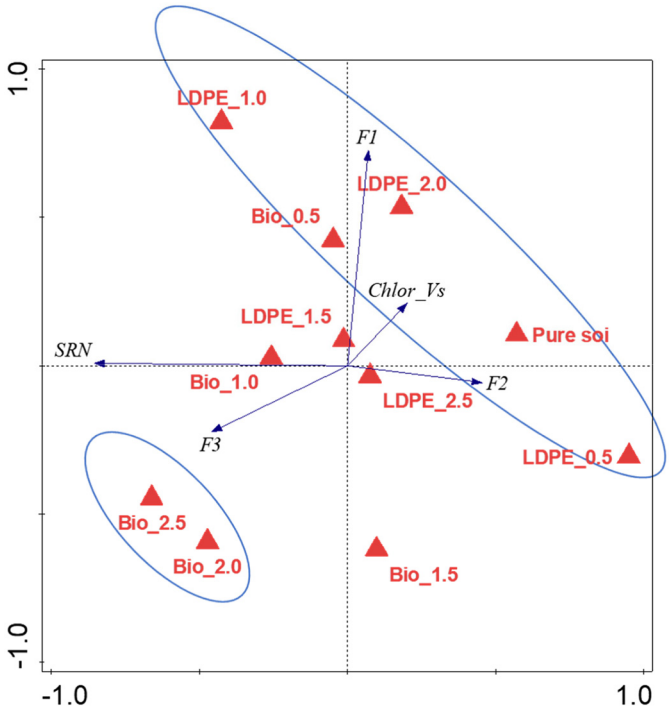

Fig. 4. Redundancy analysis ordination diagram of common bean growth parameters with treatment factors.
Pure soil: soil without microplastics;
LDPE_0.5: soil with LDPE microplastics of 0.5% ω/ω;
LDPE_1.0: soil with LDPE microplastics of 1.0% ω/ω;
LDPE_1.5: soil with LDPE microplastics of 1.5% ω/ω;
LDPE_2.0: soil with LDPE microplastics of 2.0% ω/ω;
LDPE_2.5: soil with LDPE microplastics of 2.5% ω/ω;
Bio_0.5: soil with biodegradable microplastics of 0.5% ω/ω;
Bio_1.0: soil with biodegradable microplastics of 1.0% ω/ω;
Bio_1.5: soil with biodegradable microplastics of 1.5% ω/ω;
Bio_2.0: soil with biodegradable microplastics of 2.0% ω/ω;
Bio_2.5: soil with biodegradable microplastics of 2.5% ω/ω;
Chlor_Vs: relative leaf chlorophyll content at the end of vegetative stage;
SRN: specific root nodules at the end of vegetative stage;
F1 defined as plant shoot and root biomass;
F2 defined as plant production;
F3 defined as root characteristics.

However, our research showed that all the LDPE-MPs treatments, except 0.5% LDPE-MPs, resulted in significant higher specific root nodules compared to control treatment except the treatment of 2.0%, which showed no significant difference. It seems that the presence of LDPE-MPs in the soil stimulates the forming of root nodules. Nodule number has been suggested as a proxy for biological nitrogen fixation (de Oliveira et al., 1998). Haase et al. (2007) found N-deficiency treatments could induce the formation of a significantly higher number of nodules in common bean. Therefore, the higher specific root nodules might be explained by the effect of the LDPE-MPs treatments on available N in the soil. As soil nutrient and microbial activities were not measured in current research, further research is needed to fully understand the mechanism of how LDPE-MPs affects the common bean root traits.

4.2. Effects of bio-MPs on common bean growth

Contrary to LDPE-MPs, the Bio-MPs of PBAT+PLA exerted stronger negative effects on common bean. 1.5%, 2.0% and 2.5% ω/ω showed significantly lower root and shoot biomass, 2.5% ω/ω showed significant lower leaf chlorophyll content. Several factors might account for this. Qi et al. (2018) exposed wheat to 1% ω/ω of starch-based MPs, thus resulting in a plant total biomass of 3.71 ± 0.67 g, significantly lower than the control treatment of 5.59 ± 0.47 g. A later study from Qi et al. (2020) suggested that the shifted rhizosphere bacterial communities and increased volatile compounds like dodecanal might account for the decreased total wheat biomass. Another study by Wang et al. (2020) found that soil with a concentration of 10% ω/ω PLA-MPs (100-154 μm) also had significant phytotoxic effects on maize growth as compared to PEHD, i.e. lower dry shoot and root biomass and lower chlorophyll content. They suggested that the intermediate and final metabolites degraded from PLA-MPs, which may have directly and/or indirectly affected soil properties, soil biota and soil nutrient availability, which may accounted for the inhibition on the plant biomass and leaf chlorophyll content. While contrast to the lower plant shoot and root biomass, all Bio-MPs treatments showed significantly higher specific root length and specific root nodules. As we mentioned previously, the number of common bean nodules has been suggested as an estimate of biological nitrogen fixation and positively related to N-deficiency (de Oliveira et al., 1998; Haase et al., 2007). PBAT material has been reported could increase soil rhizobacterial growth and thus competing for nutrients with plant roots (Kuzyakov and Xu, 2013; Muroi et al., 2016; van Weert et al., 2019). Therefore, in our experiment, it is plausible that in Bio-MPs treated soil, in order to overcome the competition with the soil communities, common bean's produced more specific root length and specific root nodules to allow for better nutrient transportation. However, judging by the observed decreased root and shoot biomass, the nutrient status in Bio-MPs treatments might be reduced.

4.3. Limitations and implications

In this study a wide range of MPs concentrations (0.5%, 1.0, 1.5%, 2.0% and 2.5% ω/ω dry soil weight) was used to study their effect on the growth of common bean. However, MPs concentrations reported under normal field conditions are much lower. To depict the potential subtle effects caused by MPs, it is necessary to use these relatively high concentrations as was also stated by van Weert et al. (2019). Another limitation of our study is that it was not tailored to identify degradation of MPs in soil or nutrient cycling in the soil. Of all the responses, we observed no clearer consistent dose-effects with the increased doses of MPs, which revealed the uncertainties and complexities to predict the impacts of MPs in soil-plant systems. Considering the native properties of the two materials, the effects of Bio-MPs probably come from the degraded by-products while the less pronounced effects of LDPE-MPs might attributed to its stable structure. It should also be noticed that species-species effects, i.e. micro-PS (20–500 μm, up to 10% dry sediment weight) showed no significant impacts on macrophytes in sediments (van Weert et al., 2019), while common bean specific root nodules responded to the occurrence of LDPE-MPs in sandy soil (250–1000 μm, up to 2.5% dry soil weight) in current research, which highlights that different root traits may be susceptible to different mechanisms caused by the occurrence of MPs in soil (Rillig, 2020). In addition, even though LDPE-MPs were structural stable, other properties (i.e. type, size, shapes and surface properties) should also be taken into consideration in future studies since they could also pose threat to plant growth (Rillig, 2020). A recent study by Li et al. (2020b) has evidenced uptake of 0.2 μm and 2 μm PS MPs by wheat and lettuce root. Thus urgent ecological assessments for those petroleum-based polymers are crucial as those particles will eventually degrade into smaller particles (Ng et al., 2018; Rillig et al., 2017a).

5. Conclusion

In this study we tested the hypotheses that Bio-MPs have a stronger effect on the growth of common bean (*Phaseolus vulgaris L.*)than LDPE-MPs. From the results we can conclude that this is indeed the case. LDPE-MPs showed no significant effects on shoot and root biomass, while Bio-MPs, especially at 1.5%, 2.0% and 2.5% ω/ω, significantly inhibited the root and shoot biomass Bio-MPs produced higher specific root length and specific root nodules while LDPE-MPs also showed

significant impacts on specific root nodules, suggesting a potential threat of MPs to soil-plant systems. The results presented have demonstrated that the occurrence of MPs in soil are capable of changing the plant growth, this is a fundamental understanding for future efforts to assess risks of agricultural MPs pollution in soil-plant systems. This current research, therefore, has highlighted the necessity to gain more insight into the mechanisms (i.e. dynamics of nutrient status and soil bacterial communities) underlying MPs effects on plant growth and the fate of MPs with different properties (types and size) in soil-plant systems.

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CRediT authorship contribution statement

Fanrong Meng: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Xiaomei Yang:** Conceptualization, Writing - review & editing, Supervision. **Michel Riksen:** Conceptualization, Writing-review & editing. **Minggang Xu:** Supervision. **Violette Geissen:** Conceptualization, Resources, Supervision. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. These authors contributed equally.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142516.

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