

Biodegradable microplastic type and wheat-straw addition shape soil CO₂ emissions: links to dissolved organic matter turnover and microbial carbon allocation

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Abstract

Biodegradable microplastics (BMPs) are increasingly recognized as important factors affecting soil carbon cycling. However, how BMP-induced changes in respiration relate to dissolved organic matter (DOM) transformation and microbial carbon use under crop-residue addition remains poorly resolved. Here, we combined a 120-d poly(butylene adipate-co-terephthalate) (PBAT) × wheat-straw incubation with a 64-d comparison of BMP type and dose and quantified CO₂ emission, DOM optical and molecular properties, hydrolytic enzymes, functional genes, microbial biomass and ¹³C-DOC allocation. PBAT increased cumulative CO₂ emission by 214% without wheat straw and by 161% with wheat straw. Although the proportional response was smaller with wheat straw, the absolute PBAT-associated increment was larger. PBAT also caused an early DOC increase and a transient humic-like fluorescence response, whereas day-10 DOM contained more assigned formulae but had a higher intensity-weighted nominal oxidation state of carbon and a lower molecular-lability boundary. In addition, PBAT increased the absolute abundance of several C-degradation genes, but most differences weakened after normalization to bacterial 16S rRNA gene abundance, suggesting that bacterial growth accounted for much of the increase. Among BMP treatments, 1% PHA produced the highest CO₂ emission, microbial biomass and 24-h ¹³C-DOC-derived apparent carbon use efficiency. Total ¹³C incorporation into PLFAs did not differ significantly among treatments. Under 1% PHA, however, relative allocation shifted away from Gram-positive bacteria and fungi toward actinomycetes. Collectively, these findings show that increased CO₂ emission was not consistently accompanied by higher DOC concentration, greater gene abundance per bacterial 16S rRNA gene or greater total short-term tracer assimilation. Evaluating carbon flux together with DOM composition and microbial allocation therefore provides a more complete assessment of BMP effects on soil carbon processing under controlled incubation conditions.

1. Introduction

Soil organic-carbon processing is a major determinant of terrestrial carbon storage and nutrient availability, yet it is increasingly altered by anthropogenic plastic inputs. The widespread use of biodegradable films and other short-lived products has raised concern because incomplete degradation can generate biodegradable microplastics (BMPs) in agricultural soils (Liao and Chen, 2021; Qin et al., 2021; Fan et al., 2022). PBAT, polylactic acid (PLA) and polyhydroxyalkanoates (PHA) contain hydrolysable bonds and can release compounds that interact with soil microorganisms. Given that microbial processing partitions organic carbon between CO₂ loss, biomass production and residual dissolved organic matter (DOM), even moderate BMP-induced changes may affect soil-carbon balance. This issue is especially relevant where BMP residues coexist with returned crop straw, a large and recurrent input of readily transformed carbon.

Previous studies have consistently reported that BMPs can alter soil respiration, microbial biomass and native-organic-matter mineralization, although the magnitude and even direction of the response vary with material, dose, particle size, soil properties and exposure duration (Rauscher et al., 2023; Shi et al., 2023; Jia et al., 2024; Su et al., 2024). Stable-isotope tracing shows that biodegradable-plastic carbon

can enter both CO₂ and microbial biomass, while optical and molecular analyses show that BMPs modify soil DOM (Zumstein et al., 2018; Sun et al., 2022; Zhang et al., 2023; Chen et al., 2024). On the one hand, hydrolysis products may promote microbial growth, co-metabolism and nutrient acquisition. On the other hand, slow hydrolysis, preferential use of newly supplied carbon or physical changes in the soil matrix may offset or reverse effects on native soil organic carbon (Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010; Rillig et al., 2021; Chen et al., 2025). Consequently, greater total respiration cannot be equated with positive priming, and nominal biodegradability alone cannot predict the soil response.

Most studies have examined CO₂ emission, DOM composition or microbial responses separately. Consequently, it remains unclear whether BMP-induced DOC accumulation is accompanied by greater respiratory loss, whether higher functional-gene abundance reflects bacterial growth or enrichment of specific functions, and whether BMP exposure changes total microbial assimilation of dissolved carbon or its distribution among microbial groups. These distinctions are important because each measurement represents a different aspect of carbon turnover and can respond over a different time scale. Resolving these relationships requires time-resolved measurements of CO₂ and DOM together with molecular and microbial analyses.

Accordingly, this study aimed to (i) quantify the absolute and proportional PBAT-associated CO₂ responses in the presence and absence of wheat straw, (ii) determine how changes in DOC concentration were related to optical and molecular composition, hydrolytic activity and functional-gene abundance, and (iii) assess whether BMP type and dose altered total ¹³C-DOC incorporation or its allocation among microbial groups. We hypothesized that PBAT would cause an early DOC increase followed by selective molecular transformation, that higher absolute functional-gene abundance would occur largely with bacterial growth, and that microbial-group allocation would be more responsive than total short-term tracer incorporation. To test these hypotheses, we combined a temporal PBAT × wheat-straw incubation with a type-and-dose comparison of PHA, PLA and PBAT. The former resolved changes over 120 d, whereas the latter compared respiration, biomass and tracer allocation across BMP treatments.

2. Materials and methods

2.1. Study design and analytical framework

Two complementary soil incubations were integrated within a common analytical framework. Firstly, a 120-d PBAT × maize-straw factorial incubation resolved time-dependent changes in CO₂ emission and DOM under unamended, PBAT-amended, maize-straw-amended and co-amended conditions; hydrolytic enzymes, MBC, mineral N, hemicellulose degradation and functional genes were measured to evaluate processes associated with those changes. Secondly, a 64-d BMP type × dose incubation compared PHA, PLA and PBAT at 0.1% and 1% (w/w), with emphasis on cumulative CO₂ emission, dissolved C and N pools, microbial biomass and ¹³C-DOC allocation. Thus, the factorial incubation resolved the temporal

and residue dependence of the PBAT response, whereas the BMP comparison tested its transferability across BMP types and doses.

2.2. PBAT–wheat-straw factorial incubation

Topsoil (0–20 cm) was collected from an agricultural field in Dazu District, Chongqing, China (31°53' N, 108°40' E). The region has a subtropical monsoon climate, with a mean annual temperature of 18.4°C and annual precipitation of 1194.1 mm. After plant residues and stones were removed, the soil was air-dried, passed through a 20-mesh sieve, homogenized and pre-incubated for 7 d to restore microbial activity. The loam comprised 40% sand, 45% silt and 15% clay and contained 15.2 g organic C kg⁻¹, 1.7 g total N kg⁻¹ and 127.2 mg available N kg⁻¹; its pH was 5.02 (1:2.5 soil:water). Organic C and total N were measured by elemental analysis, pH in a soil–water suspension and texture by laser diffraction. Wheat straw obtained from an agricultural-product processing facility in Jiangsu Province was dried at 40°C, ground and sieved to 96–3000 µm. It contained 369.1 g C kg⁻¹ and 5.2 g N kg⁻¹ (C:N = 71.1). PBAT particles (Fengtai Plastic Material Co., Dongguan, China) contained 558.6 g C kg⁻¹; the 20–80-µm fraction was washed with ultrapure water and dried at 40°C before use. Initial material images, PBAT FTIR spectra and particle-size distribution are provided in **Fig. S1**.

Four treatments were established on a dry-soil-mass basis: CK, unamended soil; P, soil amended with 1% PBAT; M, soil amended with 1% wheat straw; and P + M, soil amended with both 1% PBAT and 1% wheat straw. Three independent bottles were allocated to each treatment × destructive-harvest combination (four treatments × five harvests × three replicates; 60 bottles in total). Each bottle contained 40 g dry-soil equivalent in a 150-mL glass vessel and was incubated in the dark at 30°C for up to 120 d. Soil water content was adjusted to 60% of water-holding capacity and restored every 3 d with deionized water. Triplicate bottles per treatment were destructively sampled on days 10, 30, 60, 90 and 120 for the chemical and biological measurements assigned to each date.

2.3. PHA, PLA and PBAT dose-comparison incubation

Cultivated topsoil (0–20 cm) was collected after wheat harvest in Dazu, Chongqing, China. Plant residues and stones were removed, and the soil was passed through a 2-mm sieve. PHA, PLA and PBAT (Jiecheng Plastic Chemical Co., China) were added at 0.1% (L) or 1% (H) of dry soil mass, producing seven treatments: CK, PHA_L, PHA_H, PLA_L, PLA_H, PBAT_L and PBAT_H. Each treatment comprised three independent replicates. Twenty-five grams of dry-soil equivalent were placed in 125-mL serum bottles, adjusted to 60% of water-holding capacity and incubated in the dark at 25°C for 64 d. Moisture was restored every 3 d with sterile distilled water. At final harvest, subsamples were stored at 4°C for physicochemical measurements or at – 20°C for microbial analyses. Material characteristics and the complete provenance of this incubation are reported by Song et al. (2025).

2.4. CO₂ emission and cumulative mineralization

In the PBAT–wheat-straw incubation, headspace CO₂ was measured on days 1, 4, 7, 10, 13, 16, 19, 22, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120. Before each determination, bottles remaining in incubation were

opened in a fume hood for 20 min to equilibrate the headspace with ambient air, sealed with butyl-rubber stoppers and incubated for approximately 24 h. Headspace gas was withdrawn through a three-way valve, and CO₂ was quantified with an Agilent 7890A gas chromatograph equipped with a flame-ionization detector. Three empty bottles were analyzed concurrently to determine background CO₂. The type-and-dose incubation used the same ventilation–closure principle, followed by a 24-h accumulation period and analysis with an Agilent 7890B gas chromatograph. All fluxes were expressed on a dry-soil basis.

The CO₂ emission rate was calculated from the difference between the soil-containing bottle and the empty-bottle background:

$$F = \frac{(C_s - C_0) M_C V_h}{V_m m_s 10^3 \Delta t}$$

1

where F is the CO₂ emission rate (mg C kg⁻¹ h⁻¹), C_s and C_0 are headspace CO₂ concentrations in the sample and blank bottles (ppm), respectively, M_C is the molar mass of C (12 g mol⁻¹), V_h is headspace volume (L), V_m is molar gas volume at the incubation temperature and atmospheric pressure (L mol⁻¹), m_s is dry soil mass (kg), and Δt is the closure duration (h). For the PBAT–wheat-straw incubation, $V_h = 0.12$ L, $V_m = 24.86$ L mol⁻¹ at 30°C and 101.325 kPa, $m_s = 0.040$ kg and $\Delta t \approx 24$ h. Corresponding vessel-, temperature- and closure-specific values were used for the type-and-dose incubation. The resulting F values are reported as the time-resolved emission rates.

Cumulative CO₂ emission was obtained by trapezoidal integration of adjacent emission rates:

$$CE = 24 \sum_{i=1}^{n-1} \frac{F_i + F_{i+1}}{2} (t_{i+1} - t_i)$$

2

where CE is cumulative CO₂ emission (mg C kg⁻¹), F_i and F_{i+1} are consecutive emission rates (mg C kg⁻¹ h⁻¹), and t_i and t_{i+1} are consecutive sampling times (d). The factor 24 converts days to hours. These CE values underpin the cumulative and net-increment comparisons reported below.

2.5. Dissolved C and N pools and microbial biomass carbon

For the PBAT–wheat-straw incubation, water-extractable DOM was obtained from 4.0 g fresh soil at a soil:solution ratio of 1:10 (w/v). Suspensions were shaken for 12 h at 25°C, centrifuged at 4000 rpm for 20 min and passed through pre-rinsed 0.45-μm cellulose-acetate membranes. DOC was measured with a Multi N/C 3100 total organic carbon analyzer. On day 10, NH₄⁺–N and NO₃⁻–N were determined

colorimetrically in extracts of 5.0 g fresh soil. Microbial biomass carbon (MBC) was measured on days 10 and 30 by chloroform fumigation–extraction using 5.0 g fresh soil.

For the type-and-dose incubation, 3.0 g soil was extracted with 15 mL of 0.5 M K₂SO₄ by shaking for 1 h followed by filtration. DOC and total dissolved N (TDN) were measured with a Multi N/C 2100S analyzer, whereas NH₄⁺–N and NO₃[–]–N were quantified with a continuous-flow analyzer. Dissolved inorganic N (DIN) and dissolved organic N (DON), which are reported in Table 1, were calculated as:

Table 1
Endpoint DOC, DON and DIN concentrations in the BMP type-and-dose incubation.

Treatment	DOC (mg C kg ⁻¹)	DON (mg N kg ⁻¹)	DIN (mg N kg ⁻¹)
CK	174.25 ± 2.10 bc	93.87 ± 6.28 ab	5.78 ± 0.19 a
PHA_L	172.05 ± 13.35 bc	70.15 ± 1.20 c	6.16 ± 0.60 a
PHA_H	221.38 ± 11.38 a	21.67 ± 0.80 e	2.14 ± 0.01 d
PLA_L	163.63 ± 10.72 c	84.37 ± 4.72 bc	6.04 ± 0.29 a
PLA_H	192.20 ± 7.10 b	52.93 ± 1.91 d	3.30 ± 0.24 c
PBAT_L	160.35 ± 1.36 c	108.94 ± 8.30 a	6.26 ± 0.18 a
PBAT_H	226.00 ± 3.54 a	86.62 ± 9.15 bc	4.70 ± 0.15 b
Values are means ± SD (n = 3). Different lowercase letters within a column denote p < 0.05. L and H indicate 0.1% and 1% additions, respectively.			

$$\text{DIN} = \left[\text{NH}_4^+ - \text{N} \right] + \left[\text{NO}_3^- - \text{N} \right]$$

3a

$$\text{DON} = \text{TDN} - \text{DIN}$$

3b

For both incubations, the MBC values reported in the factorial-incubation supporting analysis and in the type-and-dose comparison were calculated from paired fumigated and non-fumigated extracts:

$$\text{MBC} = \frac{C_{\text{fum}} - C_{\text{nonfum}}}{k_{\text{EC}}}$$

4

where C(fum) and C(nonfum) are extractable organic C in fumigated and non-fumigated soils, respectively, and kEC is the extraction-efficiency coefficient (0.45).

2.6. PBAT surface morphology and hemicellulose degradation

PBAT surface morphology was examined by scanning electron microscopy before soil addition and after 10 d in the P and P + M treatments. Because the 20–80- μm particles could no longer be distinguished reliably from soil particles after day 30, imaging was restricted to the initial material and day-10 recovered particles. Surface alteration was therefore evaluated qualitatively and was not used as a measure of PBAT mass loss.

Soil hemicellulose was measured at the start of incubation and on day 60 by two-step acid hydrolysis followed by colorimetric quantification. Day 60 was selected because preliminary measurements indicated that the low hemicellulose concentration at day 120 could compromise quantitative accuracy. For each treatment, the degradation amount (D_h) and degradation percentage reported in **Table S4** were calculated from that treatment's own initial concentration:

$$D_h = H_0 - H_{60}$$

5a

$$D_h (\%) = \frac{H_0 - H_{60}}{H_0} \times 100$$

5b

where H_0 and H_{60} are hemicellulose concentrations at days 0 and 60, respectively.

2.7. DOM optical, fluorescence and molecular analyses

Absorbance spectra of water-extractable DOM were recorded from 200 to 600 nm at 1-nm intervals with Milli-Q water as the blank. Specific UV absorbance at 254 and 280 nm and the A_{250}/A_{365} ratio were calculated as:

$$\text{SUVA}_\lambda = \frac{A_\lambda}{b \times \text{DOC}}, \lambda = 254 \text{ or } 280 \text{ nm}; R_{250/365} = \frac{A_{250}}{A_{365}}$$

6

where $A(\lambda)$ is absorbance at wavelength λ , b is optical path length (m), DOC is expressed in mg C L^{-1} , and $R_{250/365}$ is the absorbance ratio; SUVA is reported in $\text{L mg C}^{-1} \text{ m}^{-1}$.

Excitation–emission matrices (EEMs) were acquired with a Lumina fluorescence spectrometer (Thermo Scientific, USA) at 1200 nm min^{-1} . Excitation wavelengths ranged from 200 to 500 nm and emission wavelengths from 250 to 550 nm; both slit widths were 5 nm. The matrices are displayed as two-dimensional excitation–emission contour maps, with measured fluorescence intensity encoded by color.

The archived analytical record does not document Rayleigh-scatter removal, inner-filter correction, Raman normalization or PARAFAC decomposition; the maps were therefore used only for qualitative comparison of the broad fluorescence feature, and no component scores or quantitative intensity ratios were calculated. For DOM-FTIR analysis, freeze-dried DOM was mixed with spectroscopic-grade KBr at a KBr:sample mass ratio of 100:1 and scanned from 400 to 4000 cm⁻¹ with a Nicolet iS5 FTIR spectrometer (Thermo Scientific, USA).

Day-10 DOM was subjected to PPL solid-phase extraction before molecular analysis. Bond Elut PPL cartridges (500 mg, 6 mL) were conditioned sequentially with 12 mL HPLC-grade methanol and 12 mL 0.01 M HCl. DOM extracts were acidified to pH 2, loaded by gravity at approximately 5 mL min⁻¹, rinsed with 18 mL 0.01 M HCl and Milli-Q water to remove salts, and eluted with 6 mL methanol. Eluates were stored at -20°C until analysis. Ultrahigh-resolution mass spectra were acquired with a Bruker Solarix XR 7.0 T FT-ICR-MS coupled to a negative-ion electrospray source. Samples were infused at 120 µL h⁻¹; capillary entrance and end-plate voltages were 4.0 kV and -500 V, respectively. Ions accumulated for 0.03 s and were transferred to the ICR cell with a 0.7-ms flight time. Spectra covered m/z 100–1200 and comprised 300 co-added scans at a 4-Mword size. Sodium trifluoroacetate was used for external calibration, and m/z 369.11911 was used for internal calibration.

Molecular formulae were assigned with the FTMSDeu workflow. Minimum signal-to-noise ratios were 6 for non-halogenated and 10 for halogenated monoisotopic formulae. Formulae contained 1–50 C atoms, 0–5 N atoms, 0–3 S atoms, 0–1 P atom and 0–5 Cl atoms; for formulae with C ≥ 5, 0.3 ≤ (H + Cl)/C ≤ 2.25 and 0 < O/C ≤ 1.2. Only non-negative integer DBE values and -10 ≤ DBE - O ≤ 10 were retained. Assigned formulae were classified in van Krevelen space. For each straw-matched comparison (P versus CK and P + M versus M), formulae were designated persistent when detected in both samples, produced when detected only after PBAT addition, and removed when detected only in the paired reference. Exact-mass matches to reported PBAT transformation products were treated as candidates rather than source-specific identifications.

For each molecular property X, the peak-intensity-weighted value used in Fig. 4d was calculated as:

$$\overline{X} = \frac{\sum_i I_i X_i}{\sum_i I_i}$$

7

where I_i is the intensity of formula i and X_i is its molecular mass, H/C, O/C, DBE, modified aromaticity index [AI(mod)] or nominal oxidation state of carbon (NOSC). Formula-level indices were calculated as:

$$\text{DBE} = 1 + \frac{2C - H + N + P - Cl}{2}$$

8

$$AI_{\text{mod}} = \frac{1 + C - 0.5O - S - 0.5(N + H)}{C - 0.5O - S - N}$$

9

$$\text{NOSC} = 4 - \frac{4C + H - 3N - 2O - 2S}{C}$$

10

The molecular lability boundary (MLB) was expressed as the percentage of assigned formulae with H/C ≥ 1.5 :

$$\text{MLB} (\%) = \frac{N(H/C \geq 1.5)}{N_{\text{total}}} \times 100$$

11

One DOM extract per treatment was analyzed by FT-ICR-MS. Molecular patterns, including the indices in Fig. 4 and **Table S2**, were therefore interpreted descriptively and were not subjected to inferential treatment comparisons.

2.8. Hydrolytic enzymes and microbial functional genes

β -Glucosidase activity was determined by the p-nitrophenol method using kit BC0160 (Solarbio Science & Technology, Beijing, China), and cellulase activity by anthrone colorimetry using kit ml076931 (Enzyme-linked Biotechnology, Shanghai, China). Activities were expressed per gram of dry soil per day. Functional genes were quantified in soils collected on days 10 and 120. Total DNA was extracted in triplicate from 0.8 g soil with the ALFA-SEQ Advanced Soil Kit and analyzed using a high-throughput qPCR-based quantitative microbial element-cycling platform. Targets comprised genes associated with starch degradation (amyA, amyX, sga, ipu and apu), hemicellulose degradation (abfA, xylA and manB), cellulose degradation (cex and cdh), chitin degradation (chiA and exo-chi), pectin degradation (pgu), lignin degradation (glx, lig, mnp and pox), urea degradation (ureC) and amino-sugar degradation (naglu). Primer information is provided in **Table S3**. Absolute abundance was expressed as copies g⁻¹ dry soil. Each C-degradation-gene abundance was also divided by bacterial 16S rRNA gene abundance to distinguish an increase in community size from enrichment of a function within the bacterial pool.

For the straw-matched PBAT contrasts used to relate functional-gene and CO₂ responses, log response ratios were calculated as:

$$\ln\text{RR} = \ln \left(\frac{X_{\text{amended}}}{X_{\text{reference}}} \right)$$

12

where X_{P} and X_{r} are the response values in the PBAT treatment and its corresponding reference, respectively; the paired contrasts were P versus CK and P + M versus M.

2.9. PLFA profiles, ^{13}C -DOC assimilation and carbon use efficiency

Microbial phospholipid fatty acids (PLFAs) were extracted from 2.0 g freeze-dried soil using a modified Bligh–Dyer procedure. Lipids were extracted with methanol, chloroform and citric-acid buffer; the chloroform phase was recovered, and phospholipids were separated from neutral lipids and glycolipids by silicic-acid chromatography. Phospholipids were saponified and methylated, and the resulting fatty-acid methyl esters were identified with the MIDI Sherlock system and quantified by gas chromatography. Total PLFA was the sum of all detected microbial PLFAs. Established biomarkers were grouped as Gram-negative bacteria, Gram-positive bacteria, fungi and actinomycetes.

For the substrate-allocation assay, 1.0 g fresh soil from each BMP treatment received 52 μL of sterile ^{13}C -labeled DOC containing 0.13 mg C in a 12-mL vial. The DOC was prepared from dried plant material that had been uniformly labeled with $^{13}\text{CO}_2$ in an automatically controlled gas-tight growth chamber following Yao et al. (2012). The labeled material contained 48.0% organic C, 1.23% N and had $\delta^{13}\text{C} = 557\text{‰}$; the DOC solution was passed through a 0.22- μm membrane before addition. Vials were incubated in the dark at 25°C for 24 h. Headspace CO_2 concentration and $\delta^{13}\text{C}\text{-CO}_2$ were measured with a GasBench coupled to isotope-ratio mass spectrometry. Incorporation of ^{13}C into individual PLFAs was quantified by GC–IRMS following Wang et al. (2016), and excess ^{13}C in bacterial and fungal PLFAs represented growth supported by the added plant-derived DOC.

Apparent CUE for the 24-h added-DOC assay was calculated following Karhu et al. (2022) as the fraction of recovered tracer C allocated to bacterial and fungal growth:

$$\text{CUE} = \frac{G_{\text{bacterial}} + G_{\text{fungal}}}{G_{\text{bacterial}} + G_{\text{fungal}} + R_{^{13}\text{CO}_2}}$$

13

where $G(\text{bacterial})$ and $G(\text{fungal})$ are bacterial and fungal growth derived from the added ^{13}C -DOC, respectively, and $R(^{13}\text{CO}_2)$ is respiration derived from the same tracer during the 24-h assay. Because the tracer was plant-derived DOC rather than BMP-derived carbon and the assay lasted only 24 h, this value is hereafter termed 24-h ^{13}C -DOC-derived apparent CUE; it does not quantify long-term retention of BMP carbon.

2.10. Statistical analysis

Unless otherwise stated, data are presented as means $\pm$ standard deviations of three independent replicates. Within the PBAT–wheat-straw incubation, treatment differences at each sampling date were assessed by one-way ANOVA followed by Tukey's multiple-comparison test. Main effects of PBAT and

wheat straw and their interaction were evaluated with a 2×2 factorial ANOVA. Spearman rank correlations were calculated among DOC, β -glucosidase activity, cellulase activity and CO_2 emission rate, and between log response ratios of functional genes and CO_2 emission. A power-function regression was additionally fitted between DOC and CO_2 emission rate. These analyses were performed in SPSS 26.0.

Within the type-and-dose incubation, differences among CK and the six BMP treatments were evaluated by one-way ANOVA. Tukey's HSD was used for cumulative CO_2 emission, MBC and apparent CUE, whereas Duncan's test was retained for dissolved pools and PLFA composition to match the prespecified analysis. Factorial effects of BMP type, dose and their interaction were tested within the six BMP-amended treatments by two-way ANOVA in R 4.2.2. Statistical significance was accepted at $p < 0.05$. FT-ICR-MS data from one extract per treatment were descriptive only, and no between-incubation statistical tests were conducted.

3. Results and discussion

3.1. Wheat straw increased the absolute but reduced the proportional PBAT-associated CO_2 response

PBAT particles were relatively smooth before incubation but developed visible cracks after 10 d in soil without wheat straw and in wheat-straw-amended soil (Fig. 1a–c). Because these images were qualitative, the surface changes were not used to estimate PBAT mass loss. CO_2 emission rates were higher mainly during the early and middle phases of incubation (Fig. 1d). By day 120, cumulative CO_2 emission increased from 751.1 to 2356.7 mg C kg⁻¹ after PBAT addition without wheat straw and from 1133.6 to 2961.2 mg C kg⁻¹ after PBAT addition with wheat straw (Fig. 1e). Relative to their straw-matched references, these values corresponded to proportional increases of 214% without wheat straw and 161% with wheat straw. The net PBAT-associated increment shown in Fig. 1f was higher under P + M than under P. Thus, wheat straw increased the absolute PBAT-associated CO_2 response but reduced its proportional magnitude because the M treatment had a higher respiratory baseline. PBAT, wheat straw and their interaction were significant in the factorial analysis. Detailed ANOVA results are provided in **Table S1**.

Compared with the 13–57% increases reported after four weeks of exposure to 1% PBAT (Rauscher et al., 2023), the 214% increase observed here was considerably larger. This difference may reflect the smaller particles and the longer, warmer incubation used here, although differences among soils prevent attribution to any single factor. Previous studies combining microplastics with crop residues have reported increased, decreased or unchanged respiration (Chen et al., 2020; Yu et al., 2021; Xiao et al., 2021; Yan et al., 2024; Li et al., 2024). The present results clarify one source of this variation: a residue amendment can raise the control respiration rate and thereby reduce the percentage effect of PBAT even when the absolute PBAT-associated CO_2 increase is larger. Reporting both absolute and proportional changes is therefore necessary when treatment backgrounds differ. Because emitted CO_2 was not

source-partitioned, however, these results demonstrate an increase in total CO₂ emission after PBAT addition but do not distinguish PBAT mineralization from wheat-straw decomposition or native-soil-carbon priming.

3.2. PBAT caused an early DOC increase followed by transient changes in DOM optical properties

PBAT increased DOC most strongly on day 10, by approximately 70.0 mg C kg⁻¹ without wheat straw and 67.0 mg C kg⁻¹ with wheat straw (Fig. 2a). Both differences subsequently narrowed and were small by day 120. β -Glucosidase activity also increased, with the largest response in P + M (Fig. 2b), whereas cellulase showed a smaller early increase (Fig. 2c). DOC, both enzyme activities and the CO₂ emission rate were positively associated across sampling dates and treatments (Fig. 2d). A separate power-function model explained 92% of the variation in the emission rate ($p < 0.01$). The fitted relationship is provided in **Fig. S2**. SUVA₂₅₄ and SUVA₂₈₀ increased most clearly around day 30, whereas A_{250}/A_{365} declined during the early and middle phases. These optical indices are provided in **Fig. S3**. Thus, the DOC increase was strongest at the beginning of incubation, while changes in bulk optical properties developed later and persisted for longer.

The EEM contour maps were dominated by a broad humic-like feature near Ex/Em = 330/440 nm. On day 10, this feature was visually stronger in P than in CK and in P + M than in M. The differences remained apparent on days 30 and 60, weakened by day 90 and were no longer evident by day 120 (Fig. 3). DOM-FTIR showed lower PBAT-associated peak intensities at 1381 cm⁻¹ during days 10–60, at 1635 cm⁻¹ on day 10 and at 1139 cm⁻¹ during days 10–60. The spectra are provided in **Fig. S4**. Chen et al. (2022) similarly reported increased DOM aromaticity, molecular weight and humification after PBAT addition, whereas Sun et al. (2022) found that fluorescence responses depended on material and soil. Qiu et al. (2024) further showed that individual fluorescent components can respond differently. The present time series extends these findings by showing that the fluorescence difference outlasted the largest DOC increase but was not maintained to day 120. Because the EEMs were interpreted qualitatively and fluorescence intensity also varies with DOC concentration, the results support a transient change in bulk DOM optical character rather than formation of a new compound class or long-term carbon stabilization.

3.3. PBAT-treated DOM contained more assigned formulae but showed lower nominal molecular lability

At day 10, lignin-like formulae dominated the assigned DOM and their relative contribution increased after PBAT addition, whereas the lipid-like fraction declined (Fig. 4a). CHO and CHON formulae were the principal elemental groups, and PBAT shifted their relative distribution toward CHON and away from CHO (Fig. 4b). Paired van Krevelen comparisons identified both produced and removed formulae in the carbohydrate-, protein/amino-sugar-, lipid- and lignin-like regions (Fig. 4c). PBAT-treated DOM had higher intensity-weighted molecular mass, O/C, DBE, Al(mod) and NOSC but lower H/C and MLB values (Fig. 4d). The total number of assigned formulae increased from 7800 to 8598 without wheat straw and

from 7974 to 8520 with wheat straw. Formula counts are provided in **Fig. S5**. The corresponding molecular indices are listed in **Table S2**. Because one extract per treatment was analyzed, these molecular patterns are descriptive. Nevertheless, the same combination occurred in both straw backgrounds: PBAT increased the number of detected formulae while the remaining DOM was more oxidized, more unsaturated and less nominally labile.

An increase in DOC does not necessarily mean that the remaining DOM is more labile. One possible explanation is that continued solubilization or microbial release added molecules to the dissolved pool while microorganisms preferentially used relatively reduced, H-rich molecules. The higher NOSC and lower H/C and MLB values are consistent with this interpretation. After 100 d of PE or PLA exposure, Qiu et al. (2024) likewise observed loss of relatively labile components together with greater oxidation and aromaticity. In contrast, Liu et al. (2023) reported a decline in lignin-like formulae after 120 d at 5–10% PBAT. The different direction may reflect the higher dose and longer exposure used in that study. In the present study, more assigned formulae and lower nominal molecular lability occurred at the same early sampling point, suggesting that DOM production was already accompanied by selective molecular use. However, exact-mass formulae cannot establish chemical source, and the absence of replicated FT-ICR-MS measurements prevents inferential comparison. These data therefore support selective DOM processing after PBAT addition but do not quantify PBAT mineralization.

3.4. PBAT increased C-degradation gene abundance largely in parallel with bacterial growth

PBAT increased the summed absolute abundance of C-degradation genes most strongly on day 10 (Fig. 5a). Genes associated with hemicellulose, lignin and chitin degradation showed the clearest individual increases (Fig. 5b), and their log response ratios were positively associated with the corresponding CO₂ response (Fig. 5c). Absolute ureC and naglu abundances also increased, particularly on day 10 (Fig. 5d). Bacterial 16S rRNA gene abundance and early MBC increased in parallel. These measurements are provided in **Fig. S6**. After normalization to 16S rRNA gene abundance, most C-degradation-gene differences weakened or disappeared, whereas chiA remained elevated. The normalized results are provided in **Fig. S7**. PBAT-amended soils also contained little NO₃⁻-N and less NH₄⁺-N than their straw-matched references. Mineral-N data are provided in **Fig. S8**. Consistent with the enzyme and gene responses, PBAT increased day-60 hemicellulose degradation from 20.39% to 36.65% without wheat straw and from 26.17% to 36.99% with wheat straw. The underlying concentrations and calculations are reported in **Table S4**.

The comparison between absolute and normalized gene abundance changes the interpretation of the microbial response. Absolute gene copies per gram of soil increased after PBAT addition, but most differences became small after division by bacterial 16S rRNA gene abundance. This pattern suggests that much of the increase occurred because bacterial abundance increased, rather than because the measured functions became more abundant within the bacterial community. The persistence of chiA was a more specific exception. Zhang et al. (2022) reported increased CO₂ emission despite decreases

in several C-degradation genes, also indicating that DNA copy number and measured carbon flux do not necessarily change in parallel. In the present study, higher enzyme activity and greater hemicellulose loss provide independent evidence that decomposition processes increased, although DNA-based gene abundance does not demonstrate transcription. The increases in *ureC* and *naglu*, together with lower mineral N, are consistent with greater microbial N-acquisition potential under a C-rich input (Mooshammer et al., 2014; Cui et al., 2020). However, gross N fluxes were not measured. Overall, PBAT increased bacterial abundance and the absolute number of decomposition-related genes, while selective enrichment was evident for only a limited subset of genes.

3.5. BMP type and dose differentially affected respiration, biomass and microbial ^{13}C allocation

At final harvest, the highest numerical DOC means occurred in PBAT_H ($226.00 \pm 3.54 \text{ mg C kg}^{-1}$) and PHA_H ($221.38 \pm 11.38 \text{ mg C kg}^{-1}$), whereas PBAT_L had the lowest numerical mean ($160.35 \pm 1.36 \text{ mg C kg}^{-1}$). PBAT_L also had the highest numerical DON mean ($108.94 \pm 8.30 \text{ mg N kg}^{-1}$), while PHA_H had the lowest DON ($21.67 \pm 0.80 \text{ mg N kg}^{-1}$) and DIN ($2.14 \pm 0.01 \text{ mg N kg}^{-1}$). These dissolved-pool measurements are reported in Table 1. Cumulative CO_2 emission increased by 87% under PHA_L, 665% under PHA_H and 214% under PLA_H relative to CK, but decreased by 17% under PBAT_L (Fig. 6a). MBC increased from $97.73 \pm 3.03 \text{ mg C kg}^{-1}$ in CK to $223.09 \pm 7.91 \text{ mg C kg}^{-1}$ in PHA_H (Fig. 6b). The 24-h ^{13}C -DOC-derived apparent CUE ranged from 0.19 to 0.28 and was significantly higher than CK only in PHA_H (Fig. 6c). Total ^{13}C incorporation into PLFAs did not differ significantly among treatments (Fig. 6d). However, the distribution of incorporated ^{13}C changed: PHA_H reduced the Gram-positive bacterial and fungal shares and increased the actinomycete share (Fig. 6e). Thus, PHA_H altered microbial-group allocation even though total short-term tracer incorporation did not differ significantly among treatments.

The 665% increase in CO_2 emission under PHA_H falls within the 552–1744% range of PHA-induced priming reported across three agricultural soils (Zhang et al., 2023). However, the present CO_2 was not source-partitioned and therefore cannot be classified as priming. Chen et al. (2025) reported that PLA can induce negative native-SOC priming while microbial growth and carbon retention increase, further showing that high total respiration does not necessarily indicate greater native SOC loss. More importantly, PBAT_H and PHA_H produced similarly high DOC concentrations but markedly different CO_2 emissions, indicating that DOC concentration alone was insufficient to predict respiratory loss. This result agrees with evidence that the respiratory effect of microplastic-derived DOM depends on substrate bioavailability and microbial physiology (Wang et al., 2025). Under PHA_H, higher MBC and apparent CUE accompanied high respiration, suggesting that greater short-term substrate processing supported both biomass production and CO_2 release. Nevertheless, the tracer assay measured 24-h allocation of added plant-derived DOC rather than BMP carbon and cannot indicate long-term retention. The absence of a significant treatment effect on total ^{13}C incorporation, together with the altered microbial distribution, suggests that BMP exposure changed which groups used the added DOC without increasing total short-

term microbial incorporation. The greater actinomycete share under PHA_H is consistent with the reported involvement of Actinobacteriota in BMP-associated DOM metabolism (Qiu et al., 2024).

3.6. Overall interpretation of the observed carbon responses

Across the 120-d incubation, the measured responses occurred at different times. The DOC and hydrolytic-enzyme responses were strongest early in incubation, the humic-like fluorescence difference remained apparent through day 60, and cumulative CO₂ emission continued to diverge through day 120. Day-10 molecular analysis further indicated that the number of assigned formulae increased while the remaining DOM became more oxidized and less nominally labile. At the same time, MBC, bacterial 16S rRNA gene abundance and absolute C-degradation-gene abundance increased, whereas most normalized gene responses were limited. Recent studies similarly show that microbial physiological traits regulate respiration from microplastic-derived DOM (Wang et al., 2025) and that microbial adaptation to BMPs changes DOM reactivity (Liu et al., 2025). Together, the present results are consistent with an early increase in dissolved substrates and microbial growth, followed by continued use of selected DOM fractions and sustained CO₂ release. Because molecular composition was measured only on day 10 and CO₂ was not source-partitioned, this interpretation does not identify the chemical origin of the respired carbon.

These results also explain why a single measurement can provide an incomplete assessment of BMP effects on soil carbon. Wheat straw increased the absolute PBAT-associated CO₂ increment but reduced its proportional increase. Similarly high DOC concentrations under PBAT_H and PHA_H were accompanied by markedly different CO₂ emissions. Most functional-gene responses became smaller after normalization to bacterial 16S rRNA genes, and microbial ¹³C allocation changed even though total incorporation did not differ significantly among treatments. Consequently, respiration should be interpreted together with DOM composition, microbial abundance and substrate allocation. Nevertheless, the CO₂ sources were unresolved, FT-ICR-MS lacked treatment replication, functional genes were measured at the DNA rather than transcript level, and the EEMs were interpreted qualitatively. The results therefore support increased carbon turnover and altered microbial use under the tested incubation conditions, but they do not provide a field-scale carbon budget, a source-resolved priming estimate or evidence of long-term carbon sequestration.

4. Conclusions

This study shows that BMP-induced changes in soil carbon processing cannot be evaluated from CO₂ emission or DOC concentration alone. Wheat straw increased the absolute PBAT-associated CO₂ increment even though the proportional response decreased from 214% to 161%. PBAT also caused an early DOC increase and a transient humic-like fluorescence response, while day-10 DOM contained more assigned formulae and had a higher nominal oxidation state and lower molecular lability. Most increases in absolute C-degradation-gene abundance weakened after normalization to bacterial 16S rRNA genes,

indicating that bacterial growth contributed substantially to the response. In the type-and-dose comparison, 1% PHA produced the largest CO₂ and MBC responses and the highest 24-h ¹³C-DOC-derived apparent CUE. Total tracer incorporation did not differ significantly among treatments, whereas its allocation among microbial groups shifted. Collectively, these findings show that BMPs affected carbon release, DOM transformation, microbial growth and substrate allocation to different extents. Evaluating these measurements together improves interpretation of BMP effects under controlled incubation conditions. Source-resolved CO₂ measurements, replicated molecular analyses and longer-term field studies are still needed to determine whether these responses alter native soil-carbon persistence.

Declarations

CRediT authorship contribution statement

Hongcheng Bai

Writing-original draft, Writing-review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Yan Li

Writing-review & editing, Supervision, Methodology, Investigation, Formal analysis, Software

Peili Lu

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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.

Author Contribution

Hongcheng Bai: Writing-original draft, Writing-review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. Yan Li: Writing-review & editing, Supervision, Methodology, Investigation, Formal analysis, Software. Peili Lu: Writing-review & editing, Supervision, Data curation

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Data availability

Data will be made available on request.

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Figures

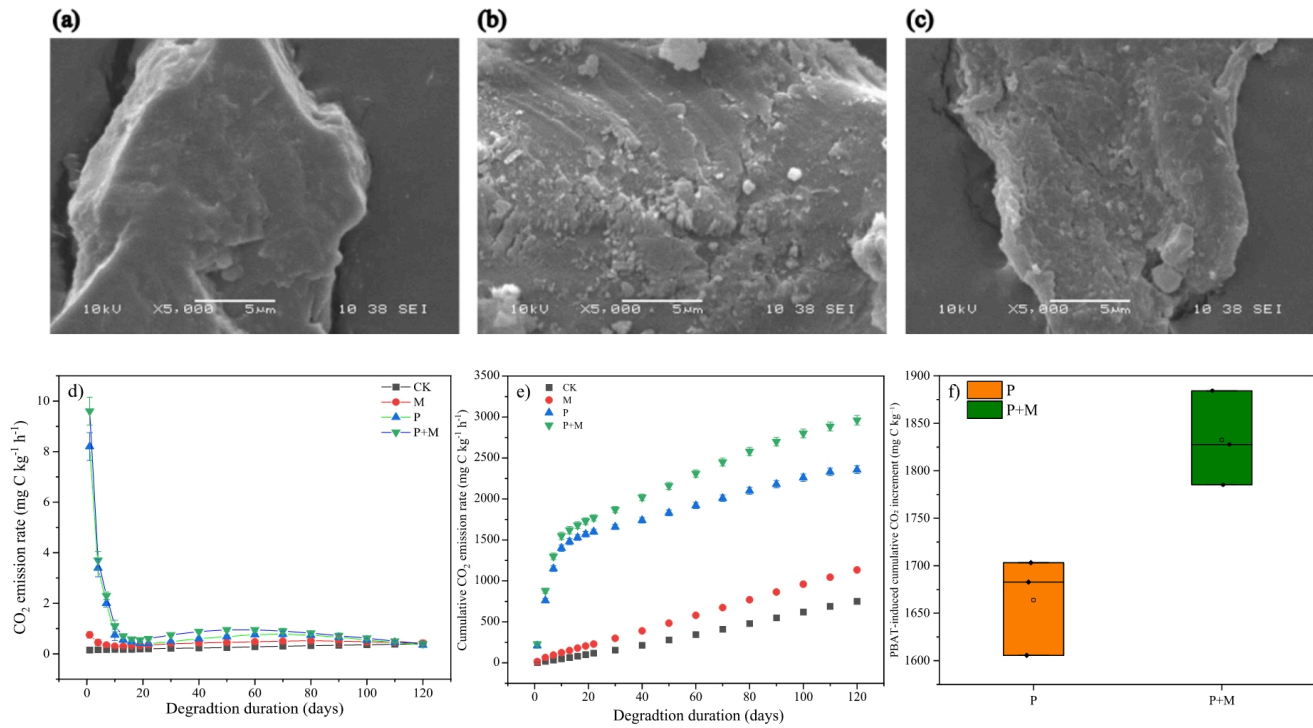


Figure 1

PBAT surface alteration and soil CO₂ emission during the 120-d PBAT-wheat-straw incubation. (a) PBAT before incubation; (b) PBAT after 10 d in soil without wheat straw; (c) PBAT after 10 d in wheat-straw-amended soil; (d) CO₂ emission rate; (e) cumulative CO₂ emission; and (f) net PBAT-associated increment, calculated as P – CK and P+M – M. CK, unamended soil; P, soil amended with 1% PBAT; M, soil amended with 1% wheat straw; and P+M, soil amended with 1% PBAT and 1% wheat straw. Points and bars are means ± SD (n = 3); different lowercase letters denote p < 0.05. Surface images are qualitative and do not represent PBAT mass loss.

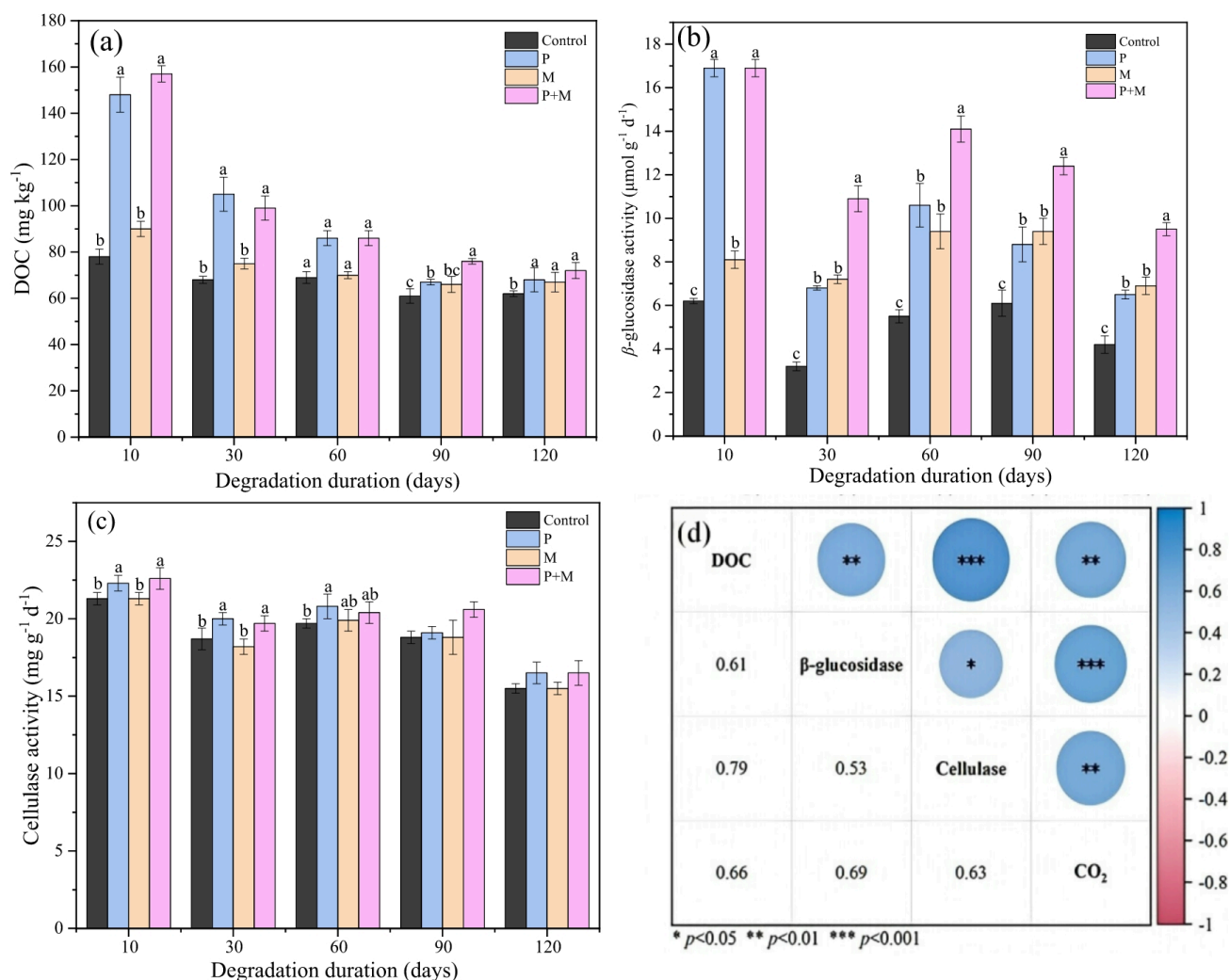


Figure 2

PBAT-induced changes in dissolved organic carbon (DOC), hydrolytic enzymes and their associations with CO₂ emission. (a) DOC; (b) β-glucosidase activity; (c) cellulase activity; and (d) Spearman correlations among DOC, enzyme activities and CO₂ emission rate. Bars are means ± SD (n = 3); different lowercase letters at a sampling time denote $p < 0.05$. Circle size and color in panel (d) represent the magnitude and direction of the correlation coefficient.

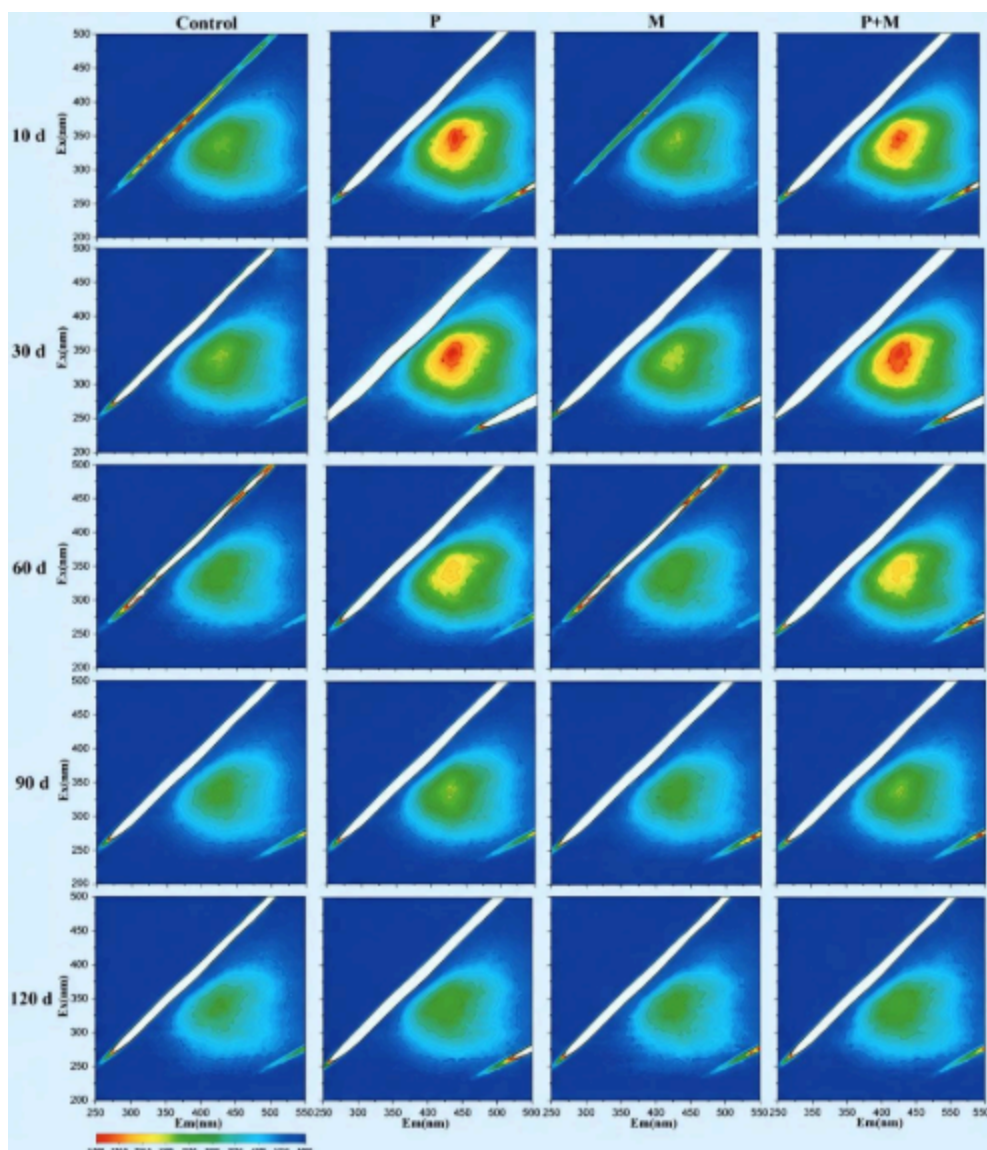


Figure 3

Two-dimensional contour representation of excitation–emission matrices for soil DOM during the 120-d PBAT–wheat-straw incubation. Rows represent days 10, 30, 60, 90 and 120; columns represent CK, P, M and P+M, respectively. Excitation and emission wavelength are shown on the two axes, and measured fluorescence intensity is encoded by color. The broad feature near Ex/Em = 330/440 nm is operationally described as humic-like. The maps were interpreted qualitatively; no PARAFAC decomposition or quantitative component comparison was performed.

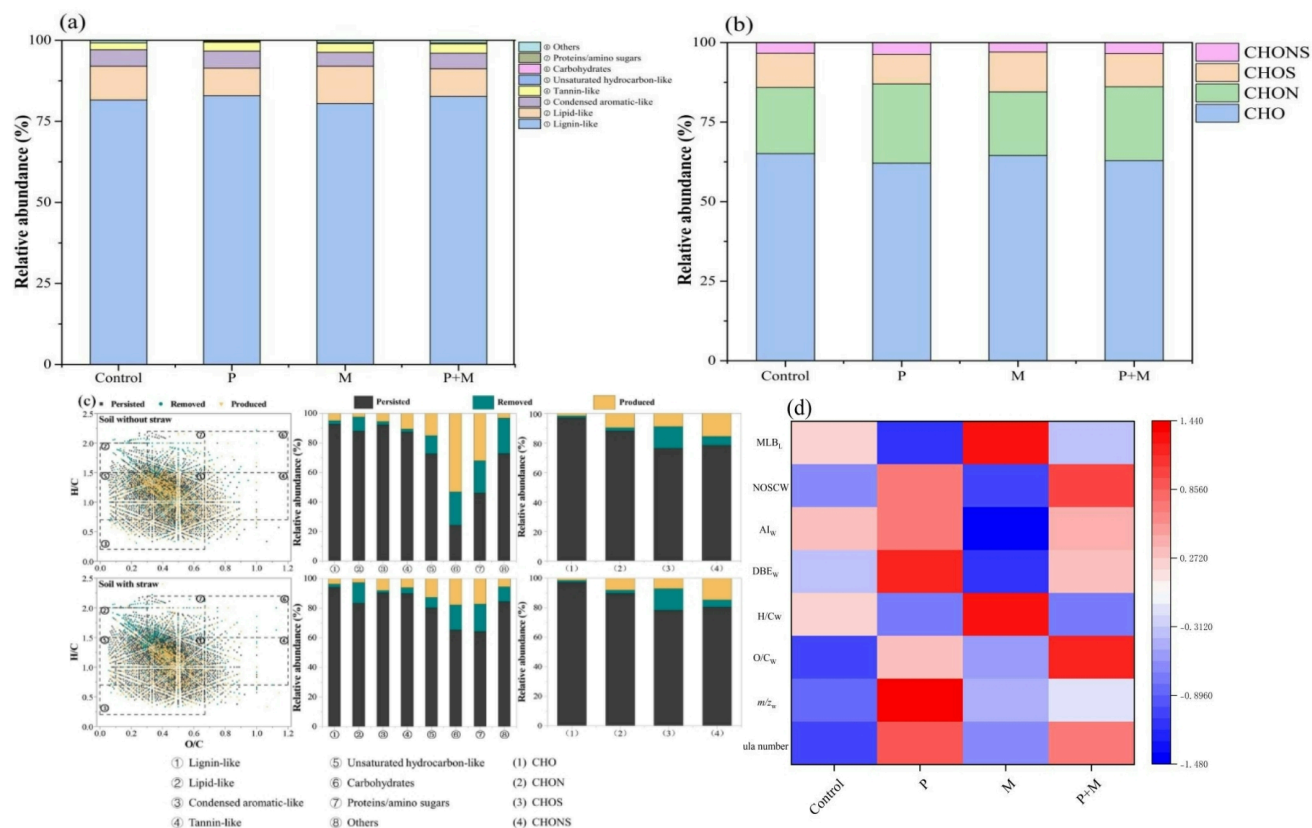


Figure 4

PBAT-associated DOM molecular turnover resolved by FT-ICR-MS on day 10. (a) Relative abundance of molecular compound classes; (b) elemental formula groups; (c) persistent, removed and produced formulae in van Krevelen space and their class distributions; and (d) Z-score-normalized molecular indices. MLB, molecular lability boundary; NOSC, nominal oxidation state of carbon; AI(mod), modified aromaticity index; DBE, double-bond equivalents. One extract was analyzed per treatment; panels are descriptive and do not imply inferential significance.

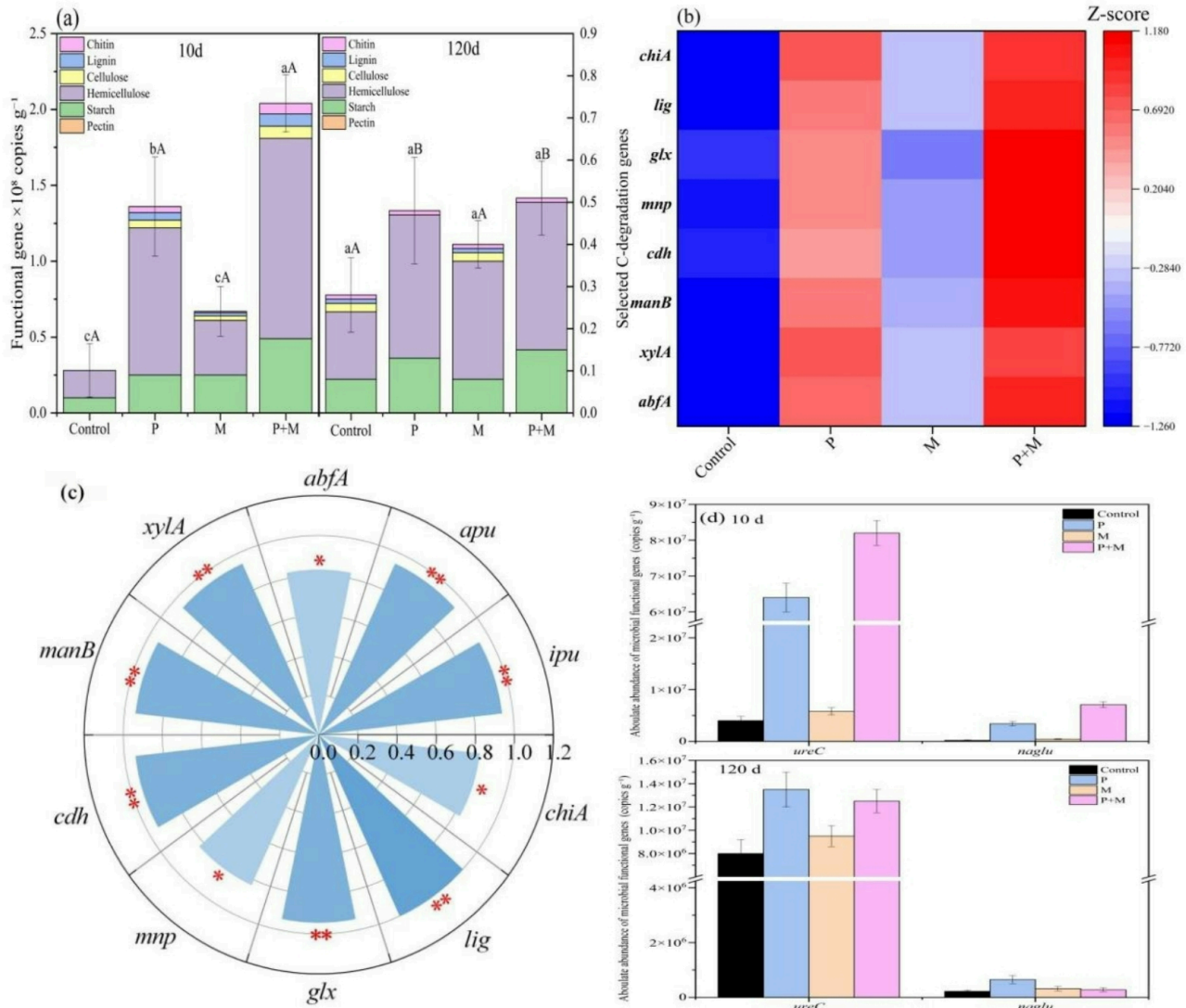


Figure 5

Functional-gene responses associated with organic-matter decomposition and N acquisition after PBAT addition. (a) Absolute abundance of C-degradation genes grouped by substrate on days 10 and 120; (b) Z-score heatmap of selected C-degradation genes; (c) correlations between gene and CO_2 log response ratios; and (d) absolute abundances of *ureC* and *naglu* on days 10 and 120. Bars are means $\pm$ SD ($n = 3$); different letters denote $p < 0.05$, and asterisks in panel (c) denote significant correlations.

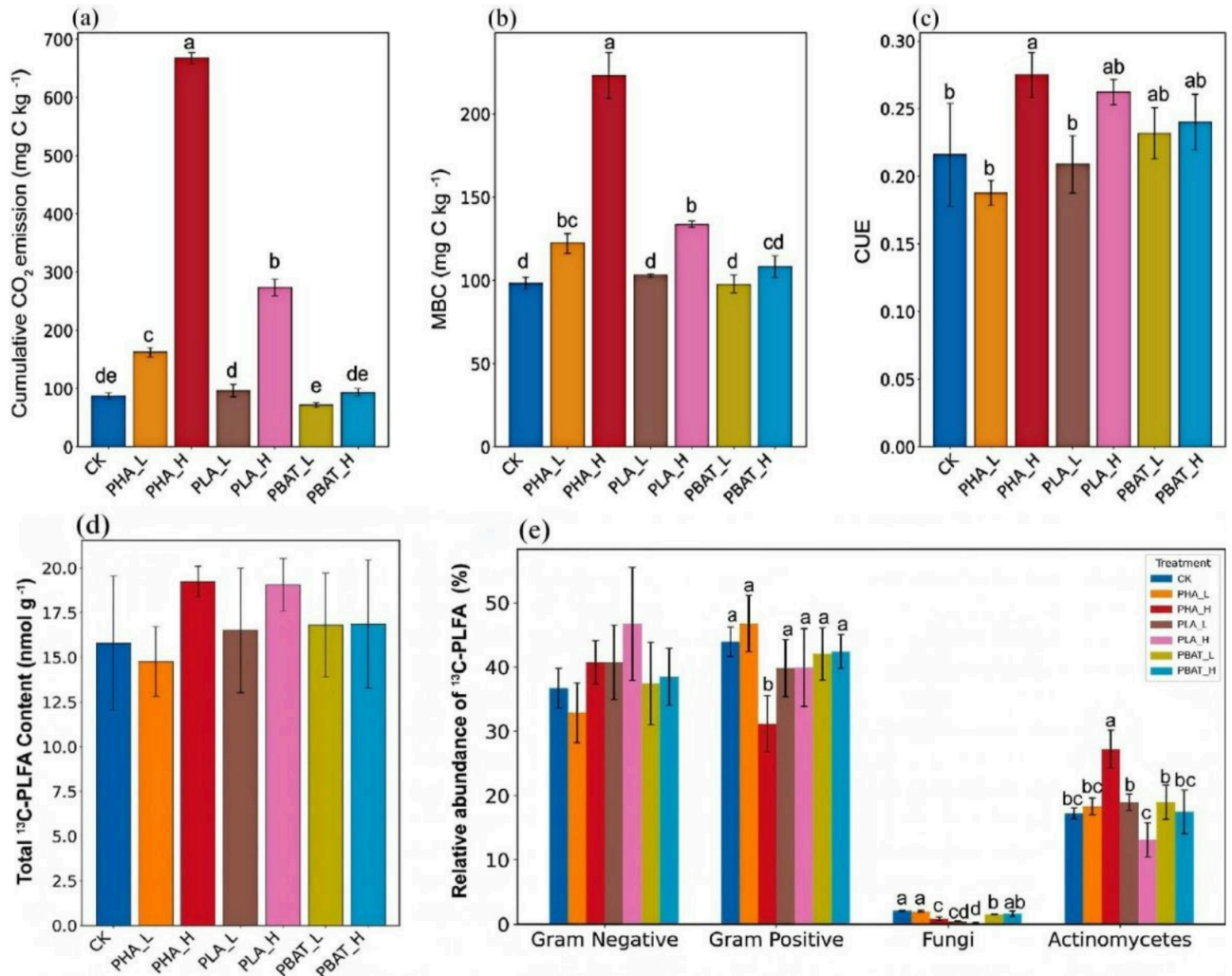


Figure 6

Effects of BMP type and dose on soil carbon flux and microbial allocation of added ^{13}C -DOC. (a) Cumulative CO_2 emission over 64 d; (b) microbial biomass carbon (MBC); (c) 24-h ^{13}C -DOC-derived apparent carbon use efficiency (CUE); (d) total ^{13}C incorporation into PLFAs; and (e) relative allocation of ^{13}C -PLFA among Gram-negative bacteria, Gram-positive bacteria, fungi and actinomycetes. L and H denote 0.1% and 1% BMP addition. Bars are means $\pm$ SD ($n = 3$); different lowercase letters denote $p < 0.05$.

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