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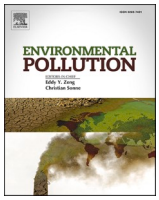

Effects of LDPE and PBAT plastics on soil organic carbon and carbon-enzymes: A mesocosm experiment under field conditions☆

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ABSTRACT

Although the effects of plastic residues on soil organic carbon (SOC) have been studied, variations in SOC and soil carbon-enzyme activities at different plant growth stages have been largely overlooked. There remains a knowledge gap on how various varieties of plastics affect SOC and carbon-enzyme activity dynamics during the different growing stages of plants. In this study, we conducted a mesocosm experiment under field conditions using low-density polyethylene and poly (butylene adipate-co-terephthalate) debris (LDPE-D and PBAT-D, 500–2000 μm (pieces), 0%, 0.05%, 0.1%, 0.2%, 0.5%, 1%, 2%), and low-density polyethylene microplastics (LDPE-M, 500–1000 μm (powder), 0%, 0.05%, 0.1%, 0.5%) to investigate SOC and C-enzyme activities (β-xylosidase, cellobiohydrolase, β-glucosidase) at the sowing, seedling, flowering and harvesting stages of soybean (*Glycine Max*). The results showed that SOC in the LDPE-D treatments significantly increased from the flowering to harvesting stage, by 12.69%–13.26% ($p < 0.05$), but significantly decreased in the 0.05% and 0.1% LDPE-M treatments from the sowing to seedling stage ($p < 0.05$). However, PBAT-D had no significant effect on SOC during the whole growing period. For C-enzyme activities, only LDPE-D treatments inhibited GH (17.22–38.56%), BG (46.7–66.53%) and CBH (13.19–23.16%), compared to treatment without plastic addition, from the flowering stage to harvesting stage. Meanwhile, C-enzyme activities and SOC responded non-monotonically to plastic abundance and the impacts significantly varied among the growing stages, especially in treatments with PBAT-D ($p < 0.05$). These risks to soil organic carbon cycling are likely mediated by the effects of plastic contamination and degradation soil microbe. These effects are sensitive to plastic characteristics such as type, size, and shape, which, in turn, affect the biogeochemical and mechanical interactions involving plastic particles. Therefore, further research on the interactions between plastic degradation processes and the soil microbial community may provide better mechanistic understanding the effect of plastic contamination on soil organic carbon cycling.

1. Introduction

Plastic mulching strongly boosts agricultural productivity in arid and semi-arid areas as they increase soil temperature and moisture, among other benefits, thereby improving crop yield and quality (Kasirajan and Ngouajio, 2012; Zhang et al., 2018a). However, due to inadequate recycling and management, the widespread use of plastic films has led to severe plastic pollution in agricultural soils (Li et al., 2022). Low density polyethylene (LDPE) plastic film, the most commonly used plastic mulch and a major source of plastic pollution, may persist in agricultural soil, posing a threat to sustainable agriculture and food security (Nizzetto et al., 2016; Qi et al., 2018). To mitigate LDPE plastic pollution, biodegradable plastic films, such as poly (butylene adipate-co-terephthalate) (PBAT) and polylactic acid (PLA), have been developed as alternatives (Goldberger et al., 2019; Sintim et al., 2019). However, recent studies indicate that PBAT/PLA is prone to negatively

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impacting soils, such as the release of phthalates and more micro-particles (Boots et al., 2019; Han et al., 2021; Hu et al., 2022; Nizzetto et al., 2024), leading to more concerns about its use in future.

Soil organic carbon (SOC) is a crucial indicator of soil nutrient supply and crop production (Yang et al., 2021). Research has indicated that carbon-rich materials introduced into the soil, such as foam, biochar, organic dyes, etc., may release carbon into soils, contributing to the increasing in soil organic carbon pools (Li et al., 2024a; Schwichtenberg et al., 2020; Siddique et al., 2022). As a high-molecular polymer with about 80% carbon, the potential contribution of plastic debris to the SOC pool has attracted attention recently (Rillig, 2012; Rillig, 2018). It has been reported that plastic residues can directly affect SOC pools by releasing carbon into the soil (Guo et al., 2023), and indirectly affect SOC transformation by altering the soil structure and microbial communities (Rillig et al., 2021), depending on the characteristics of the plastic residues (e.g., type, size and abundance) (de Souza Machado et al., 2018; Lehmann et al., 2021). It is reported that LDPE debris can inhibit carbon exchange between soil and atmosphere, as well as can hinder soil water migration and infiltration to reduce the leaching of DOC, thereby reducing soil carbon loss (Santini et al., 2022; Zhang et al., 2022a). Meanwhile, Low density polyethylene microplastics (LDPE-M) can accelerate the mineralization of SOC by undermining the stability of soil aggregate (Lehmann et al., 2021), promoting oxygenic microenvironments, and DOM electron transport capability (Shi et al., 2023). Unlike LDPE-M, biodegradable microplastics participate in the biochemical cycle of SOC as available substrates for soil microorganisms, increasing microbial biomass carbon (MBC), and become further incorporated into persistent soil organic matter as the microorganisms die (Ding et al., 2021). Plastic residues may alter the soil organic carbon pool by influencing soil processes, including soil microbial activities, and these interactions and their intensities may strongly depend on the type of plastic residues.

It has been reported that soil enzyme activities are extremely sensitive to exogenous pollutants, such as heavy metals (Aponte et al., 2020), pesticides (Riah et al., 2014) and plastics (Yi et al., 2021). A meta-analysis from Zhang et al. (2022a) showed that in the plastic-contaminated soil, soil enzyme activities were effected significantly, ranging from 37% to 441%. Soil enzymes such as β-xylosidase (GH), β-glucosidase (BG) and cellobiohydrolase (CBH) are representative of C-enzymes in the soils (Wolińska and Stępniewska, 2012). Shah et al. (2023) reported that 10% polystyrene (PS), polyvinyl chloride (PVC) and LDPE microplastic inhibited BG activities in maize soils at flowering stage, in accordance with the findings from Zang et al. (2020). However, Mazzon et al. (2022) observed that 0.1%, 1% and 10% Mater-Bi debris significantly increased BG activities in loamy soil, with the effects that were independent of the plastic dose added. Yu et al. (2023) observed that PBAT-MPs stimulated GH, BG and CBH activities in soybean-planted soils at the flowering and harvesting stage, while inhibiting GH, BG and CBH activities in maize-planted soils at the harvesting stage. These results indicate that in different soil-plant systems and growth stages, the responses of GH, BG, and CBH activities to plastic contamination would differ. However, the dynamics of soil enzyme activities across the whole cultivation period of plants have not been fully reported, which may lead to an under/over estimation of the impact of plastic contamination on enzyme activity. Therefore, more work needs to be done on the response of C-enzyme activities to plastic contamination during the various growth stages of plants. In this present study, we conduct a mesocosm experiment under field conditions to investigate SOC and C-enzyme changes in soils contaminated by plastic debris generated from traditional LDPE and PBAT films, as well as microplastics from LDPE, during the whole growth period of soybean. The objectives of this study were: 1) to quantify the changes to SOC and C-enzymes at the various growth stages of soybean; 2) to compare the responses of SOC and C-enzymes to various plastic contamination scenarios (plastic type, size and abundance). In addition, we also measured the plastic mass in the soil after the crop harvest, and compared the morphology of plastic residues with scanning electron microscopy before and after planting, in order to understand the aging/degradation of plastics during the period of plant growth. The findings of this study contribute towards the understanding of the soil carbon cycle under soil contamination by plastics.

2. Materials and methods

2.1. Study site and materials

A mesocosm experiment under field conditions was conducted at the Caoxinzhuang experimental field station of Northwest A&F University (34°31′N, 108°10′E). The average annual temperature is 14.5 °C, with 635.1–663.9 mm of annual precipitation. Soil was collected from surrounding field with organic carbon content of 9.2 g kg^{-1}, and total nitrogen content of 0.8 g kg^{-1}, and available phosphorus content of 1.5 mg kg^{-1}. To minimize plastic contamination from environmental sources, clay pots with 24 cm top diameter, 20 cm bottom diameter and 24 cm height, were used as experimental containers. Soybean (*Glycine Max (Linn). Merr*., QT834) bred by College of Agronomy, Northwest A&F University, was used. Two types of plastic film were used: low density polyethylene (LDPE) film produced by Xifeng Agricultural Technology Service Co., Ltd., China and biodegradable film which consists of 98% Poly (butylene adipate-co-terephthalate) (PBAT) and 2% polylactic acid (PLA) provided by Qingtian Plastic Products Co., Ltd., China. Low density polyethylene microplastics (LDPE-M, 500–1000 μm) was purchased from Dongguan Zhonglian Plasticizing Co., Ltd., China. To simulate the field size of plastic debris, two types of plastic film were cut manually with scissors, with diameter of 2000 μm, 1000 μm, 500 μm and then mixed in a 1:1:1 ratio before adding into soil. Taking plastic type and size into account, we selected LDPE and PBAT debris (LDPE-D and PBAT-D) to compare the effects on different plastic type but with the same size, while we selected LDPE-D and LDPE-M to compare the effects on different plastic size but the same type. After the plastic mixture was prepared, we added it into a clay pot together with the test soil, and then mixed thoroughly.

2.2. Experiment setup

The contents of LDPE debris (LDPE-D) and PBAT debris (PBAT-D) in soil were 0.05%, 0.1%, 0.2%, 0.5%, 1% and 2%, and the contents of LDPE-M in soil were 0.05%, 0.1%, 0.2% and 0.5% according to our previous studies (Qi et al., 2020; Chen et al., 2022b). In total, 17 treatments were conducted including the control without plastic addition. In each treatment, 5 replicates were considered. To avoid polluting the surrounding soil, clay pots with 8.8 kg soil mixed with plastics were buried into the field to simulate no-tillage during the winter season (October 2020–March 2021). During the whole winter, a nylon-mesh with 150 μm was used to avoid field animals and plastic drift. Before sowing, we cleaned up the surrounding weeds. Afterwards, 2 L water was added to each pot for overnight and six soybean seeds were sown per pot. Two seedlings were left in each pot for experimental observation and water was added depending on the weather (2–4 times per week).

2.3. Soil sampling

Soil samples were taken at four different growing stages: sowing, seedling, flowering and harvesting. 50 g soil was collected from each pot at 0–15 cm by a small steel auger at the sowing, seedling and flowering stages, and the same amount of soil was taken in the harvesting by removing the whole plant and soil out of the pot. After taking soil samples at the harvesting stage, plant residuals were removed and the rest soil was re-filled into the same pot which was reburied into the field for further study. After sampling, each soil sample was divided into two portions: one was stored at 4 °C in order to test soil enzyme activities;

another one was air-dried for SOC analysis. All the collected samples (soil and plant) were packed in paper bags till analysis.

2.4. Sample analysis

2.4.1. Plastic extraction, mass calculation and morphology

The LDPE-D and PBAT-D were collected with metal tweezers after harvesting. Then, the debris was wrapped in a nylon-mesh (aperture <3 μm) and soaked in 30% H_2O_2 and 20% diluted H_2SO_4 for 10 h to remove impurities. The microplastics from soil were extracted through a modified density flotation method (Chen et al., 2022b). Briefly, 5 g of air-dried soil was extracted with 50 mL of saturated $CaCl_2$ solution, and the suspension was then filtered 4–5 times with slow quantitative filter paper (aperture <3 μm). The filter paper was folded and dried at 45 °C for 16 h, and the suspension was transferred to a 600 meshes nylon-mesh. Then, the nylon mesh was fixed and soaked in 30% H_2O_2 and 20% diluted H_2SO_4 for 10 h to remove impurities. Afterwards, the nylon-mesh was washed with deionized water and then dried in an oven at 40 °C for 24 h. The plastic debris was washed with deionized water and then dried and weighted. The mass of microplastics were estimated based on the empirical model established by Zhang et al. (2018b). Aluminum cups and glass bottles were used for plastic extraction and measurement in order to avoid any contamination during the sample processing. The morphology of plastic debris and microplastics were scanned by Nova Nano-SEM 450. The specific parameters were: magnification at 3000 × , working voltage at 5.00 kV, working distance at 4.9 mm, and length scale at 50 μm.

2.4.2. SOC and C-enzyme activities

The potassium dichromate oxidation method was used to determine SOC (Mebius, 1960). In brief, 0.5–1.0 g of air-dried soil was placed in a glass tube, and then 5 mL $K_2Cr_2O_7$ (0.80 mol L^{-1}) and 5 mL sulfuric acid (98%) were added before heating in paraffin oil at 180 °C for 5 min. After tube cool down, 3 drops of o-phenanthroline indicator were added and the solution was titrated with $FeSO_4$ (0.20 mol L^{-1}) until it turned red in color. SOC was thus calculated based on the volume of $FeSO_4$ (Mebius, 1960). A fluorescence microplate enzyme assay was used to analyze soil enzyme activities (DeForest, 2009). Three carbon enzymes (β-glucosidase (BG), β-xylosidase (GH) and cellobiohydrolase (CBH)) were assayed using their respective fluorescent substrates and standards. To determine those enzymes, 3 g soil were added to 125 mL of Tris-HCl buffer (pH = 8.25), stirred thoroughly, and allowed to settle for 10 min to prepare soil suspension, and then 150 μL of soil suspension was drawn into the 96-well enzyme label plate. Separate plates were prepared for 4-methylumbelliferone standard curves for each sample. 50 μL of appropriate standards and substrates were added to each column of the enzyme label plate. The plates were sealed and vibrated to mix the contents. The plates were incubated for 4 h at 25 °C in an incubator, after which fluorescence values were measured at 365 nm excitation wavelength and 450 nm emission wavelength.

2.5. Statistics

The mean values and standard deviations of SOC and C-enzyme activities for each treatment were calculated. All statistical analysis was performed using IBM SPSS Statistics 26.0. Data normality distribution analysis was performed using the Shapiro-Wilk test. The indexes in different growing stages and treatments were compared with one-way ANOVA. Based on the test of homogeneity using Levene's test, the significance of each indicator amongst different treatments was tested with Duncan's New Multiple Range Test at a level of 0.05. The test of fixed effects is used to quantify the correlations among plastic properties (type, size and abundance), SOC and C-enzyme activities. Correlation analysis is used to quantify the relationship between SOC and carbon enzyme activities, with the test level of 0.01 and 0.05. Furthermore, the change in C-enzyme activities during the seedling, flowering, and harvesting stages relative to the sowing stage were calculated, aiming to assess the dynamic variations of carbon enzyme activities throughout the growth stages of soybeans.

3. Results

3.1. Mass loss and morphology changes of plastic before and after planting

Plastic concentrations were monitored after soybean harvesting. The results showed that LDPE-D treatments had the least plastic mass loss, ranging from 4.56% to 9.68%. The PBAT-D treatments had the highest loss of plastic mass, ranging from 26.16% to 87.20%. The plastic mass loss of LDPE-M treatments was in between the first two, ranging from 7.60% to 17.69% (Fig. 1).

After checking the plastic loss, we scanned the extracted plastic particles with SEM. Before planting, the LDPE-D had relatively clean, smooth surfaces with prominent linear graining; the surfaces of the PBAT-D had many bulbous protrusions; and the surface of LDPE-M was relatively dense with shining surface layer. After soybean planting, the LDPE-D surfaces appeared scratched, but no significant changes in morphology occurred. The PBAT-D surfaces underwent significant changes, with their bubbles completely popped, while numerous cracks and corrosion holes appeared. The LDPE-M surface became rough and developed micropores with hollow structure (Fig. 2).

3.2. SOC changes in different treatments

In treatments with LDPE-D, SOC content increased 12.69–13.26% at sowing stage, 3.24–7.56% at the seedling stage, 6.48–11.86% at the flowering stage, and 7.94–12.99% at the harvesting stage, compared to control, respectively, while no significant difference was observed among different treatments in each growing stage (Table 2). In treatments with PBAT-D, SOC increased significantly in treatments with ≥0.2% PBAT-D at the sowing stage and the seedling stage, compared to control, ranging 3.94–11.04% and 7.08–9.93%, respectively ($p < 0.05$). At the flowering and harvesting stages, the addition of PBAT-D linearly increased SOC with the increasing PBAT-D level, significant differences were observed between each PBAT-D dose and the control ($p < 0.05$). In treatments with LDPE-M, SOC content increased 16.87–33.89% at the

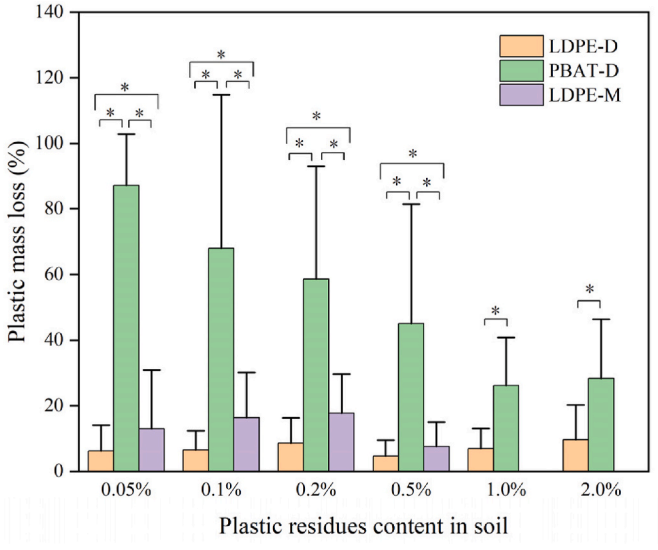

Fig. 1. The mass loss of plastic residues in soil before and after soybean planting. (LDPE-D: Polyethylene debris; PBAT-D: Poly (butylene adipate-co-terephthalate) debris; LDPE-M: Polyethylene microplastics. * indicates significant difference.).

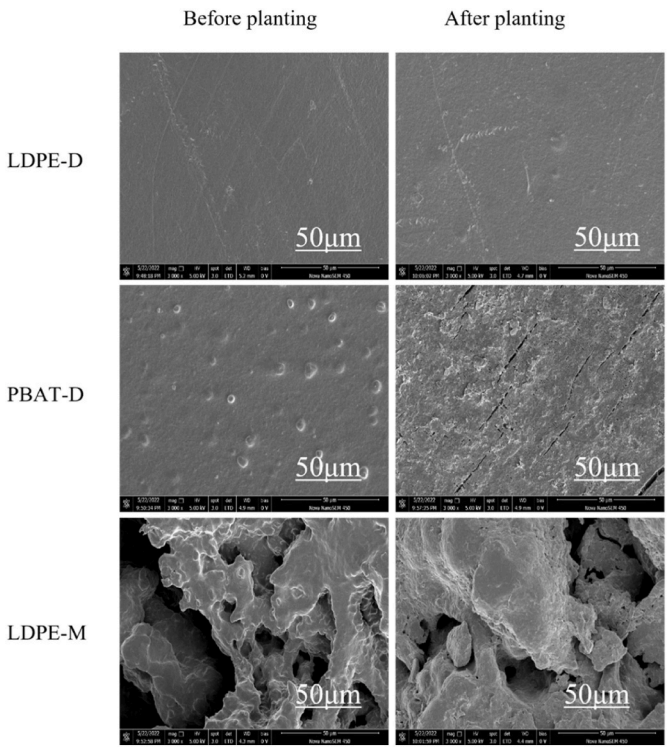

Fig. 2. Plastic surfaces made by scanning electron microscope.

sowing stage, 5.58–10.27% at the seedling stage, 9.53–10.73% at the flowering stage, and 8.14–13.75% at the harvesting stage, compared to control, respectively, whereas no statistically significant difference was observed among different treatments at the seedling, flowering and harvesting stages. At the sowing stage, SOC content among LDPE-M treatments decreased significantly with increasing levels of LDPE-M ($p < 0.05$).

We also compared the SOC dynamics throughout the sowing stage, seedling stage, flowering stage and the harvesting stage for each treatment. For LDPE-D treatments, SOC across the sowing stage, seedling stage and flowering stage showed no significant differences in each treatment, while they all significantly lower than on harvesting stage except 0.2% LDPE-D treatment ($p < 0.05$). For PBAT-D treatments, SOC at the sowing stage, seedling stage, flowering stage and harvesting stage showed no significant difference in 0.05%, 0.1% and 0.5% PBAT-D treatments. In 0.2% PBAT-D treatments, SOC significantly increased 6.64%, 6.02% and 6.06% respectively at the seedling, flowering and harvesting stage ($p < 0.05$). In 1% PBAT-D treatments, SOC significantly increased 6.62%, 10.75% and 11.11% respectively at the seedling, flowering and harvesting stage compared to the sowing stage ($p < 0.05$). In 2% PBAT-D treatments, SOC significantly increased 1.30%, 7.23% and 10.81% respectively at the seedling, flowering and harvesting stage compared to the sowing stage ($p < 0.05$). For LDPE-M treatments, at the seedling, flowering and harvesting stages, SOC significantly decreased by 16.75%, 17.28%, 10.50% respectively in 0.05% LDPE-M treatment, and by 16.06%, 14.57%, 7.17% respectively in 0.1% LDPE-M treatment, compared to the sowing stage ($p < 0.05$). In treatments of 0.2% and 0.5% LDPE-M, SOC was no significant difference in four observation stages (Table 1).

3.3. Carbon-enzyme activities in different treatments

Regarding to control treatment at sowing stage, the GH activities significantly decreased by 45.80% at seedling stage, and by 14.49% in flowering stage, while increased by 1.64% at harvesting stage (Fig. 3a, $p < 0.05$). The BG activities significantly decreased by 49.76% at seedling stage, and by 8.07% at flowering stage, while increased by 2.76% at harvesting stage (Fig. 3b, $p < 0.05$). The CBH activities significantly decreased by 63.45% at seedling stage, while increased by 1.06% at flowering stage, and by 23.58% at harvesting stage (Fig. 3c, $p < 0.05$).

In treatments with LDPE-D at sowing stage, the GH activity significantly decreased 63.60–77.53% in the seedling stage, while significantly increased by 15.77–57.90% at the flowering stage. At the harvesting stage, the GH activity significantly decreased by 6.98%, 4.24% and 5.71% in 0.2%, 0.5% and 1% LDPE-D treatments, while significantly increased by 25.12% and 7.67% in 0.05% and 0.5% LDPE-D treatments, respectively (Fig. 3a, $p < 0.05$). Compared to the sowing stage, the BG activity significantly decreased by 58.81–73.23% in the seedling stage, while significantly increased by 29.65–40.21% in flowering stage. In the harvesting stage, the BG activity significantly decreased by 6.49–35.01% (Fig. 3b, $p < 0.05$). Compared to the sowing stage, the CBH activity significantly decreased by 49.36–88.43% in the seedling stage. In the flowering stage, the CBH activity significantly decreased by 16.86% 8.08% and 7.38% in 0.1%, 0.2% and 0.5% LDPE-D treatments, respectively, while significantly increased by 20.40% and 16.18% in 0.05% and 2% LDPE-D treatments, respectively. In the harvesting stage,

Table 1
Soil organic carbon content (mg kg^{-1}) at different growth stages of treatments.

Treatment	Sowing		Seedling		Flowering		Harvesting	
Control-0%	8.68 ± 0.18	cC	8.77 ± 0.13	cBC	8.98 ± 0.18	cABC	9.38 ± 0.28	bA
LDPE-D-0.05%	9.71 ± 0.15	abB	9.58 ± 0.13	bcB	9.61 ± 0.13	bcB	10.59 ± 0.11	aA
LDPE-D-0.1%	9.57 ± 0.15	bBC	9.69 ± 0.10	aB	9.44 ± 0.22	aB	10.34 ± 0.18	aA
LDPE-D-0.2%	9.73 ± 0.21	abAB	9.50 ± 0.26	abB	9.67 ± 0.16	abAB	10.12 ± 0.30	aA
LDPE-D-0.5%	9.70 ± 0.16	abB	9.47 ± 0.02	abB	9.44 ± 0.22	abB	10.29 ± 0.12	aA
LDPE-D-1.0%	10.00 ± 0.20	aAB	9.66 ± 0.14	aBC	9.60 ± 0.16	aC	10.26 ± 0.22	aA
LDPE-D-2.0%	9.79 ± 0.19	abB	9.61 ± 0.14	aB	9.81 ± 0.18	aB	10.67 ± 0.41	aA
Control-0%	8.68 ± 0.18	cC	8.77 ± 0.13	cBC	8.98 ± 0.18	cABC	9.38 ± 0.28	bA
PBAT-D-0.05%	8.87 ± 0.18	bcA	8.90 ± 0.27	bA	8.88 ± 0.36	dA	9.28 ± 0.07	cA
PBAT-D-0.1%	9.18 ± 0.31	abAB	8.98 ± 0.16	bB	9.29 ± 0.12	cAB	9.50 ± 0.37	cA
PBAT-D-0.2%	9.03 ± 0.28	bcB	9.62 ± 0.23	aA	9.56 ± 0.16	cA	9.57 ± 0.06	cA
PBAT-D-0.5%	9.19 ± 0.22	abA	9.65 ± 0.56	aA	9.92 ± 0.06	bA	9.85 ± 0.66b	cA
PBAT-D-1.0%	9.28 ± 0.45	abB	9.88 ± 0.05	aA	10.26 ± 0.05	aA	10.28 ± 0.35	abA
PBAT-D-2.0%	9.64 ± 0.39	aB	9.75 ± 0.11	aB	10.33 ± 0.10	aA	10.68 ± 0.26	aA
Control-0%	8.68 ± 0.18	cC	8.77 ± 0.13	cBC	8.98 ± 0.18	cABC	9.38 ± 0.28	bA
LDPE-M-0.05%	11.63 ± 0.30	aA	9.68 ± 0.44	aC	9.61 ± 0.25	aC	10.40 ± 0.07	aB
LDPE-M-0.1%	11.30 ± 0.31	aA	9.49 ± 0.34	abC	9.65 ± 0.13	aC	10.48 ± 0.36	aB
LDPE-M-0.2%	10.15 ± 0.28	bAB	9.91 ± 0.17	aAB	9.70 ± 0.19	aB	10.14 ± 0.12	aAB
LDPE-M-0.5%	10.28 ± 0.71	bAB	9.54 ± 0.15	abB	9.71 ± 0.19	aAB	10.66 ± 0.58	aA

Note: Lowercase letter means significant differences among plastic levels in each sampling time; capital letter means significant differences in each individual treatment among different growth stages.

Table 2
Correlation analysis of SOC and C-enzyme activities with treatment factors.

Indices	Factors	*F-value*		
		Sowing-Seedling	Sowing-Flowering	Sowing-Harvesting
SOC	Type	13.80[b]	39.11[b]	0.82
	Size	25.55[b]	37.36[b]	40.96[b]
	Abundance	4.56[a]	2.68	3.29
	Abundance × Type	2.09	2.97	2.66
	Abundance × Size	5.22[a]	5.50[a]	8.03[a]
GH	Type	32.60[b]	1370.20[b]	4.45[a]
	Size	30.19[b]	1.33	311.04[b]
	Abundance	7.37[b]	13.23[b]	2.44
	Abundance × Type	7.24[b]	4.10[a]	1.49
	Abundance × Size	12.49[b]	32.40[b]	7.30[a]
BG	Type	13.47[b]	1813.99[b]	14.52[b]
	Size	0.11	732.38[b]	1035.94[b]
	Abundance	9.90[b]	12.39[b]	7.88[b]
	Abundance × Type	10.67[b]	17.97[b]	6.36[b]
	Abundance × Size	3.18[a]	1.61	2.96[a]
CBH	Type	25.32[b]	55.05[b]	0.39
	Size	65.08[b]	0.62	1.42
	Abundance	24.91[b]	6.66[b]	4.58[b]
	Abundance × Type	3.17[a]	8.46[b]	5.14[b]
	Abundance × Size	11.45[b]	21.20[b]	0.04

Note: SOC: soil organic carbon; GH: β-xylosidase; BG: β-glucosidase CBH: cellobiohydrolase.
[a] $p < 0.05$.
[b] $p < 0.01$.

the CBH activity significantly decreased by 12.13–43.02% (Fig. 3c, $p < 0.05$).

In treatments with PBAT-D at the sowing stage, the GH activity significantly decreased 50.99–77.19% in the seedling stage, and by 6.85–30.45% in the flowering stage. In the harvesting stage, the GH activity significantly decreased by 8.03%, 17.70% and 13.37% in 0.1%, 0.2% and 1% PBAT-D treatments, while significantly increased by 5.61% in 2% PBAT-D treatments (Fig. 3d, $p < 0.05$). Compared to sowing stage, the BG activity significantly decreased by 48.43–69.19% in the seedling stage, and by 7.39–44.95% in the flowering stage, and by 2.25–34.40% in the harvesting stage (Fig. 3e, $p < 0.05$). Compared to sowing stage, the CBH activity significantly decreased by 70.05–90.89% in the seedling stage, and by 10.08–36.15% in the flowering stage. In the harvesting stage, the CBH activity significantly decreased by 20.06% and 6.90% in 0.5% and 2% PBAT-D treatments, respectively, while significantly increased by 25.90% and 18.79% in 0.05% and 1% PBAT-D treatments, respectively (Fig. 3f, $p < 0.05$).

In treatments with LDPE-M at the sowing stage, the GH activity significantly decreased 52.53–71.98% in the seedling stage, while significantly increased by 5.21–65.84% in the flowering stage, by 69.09–103.89% in the harvesting stage (Fig. 3g, $p < 0.05$). Compared to sowing stage, the BG activity significantly decreased by 55.35–69.13% in the seedling stage, and by 11.40–19.39% in the flowering stage, while significantly increased by 57.00–69.73% in the harvesting stage (Fig. 3h, $p < 0.05$). Compared to sowing stage, the CBH activity significantly decreased by 81.75–82.91% in the seedling stage. In the flowering stage, the CBH activity significantly decreased by 18.95% and 11.05% in 0.05% and 0.2% LDPE-M treatments respectively, while significantly increased by 12.04% in 0.5% LDPE-M treatments. In harvesting stage, the CBH activity significantly increased by 53.41–73.44% in LDPE-M treatments compared to control (Fig. 3i, $p < 0.05$).

3.4. Correlation analysis

The SOC dynamic was significantly affected by plastic size and type from the sowing stage to seedling stage ($p < 0.01$), while significantly affected by abundance × size from the seedling to flowering stage ($p < 0.05$) (Table 2).

From the flowering stage to harvesting stage, the SOC dynamic was significantly affected by plastic type ($p < 0.01$). The activities of GH were significantly affected by plastic size and type from the sowing stage to seedling stage ($p < 0.01$), and significantly affected by plastic type from the seedling stage to flowering stage ($p < 0.01$). From the flowering stage to harvesting stage, the activities of GH were significantly affected by plastic size ($p < 0.01$). The plastic type, abundance and abundance × type had significant effects on BG activities from the sowing stage to seedling stage, while type and size become the most significantly influencing factor from the seedling stage to flowering stage ($p < 0.01$). From the flowering stage to harvesting stage, the size of plastic has a significant effect on BG activities ($p < 0.01$). The activities of CBH were significantly affected by type, size and abundance from the sowing stage to seedling stage ($p < 0.01$), and by type, abundance and abundance × size from the seedling stage to flowering stage ($p < 0.01$). The abundance and abundance × type have significant effects on CBH activities from the flowering stage to harvesting stage ($p < 0.01$). We did not find any impact due to the interaction term abundance × size × type.

4. Discussion

4.1. Effects of plastic-contamination on soil organic carbon

Previous research has shown that plastic residues affect SOC content, and that this effect varies with their type, size and abundance (Lehmann et al., 2021; Rillig et al., 2021). According to our study, we observed the changes of plastic mass loss (Fig. 1) and morphology (Fig. 2) before and after the experiment. Comparing to LDPE-D and LDPE-M, PBAT showed significant mass loss, in accordance with other findings (Meng et al., 2022). Meanwhile, LDPE-M seems degraded faster than LDPE-D regarding to the mass loss. It is reported that small-sized LDPE-M leads to higher CO_2 emission (Huo et al., 2024) and stronger activities related to carbon-associated processes, such as carbon turnover and its mineralization, than larger-sized LDPE-M (Zang et al., 2020). Those findings indicate that plastic may release carbon during its weathering and aging (Li et al., 2022), which may eventually affect soil organic carbon storage. To compare the findings of different studies, we reviewed and summarized those findings in Table S2. Cao et al. (2023) indicated that PE-D and PBAT-D (2 × 3 cm) released carbon into the soil and that PBAT released about 100-fold more SOC than PE. Santini et al. (2022) reported that SOC content increased by 4.41% and 3.71%, in treatment with LDPE and biodegradable plastic debris, respectively. Zhao et al. (2021) found that LDPE debris increased the SOC by 4.26–17.13% while Chen et al. (2024) observed that PBAT significantly increased SOC by 116.0–191.1%. Our research indicates similar findings, though the variations were observed across different growing stages and treatments (Table 1). In the LDPE-D treatments, the changes of SOC were not significant before the flowering stage, whereas it significantly increased from the flowering stage to the harvesting stage ($p < 0.05$). In the PBAT-D treatments, SOC exhibited a slight increase with the progression of growth stages. It has been reported that plastic with varying physicochemical properties induces various effects on SOC (Zhang et al., 2022a). LDPE-D has a highly stable hydrocarbon chain structure, which leads to long-term persistence in the soil and long-term negative impacts on soil structure (i.e. porosity, connectivity, aeration and migration path of capillary water) (Ma et al., 2023; Qi et al., 2020). This might inhibit the mineralization and leaching of SOC and thereby promote the sequestration of SOC (Fukumasu et al., 2022; Rabot et al., 2018). Compared to LDPE-D, the structure of PBAT-D makes it easier for it to fragment and degrade in soil (Meng et al., 2022). Related studies

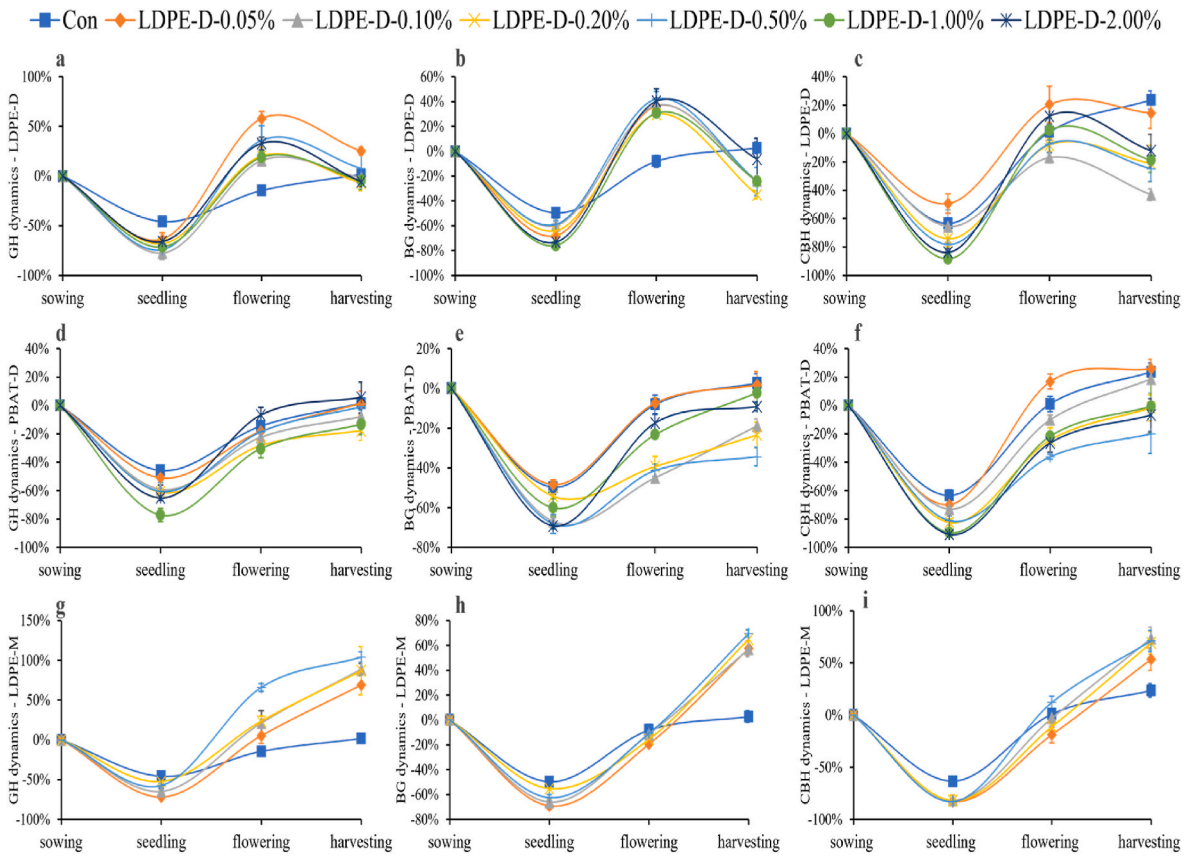

Fig. 3. Soil C-enzyme dynamics in different treatments (a–c: GH, BG and CBH dynamics in LDPE-D treatments, respectively; d–f: GH, BG and CBH dynamics in PBAT-D treatments, respectively; g–i: GH,BG and CBH dynamics in LDPE-M treatments, respectively; LDPE-D: Polyethylene debris; PBAT-D: Poly (butylene adipate-co-terephthalate) debris; LDPE-M: Polyethylene microplastics; GH: β-xylosidase; BG: β-glucosidase CBH: cellobiohydrolase).

indicate that PBAT and PLA debris tends to accumulate a large number of soil microorganisms on their surface, which then consume carbon from both PBAT and PLA, converting it into their own biomass (Meng et al., 2023). Subsequently, these organic matter may flow into the soil carbon pool along with the death and decomposition of the microorganisms (Ding et al., 2021). In this study, the mass loss of PBAT-D reached 28.32–87.20%, which is significantly higher than LDPE-D (Fig. 1). Thus, we infer that the changes of SOC dynamics in PBAT-D treatments result from carbon input from the biodegradation processes of the plastics. Thus, the LDPE-D increased SOC content by inhibiting the SOC loss, whereas PBAT-D enhanced SOC content through C input from its degradation and fragmentation.

As mentioned above, plastic debris can positively affect SOC (Cao et al., 2023; Santini et al., 2022; Zhao et al., 2021). However, once it breaks down into microplastics, the impacts may change. Shi et al. (2023) observed that PE microplastics decreased soil SOC concentrations by 4–18%. Cao et al. (2023) found that the addition of LDPE-M led to a threefold increase in soil CO_2 emissions, with almost all of the emitted CO_2 originating from DOC. This indicates that the input of microplastics enhances the mineralization of soil organic carbon SOC. In this study, we also observed a significant decrease of SOC from the sowing stage to seedling stage in 0.05% and 0.1% LDPE-M treatments ($p < 0.05$). Unlike LDPE-D, LDPE-M negatively affected SOC which may result from altering the spatial arrangement of soil grains and the pore network, thereby disrupting the stability of soil aggregates and reducing their physical protection of SOC (Lehmann et al., 2021). In addition, recent studies have shown that the hydrophobic surface of LDPE-M could modify the O_2 microenvironment of the soil, and create an oxygenated porous habitat surrounding the microplastics. This may lead to a decrease of SOC (Shi et al., 2023). However, after the flowering stage, an increase of SOC was observed (Table 1). This result was similar to the finding reported by Meng et al. (2022), that the addition of microplastic decreased SOC content from day 0–46 in soybean soils while increasing it after day 46. This may be because mature vegetation can alleviate SOC loss in microplastic-contaminated soil (Hou et al., 2024). However, the SOC content after the flowering stage was still lower than sowing stage, which suggests that the negative effects of LDPE-M on SOC remain important.

It has also been reported that different abundances of plastic residues induce a diverse range of responses by SOC. For non-biodegradable plastic debris, Gao et al. (2021) reported that 3%, 6% and 18% LDPE addition increased DOC by 54.72% and 61.79% and 74.29%, respectively. Zhang et al. (2022b) found that 5% LDPE had no significant effect on SOC, but that 10% and 15% LDPE increased SOC by 13.62% and 7.81% in clay, respectively. However, in this study, the abundance of LDPE-D was found to have no significant effect on SOC dynamic. This may be attributed to the plastic abundance (3–18%) used in their study, which was much higher than what we used. Hence, under low LDPE-D abundance (≤2%), these differences may not occur. As for biodegradable plastic debris, an increase in the abundance of PBAT-D enhances its impacts on SOC dynamics, especially in the 1% and 2% treatments. These results are similar to the findings reported by Chen et al. (2022a), that soils treated with 10% PBAT were approximately 50% higher in SOC than soils treated with 5% PBAT. This may be because the higher abundance of biodegradable plastics leads to more input of carbon into the soil (Ding et al., 2021). In addition, low abundances (0.05% and 0.1%) of LDPE-M had a significant negative effect on SOC dynamics, while high abundance (0.2% and 0.5%) LDPE-M had no significant effect on SOC dynamics. This may be because a small amount of LDPE-M can lead to a “priming effect”, stimulating microbial activity in the soil

and accelerating the mineralization of SOC (Huo et al., 2024; Zhang et al., 2024), whereas excessive amounts of LDPE-M may be toxic to the microbial organisms thereby inhibiting this effect (Qiu et al., 2022; Sendra et al., 2019). In summary, there are significant differences in the responses of SOC to plastic pollution characteristics (types, sizes, and abundances). It is recommended that future studies should comprehensively consider factors such as the properties of the plastics, and environmental conditions, to better understand the impact of plastic pollution on soil organic carbon.

4.2. Effects of plastic contamination on soil C-enzymes

The accumulation of plastic residues may alter the soil C-enzyme activities, and this effect varies on their types, sizes and abundances (Liu et al., 2023a; Liu et al., 2022). It has been reported that 10% polystyrene (PS), polyvinyl chloride (PVC) and LDPE debris inhibited BG activities by 35.68%, 30.67% and 25.76%, respectively in maize soils at the flowering stage (Shah et al., 2023). Zang et al. (2020) studied the effects of two non-degradable microplastics on enzyme activities in bulk soil with wheat and showed that 5% and 20% PVC-MPs addition inhibited the activities of BG and GH by 16–43 %, while 1%, 5%, 10% and 20% PE-MPs had no significant effect on the activities of BG, GH, and CBH. In contrast, Yu et al. (2023) observed that C-enzyme activities (include BG, GH and CBH) in rhizosphere soil at the harvesting stage increased significantly with increasing levels of PBAT-MPs, ranging from 7.83 ± 1.69 nmol g^{-1} h^{-1} to 28.17 ± 14.33 nmol g^{-1} h^{-1} ($p < 0.05$). In our study, the enzyme activities were affected by the plastic contamination of soils, which is consistent with previous studies (Awet et al., 2018; Guo et al., 2021). The C-enzyme activities are dynamic across the different growing stages (Fig. 3). Specifically, the C-enzyme activities significantly decreased from the sowing stage to the seedling stage in control treatment but increased later ($p < 0.05$). Among the plastic treatments, only LDPE-D changed this trend. PBAT-D and LDPE-M resulted in the same dynamics as under control but with a larger amplitude (Table S1). These results emphasize the importance of looking into dynamics across the whole cultivation period rather than single growth stages. Otherwise, the impacts of plastic contamination on enzyme activities may be overestimated. The disruption of C-enzyme activity dynamics by plastic residues may be due to their alteration of soil microbial communities (Stegarescu et al., 2021; Yang et al., 2018). Depending on the type and size of the plastic residues, differences exist in their impacts on soil microbial communities. For example, different plastic surfaces will enrich different microbial communities, which will lead to the proliferation of certain dominant populations and thus affect the secretion of soil enzymes (Meng et al., 2023; Ogonowski et al., 2018). From the results, it appears that LDPE-D has a stronger disruptive effect on C-enzyme activity dynamics, which could be attributed to the interactions between plastic type and size (Table 2). More important, soil C-enzymes can degrade cellulose and hemicellulose into glucose, which is of great significance for the utilization and conversion of soil organic matter (Moreno et al., 2017). The alteration of soil enzyme activity by plastic residues may indirectly impact the content of soil available nutrients (Liu et al., 2023b), which further affects the growth of plant roots (Yu et al., 2023). Therefore, the effects of plastic residues on soil enzyme activity dynamics need to be further studied in different soil-plant systems.

The dynamics of C-enzyme activities were significantly influenced by plastic abundance (Table 2). Similarly, Yu et al. (2023) also observed nonmonotonic responses between plastic abundance and soil enzyme activities. This may be due to the nonmonotonic effects of plastic abundance on soil structure (Qi et al., 2020) and soil microbial communities (de Souza Machado et al., 2019). As a result, it is difficult to concisely summarize how abundance impacts the effects of various types of plastic residues on soil enzyme activities. de Souza Machado et al. (2018) suggested that the addition of plastic residues to the soil would affect multiple interacting soil processes, such that the overall effect would be uncertain, and that it would be challenging to make a definitive conclusion. Therefore, further research is needed to explore more the effects of plastic pollution on soil microbial communities. This is evident in the recent emergence of the new research field on the microplastome (Li et al., 2024b).

In addition, SEM showed that the surface morphology of LDPE-D hardly changed after soybean planting, in line with findings from previous studies (Mumtaz et al., 2010; Ohtake et al., 1998) which indicated that the degradation rate of LDPE-D is low. Therefore, we believe that the changes in SOC and C-enzyme activity in the LDPE-D treatment mainly arose from the indirect effects of LDPE-D, such as its negative effects on soil pore properties and capillary water pathways (de Souza Machado et al., 2018; Qi et al., 2020). In contrast, the surfaces of PBAT-D became obvious degraded, to a much higher degree than LDPE-D (Fig. 2). A large number of soil microorganisms may be attached to the surfaces of PBAT-D in the soil, which will facilitate the biodegradation of PBAT-D and the release of a large amount of DOC into the soil (Cao et al., 2023; Ding et al., 2021; Meng et al., 2023). Therefore, the changes to SOC and C-enzyme activities under PBAT-D treatment depends on its enrichment of soil microorganism communities, and the input of carbon to the soil resulting from its degradation. For LDPE-M, numerous micropores was observed on its surfaces after planting, compared to LDPE-D. In combination with its smaller particle sizes, this means that LDPE-M has a higher specific surface area than LDPE-D, which may be more conducive to microbial colonization, leading to the formation of a “plastisphere” (Rillig et al., 2024). Recent research has showed that the microorganisms enriched on the surfaces of microplastics are primarily carbon-degrading microbes, such as *Proteobacteria*, *Actinobacteriota*, and *Bacteroidota* (Qiu et al., 2024), which contribute to the activities of GH, BG, and CBH in the soil (Pathan et al., 2015; Zhang et al., 2022b), and further accelerates SOC mineralization. These results support our findings that the effects of plastic contamination on SOC and enzyme activities depends on plastic type and size. Further research is needed to explore the relationship between plastic aging and the biogeochemical properties of the soil.

5. Conclusion

Plastic contamination, especially in agricultural soils, has increasingly become recognized as an important environmental pollution issues. We conducted a field experiment and showed that LDPE-D, PBAT-D and LDPE-M had noticeable effects on SOC and C-enzyme activity dynamics throughout the various growth stages of soybean. Specifically, LDPE/PBAT residues positively affected SOC while LDPE microplastics had the opposite effect. Plastics contamination altered C-enzyme activities differently under different treatments, but only LDPE-D significantly altered the dynamics of carbon enzymes. Meanwhile, SOC and C-enzyme activities have nonmonotonic responses to the abundance of plastic residues. Furthermore, the occurrence of positive effects during any particular growth stage of a plant does not preclude negative effects during other growth stages, relative to the control treatment. As discussed, these outcomes are likely mediated by the effects of the plastic contamination on the soil microbial community, which is differentiated according to plastic type as they are subject to highly different weathering and degradation processes. The processes underlying these effects are dynamic, and sensitive to the stage of crop growth and to soil and microbe community properties, which precludes the making of a general conclusion on the overall effects of plastic contamination. Nevertheless, our findings suggest that the soil carbon cycle is likely disrupted differently by various forms of plastic contamination, and provide further understanding on the effects of plastic pollution on the organic carbon pools of agricultural soils.

CRediT authorship contribution statement

Xinkai Jia: Writing – review & editing, Writing – original draft,

Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yu Yao:** Writing – review & editing, Investigation. **Gaowei Tan:** Writing – review & editing. **Sha Xue:** Resources. **Mengjuan Liu:** Resources. **Darrell W.S. Tang:** Writing – review & editing, Supervision. **Violette Geissen:** Conceptualization. **Xiaomei Yang:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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