



# Effects of plastic particles on germination and growth of soybean (*Glycine max*): A pot experiment under field condition<sup>☆</sup>

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## ABSTRACT

Plastic residues have become a serious environmental problem in areas where agricultural plastic film are used intensively. Although numerous of studies have been done to assess its impacts on soil quality and crop yields, the understanding of meso-plastic particles effects on plant is still limited. In this study, low density polyethylene (PE) and biodegradable plastic (Bio) mulch film were selected to study the effects of meso-plastic debris on soybean germination and plant growth with the accumulation levels of 0%, 0.1%, 0.5% and 1% in soil (w: w, size ranging 0.5–2 cm) by a pot experiment under field condition. Results showed that the germination viability of soybean seeds was reduced to 82.39%, 39.44% and 26.06% in the treatments with 0.1%, 0.5% and 1% added plastic debris compared to the control (CK), respectively, suggesting that plastic residues in soil inhibit the viability of soybean seed germination. The plastic debris had a significant negative effect on plant height and culm diameter during the entire growth stage of soybean. Similarly, the leaf area at harvest was reduced by 1.97%, 6.86% and 11.53% compared to the CK in the treatments with 0.1%, 0.5% and 1% plastic debris addition, respectively. In addition, the total plant biomass under plastic addition was reduced in both the flowering and harvesting stages, compared to the CK. For the different type of plastic residues, plant height, leaf area and root/shoot ratio at group PE were significantly lower than those of groups treated by Bio. In conclusion, PE debris had a greater negative effects on plant height, culm diameter, leaf area and root/shoot ratio while Bio debris mainly showed the adverse effects on germination viability and root biomass especially at the flowering stage. Therefore, further research is required to elaborate plastic particles' effects on different stages of crops and soil quality.

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## 1. Introduction

Along with the continuous development of the plastics industry, plastic products have become widely used in industry, agriculture

and daily life (Xu et al., 2020). Plastic is essential in nearly all aspects of life as most human activity nowadays is based on or influenced by plastics and plastic products (Geyer et al., 2017). Plastic waste, which comprises a considerable proportion of urban municipal solid waste, is a particularly major concern (Sanjana and Yogendra, 2020). It is estimated that around 12,000 Mt of plastic waste will be in landfills or in the natural environment by 2050 (Geyer et al., 2017). The global use of plastic mulch films in agriculture, which includes films used in greenhouses, mulching, and silage, has increased dramatically from 4.4 million tons in 2012 to 7.4 million tons in 2019 (Brodhagen et al., 2017; Gao et al., 2019;

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Sintim and Flury, 2017). Plastic film is used for cash crops and grain crops due to its diverse benefits to crop yields and quality (Gao et al., 2019; Zeng et al., 2013). However, plastic residues left in the soil has become a serious environmental issue that affects plant growth, soil quality, and surrounding ecosystems in the long-term (Jambeck et al., 2015; Wan et al., 2019). It has been reported that soil organic carbon and nitrogen stocks of farmlands have been depleted by plastic film residues, leading to low soil fertility, and increased greenhouse gas emissions (Cuello et al., 2015; Lee et al., 2019; Ma et al., 2018). Large amounts of plastic residuals affect soil permeability, water content, seed moisture and nutrient absorption, thereby disrupting crop seed germination and crop growth (Kasirajan and Ngouajio, 2012; Yan et al., 2014). Therefore, plastic film use in agriculture, as well as other plastic products in daily life, have become a great concern, especially regarding the effects of its residues in ecosystems (Horton et al., 2017; Ramos et al., 2015).

As an important raw material, polyethylene is frequently used to produce plastic film, which is difficult to decompose either by weathering or biodegradation (Liu et al., 2014). Once in the soil, the physical fragmentation and slow aging process of the plastic threatens the ecosystems either by separation into smaller plastic particles, or through the release of toxins such as bisphenol A (BPA), additives, and adsorbed compounds (Sajiki and Yonekubo, 2003). According to the Guidance on Monitoring of Marine Litter in European Seas, plastics are differentiated into macro-plastics (>2.5 cm), meso-plastics (0.5–2.5 cm), large micro-plastics (0.1–0.5 cm), and small micro-plastics (<1 mm) and nano-plastics (<100 nm) by the European Marine Strategy Framework Directive Working Group on Good Environmental Status Seas (European Commission et al., 2013). In particular, smaller plastic particles, such as microplastics and nanoplastics are more harmful to the environment as they act as vectors of other pollutants (Besseling et al., 2014; da Costa et al., 2016; Teuten et al., 2007), leading to high risks of uptake by plant roots (Engler, 2012; Graham and Thompson, 2009; Li et al., 2020). Furthermore, diphtalate (2-ethylhexyl)-phthalate (DEHP), the most widely used plastic additive, has been shown to affect biochemical functioning of humans and wildlife (Molino et al., 2019). Compared to polyethylene plastic film (PE), biodegradable plastic film is a promising product in light of environmental issues and “white pollution” in farmland ecosystems (Moreno et al., 2017; Ren, 2003; Sintim et al., 2019a, 2019b), as it decomposes into water, carbon dioxide, and microbial biomass. Potentially, it is a promising solution to address the accumulation of PE debris in farming soils, the costs and uncertainty regarding its effects on soil and environment are the main barriers to wider adoption (Yan et al., 2014; Yang et al., 2015).

Although some studies have been investigated plastic debris impacts on soil quality and crop yields, the understanding of their impacts on soil-vegetation ecosystem in the terrestrial environment is still limited (Rillig, 2012; Zhang et al., 2018; Zhou et al., 2018). So far, little is known about the effects of plastic mulch on plant growth and their breakdown into micro- and nanometer-sized fragments (Cao et al., 2017; Ng et al., 2018). At present, the majority of research performed in this area is focused on the quantity of large pieces of plastic in the soil, while little information is available on the effects of small plastic debris, especially after long periods of accumulation, on the growth and development of vegetation in order to obtain deeper insight into the impacts of the particles on the soil-plant systems, especially on *Leguminosae* sp. (Accinelli et al., 2019, 2020). Therefore, in this present study, we focus on the types and quantity of plastic debris in soil, with the aim of simulating fields ‘white pollution’ (Chandran et al., 2020; Yang et al., 2020), in order to investigate effects of meso-plastic residues on plant germination and growth properties. We

conducted pot experiments with soybeans under field conditions and two important growth stages (flowering and harvesting) are taken to study the effects of plastic debris (types or levels) on plant growth and to obtain the potential insights regarding to biodegradable plastic application in agriculture.

## 2. Materials and methods

### 2.1. Study site and materials

The experiment was conducted at the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, a semiarid area of Guanzhong Plain, China (34°18'N, 108°03'E). The average annual temperature is 12.9 °C, with 635.1 mm of annual precipitation. Local soybean (*Glycine max*), cultivated No.290 from Yangling, was selected as a model plant, with average seed weight of  $0.23 \pm 0.02$  g. Loess soil, taken from farmland (0–30 cm) in Yangling, was air-dried and sieved at 10 mm. PVC pot with 24 cm opening diameter, 19.5 cm base diameter and 26 cm height, was used in the experiment. Two types of plastic mulch films were used: polyethylene mulch film (PE) produced by Xifeng Agricultural Technology Service Co. Ltd., China and biodegradable mulch film (Bio) consisting of poly (butylene adipate-co-terephthalate) and poly (lactic acid) provided by Qingtian Plastic Products Co., Ltd., China. In order to simulate plastic debris under field conditions, 2 cm\*2 cm, 1 cm\*1 cm and 0.5 cm\*0.5 cm sizes of debris were cut by scissors manually.

### 2.2. Experiment setup

The contents of different types of plastic debris in soil were 0%, 0.1%, 0.5% and 1% (weight to weight, w:w). The six treatments and a control treatment without plastic debris addition, are shown in Table 1. Six pots were conducted for each treatment and three of them (as replicates) were grouped and harvested at two sampling stages.

In each pot, a piece of nylon filter (600 cm<sup>2</sup>) with a diameter of 250 mesh was placed at the bottom and then 6.5 Kg soil mixed with plastic debris was filled. 1.3 mL water was added in each pot for overnight to make sure water could distribute evenly, keeping 20% water content, equally to the observed field capacity (FC). 5 soybean seeds were sowed in each pot and then 500 g of nutrient matrix, with 60% moisture content, were added on the surface soil (2 cm layers). During the first 4 weeks, all the seedlings were observed and then only 2 of the strongest plants were kept for samplings in each pot. During the plant germination and growing stage, water content was controlled by weight and irrigation was conducted if the water content lower than 12% (60% of FC). We harvested the soybean at two time points, the flowering stage (around 2 months) and harvesting stage (around 4 months), in order to examine the effects of our experiments on both vegetative and reproductive growth.

**Table 1**  
Experiment design and treatments.

Treatments	Type of plastics	Contents
CK	—	0%
T1	PE	0.1%
T2	PE	0.5%
T3	PE	1.0%
T4	Bio	0.1%
T5	Bio	0.5%
T6	Bio	1.0%

Notes: PE means polyethylene mulch film; Bio means biodegradable mulch film.

### 2.3. Observation indices

The germination viability and germination rate are used to identify the germination of seeds, while plant height, culm diameter, leaf area are used for identifying the growth of plants. Germination viability (GV) and final germination rate (GR) of soybean seeds are the percentage of germinating seeds related to number of soybean seeds after three days and seven days, respectively (Li et al., 2013). The germination viability and germination rate are calculated as reported by (Yue et al., 2019), as follows:

$$GV = \sum N_3 / N_0 \times 100\% \quad [1]$$

$$GR = \sum N_7 / N_0 \times 100\% \quad [2]$$

Where  $N_3$  is the number of seeds germinated in the 3rd day;  $N_7$  is the number of seeds germinated in the 7th day;  $N_0$  is the total number of seeds).

Plant height was measured as the distance from the base of the plant to the top of the main stem by a ruler every 10 days during the growth period. Culm diameter was measured at the first elongation node's middle with a MNT-1500 I (GB/T1214.2–1996) electronic vernier caliper. To calculate the leaf area, mature leaves were selected and measured by camera. Then, the ImageJ software was used to extract the leaf area through establishing a scale. In order to ensure the accuracy of the calculated results, we adjusted the known distance to 1 cm and ensured a pixel aspect ratio of 1.0. Biomass (shoot and root) were collected after samples drying at 70 °C till constant weight per plant (12 h).

### 2.4. Statistical analysis

Data was collected and analysed by each individual treatment. All errors are indicated as standard deviations (SD), and results are expressed as mean values and SD. A one-way ANOVA was performed to compare differences in the measured variables arising from different treatments, while a two-way ANOVA was carried out to analyze the significance of interactions between type of plastic and concentration of plastic. Duncan's new multiple range method (SSR) was to determine the mean values of different treatments. Redundancy analysis (RDA) was used to study the relationships between the observed soybean growth factors. Also, variance inflation factor (VIF) analysis was used to exclude highly correlated explanatory variables. The R packages "vegan" and "ggplot2" were used to generate the RDA ordination diagram. Origin 9.0 was employed for visualization.

## 3. Results

### 3.1. Plant germination

The characteristics of seed germination presented differently among the treatments (Table 2). The results showed that different concentrations of PE and Bio had significant effects on the germination viability (GV). Specifically, GV was significantly affected by plastic debris addition and was negatively related to plastic concentrations. In PE treatments, GV decreased significantly in the treatment with 0.5% and 1% of debris addition. In Bio treatments, GV significantly inhibited in all treatments, especially in the treatment with 1% of Bio addition. To be contrary, germination rate (GR) wasn't significantly affected neither by plastic types nor by different concentrations of plastic in soil. Interestingly, the highest GR was 97%, observed in T6 with 1% of biodegradable plastic

**Table 2**

Effects of different plastic treatments on germination of soybean seeds.

Treatments	GV (100%)	GR (100%)
CK	0.70 ± 0.23a	0.82 ± 0.21 ab
T1	0.67 ± 0.16 ab	0.90 ± 0.11 ab
T2	0.23 ± 0.08de	0.77 ± 0.23 ab
T3	0.27 ± 0.16de	0.90 ± 0.17 ab
T4	0.50 ± 0.17bc	0.90 ± 0.17 ab
T5	0.33 ± 0.21cd	0.73 ± 0.21 b
T6	0.10 ± 0.17e	0.97 ± 0.08a

Notes: GV: germination viability; GR: germination rate. Different lowercase letters indicate the significant differences between treatments ( $P < 0.05$ ). All of the data are presented as means ± SD.

addition, while the lowest GR was 73%, observed in T5 with 0.5% of biodegradable plastic addition. Furthermore, the similar GR was also observed in the treatment with 0.5% of PE plastic addition.

### 3.2. Plant growth characteristics

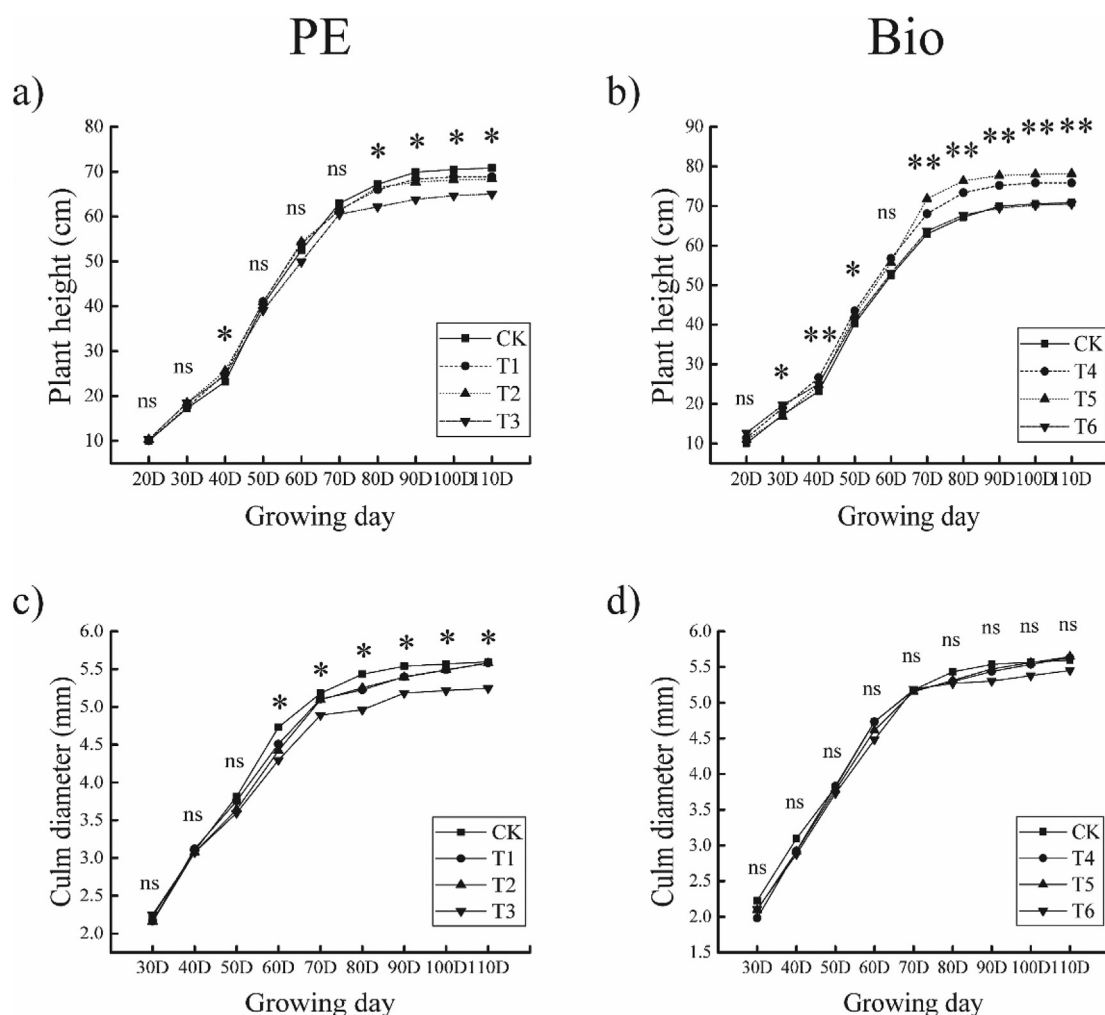
#### 3.2.1. Plant height and culm diameter

During the growth period of soybean, plant height first increased and then tended to be stable after the flowering stage (Fig. 1, a-b). Although different levels of plastic addition affected plant height, the variation differed between types of plastic significantly. PE treatments showed significant differences on the 80th day across different concentrations while the Bio treatment showed a difference on the 30th day. Compared with CK, the plant height was inhibited by different concentrations of plastic residues in PE treatments, and the inhibition was enhanced with the increasing of plastic concentration after 80 days, obviously in T3 with 1% of plastic addition. In group treatments of Bio, however, the addition of different concentration of biodegradable plastics improved the plant height significantly after seedling stage (20 days) in T4 and T5, except in day 60. Specifically, before flowering stage (60 days), the levels of PE addition had no significant effects on plant height but the differences were observed afterwards, with the increasing level of PE addition, plant height decreased significantly. To be contrast, in the Bio group, the lower levels of plastic addition (T4 and T5) stimulated plant height than that in T6, with the similar plant height in CK after flowering stage. Thus, in the harvesting stage, compared with PE treatments, the presence of biodegradable plastics significantly enhanced the plant height by 10.2%, 14.4%, 8.5% at the level of 0.1%, 0.5%, 1% of plastic addition, respectively.

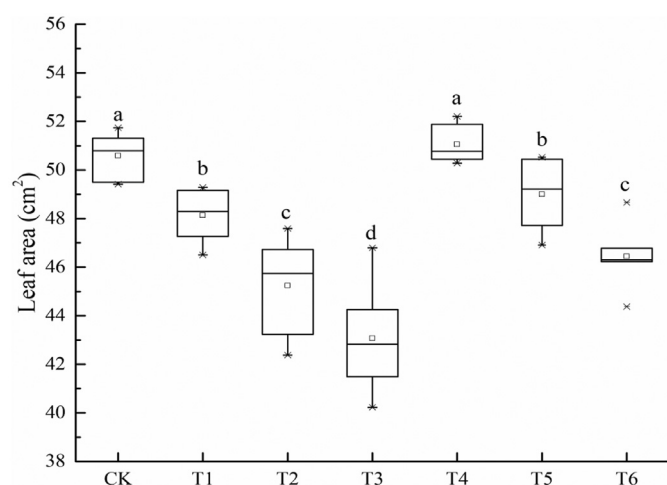
Culm diameter changed differently among treatments with different types of plastic addition (Fig. 1, c-d). The significant differences of culm diameter were observed after 60 days (flowering stage) in PE treatments while no significant difference of plant culm diameter was determined during the whole growth of plant in Bio treatments. In general, comparing with control, no matter in PE or Bio group of treatments, with the increasing of plastic debris addition, culm diameter was getting slimmer at harvesting stage, especially with  $5.25 \pm 0.52$  mm in T3. Concerning the types of plastic, culm diameter was slightly higher in Bio treatments with the same level of addition in PE treatments but no significant difference was tested.

#### 3.2.2. Leaf area

Leaf area was measured during the vegetation stage (Fig. 2). The results showed that with the plastic addition, leaf area decreased significantly, especially at the highest addition level of 1%. In PE treatments, leaf area was  $48.14 \pm 1.09$  cm<sup>2</sup>,  $45.24 \pm 2.03$  cm<sup>2</sup> and  $43.07 \pm 2.37$  cm<sup>2</sup>, in T1, T2 and T3, respectively, significantly differed from the CK ( $50.59 \pm 0.96$  cm<sup>2</sup>). In Bio treatments, the



**Fig. 1.** Plant height and culm diameter in all the treatments during soybean growth (a) Plant height in group PE; b) Plant height in group Bio; c) Culm diameter in group PE; d) Culm diameter in group Bio.) Asterisks represent significant differences (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; ns: no significant against the control).



**Fig. 2.** Leaf area in different treatments. Different lowercase letters indicate the significant differences between treatments ( $P < 0.05$ ). All of the data are presented as means  $\pm$  SD.

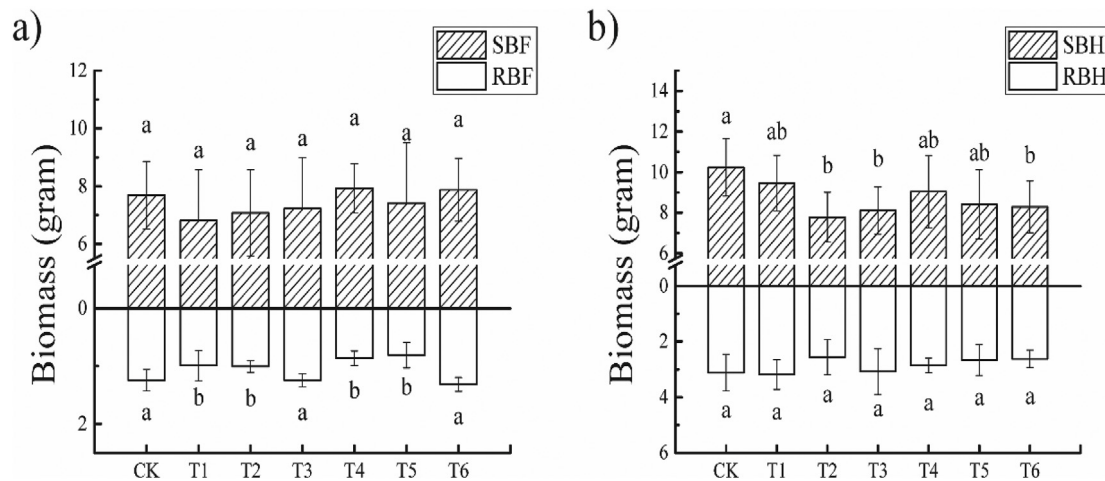
smallest leaf area was observed in T6 ( $46.44 \pm 1.37$  cm<sup>2</sup>) but no significant difference was observed between CK and T4. Comparing

with PE treatments, Bio treatments promoted the leaf area significantly at the same level of plastic addition and the presence of biodegradable plastic significantly enhanced leaf area by 6.07%, 8.33%, 12.17% at 0.1%, 0.5%, 1% levels, respectively.

### 3.3. Plant biomass

The shoot and root biomass showed differently in the treatments at flowering and harvesting stage (Fig. 3). At the flowering stage, no significant difference of shoot biomass was observed among treatments while the significant differences of root biomass were tested in T1, T2, T4, and T5, with the comparison of CK. At the harvesting stage, however, the shoot biomass varied significantly in T2, T3 and T6 but no significant difference of root biomass was observed among all treatments. Compared to PE treatments, the shoot biomass increased slightly in Bio treatments both at the flowering stage and the harvesting stage while the root biomass in Bio treatments only differed at the flowering stage, slightly lower than that in the same level of PE treatments.

Hence, the total plant biomass and shoot/root ratio were calculated and shown in Table 3. Interestingly, the results showed that the significant differences of plant biomass were observed among treatments at the harvesting stage but none at the flowering stage. The highest of plant biomass was found in CK and declined



**Fig. 3.** Shoot and root biomass of all the treatments at the flowering stage and the harvesting stage: a) the flowering stage; b) the harvesting stage. Different lowercase letters indicate significant differences between treatments ( $P < 0.05$ ). All of the data are presented as means  $\pm$  SD.

**Table 3**

Total plant biomass and root/shoot ratio of soybeans for all treatments at the flowering stage and the harvesting stage.

Treatment	TBF	RSBF	TBH	RSBH
CK	8.93 $\pm$ 1.33a	0.16 $\pm$ 0.01 ab	13.35 $\pm$ 1.96a	0.30 $\pm$ 0.04a
T1	7.80 $\pm$ 1.95a	0.15 $\pm$ 0.04 abc	12.64 $\pm$ 1.66 ab	0.34 $\pm$ 0.06a
T2	8.08 $\pm$ 1.56a	0.15 $\pm$ 0.02bc	10.34 $\pm$ 1.45c	0.33 $\pm$ 0.10a
T3	8.52 $\pm$ 1.82a	0.19 $\pm$ 0.05a	11.18 $\pm$ 1.55bc	0.38 $\pm$ 0.12a
T4	8.80 $\pm$ 0.90a	0.11 $\pm$ 0.02c	11.89 $\pm$ 1.80 abc	0.33 $\pm$ 0.09a
T5	8.22 $\pm$ 2.42a	0.12 $\pm$ 0.05c	11.08 $\pm$ 1.61bc	0.33 $\pm$ 0.11a
T6	9.19 $\pm$ 1.17a	0.17 $\pm$ 0.02 ab	10.91 $\pm$ 1.32bc	0.32 $\pm$ 0.06a

Notes: Different lowercase letters indicate significant differences between treatments ( $P < 0.05$ ). All of the data are presented as means  $\pm$  SD (TBF: total biomass at the flowering stage; RSBF: root/shoot ratio at the flowering stage; TBH: total biomass at the harvesting stage; RSBH: root/shoot ratio at the harvesting stage).

with the plastic addition, especially in T2 at the harvesting stage. Meanwhile, with the increasing level of plastic addition, either PE or Bio plastics, the root/shoot ratio declined in the treatments with 0.1% (T1 and T4) and 0.5% (T2 and T5) of plastic addition and slightly increased in the treatments with 1% (T3 and T6) of plastic addition at the flowering stage. However, no significant difference of root/

shoot ratio was observed among all the treatments at the harvesting stage.

### 3.4. The relationships between treatment factors and soybean growth parameters

The results showed that both types and levels of plastic affected plant growth significantly (Table 4). The concentration of plastic had significant effects on most of the observed parameters, and explains 51.23%, 48.90% and 52.49% of the variability in the GV, LAH and RBF respectively. The type of plastic had significant effects on the PHH, LAH and RSBF, which explains 48.17%, 34.61% and 11.96% of the variability respectively. Furthermore, almost all of the measurements were not affected by the interaction between plastic type and concentration except leaf area. Leaf area was significantly affected by factors (type, concentration and interaction between type and concentration) with only 14.76% of residuals at the harvesting stage. Regarding to germination rate, culm diameter and plant biomass, the two factors (type/concentration) and their interactions explained less than half of the variability of these parameters.

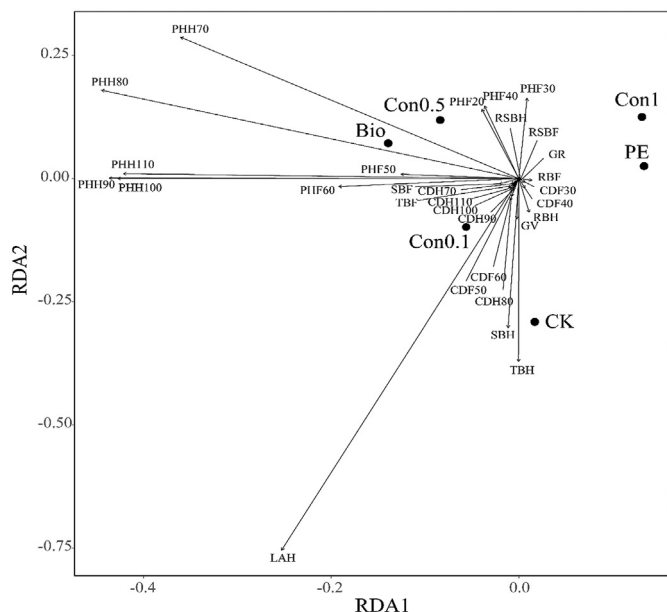
A principal component analysis to determine the explanatory

**Table 4**

$p$ -Value and contribution of independent factors (type of plastics and concentration of plastic residues) and their interactions to growth parameters studied by two way ANOVA.

	Type		Concentration		Type*Concentration		Residual
	%	Sig.	%	Sig.	%	Sig.	
GV	2.66	ns	51.23	<0.001	7.00	ns	39.11
GR	0.09	ns	20.71	0.03	1.45	ns	77.75
PHH	48.17	<0.001	20.12	<0.001	2.81	ns	28.90
CDH	3.03	ns	15.55	ns	1.24	ns	80.18
LAH	34.61	<0.001	48.90	<0.001	1.71	0.04	14.76
SBF	5.16	ns	0.65	ns	1.09	ns	93.10
RBF	3.93	ns	52.49	<0.001	3.27	ns	40.31
TBF	3.43	ns	3.41	ns	1.19	ns	91.97
RSBF	11.96	0.02	29.04	<0.01	1.01	ns	57.99
SBH	0.20	ns	12.49	ns	2.13	ns	85.18
RBH	3.86	ns	8.51	ns	4.48	ns	83.15
TBH	0.09	ns	17.21	0.05	3.69	ns	79.01
RSBH	1.97	ns	1.15	ns	2.59	ns	94.29

Type: type of plastic; Concentration: concentration of plastic; GV: germination viability; GR: germination rate; PHH: plant height at the harvesting stage; CDH: culm diameter at the harvesting stage; LAH: leaf area at the harvesting stage; SBF: shoot biomass at the flowering stage; RBF: root biomass at the flowering stage; TBF: total biomass at the flowering stage; RSBF: root/shoot ratio at the flowering stage; SBH: shoot biomass at the harvesting stage; RBH: root biomass at the harvesting stage; TBH: total biomass at the harvesting stage; RSBH: root/shoot ratio at the harvesting stage.



**Fig. 4.** Redundancy analysis ordination diagram of plant growth parameters with treatment factors (GV: germination viability; GR: germination rate; PHF: plant height at the flowering stage; PHH: plant height at the harvesting stage; CDF: culm diameter at the flowering stage; CDH: culm diameter at the harvesting stage; LAH: leaf area at the harvesting stage; SBF: shoot biomass at the flowering stage; RBF: root biomass at the flowering stage; TBH: total biomass at the flowering stage; RSBH: root/shoot ratio at the flowering stage; TBH: total biomass at the harvesting stage; RSBH: root/shoot ratio at the harvesting stage).

variables (types of plastics: Bio, PE; concentrations of plastics: 0%, 0.1%, 0.5%, 1%) effects on response variables was performed (Fig. 4). In general, the explanatory variables were able to explain 100% of total variance, and RDA1 contributes 91.42% to the parameter-factor relationships. The PE and Bio treatments showed the opposite effects in the principal component analysis. The factor Bio positively correlated with shoot biomass and total biomass at the flowering stage, leaf area, plant height, and other parameters that impact plant growth. The length of the arrows indicate the strength of the relationship between the explanatory variables and measured outcomes. Furthermore, the variable culm diameters, which were clustered together, showed no significant relationship among the treatments. Concerning the levels of plastic addition, the treatments with 0%, 0.1%, 0.5% and 1% plastics debris addition distributed in four different directions indicating that the level of plastic debris has great impacts on soybean seed germination and plant growth. The ordination diagram suggests plant biomasses at the harvesting stage and germination viability were significantly positively affected by the conditions without plastic residues in soil.

## 4. Discussion

### 4.1. Effects of plastic debris on soybean germination

Seed germination is a critical stage during the initiation of the soybean lifecycle and is strongly affected by soil properties and environmental stress, such as salt stress (Chen et al., 2019). During germination, seeds and seedlings are most vulnerable to substance in soil because of water adsorption through the pores of the seed (Parkpian et al., 2002). The study showed that the plastic particles (500 and 4800 nm) could slow down water uptake and thus delaying seed germination (Bosker et al., 2019). According to this present study, we observed that with an increase in concentration

of plastic debris, the inhibition effect of different types of plastics on soybean seed germination viability was enhanced, but no significant effect on the seed germination was observed after seven days (Table 1). It is clear that concentration of plastics directly affects the crop by changing the soil environment, thereby delaying the germination of soybean seeds (El-Darier and Youssef, 2000), especially in the treatments with biodegradable plastic addition. Consistent with the results of Balestri, who performed a *Lepidium sativum* seed germination test, a significant number of seedlings showed developmental abnormalities or reduced seedling growth when plastic was present (Balestri et al., 2019). Similarly, Pflugmacher et al. (2020) found that a lower germination rate (by 15%, 30%, and 65%) observed, compared to untreated controls, after new polycarbonate material directly mixed into the substrate in the ratio of 0.1%, 1.0%, and 10% (w/w) (Pflugmacher et al., 2020). Comparing with the GV in CK ( $0.70 \pm 0.23$ ), our results, with 0.1%, 0.5% and 1.0% PE residues in the soil, decreased in germination rate at three days of 5.63%, 67.61%, and 61.97%, respectively. Meanwhile, comparing with the GR in CK ( $0.82 \pm 0.21$ ), with 0.1%, 0.5% and 1.0% biodegradable plastic residues in the soil, GR decreased by 29.58%, 53.52%, and 85.92%, respectively. However, Lian et al. (2020) found that polystyrene had no discernible effect on seed germination rate of wheat with the nanoplastics addition in the cultivated solution at  $0.01 \text{ mg L}^{-1}$  to  $10 \text{ mg L}^{-1}$  levels. Thus, in order to well-understand of plastic residues effects on plant germination in the soil-plant system, further studies on different sizes and types of plastics, as well as the different abundant of plastic residue are required.

### 4.2. Effects of plastic debris on soybean growth

Plastic residues in soil has a negative effect on plant growth (Boots et al., 2019; de Souza Machado et al., 2019; Qi et al., 2020a). It may be caused by the agglomeration of plastics after ageing and degradation in the soil system. The agglomeration of plastics negatively affected plant roots or their symbionts, potentially inhibiting plant growth (Galloway et al., 2017). Meanwhile, bio-plastic could also affect soil biota, such as earthworms, and soil biophysical properties, including bulk density, soil aggregation and water holding capacity (de Souza Machado et al., 2018; Lwanga et al., 2017) which indirectly impact nutrient cycling and plant productivity. Qi et al. (2018) showed that biodegradable plastic mulch residues (37.1% pullulan, 44.6% polyethylene terephthalate and 18.3% polybutylene terephthalate (PBT) stimulated wheat height, with improvement of 10.4% at 2 months and 2.1% at 4 months sampling, respectively, compared to the treatment with PE plastic mulch residues (particle size around  $0.36 \text{ cm}^2$ ). Xin et al. (2014) reported that maize heights were 3.3%, 11.9% and 16.8% lower in the treatment with plastic residuals of 180, 360 and  $720 \text{ kg hm}^{-2}$  at the seedling stage, respectively, than those without residual treatment. A significant difference on the leaf area of maize was also observed if the accumulative plastic residues increasing in soil. In this present study, the different concentrations of plastic residues addition in soil, ranging from 0.1% to 1%, have shown the different effects on soybean growth at the flowering stage and harvesting stage. In PE treatments, the plant height improved before the flowering stage and then decreased significantly after 80 days, especially in the treatment with 1% of plastic debris addition (Fig. 1a). In Bio treatments, however, the plant height enhanced significantly almost in the whole growing period, from the seedling stage to the harvesting stage, except for the treatment with 1% plastic addition which showed the similar height in the control treatment (Fig. 1b). Furthermore, with the increasing of PE addition, plant height decreased significantly while the opposite findings observed in Bio treatments. Those findings indicate that the biodegradable plastic could positively improve the plant height in

the level of lower than 1%, while the accumulation of polyethylene plastic debris in soil could inhibit plant growth significantly, especially after the flowering stage, in the accordance with the findings of Qi et al. (2018). Regarding to plant culm diameter, Bio macro-plastic residues negatively affected wheat culm diameter but no effect observed in the treatment with PE macro-plastic treatments (Qi et al., 2018). In the present study, however, with the increasing addition of PE, culm diameter slided significantly after the flowering stage while no significant difference has been observed but slightly slided during the whole period of plant growing in the treatments with Bio debris. Although the same material (such as PE, 1%) used in the similar study (pot experiment), factors such as plant species, experiment conditions and particle size, would all influence the results leading to difficulties to make general conclusion and implications to others. By the way, it is known that plant height and culm diameter are associated with lodging resistance. Interestingly, with the increasing of PE debris in soil, plant height inhibited and culm diameter slided significantly, suggesting the risk of plant lodging, as well as the lower productivity (plant biomass). Meanwhile, with the increasing of Bio addition in soil, plant height increased significantly but the culm diameters slided slightly which indicates the higher risk of plant lodging than that in the treatment of PE. Unfortunately, the knowledge of the effects of plastic debris on plant culm diameter and lodging resistance is rare studied. Further studies might be needed to link the plant traits associated with lodging resistance with the accumulation of plastic debris.

Meng et al. (2020) reported that leaf area decreased significantly in the treatment with biodegradable polylactic acid microplastic addition but increased in the treatment with PE microplastic addition. However, in this present study, we found that with the plastic addition (either PE or Bio), leaf area decreased significantly, especially at the highest addition level of 1% of PE. The opposite findings in PE treatment resulted from the testing soil (loess soil), cultivation conditions (warm summer, 25–39 °C) and plastic particle size (meso-plastics), plant species (soybean) while the same findings observed in the biodegradable plastic treatment showed a clear message of reducing leaf area which potentially affects plant chlorophyll content and photosynthesis (Qi et al., 2018). In addition, plastic residues may affect soybean growth by changing the porosity of the soil environment, and affect rhizobia in soybean roots (Kaushal and Wani, 2016; Wang et al., 2020). It is reported that the biomass of cotton decreased during the boll development and opening stages but the root/shoot ratio rose during the boll opening stage when the level of film residues (particle size ranging from 0 to 200 cm<sup>2</sup>) increased from 0.0028% to 0.28% (Dong et al., 2015). Qi et al. (2018) showed that shoot and root biomass inhibited in the treatment with biodegradable macro-plastic addition at first 2 months of wheat growing but no difference observed after 4 months harvesting. No significant difference of shoot biomass was observed while root biomass reduced significantly in the treatment with PE macro-plastic both in 2 and 4 months. In the present study, however, shoot biomass changed slightly at the flowering stage (around 2 months) while it significant differed at the harvesting stage, especially in the treatments with higher content of plastic debris both in PE treatments and Bio treatments (Fig. 3). Meanwhile, the root biomass inhibited significantly at the flowering stage, especially in treatment with 0.1% and 0.5% of plastic debris addition while reached the similar amount at the harvesting stage. The differences of findings about biodegradable plastic indicate that different model plant used in the experiment might conclude different information which needs more studies to elaborate plastic debris effects on plant growth. Interesting, no significant difference of the total plant biomass at harvesting stage was observed neither in the study of Qi et al. (2018) nor in the present study indicating

that the effects of plastic debris on plant biomass should be paid more attention during the plant growing stage.

A field experiment conducted to study the degradation of six various mulch materials (i.e. vegetable starch, polylactic acid plastic films or paper mulches) and the results showed that PE differed significantly from the biodegradable plastic, as it exhibits stronger puncture resistance values (Muroi et al., 2016). It implies that PE debris is persistent in soil which strongly impacts soil quality (Yang et al., 2020). Thus, biodegradable plastic film might be the promising alternative materials to reduce the plastic pollution in farming land. Qi et al. (2020b) stated that adoption of biodegradable films would be possible to reduce soil plastic pollution but the effects on soil quality and soil microbial activities needs further concern. Plastic particles such as macro-plastics, meco-plastics, micro/nano-particles, are accumulated, fragmented, aged in soil and potentially enter into the food chain (Larue et al., 2012; Rico et al., 2011; Tu et al., 2020). Given the rapidly increasing accumulation of plastic debris in soils worldwide, there is an urgent need to better understand the impact of these residues on the complex interactions that take place in soil-plant systems (Barnes et al., 2009). Our study confirmed that the accumulation of plastic debris in soil could affect plant growth, especially in the early stage under the treatment with biodegradable plastic addition. However, factors such as soil types, plant species, climate conditions, as well as agriculture management practices, might combine together to affect plant growth in the field. Therefore, the results and data obtained from this experiment serves as a reference for future large scale of field studies. The mechanisms of interaction between plastic debris and soybean growth remain to be further studied either on the feedback of interaction or on the risk threshold of plastic levels, as well as the balance of plant lodging resistance.

## 5. Conclusion

In order to reduce the “white pollution” in agricultural fields, points of consideration include whether biodegradable plastic mulch can be used as an alternative to polyethylene plastic mulch, and whether plastic residue concentrations affect seed germination and plant growth. From the results of this experiment, the key message can be concluded as following:

- 1) The type of plastic debris (polyethylene or biodegradable) significantly inhibited germination viability and the inhibitory effect of these two types of plastics increased with concentration. No significant difference of germination rate was observed in the treatments with plastic addition while differences in concentration produced a greater variation in outcomes.
- 2) Plastic-induced negative effects on soybean plant growth were wide-ranging, including plant height, culm diameter, leaf area, and biomass production. Compared to polyethylene debris, the biodegradable debris showed stronger positive effects on soybean height and weaker negative effects on leaf area, culm diameter and biomass. Furthermore, the interaction between plastic types and concentrations had a significant effect on the plant height and leaf area, but no significant effect on the culm diameter and other biomass indices. In conclusion, our results indicate that comparing with polyethylene, the biodegradable plastics might be the promising alternative materials applied in mulch-farming system while the negative effects of its debris on plant growth need to be further studied.

## CRedit author statement

Bintao Li: Conceptualization, Methodology, Formal analysis,

Investigation, Writing – original draft, Writing – review & editing, Visualization. Shan Huang: Conceptualization, Methodology, Investigation, Writing – original draft. Haoming Wang: Methodology, Software, Formal analysis, Investigation, Writing – original draft, Visualization. Mengjuan Liu: Conceptualization, Resources, Supervision. Sha Xue: Conceptualization, Investigation, Resources. Darrell Tang: Resources and language editing. Wanli Cheng: Investigation, Resources. Tinglu Fan: Investigation, Resources. Xiaomei Yang: Conceptualization, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## 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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