Macro- and micro- plastics in soil-plant system: effects of plastic mulch film residues on wheat (Triticum aestivum) growth.

Qi, Y.; Yang, Xiaomei; Pelaez, A.M. ; Huerta Lwanga, E.; Beriot, N.; Gertsen, H.; Garbeva, P.V.; Geissen, V.

published in
Science of the Total Environment
2018

DOI (link to publisher)
10.1016/j.scitotenv.2018.07.229

document version
Publisher's PDF, also known as Version of record

citation for published version (APA)
Qi, Y., Yang, X., Pelaez, A. M., Huerta Lwanga, E., Beriot, N., Gertsen, H., Garbeva, P. V., & 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*(15 december 2018), 1048-1056. https://doi.org/10.1016/j.scitotenv.2018.07.229

Science of the Total Environment 645 (2018) 1048–1056

Contents lists available at ScienceDirect

Science of the Total Environment

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

Macro- and micro- plastics in soil-plant system: Effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth☆

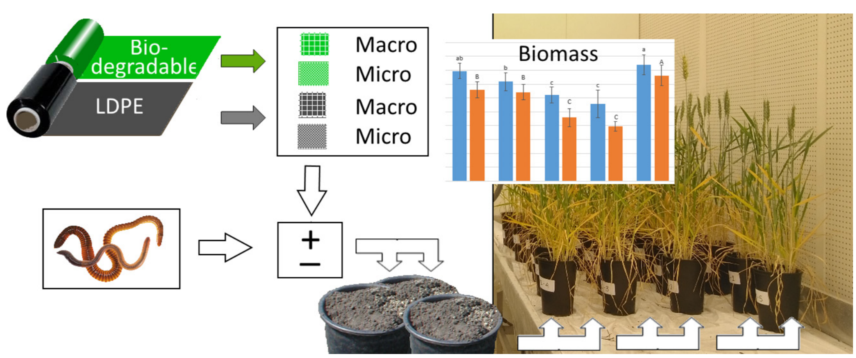
Yueling Qi [a,b,*], Xiaomei Yang [a,c], Amalia Mejia Pelaez [d], Esperanza Huerta Lwanga [a,e], Nicolas Beriot [a], Henny Gertsen [a], Paolina Garbeva [b], Violette Geissen [a]

[a] *Soil Physics and Land Management Group, Wageningen University & Research, Droevendaalsesteeg 4, 6708PB Wageningen, the Netherlands*
[b] *Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), 6700 AB Wageningen, the Netherlands*
[c] *College of Natural Resources and Environment, Northwest A&F University, 712100 Yangling, China*
[d] *Escuela Agrícola Panamericana, PO Box 93, Km 30 road from Tegucigalpa to Danli, Yeguare Valley, Municipality of San Antonio de Oriente. Francisco Morazan, Honduras.*
[e] *Agroecología, El Colegio de la Frontera Sur, Unidad Campeche, Av Poligono s/n, Ciudad Industrial, Lerma, Campeche, Mexico*

HIGHLIGHTS

- This is the first experimental study about effects of microplastics on wheat growth.
- Both plastic residues and soil organisms were studied in this soil-plant system.
- Biodegradable plastic residues showed stronger negative effects than polyethylene.
- Earthworms alleviated the impairments in wheat made by addition of plastic residues.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 May 2018
Received in revised form 16 July 2018
Accepted 17 July 2018
Available online xxxx

Editor: Shuzhen Zhang

Keywords:
Microplastics
Plastic residues
Biodegradable mulch film
Plant growth
Agroecosystem

ABSTRACT

Plastic residues have become a serious environmental problem in the regions with intensive use of plastic mulching. Even though plastic mulch is widely used, the effects of macro- and micro- plastic residues on the soil-plant system and the agroecosystem are largely unknown. In this study, low density polyethylene and one type of starch-based biodegradable plastic mulch film were selected and used as examples of macro- and micro- sized plastic residues. A pot experiment was performed in a climate chamber to determine what effect mixing 1% concentration of residues of these plastics with sandy soil would have on wheat growth in the presence and absence of earthworms. The results showed that macro- and micro- plastic residues affected both above-ground and below-ground parts of the wheat plant during both vegetative and reproductive growth. The type of plastic mulch films used had a strong effect on wheat growth with the biodegradable plastic mulch showing stronger negative effects as compared to polyethylene. The presence of earthworms had an overall positive effect on the wheat growth and chiefly alleviated the impairments made by plastic residues.

☆ Declarations of interest: none.

* Corresponding author at: Soil Physics and Land Management Group, Wageningen University & Research, Droevendaalsesteeg 4, 6708PB Wageningen, the Netherlands; Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), 6700 AB Wageningen, the Netherlands.
E-mail addresses: yueling.qi@wur.nl Y.Qi@nioo.knaw.nl (Y. Qi).

https://doi.org/10.1016/j.scitotenv.2018.07.229
0048-9697/© 2018 Published by Elsevier B.V.

1. Introduction

Microplastic pollution has been a hot topic since 2004 when Thompson et al. (2004) published a paper describing the distribution of microscopic plastic debris in seawater (Thompson et al., 2004). It is widely accepted that microplastics in aquatic ecosystems are serious threats that can have potentially negative effects on marine ecosystems, aquatic organisms and even human health (Sharma and Chatterjee, 2017; Syberg et al., 2015; Wright et al., 2013). Even though the term microplastic was already used in 1990 by Ryan and Moloney in their paper concerning surveys of South African beaches (Ryan and Moloney, 1990), 'microplastic' is still a poorly defined term without a universal standard so far (Law and Thompson, 2014). At present, the majority of the research performed in this area is focused on microplastics between 5 mm or 1 mm in size (Arthur et al., 2009; Browne et al., 2007; GESAMP, 2015; Verschoor, 2015).

Although soil, especially agricultural land, has become a major sink for microplastics (Browne et al., 2011; Mahon et al., 2017; Nizzetto et al., 2016a; Nizzetto et al., 2016b; Rillig, 2012; Zubris and Richards, 2005), most of the research done so far has been focused on microplastics in the aquatic ecosystem (Auta et al., 2017; Cole et al., 2011; Duis and Coors, 2016; Eerkes-Medrano et al., 2015; Koelmans et al., 2014; Koelmans et al., 2017; Nizzetto et al., 2016b). Plastics, especially polyethylene, are intensively used as mulch film in agriculture with the aim of improving the soil climate thus making it more beneficial to plant growth and increasing water use efficiency in (semi-) arid regions (Ekebafe et al., 2011). The current global usage of plastic mulch films is enormous and has been increasing in recent years (Brodhagen et al., 2017; Research, 2013). China has the biggest plastic mulch film usage worldwide with 19.8 million hectares of agricultural land covered by plastic mulch film (Changrong et al., 2014; Liu et al., 2014). Although the use of plastic mulch has numerous economic benefits, one devastating side effect is that the plastic is left in the soil after harvest (Brodhagen et al., 2017). Any attempts to recycle the plastic residues have been hampered by practical difficulties and high costs (Brodhagen et al., 2017; Kasirajan and Ngouajio, 2012; Steinmetz et al., 2016). Year after year new plastic residue is added to the soil and this constant accumulation, coupled with traditional tillage practices, results in a huge amount of mega-, macro- and micro- plastic particles being incorporated into the agricultural soils (Changrong et al., 2014; Liu et al., 2014; Rillig et al., 2017a; Steinmetz et al., 2016). The environmental concerns stemming from residual mulch film has aroused the interest of scientists and studies have shown that mulch film residues can reduce soil quality and crop production (Dong et al., 2015; Jiang et al., 2017; Zhang et al., 2016). Even though biodegradable plastic mulch films were invented in an attempt to decrease plastic residues in agricultural land and touted as promising alternatives to traditional polyethylene mulch films, these seemingly more environmentally friendly films have aroused debate concerning their use (Changrong et al., 2014; Moreno et al., 2017; Ren, 2003; Sintim and Flury, 2017; Yang et al., 2014).

In recent years, soil scientists have made progress in researching microplastics in terrestrial ecosystems and new techniques for quantifying and identifying microplastics in the soil have been developed, applied and debated (Bläsing and Amelung, 2018; Claessens et al., 2013; Elert et al., 2017; Zhang et al., 2018). However, there are still only a few studies that have been focused on the effect of microplastics in the terrestrial environment (Chen, 2016; Zhang et al., 2018; Zhou et al., 2018). The presence of microplastics in the soil could change soil properties and microplastics may be transported by soil organisms or act as vectors for other soil pollutants (Hodson et al., 2017; Liu et al., 2017; Maass et al., 2017; Rillig et al., 2017b; Zhu et al., 2018a). Recently, Huerta Lwanga et al. completed a series of research projects concerning microplastics in soil which examined the effects on earthworms on plastics in soil, transferability of plastics in a terrestrial food chain and the possibility of restoring microplastic-polluted soils using bacteria. (Huerta Lwanga et al., 2016; Huerta Lwanga et al., 2017a; Huerta Lwanga et al., 2017b; Lwanga et al., 2018). Zhu et al. proved that microplastics can disturb the collembolan gut microbiota and enhance the diversity of gut bacteria (Zhu et al., 2018b). Even though there is a growing concern about the microplastic pollution in terrestrial ecosystems, so far there has been no experimental research concerning both macroplastics and microplastics in the soil-plant system and the effects that this could have on plant growth (Cao et al., 2017; Ng et al., 2018; Nizzetto et al., 2016a; Nizzetto et al., 2016b; Rillig, 2012).

With this current research, we aimed to take the first steps towards filling the gaps left by past studies and focused on the previously neglected area of research concerning microplastics in the soil-plant system. Here, we tested the effects of two different sizes of polyethylene and biodegradable plastic mulch film residues in a soil system with and without the presence of earthworms. Both earthworms and plastic residues are known to alter soil properties and they are likely to interact through various mechanisms (Bertrand et al., 2015; Cao et al., 2017; Huerta Lwanga et al., 2016; Huerta Lwanga et al., 2017a; Rillig et al., 2017b; van Groenigen et al., 2014). In the present study, we performed a greenhouse pot experiment using wheat (*Triticum aestivum*) as a model plant and low-density polyethylene and a starch-based biodegradable plastic with realistic filed concentration of 1% (w/w) as the applied plastic residues (Chen, 2016; Tao et al., 2012; Zhang et al., 2018; Zhang et al., 2015). The experiment was performed with and without *Lumbricus terrestris* as the model earthworm. We hypothesized that the type (polyethylene/biodegradable) and the size (macro-/micro-) of the plastic residues as well as the presence or absence of earthworms effect plant growth and these effects are interactive.

2. Materials and methods

2.1. Experimental design

2.1.1. Facilities and soil

A pot experiment was conducted to investigate the effects of different types and sizes of plastic mulch film residues on wheat (*Triticum aestivum*) in a climate chamber (Klima C7) at Unifarm, Wageningen University & Research (WUR), the Netherlands. We harvested the wheat at two time points (after 2 and 4 months) in order to examine the effects of our experiments on both vegetative and reproductive growth. The sandy soil used in this study was obtained from the agricultural land in Wageningen, the Netherlands, collected by Unifarm, WUR. The soil consisted of 87% sand, 12% slit and 1% clay with an organic matter content of 4% (More information about the soil properties are presented in Fig. S1). Before use, the air-dried soil was sieved through a 2 mm steel sieve.

2.1.2. Plastic materials

Two types of plastic mulch films were applied in this experiment: (1) low-density polyethylene (LDPE) and (2) starch-based biodegradable plastic (Bio). The biodegradable plastic film consisted of 37.1% Pullulan, 44.6% Polyethylene Terephthalate (PET) and 18.3% Polybutylene Terephthalate (PBT).

To obtain macroplastics (Ma), pieces of plastics were cut on a hard wooden board using sharp blades and scissors. The same procedures were carried out for both types of plastic films. After cutting, we randomly chose 100 pieces of plastic from each sort and measured their widths and lengths. For LDPE Ma, the average length was 6.92 $\pm$ 1.47 mm and the average width was 6.10 $\pm$ 1.39 mm. For Bio Ma, the average length was 6.98 $\pm$ 1.61 mm and the average width was 6.01 $\pm$ 1.31 mm.

To obtain microplastics (Mi), the plastics were first cut into pieces, frozen with liquid nitrogen and then ground into a powder. After grinding, the resulting powder was sieved through 1 mm, 500 μm, 250 μm and 50 μm sieves in order to divide the plastics into size categories. We mixed the selected categories of plastic using the following ratio:

12.5% of 1 mm to 500 μm, 62.5% of 500 μm to 250 μm and 25% of 250 μm to 50 μm. For LDPE Mi and Bio Mi, all the processing procedures were the same.

In this study, we used 1% (w/w) content as the practicable and instructive setting to simulate the plastic mulch film residues in agricultural soil according to field survey and literature review (Chen, 2016; Tao et al., 2012; Zhang et al., 2018; Zhang et al., 2015).

2.1.3. Wheat seeds and pots

Wheat seeds (*Triticum aestivum*) were provided by Unifarm, WUR. Before being sowed, the mass of each seed was measured and only the seeds with a mass between 0.04 g and 0.05 g were used. The germination rate in this experiment was high at >80% (Table S1). The pots used in the experiment were 18 cm high with a diameter of 10 cm at the bottom and 13 cm at the top and had a volume of 2 L.

2.1.4. Earthworms and litter

We used the anecic earthworm *Lumbricus terrestris* in this study. *L. terrestris* were purchased from the Star Food Company (Barneveld, the Netherlands). From the 300 earthworms we received, we selected 100 adults with average weight of 3.72 ± 0.55 g. The biomass and mortality of earthworms were checked at the final harvest (Table S2). The commonly consumed plant litter for L. *terrestris* is *Populus nigra*, so we collected the leaves of this plant from natural areas in Wageningen, the Netherlands. These leaves were carefully cleaned and dried at 60 °C.

2.1.5. Treatments and replicates

Three factors were taken into consideration while planning the experiment: types of plastics (LDPE/Bio), sizes of plastic residues (Ma/Mi) and presence or absence of earthworms (WE: With Earthworms/NE: No Earthworms). A factorial experiment design 2^3 (three factors with two levels) was adopted. In addition, two control treatments without any plastic residues were also examined. Overall the experiment consisted of 10 treatments (Table 1). Ten replicates were made for each treatment and a total of 100 pots of wheat were cultivated.

2.2. Setting up and growth conditions

2.2.1. Setting up

For each pot, 1500 g of sieved soil and 15 g of plastic material (except for the two Control treatments with no plastic) were weighed and manually mixed with 150 g of water. Before filling the pot with this mixture, a piece of geotextile was placed in the bottom of each pot to prevent earthworms from escaping and to let air and water flow freely. After all the pots were filled, the soil moisture was unified to 15% similar to the soil field capacity. All the pots were allowed to settle down for a period of one week before wheat seeds were sowed.

Ten days after sowing, two adult earthworms were added to the pots used in the WE treatment group to avoid the possibility of the worms eating the seeds before germination (Fründ et al., 2010). Around 12 g of litter (12.08 ± 0.06 g) was added to the surface of each pot and water was sprayed on the litter to make it moist.

Table 1
Treatments setting for the experiment.

Group	Treatments	TYPE		SIZE	
		LDPE	Bio	Macro	Micro
WE	LDPE-Ma	√		√	
	LDPE-Mi	√			√
	Bio-Ma		√	√	
	Bio-Mi		√		√
	Control	/	/	/	/
NE	LDPE-Ma	√		√	
	LDPE-Mi	√			√
	Bio-Ma		√	√	
	Bio-Mi		√		√
	Control	/	/	/	/

2.2.2. Cultivation of wheats

Five seeds were sowed in each pot and after two weeks of growth, 3 seedlings per pot were selected and retained for the experiment. The following controlled conditions were applied: temperature was set at 22 °C during the day and 17 °C during the night, day/night photoperiod (14/10 h) with a light intensity of 300 μmol m^{-2} s^{-1} and a relative humidity of 70% for both day and night. The pots were watered weekly with tap water and the soil moisture was kept at around 12% to 18% with respect to weight. 100 mL of a nutritive solution was added to each pot once a week during the fifth week and the tenth week after the seeds were sowed. Reagents and concentrations of compounds in the nutritive solution are presented in Fig. S2. Pots were randomly placed within the climate chamber and their positions were shifted once a month.

2.3. Measurements of wheat growth parameters

Plant heights were measured regularly from the 14th day after seeds were sowed to the 139th day using a steel tape measure. The number of tillers were counted and recorded from the 20th day until the 139th day and the fruits were counted and recorded regularly from the 61st day until the 139th day.

The plants were harvested at two time points. For each treatment, five replicates were harvested at 2 months (61st day) when the flag leaf appeared and the wheat started to bear fruit. The remaining 5 replicates were further cultivated and harvested at 4 months (139th day) after mature wheat grains had developed. Plants were separated into shoots and roots at the 2 months harvest and shoots, fruits and roots at the 4 months harvest. Dry biomasses were recorded after drying at 70 °C to a constant weight.

For the 2 months harvest, the stem diameter, number of leaves, leaf area and relative chlorophyll content were measured and recorded. Stem diameters were measured using a vernier caliper. Leaf areas were measured using the LI-3100C Laboratory Leaf Area Meter (LI-COR Biosciences, USA). Relative chlorophyll content was measured using SPAD-502plus (Minolta, USA) at the middle and tip of three fully developed leaves on 61st day for all three plants in each pot.

2.4. Statistical analysis

All statistical data analyses were performed using IBM SPSS Statistics 23 and CANOCO 5. Values from observations were recorded for each plant and were then averaged for each pot. All errors are indicated as standard deviations. The data were screened for normal distribution using q-q plots and Shapiro-Wilk tests and homogeneity of variance using Levene's test. Comparisons among treatments were performed by two independent one way ANOVA and followed by Tukey HSD test at the $p < 0.05$ level (group WE/group NE). When data violated the assumption of homogeneity of variances, a Welch ANOVA and a Games-Howell test were carried out. Comparisons between WE and NE groups were performed by Independent-Samples *t*-Test at the $p < 0.05$ level. The effects of all three factors (type of plastics, size of residues and earthworms) and their interactive effects were tested using a three way ANOVA. The contributions of the factors and their interactions on the parameters were calculated by dividing their sum of squares by the total sum of squares. The relationships between the treatment factors and the plant growth parameters were identified through Redundancy Analysis by CANOCO 5. 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.

3. Results

3.1. Wheat development: plant height, number of tillers and fruits during the growth process

3.1.1. Plant height

The Bio Ma and Bio Mi addition inhibited wheat growth with respect to plant height, while the addition of LDPE Ma and LDPE Mi showed no clear effects relative to the Control during the tillering stage of growth (around 14th day until 40th day) (Fig. 1a, Fig. 1b). During the stem extension stage (around 40th day until 68th day), wheat plants in Bio-Ma and Bio-Mi treatments entered a rapid elongating period (Fig. 1a, Fig. 1b). At the 2 months harvest, wheat plants in the WE group showed no significant difference among treatments (Table S3). In group NE, wheat plants in the treatment Bio-Ma (491 ± 35.02 mm) had the highest plant height and those in the LDPE-Ma (415 ± 27.40 mm) treatment had the lowest plant height but none of the treatments showed significant differences from the Control (451 ± 30.89 mm) (Table S3). At the 4 months harvest, the height of wheat plants in all treatments turned out to be similar and wheat plants in group NE (584 ± 27.86 mm) had similar plant heights as group WE (578 ± 30.48 mm) (Table S3, Table S4).

3.1.2. Number of tillers

Wheat in most of the treatments started tillering from the 20th day on, while wheat in the Bio-Ma and Bio-Mi treatments in group NE had a two week delay in tillering compared to the other treatments (Fig. 1c, Fig. 1d). Overall, the number of tillers per plant grew stably during the process and no significant differences among treatments in group WE at the 4 months harvest were seen (Table S3). For the NE group, the number of tillers of wheat in the Control (5.5 ± 0.67) = LDPE-Ma (5.5 ± 0.71) = LDPE-Mi (5.5 ± 0.32) > Bio-Mi (4.2 ± 0.58) = Bio-Ma (4.1 ± 0.37) at the 4 months harvest (Table S3). At the final harvest, the wheat plants in group WE (6.0 ± 1.22) had significantly more tillers than those in group NE (5.0 ± 0.86) (Table S4).

3.1.3. Number of fruits

From 61st day to 75th day, most of the wheat plants entered the booting and heading stages and only a few fruits appeared (Fig. 1e, Fig. 1f). The number of fruits per plant then rapidly increased from 75th day to 89th day and it slowly increased between 89th day and 117th day (Fig. 1e, Fig. 1f). From 117th day on, the number of fruits per plant became stable and then the final ripening stage began (Fig. 1e, Fig. 1f). At the 4 months harvest, wheat plants in group WE had borne a similar number of fruits; in group NE, wheat plants in the treatment Bio-Ma (2.8 ± 0.16) bore significantly less fruits than those in treatments LDPE-Ma (3.4 ± 0.30) and LDPE-Mi (3.6 ± 0.30), but none of them showed a significant difference from the Control (3.7 ± 0.76) or Bio-Mi (2.9 ± 0.45) (Table S3). On average, wheat plants in group NE (3.3 ± 0.55) bore significantly less fruits than those in group WE (4.0 ± 0.68) (Table S4).

3.2. Plant biomass and its allocation: effects of plastic residues, earthworms and their interactions

3.2.1. Shoot biomass and root biomass

At the 2 months harvest, both in group WE and NE, shoot biomass was significantly lower in treatments Bio-Ma and Bio-Mi and there

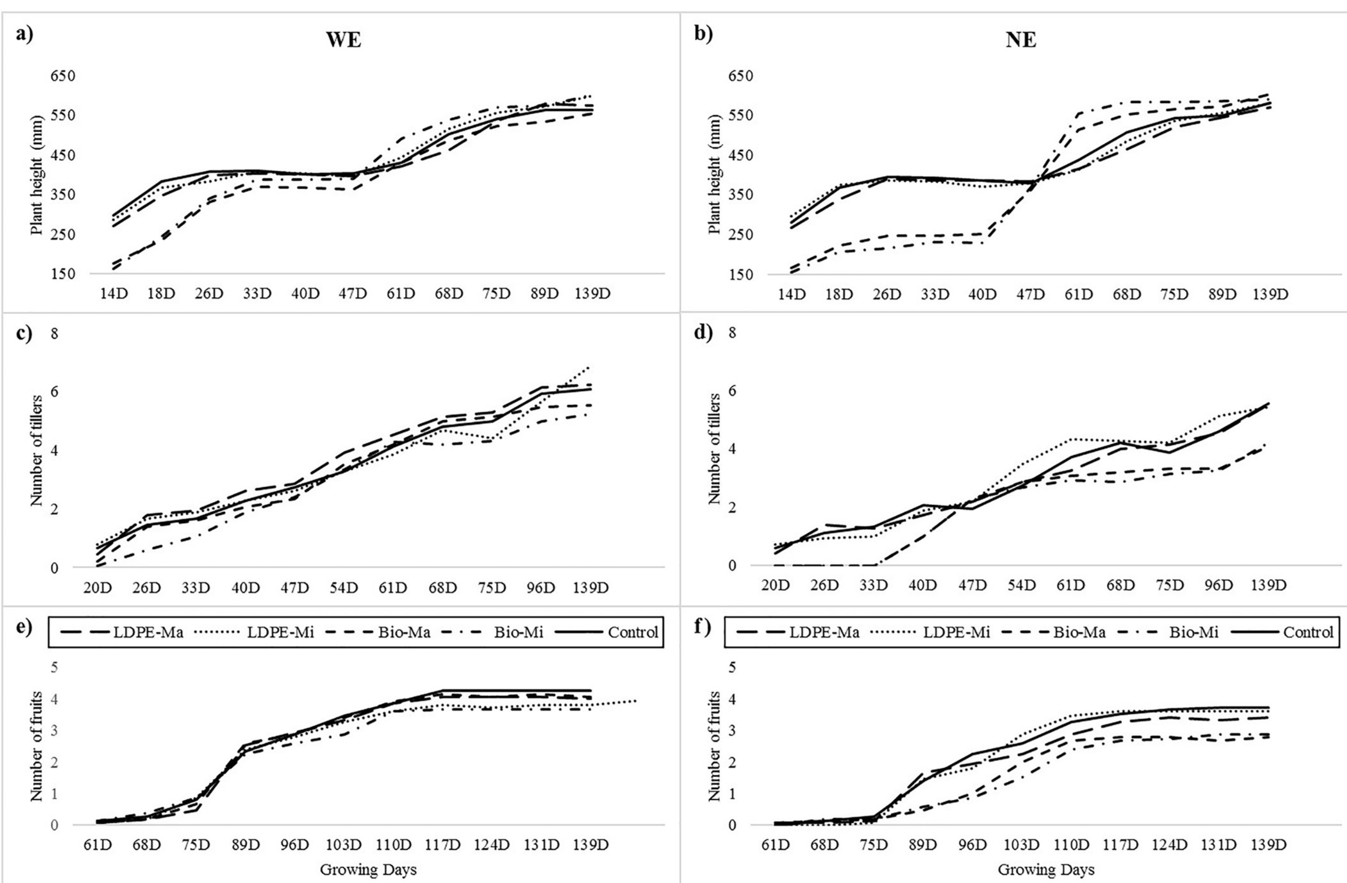


Fig. 1. Plant height, number of tillers and fruits in the process of wheat growth; a) plant height for treatments in group with earthworms; b) plant height for treatments in group no earthworms; c) number of tillers for treatments in group with earthworms; d) number of tillers for treatments in group no earthworms; e) number of fruits for treatments in group with earthworms; f) number of fruits for treatments in group no earthworms.

was no significant difference in treatments LDPE-Ma and LDPE-Mi compared to the Control (Fig. 2a). At the 4 months harvest, in group WE, only wheat plants in treatment Bio-Mi had significantly lower shoot biomass than in the Control. In group NE, only the treatment Bio-Ma had significantly lower shoot biomass than LDPE-Mi (Fig. 2b).The presence of earthworms significantly enhanced the shoot biomass by 19.9% at the 2 months harvest and 18.6% at the 4 months harvest (Table S4).

There was no significant difference in root biomass in group WE relative to the Control in either harvest, but in group NE, with addition of plastic residues, all the wheat plants had significantly lower root biomass than the Control at the 2 months harvest (Fig. 2c, Fig. 2d). The presence of earthworms significantly increased root biomass by 22.3% at the 2 months harvest and the root biomass in group WE (2.082 ± 0.494 g) was similar to group NE (1.921 ± 0.476 g) at the 4 months harvest (Table S4).

3.2.2. Total biomass, fruit biomass and root/shoot ratio

Total plant biomass was significantly reduced by the addition of plastic residues and the Bio-Mi treatment in group NE had the lowest biomass value at both the 2 months harvest (2.633 ± 0.220 g) and the 4 months harvest (7.478 ± 1.015 g) (Table S5, Table S6). For the WE group, the plant total biomass in treatments Bio-Ma (4.135 ± 0.382 g) and Bio-Mi (3.710 ± 0.671 g) were significantly lower than the Control (5.593 ± 0.471 g) at the 2 months harvest but no significant difference was found among treatments at the 4 months harvest (Table S5, Table S6). The presence of earthworms significantly increased the total biomass for wheat by 20.9% at the 2 months harvest and 26.2% at the 4 months harvest (Table S4).

Fruit biomass in Bio-Ma and Bio-Mi were significantly lower than in the Control in group NE and the addition of plastic residues exerted no significant effect on fruit biomass in group WE (Table S6). Wheats in group WE (4.857 ± 0.459 g) had significantly higher fruit biomass than group NE (3.383 ± 0.401 g) (Table S4).

At the 2 months harvest, wheat plants in Bio-Mi had the highest root/shoot ratio (R/S) (0.93 ± 0.172 in WE and 0.87 ± 0.127 in NE) and wheat plants in LDPE-Mi had the lowest R/S (0.54 ± 0.083 in WE and 0.55 ± 0.079 in NE) (Table S5). At the 4 months harvest, wheat plants in Bio-Ma had the highest R/S (0.30 ± 0.057) and wheat plants in the Control had the lowest R/S (0.20 ± 0.043) in group WE, but no significant difference was found among treatments in group NE (Table S6). The presence of earthworms had no significant effect on R/S at the 2 months harvest but significantly decreased R/S (0.24 ± 0.060 in WE and 0.29 ± 0.067 in NE) at the 4 months harvest (Table S4).

3.2.3. Type and size of plastic residues, earthworms and their interactive effects on wheat biomass

The type of plastic had significant effects on almost all of the biomasses parameters except root biomass and R/S at the 4 months harvest and it explained 63.88%, 52.07% and 47.77% of the variability in the shoot biomass, total biomass and R/S at the 2 months harvest, respectively (Table 2). The size of plastic residues only had significant effects on the root biomass and total biomass at the 2 months harvest which explained 9.55% and 4.14% of the variability found (Table 2). The presence of earthworms had significant effects on plant biomasses but not on R/S at both the 2 months and the 4 months harvest (Table 2). Root biomass and R/S at the 2 months harvest were significantly affected by the Type × Size interaction and root biomass and R/S at the 4 months harvest were significantly affected by the Size × EW interaction. Neither Type × EW nor Type × Size × EW interactions had significant effects on the plant biomasses parameters. For root biomass and R/S at the 4 months harvest, the three factors and their interactions explained less than half of the variability according to the residual contributions to these parameters.

3.3. Parameters of wheat vegetative growth: leaf area, number of leaves, relative chlorophyll content and stem diameter

In group NE, plants in treatments Bio-Ma and Bio-Mi had significantly smaller leaf areas than the Control. The addition of LDPE residues had no significant effects on leaf area compared to the Control (Fig. 3a). In group WE, plants in treatments LDPE-Ma (240.6 ± 33.96 cm^2) had the largest leaf area, followed by the Control (196.7 ± 25.32 cm^2) =

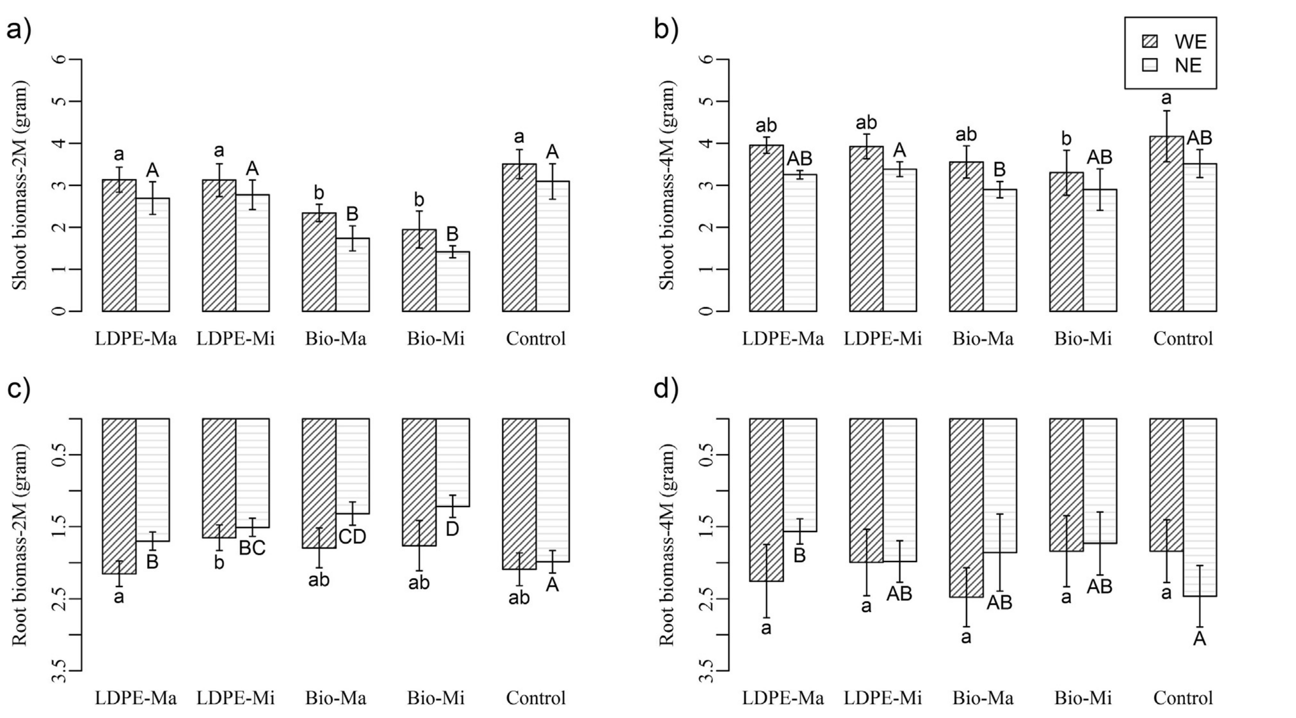


Fig. 2. Shoot biomass and Root biomass of all the treatments at 2 months and 4 months harvests; a) shoot biomass for all the treatments at 2 months harvest; b) shoot biomass for all treatments at 4 months harvest; c) root biomass for all treatments at 2 months harvest; d) root biomass for all treatments at 4 months harvest. (2 M: 2 months harvest, 4 M: 4 months harvest).

Table 2
p-Value and contribution of independent factors (type of plastics, size of plastic residues and earthworms) and their interactions to plant biomass parameters studied by three way ANOVA.

	Type		Size		EW		Type ∗ Size		Type ∗ EW		Size ∗ EW		Type ∗ Size ∗ EW		Residual
	%	Sig.	%	Sig.	%	Sig.	%	Sig.	%	Sig.	%	Sig.	%	Sig.	%
SB2M	63.88	<0.001	1.44	ns	12.85	<0.001	2.14	ns	0.41	ns	0.09	ns	0.00	ns	19.19
RB2M	12.10	0.001	9.55	0.004	37.43	<0.001	4.45	0.04	2.60	ns	0.81	ns	2.00	ns	31.06
TB2M	52.07	<0.001	4.14	0.01	24.10	<0.001	0.10	ns	1.14	ns	0.31	ns	0.29	ns	17.84
RS2M	47.77	<0.001	0.00	ns	0.56	ns	14.47	0.001	0.09	ns	0.00	ns	0.83	ns	36.18
SB4M	23.59	<0.001	0.16	ns	35.90	<0.001	0.86	ns	0.25	ns	1.12	ns	0.06	ns	38.06
FB	3.93	0.01	0.04	ns	76.82	<0.001	0.84	ns	0.87	ns	0.61	ns	0.32	ns	16.57
RB4M	0.08	ns	2.64	ns	14.48	0.013	5.98	ns	0.01	ns	9.95	0.036	0.19	ns	66.67
TB4M	6.50	0.008	0.25	ns	64.05	<0.001	2.41	ns	0.16	ns	0.71	ns	0.14	ns	25.78
RS4M	7.36	ns	1.84	ns	4.91	ns	3.68	ns	0.61	ns	9.82	0.045	0.00	ns	71.17

Type: type of plastics; Size: size of plastic residues; EW: earthworms; SB2M: shoot biomass at 2 months harvest; RB2M: root biomass at 2 months harvest; TB2 M: total biomass at 2 months harvest; RS2M: root/shoot ratio at 2 months harvest; SB4M: shoot biomass at 4 months harvest; RB4M: root biomass at 4 months harvest; TB4 M: total biomass at 4 months harvest; RS4M: root/shoot ratio at 4 months harvest; FB: fruit biomass.

LDPE-Mi (190.2 ± 13.30 cm^2) > Bio-Ma (147.1 ± 13.88 cm^2) = Bio-Mi (127.7 ± 9.56 cm^2) (Table S7). The presence of earthworms significantly increased the leaf area of the wheat plants in group WE (180.5 ± 44.89 cm^2) compared to group NE (153.4 ± 35.11 cm^2) (Table S4).

In both groups WE and NE, plants in treatments Bio-Ma and Bio-Mi had significantly fewer leaves compared to the Control and plants in LDPE-Ma and LDPE-Mi had a similar number of leaves as the Control (Fig. 3b). Wheat plants in group WE (20.2 ± 4.28) had significantly more leaves than those in group NE (17.0 ± 3.29) (Table S4).

Plants did not differ significantly in their relative chlorophyll content among treatments in both groups, but group WE (46.2 ± 2.75) had a significantly higher value than group NE (42.3 ± 2.93) (Fig. 3c, Table S4).

Wheat plants in Bio-Mi had the thinnest stems and the plants in LDPE-Ma and LDPE-Mi had a similar stem diameter as the Control in both groups (Fig. 3d). Wheat plants in Bio-Ma had comparable stem diameters to the Control in group WE and significantly thinner stems than plants in the Control in group NE (Fig. 3d). Stem diameters of wheat plants in group WE (3.58 ± 0.251 mm) and NE (3.42 ± 0.390 mm) showed no significant difference (Table S4).

3.4. The relationships of treatment factors with wheat growth parameters

The relationships among the measured parameters of wheat growth and treatment factors (plastic residues: LDPE-Ma, LDPE-Mi, Bio-Ma, Bio-Mi and Control, earthworms: WE and NE) is described in an ordination diagram (Fig. 4). The Monte Carlo permutation tests indicated significant differences among all canonical axes ($p < 0.01$) and the first axis explained 54.91% of the variation in the parameter-factor relationships (Table S8). The groups WE and NE are completely opposed in the factorial plan and factor WE stand in the positive direction of fruit biomass, relative chlorophyll content, total biomass and other parameters. For treatment factors of plastic residues, Bio-Ma and Bio-Mi clustered together, while LDPE-Ma, LDPE-Mi and the Control clustered together in the opposite direction. Plant height and root/shoot ratio clustered together in the opposite direction of other plant growth parameters.

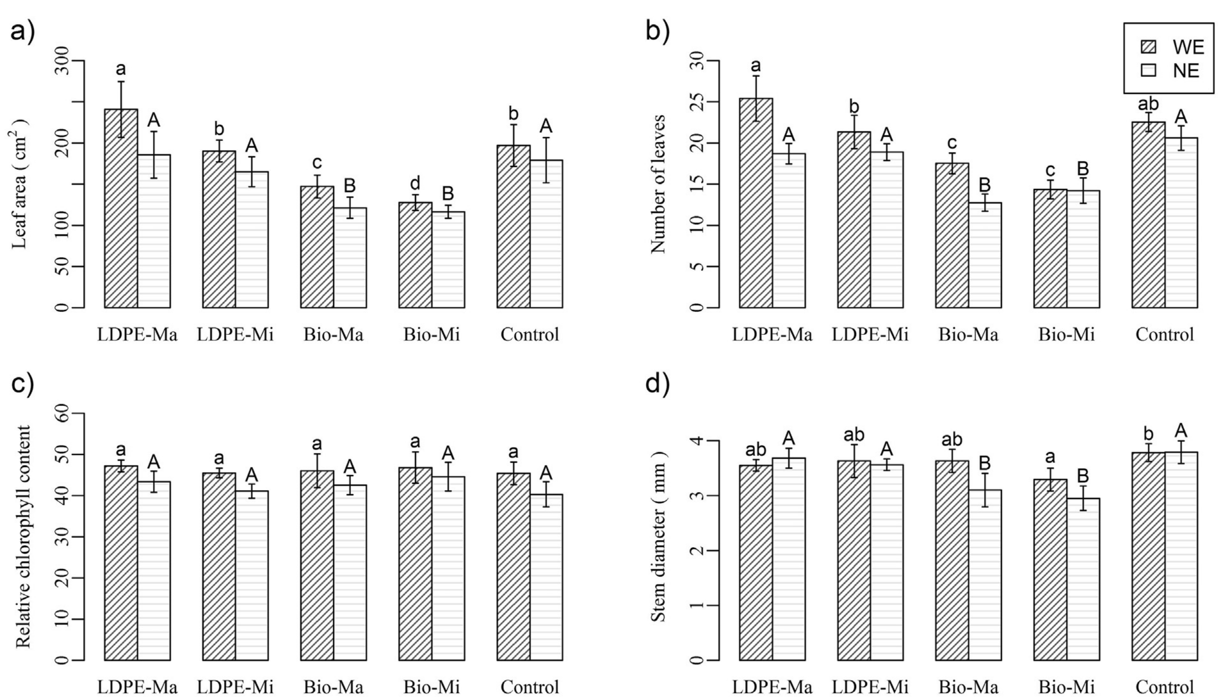


Fig. 3. Leaf area, number of leaves, relative chlorophyll content and stem diameter for all treatments; a) leaf area for all treatments; b) number of leaves for all treatments; c) relative chlorophyll content for all treatments; d) stem diameter for all treatments.

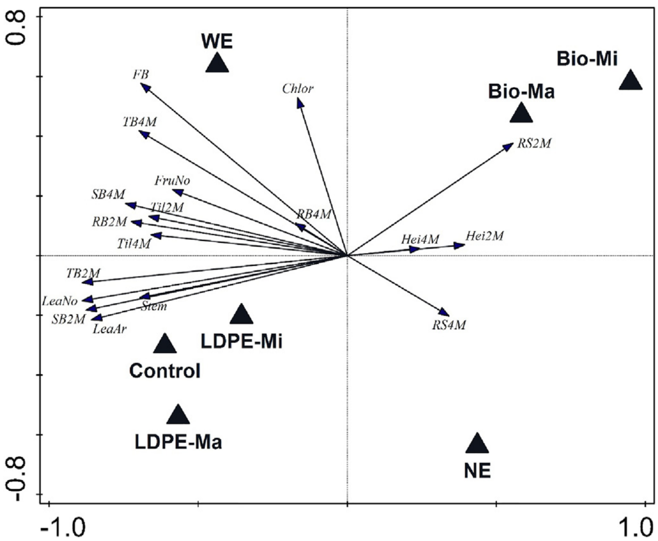


Fig. 4. Redundancy analysis ordination diagram of plant growth parameters with treatment factors (Hei2M: plant height at 2 months harvest; Hei4M: plant height at 4 months harvest; Til2M: number of tillers at 2 months harvest; Til4M: number of tillers at 4 months harvest; FruNo: number of fruits; SB2M: shoot biomass at 2 months harvest; RB2M: root biomass at 2 months harvest; TB2 M: total biomass at 2 months harvest; RS2M: root/shoot ratio at 2 months harvest; SB4M: shoot biomass at 4 months harvest; RB4M: root biomass at 4 months harvest; TB4 M: total biomass at 4 months harvest; RS4M: root/shoot ratio at 4 months harvest; FB: fruit biomass; LeaAr: leaf area; LeaNo: number of leaves; Chlor: relative chlorophyll content; Stem: stem diameter.)

4. Discussion

Looking back at the original hypotheses, several key findings emerged from this study: 1) type of plastic mulch films has strong effects on wheat growth with the biodegradable film showing stronger negative effects compared to polyethylene; 2) size of plastic residues has weak effects on wheat growth with microplastics showing more negative effects than macroplastics; 3) presence/absence of earthworms has strong effects on plant growth and the presence of earthworms positively altered wheat growth status and chiefly alleviated the impairments made by plastic residues; 4) neither the interactions between two factors nor the interaction among three factors is notable in this experiment.

In this study, we only used one type of starch-based biodegradable plastic mulch film and one low density polyethylene film. This specific type of biodegradable plastic mulch film residue showed more severe effects on wheat growth than the polyethylene film in both macro and micro sizes. This result is admissible when the composition of this biodegradable film (37.1% Pullulan, 44.6% PET and 18.3% PBT) has been taken into consideration. Even though deeper investigations should be conducted in order to study the underlying mechanisms, the plausible explanation that PET and PBT may have more negative effects on soil-plant system than LDPE could be drawn based on this study as well as other studies (Muroi et al., 2016; O'Hara et al., 2013; Parvathy et al., 2014). Even this type of biodegradable plastic should not be used to represent all the biodegradable plastic mulch films, it is merely one of the widely used types currently on the market (van den van den Oever et al., 2017). Despite all of the doubts about the cogency and rigor of these films, great expectations have been placed on these biodegradable plastic mulch films with the aim of solving plastic pollution in agricultural land (Kasirajan and Ngouajio, 2012; Moreno et al., 2017; Ren, 2003; Sintim and Flury, 2017; Yang et al., 2014). Based on the results of this study, we should not be too optimistic about using biodegradable plastic mulch films in agriculture without first conducting in-depth studies. Also, with the current boom in the bioplastics market, newly developed biodegradable plastics have been applied as agricultural mulch films. Hence, the different types of synthetic polymers and bioplastics should be closely studied for their occurrence, fate and ecological effects in the soil and for their effects on the soil-plant system.

According to this study, macro- or micro- sizes of plastic residues showed slightly differing effects on wheat growth and microplastics showed more negative effects than macroplastics. So far, most research concerning LDPE mulch film residues have focused on larger sizes (length > 2 cm) (Zhang et al., 2016). Dong et al. (2015) applied a mixture of three size classes of film residues (0–25 cm^2:25–100-cm^2:100–200 cm^2 = 1:1:1) onto soil with a range of densities from 0 to 2000 kg hm^{-2} (representative for field after 141 years of mulching) and they found that cumulative residue could decrease cotton yield after 121 years of mulching (Dong et al., 2015). Tao et al. (2012) conducted a pot experiment using horse bean mixed with three molecular weights (2000, 5000 and 10,000) of LDPE powder at different cumulants (0.0028%, 0.028%, 0.14% and 0.28% representing for 1, 10, 50 and 100 years). They found that accumulated LDPE powder may have the potential to improve the soil microenvironment (Tao et al., 2012). Zhang et al. (2015) had similar results from a field experiment using corn and found that large amounts of accumulated LDPE residues (with the maximum content of 0.35%) may improve soil fertility (Zhang et al., 2015). Looking back at our results, with 1% LDPE residues in the soil, the growth of wheat plants was negatively affected. Thus, in order to have a comprehensive understanding of plastic residues in the soil-plant system, further studies need to examine a range of different sizes (from 5 mm to 5 cm) and different concentration (from 0.2% to 1%) of plastic residue. For future in-depth research, a range of different microplastics contents could be applied to soil to scrutinize the threshold values of no effect, slight effect and severe effect and lay foundations for the ecological risk assessment of microplastics in the terrestrial ecosystem.

From this study, it is clear that the presence of earthworms positively altered wheat growth status and chiefly alleviated the impairments caused by plastic residues. Compared to other relevant studies, the mortality of earthworms is relatively high in this study (Cao et al., 2017; Huerta Lwanga et al., 2016; Huerta Lwanga et al., 2017a), which may indicate that it is not wise to study both plants and fauna in one experiment. Considering the limited space in the pot, the growing roots and earthworms may have competed with each other which makes it very difficult to study this interaction mechanism profoundly (Blouin et al., 2013; van Groenigen et al., 2014). Still, the results gave some hints to the effects of plastic residues on earthworms in the soil-plant system. Even though earthworms in the treatments with biodegradable plastic residues (Bio-Ma and Bio-Mi) lost more weight than those in the treatments with low density polyethylene residues (LDPE-Ma and LDPE-Mi), newly born earthworms were only found in Bio-Ma and Bio-Mi and the Control. We could speculate that this biodegradable plastic had more effects on the growth of earthworms, while LDPE have more effects on their breeding. Based on this experiment and other relevant studies, we suggest that deliberate equipment should be developed in order to learn more about the interactions between plants and soil fauna in the soil-plant system (Huerta Lwanga et al., 2016; Huerta Lwanga et al., 2017a). In addition, studying different species of earthworms in the soil-plant system is needed in order to learn more about soil organisms interacting with plastic residues.

For the doubts at present, the underlying reason why the wheat growth was effected has not been traced. One possible explanation could be that the micro- and macro- plastic residues in the soil altered the soil properties. In addition, microorganisms, rhizosphere bacteria in particular, play an important role in plant growth (Kaushal and Wani, 2016). Therefore, an investigation into the structure and the diversity of the microbial community may shed light on this as well.

In the long term, the microplastics currently found in the soil have a large chance of forming nanoplastics (Lambert and Wagner, 2016; Lwanga et al., 2018). Taking the nanoplastics found in aquatic ecosystem as references, nanoplastics have been shown to have an effect on the feeding behavior, growth, and reproduction of several aquatic organisms (Besseling et al., 2014; Wegner et al., 2012) as well as effects on the growth and/or photosynthesis of algae (Bhattacharya et al., 2010; Sjollema et al., 2016). With comparable properties to other nanoparticles, the nanoplastics may be transferred and accumulate in plants which have the risk of being ingested by humans (Larue et al., 2012; Rico et al., 2011). Hence, studying microplastics in the agricultural soil is of crucial importance to the ecological environment and human health.

Overall, our study revealed that macro- and micro- plastic residues of polyethylene and biodegradable mulch films have negative effects on both above-ground and below-ground parts of wheat and affect both vegetative and reproductive growth. Undoubtedly, more research is urgently needed in order to fully understand the effects of microplastics on the soil-plant system and the agroecosystem.

Acknowledgments

Funding for this research came from the Fundamental Research Funds for the Central Universities (Z109021717), the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, China (A314021402–1702) and the EU Horizon 2020 project (ISQAPER: 635750). We are thankful for the financial support from the China Scholarship Council (CSC: 201604910510). Many thanks to Rinie Verwoert, Taede Stoker and Gerrit Stunnenberg for help cultivating plants in the climate chamber at Unifarm, WUR. Thanks to Harm Gooren and Piet Peters for help in transporting materials and setting up this experiment. Thanks to Bert Meurs for sharing the chlorophyll meter SPAD 502 plus. Thanks to Coleen Carranza for helping draw the graphs. Yueling Qi would like to thank Sanna Kosh for the company and support during the trial stage of this study. This is NIOO-KNAW publication number 6554.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.07.229.

References

Arthur, C., Baker, J.E., Bamford, H.A., 2009. Proceedsings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008. University of Washington Tacoma, Tacoma, WA, USA.

Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. Environ. Int. 102, 165–176.

Bertrand, M., Barot, S., Blouin, M., Whalen, J., de Oliveira, T., Roger-Estrade, J., 2015. Earthworm services for cropping systems. A review. Agron. Sustain. Dev. 35, 553–567.

Besseling, E., Wang, B., Lurling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ. Sci. Technol. 48, 12336–12343.

Bhattacharya, P., Lin, S.J., Turner, J.P., Ke, P.C., 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J. Phys. Chem. C 114, 16556–16561.

Bläsing, M., Amelung, W., 2018. Plastics in soil: analytical methods and possible sources. Sci. Total Environ. 612, 422–435.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., et al., 2013. A review of earthworm impact on soil function and ecosystem services. Eur. J. Soil Sci. 64, 161–182.

Brodhagen, M., Goldberger, J.R., Hayes, D.G., Inglis, D.A., Marsh, T.L., Miles, C., 2017. Policy considerations for limiting unintended residual plastic in agricultural soils. Environ. Sci. Pol. 69, 81–84.

Browne, M.A., Galloway, T., Thompson, R., 2007. Microplastic—an emerging contaminant of potential concern? Integr. Environ. Assess. Manag. 3, 559–561.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., et al., 2011. Accumulation of microplastic on shorelines Woldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179.

Cao, D., Wang, X., Luo, X., Liu, G., Zheng, H., 2017. Effects of polystyrene microplastics on the fitness of earthworms in an agricultural soil. IOP Conference Series: Earth and Environmental Science. 61.

Changrong, Y., Wenqing, H., Neil, C., 2014. Plastic-film mulch in Chinese agriculture: importance and problems. World Agric. 4, 32–36.

Chen, H., 2016. Synergistic Effects of Microplastic and Glyphosate on Soil Microbial Activities in Chinese Loess Soil.

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull. 70, 227–233.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597.

van den Oever, M., Molenveld, K., van der Zee, M., Bos, H., 2017. Bio-based and biodegradable plastics: facts and figures: focus on food packaging in the Netherlands. Wageningen Food & Biobased Research.

Dong, H.D., Liu, T., Han, Z.Q., Sun, Q.M., Li, R., 2015. Determining time limits of continuous film mulching and examining residual effects on cotton yield and soil properties. J. Environ. Biol. 36, 677–684.

Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28 (1).

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82.

Ekebafe, L., Ogbeifun, D., Okieimen, F., 2011. Polymer applications in agriculture. Biokemistri 23.

Elert, A.M., Becker, R., Duemichen, E., Eisentraut, P., Falkenhagen, J., Sturm, H., et al., 2017. Comparison of different methods for MP detection: what can we learn from them, and why asking the right question before measurements matters? Environ. Pollut. 231, 1256–1264.

Fründ, H.-C., Butt, K., Capowiez, Y., Eisenhauer, N., Emmerling, C., Ernst, G., et al., 2010. Using earthworms as model organisms in the laboratory: recommendations for experimental implementations. Pedobiologia 53, 119–125.

GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment. Reports and Studies. 90. IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, London.

van Groenigen, J.W., Lubbers, I.M., Vos, H.M., Brown, G.G., De Deyn, G.B., van Groenigen, K.J., 2014. Earthworms increase plant production: a meta-analysis. Sci. Rep. 4, 6365.

Hodson, M.E., Duffus-Hodson, C., Clark, A., Prendergast-Miller, M., Thorpe, K.L., 2017. Plastic bag derived-microplastics as a vector for metal exposure in terrestrial invertebrates. Environ. Sci. Technol. 51, 4714–4721.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., et al., 2016. Microplastics in the terrestrial ecosystem: implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). Environ. Sci. Technol. 50, 2685–2691.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., et al., 2017a. Incorporation of microplastics from litter into burrows of *Lumbricus terrestris*. Environ. Pollut. 220, 523–531.

Huerta Lwanga, E., Mendoza Vega, J., Ku Quej, V., JLA, Chi, Sanchez Del Cid, L., Chi, C., et al., 2017b. Field evidence for transfer of plastic debris along a terrestrial food chain. Sci. Rep. 7, 14071.

Jiang, X.J., Liu, W., Wang, E., Zhou, T., Xin, P., 2017. Residual plastic mulch fragments effects on soil physical properties and water flow behavior in the Minqin oasis, northwestern China. Soil Tillage Res. 166, 100–107.

Kasirajan, S., Ngouajio, M., 2012. Polyethylene and biodegradable mulches for agricultural applications: a review. Agron. Sustain. Dev. 32, 501.

Kaushal, M., Wani, S.P., 2016. Rhizobacterial-plant interactions: strategies ensuring plant growth promotion under drought and salinity stress. Agric. Ecosyst. Environ. 231, 68–78.

Koelmans, A.A., Gouin, T., Thompson, R., Wallace, N., Arthur, C., 2014. Plastics in the marine environment. Environ. Toxicol. Chem. 33, 5–10.

Koelmans, A.A., Kooi, M., Law, K.L., van Sebille, E., 2017. All is not lost: deriving a top-down mass budget of plastic at sea. Environ. Res. Lett. 12.

Lambert, S., Wagner, M., 2016. Characterisation of nanoplastics during the degradation of polystyrene. Chemosphere 145, 265–268.

Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A.M., et al., 2012. Accumulation, translocation and impact of TiO_2 nanoparticles in wheat (*Triticum aestivum* spp.): influence of diameter and crystal phase. Sci. Total Environ. 431, 197–208.

Law, K.L., Thompson, R.C., 2014. Microplastics in the seas. Science 345, 144–145.

Liu, E., He, W., Yan, C., 2014. 'White revolution' to 'white pollution'—agricultural plastic film mulch in China. Environ. Res. Lett. 9, 091001.

Liu, H., Yang, X., Liu, G., Liang, C., Xue, S., Chen, H., et al., 2017. Response of soil dissolved organic matter to microplastic addition in Chinese loess soil. Chemosphere 185, 907–917.

Lwanga, E.H., Thapa, B., Yang, X., Gertsen, H., Salánki, T., Geissen, V., et al., 2018. Decay of low-density polyethylene by bacteria extracted from earthworm's guts: a potential for soil restoration. Sci. Total Environ. 624, 753–757.

Maass, S., Daphi, D., Lehmann, A., Rillig, M.C., 2017. Transport of microplastics by two collembolan species. Environ. Pollut. 225, 456–459.

Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R., et al., 2017. Microplastics in sewage sludge: effects of treatment. Environ. Sci. Technol. 51, 810–818.

Moreno, M.M., González-Mora, S., Villena, J., Campos, J.A., Moreno, C., 2017. Deterioration pattern of six biodegradable, potentially low-environmental impact mulches in field conditions. J. Environ. Manag. 200, 490–501.

Muroi, F., Tachibana, Y., Kobayashi, Y., Sakurai, T., Kasuya, K., 2016. Influences of poly(butylene adipate-co-terephthalate) on soil microbiota and plant growth. Polym. Degrad. Stab. 129, 338–346.

Ng, E.-L., Huerta Lwanga, E., Eldridge, S.M., Johnston, P., Hu, H.-W., Geissen, V., et al., 2018. An overview of microplastic and nanoplastic pollution in agroecosystems. Sci. Total Environ. 627, 1377–1388.

Nizzetto, L., Futter, M., Langaas, S., 2016a. Are agricultural soils dumps for microplastics of urban origin? Environ. Sci. Technol. 50, 10777–10779.

Nizzetto, L., Langaas, S., Futter, M., 2016b. Pollution: do microplastics spill on to farm soils? Nature 537 (488–488).

O'Hara, L.E., Paul, M.J., Wingler, A., 2013. How do sugars regulate plant growth and development? New insight into the role of trehalose-6-phosphate. Mol. Plant 6, 261–274.
Parvathy, P.C., Jyothi, A.N., John, K.S., Sreekumar, J., 2014. Cassava starch based superabsorbent polymer as soil conditioner: impact on soil physico-chemical and biological properties and plant growth. Clean: Soil, Air, Water 42, 1610–1617.
Ren, X., 2003. Biodegradable plastics: a solution or a challenge? J. Clean. Prod. 11, 27–40.
Research TM, 2013. Agricultural films (LDPE, LLDPE, HDPE, EVA/EBA, reclaims and others) market for greenhouse, mulching and silage applications - global industry analysis, size, share, growth, trends and. Forecast 2013–2019.
Rico, C.M., Majumdar, S., Duarte-Gardea, M., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2011. Interaction of nanoparticles with edible plants and their possible implications in the food chain. J. Agric. Food Chem. 59, 3485–3498.
Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? Environ. Sci. Technol. 46, 6453–6454.
Rillig, M.C., Ingraffia, R., AAD, Machado, 2017a. Microplastic incorporation into soil in agroecosystems. Front. Plant Sci. 8.
Rillig, M.C., Ziersch, L., Hempel, S., 2017b. Microplastic transport in soil by earthworms. Sci. Rep. 7.
Ryan, P., Moloney, C., 1990. Plastic and other artefacts on South African beaches: temporal trends in abundance and composition. S. Afr. J. Sci. 86, 450–452.
Sharma, S., Chatterjee, S., 2017. Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environ. Sci. Pollut. Res. 24, 21530–21547.
Sintim, H.Y., Flury, M., 2017. Is biodegradable plastic mulch the solution to agriculture's plastic problem? Environ. Sci. Technol. 51, 1068–1069.
Sjollema, S.B., Redondo-Hasselerharm, P., Leslie, H.A., MHS, Kraak, Vethaak, A.D., 2016. Do plastic particles affect microalgal photosynthesis and growth? Aquat. Toxicol. 170, 259–261.
Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Troger, J., et al., 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? Sci. Total Environ. 550, 690–705.
Syberg, K., Khan, F.R., Selck, H., Palmqvist, A., Banta, G.T., Daley, J., et al., 2015. Microplastics: addressing ecological risk through lessons learned. Environ. Toxicol. Chem. 34, 945–953.
Tao, Z., Cao, X., Luo, X., Li, X.D., Zhou, Z., 2012. Responses of three enzyme activities to lower molecular weight polyethylene added in pot-cultured horse bean soil. Chin. J. Soil Sci. 43, 1104–1110.
Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W., et al., 2004. Lost at sea: where is all the plastic? Science 304 (838–838).
Verschoor, A., 2015. Towards a definition of microplastics: considerations for the specification of physico-chemical properties. RIVM Letter Report 2015–0116.
Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A., 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ. Toxicol. Chem. 31, 2490–2497.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.
Yang, N., Sun, Z.-X., Feng, L.-S., Zheng, M.-Z., Chi, D.-C., Meng, W.-Z., et al., 2014. Plastic film mulching for water-efficient agricultural applications and degradable films materials development research. Mater. Manuf. Process. 30, 143–154.
Zhang, Z., Luo, X., Fan, Y., Wu, Q., 2015. Cumulative effects of powders of degraded PE mulching-films on chemical properties of soil. Environ. Sci. Technol. 38, 115–119.
Zhang, D., Liu, H.B., Hu, W.L., Qin, X.H., Ma, X.W., Yan, C.R., et al., 2016. The status and distribution characteristics of residual mulching film in Xinjiang, China. J. Integr. Agric. 15, 2639–2646.
Zhang, S., Yang, X., Gertsen, H., Peters, P., Salanki, T., Geissen, V., 2018. A simple method for the extraction and identification of light density microplastics from soil. Sci. Total Environ. 616–617, 1056–1065.
Zhou, Q., Zhang, H., Fu, C., Zhou, Y., Dai, Z., Li, Y., et al., 2018. The distribution and morphology of microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea. Geoderma 322, 201–208.
Zhu, D., Bi, Q.F., Xiang, Q., Chen, Q.L., Christie, P., Ke, X., et al., 2018a. Trophic predator-prey relationships promote transport of microplastics compared with the single *Hypoaspis aculeifer* and *Folsomia candida*. Environ. Pollut. 235, 150–154.
Zhu, D., Chen, Q.L., An, X.L., Yang, X.R., Christie, P., Ke, X., et al., 2018b. Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition. Soil Biol. Biochem. 116, 302–310.
Zubris, K.A.V., Richards, B.K., 2005. Synthetic fibers as an indicator of land application of sludge. Environ. Pollut. 138, 201–211.