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The Degradation of Polyethylene by *Trichoderma* and Its Impact on Soil Organic Carbon

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Abstract: Polyethylene mulching film, which is widely utilized in arid and semi-arid agriculture, leaves residual pollution. A novel approach to addressing this issue is microbial degradation. To screen the strains that degrade polyethylene efficiently and clarify the effect of degrading strains on the turnover of soil organic carbon, a polyethylene-degrading fungus PF2, identified as *Trichoderma asperellum*, was isolated from long-time polyethylene-covered soil. Strain PF2 induced surface damage and ether bonds, ketone groups and other active functional groups in polyethylene, with 4.15% weight loss after 30 days, where laccase plays a key role in the degradation of polyethylene. When applied to soil, the *Trichoderma*-to-soil weight ratios were the following: B1: 1:100; B2: 1:200; B3: 1:300 and B4: 1:400. *Trichoderma asperellum* significantly increased the cumulative CO_2 mineralization and soil organic carbon mineralization in the B1 and B2 treatments compared with the control (B0). The treatments B1, B3 and B4 increased the stable organic carbon content in soil. An increase in the soil organic carbon content was observed with the application of *Trichoderma asperellum*, ranging from 27.87% to 58.38%. A positive correlation between CO_2 emissions and soil organic carbon was observed, with the soil carbon pool management index (CPMI) being most correlated with active organic carbon. *Trichoderma* treatments improved the CPMI, with B3 showing the most favorable carbon retention value. Thus, *Trichoderma asperellum* not only degrades polyethylene but also contributes to carbon sequestration and soil fertility when applied appropriately.

Keywords: *Trichoderma*; polyethylene; degradation; soil organic carbon; mineralization

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1. Introduction

The production of plastics has increased to 6.3 billion tons in recent decades [1], with polyethylene accounting for over 25 million tons of production annually [2]. Polyethylene is widely used in various fields due to its good stability, corrosion resistance and low cost [3]. Its widespread use in agriculture, particularly in the form of film mulch, has been instrumental in enhancing water resource utilization, nutrient uptake and crop yields, thereby contributing to national food security [4,5]. However, the recalcitrant nature of polyethylene, characterized by its high molecular weight and stable molecular structure, poses a formidable environmental challenge. The thinning of plastic films has further complicated their recycling, leading to the accumulation of residues in soil that negatively impact soil fertility and crop development [6,7]. The degradation of these films into microplastics exacerbates soil pollution, with these microplastics potentially entering the food chain and posing a risk to human health [8,9].

Traditional methods of managing discarded polyethylene films, such as landfilling and incineration, are not only resource intensive but also contribute to secondary pollution and encroachment on ecological habitats [10]. Biodegradable mulching films have emerged as an alternative, offering some relief from the environmental impact of polyethylene [11].

However, their high cost and potential long-term ecological effects are serious concerns [12]. Microbial degradation is a promising solution, with microorganisms capable of adhering to polyethylene surfaces and secreting extracellular enzymes being used to decompose the polymer into carbon dioxide and water [3,13]. This approach is not only operationally straightforward but also cost effective and free from secondary pollution [3].

Research has increasingly focused on the identification and isolation of microorganisms capable of degrading polyethylene, with several strains isolated from diverse environments, including soil and marine ecosystems, being able to utilize polyethylene as their sole carbon source [14,15]. Notably, certain fungal species, such as *Aspergillus*, have demonstrated superior degradation capabilities due to their hyphae, which can effectively adhere to and penetrate polyethylene films [16]. According to Birolli et al. [17], the genus *Cladosporium* sp. is capable of breaking down a range of polycyclic aromatic hydrocarbons and polyurethanes. Mixed fungi composed of *Alternaria* sp. and *Trametes* sp. isolated from landfill sites were effective at degrading low-density polyethylene in [18]. The marine fungus *Alternaria alternata* has been shown to have polyethylene-degrading capabilities, and the molecular mechanisms that it uses for plastic degradation, including laccase and peroxidase, have been determined via transcriptomics [19]. Enzymes like laccase and manganese peroxidase are vital in this degradation process, with laccase being capable of degrading high-molecular-weight polyethylene using mediator systems [17,18]. Despite the identification of various polyethylene-degrading strains, there remains a need for the identification of more effective and efficient degrading strains to meet current demands.

The impacts of polyethylene film on soil are multifaceted, especially when used in agriculture, as their approximately 90% carbon content significantly alters soil properties. The question of whether microbial agents can remediate soil degradation caused by polyethylene films remains unknown. Microbial agents such as *Bacillus*, *Streptomyces* and *Trichoderma reesei* are recognized for their ability to enhance soil nutrient transformation and improve the soil structure and environment [20]. *Trichoderma* is a significant biocontrol fungus known for optimizing soil microbial communities and activating soil nutrients [21,22]. When combined with organic fertilizers, *Trichoderma* has been shown to increase soil organic carbon content, improve soil structure and enhance soil fertility [21,22]. However, the influence of varying amounts of *Trichoderma* on soil organic carbon turnover, especially in loamy fluvo-aquic soils, remains unclear.

Hence, this study will bridge this gap by screening for strains capable of efficiently degrading polyethylene, focusing on *Trichoderma* species. Subsequently, we investigate the effects of different application rates of *Trichoderma* on soil organic carbon dynamics, studying the potential of this fungus to mitigate the environmental impacts of polyethylene residues in agricultural soils.

2. Materials and Methods

2.1. Experiment 1: Isolation and Screening of Polyethylene-Degrading Bacteria

2.1.1. Preparation of Polyethylene and Culture Medium

The polyethylene film was purchased from a local market, cut into 4 cm × 4 cm square pieces and accurately weighed, and then the pieces were placed in a 10 mL sterile centrifuge tube and soaked in 2% sodium dodecyl sulfate, 75% ethanol and 95% ethanol for 4 h respectively. After soaking, the film was washed three times with sterile water, the moisture adhered to the polyethylene surface was absorbed with sterile filter paper and then irradiated using the UV lamp for sterilization for 15 min.

Soil samples were collected from farmland that was covered with polyethylene film for more than 10 years. Visible debris was meticulously removed from the soil, which was then carefully transferred into sterile plastic bags for transportation to the laboratory under aseptic conditions.

The liquid inorganic salt medium (Coolaber Co., Ltd., Beijing, China) and solid inorganic salt medium (Coolaber Co., Ltd., Beijing, China) were prepared. The potato dextrose medium was made of potato, glucose and agar, with a natural pH. The trace carbon source

medium was made of yeast extract, NH_4SO_4, trace elements and agar powder. The seed liquid culture medium contained potato (200 g), glucose (20 g), peptone (5 g), $MgSO_4 \cdot 7H_2O$ (1 g), KH_2PO_4 (2 g) and vitamin B1 (50 mg); all these reagents were dissolved in 1000 mL distilled water.

2.1.2. Isolation, Screening and Identification of Polyethene-Degrading Strain

The collected soil sample (5 g) was suspended in 45 mL of sterile normal saline and shaken at 150 rpm for 30 min to prepare the soil diluent. Then, 20 μL of soil diluent was transferred in 100 mL of inorganic salt medium with 1% polyethylene as the sole carbon source. Meanwhile, 20 μL of soil diluent was transferred into 100 mL of inorganic salt medium without polyethylene film. The inoculated medium was incubated at 28 °C and 150 rpm for 10 days in the dark. Then, we discarded the polyethylene film and diluted the culture solution with a sterile normal saline gradient. A total of 0.2 mL of the dilution solution was inoculated on potato glucose medium using the coating method for culturing to obtain the pure culture of the strain. The obtained strains were inoculated into the solid inorganic salt medium with 4 cm × 4 cm polyethylene using the coating method and placed in a 28 °C constant-temperature incubator to screen out strains with polyethylene-degrading ability.

2.1.3. Molecular Identification of Polyethene-Degrading Strain

The polyethene-degrading strains were identified using molecular biological methods. The total DNA of the screened strains was extracted with the column fungal genomic DNA Extraction Kit (Solarbio Life Sciences Co., Ltd., Beijing, China), and its sequences were amplified with the universal primers ITS1 (5′-TCCGTACCTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATGC-3′). The polymerase chain reaction (PCR) products were detected using agarose gel electrophoresis, and the qualified PCR products were sent to Shanghai Yuanshen Biomedical Technology Co., Ltd. (Shanghai, China) for sequencing. The sequencing results were analyzed using Bioedit v7.7.1.0 software (Tom Hall, Vista, CA, USA), and the sequences with high confidence were intercepted. The intercepted sequences were compared with the sequences in the NCBI-BLAST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 8 December 2023). The strains were identified according to the similarity of their sequences with the alignment results. The phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA ver. 7.0) software (www.megasoftware.net, accessed on 15 December 2023) using the neighbor joining method to analyze the genetic relationships between strains.

2.1.4. Degradation Efficiency of Polyethylene-Degrading Bacteria

The purified strain was inoculated into the liquid inorganic salt medium containing polyethylene film at a 5% volume ratio under sterile conditions. After a 30-day incubation at 28 °C and 150 rpm, the weight loss of the polyethylene film was measured to assess degradation. The surface morphology of the degraded polyethylene was examined with the in situ tensile scanning electron microscope Tescan Mira 3 (Tescan Group, Brno-Kohoutovice, Czech Republic). The Fourier transform infrared spectrometer Nicolet 6700 (Thermo Scientific, Waltham, MA, USA) was used to analyze the film's chemical structure and functional group changes within a frequency range of 900–4000 cm^{-1}.

2.1.5. Laccase Activity

The activated *Trichoderma asperellum* was incubated in a seed liquid medium and cultured at 28 °C and 150 rpm for 5 days. When many mycelial balls appeared in the liquid medium, it was filtered with 4 layers of gauze and centrifuged at 10,277× *g* for 15 min using a centrifuge Happy-T16 (Fudi Machinery Corporation, Jinan, China). The supernatant obtained was the crude laccase solution. Laccase activity was determined using the guaiacol method [17]. Indeed, we took 3 mL of 10 rpm sodium acetate buffer (pH = 4.6), 1 mL of crude enzyme solution and 1 mL of 2 mmol L^{-1} guaiacol as the mixture

and incubated it at 28 °C for 15 min. The absorbance of the reaction system was measured at a wavelength of 465 nm with the UV-2600i Spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

2.2. Experiment 2: Effect of the Degradable Strain on Soil Organic Carbon

2.2.1. Soil Sample and Microbial Strain

The soil sample was collected from a farmland that has been cultured for more than 50 years in Xunxian county (114°40′ E, 34°40′ N), Henan Province. The site has a warm, semi-humid climate with a mean annual precipitation rate of about 650 mm. The soil type is a typical loamy fluvo-aquic soil with a pH of 7.8. The surface soil samples (0–20 cm) were collected using the five-point sampling method and passed through a 2 mm soil screen to remove the visible plant roots, sand, gravel and other debris. Soil samples were naturally air dried at room temperature. The surface soil organic carbon content was 8.57 g kg^{-1}, and the total nitrogen content was 0.78 g kg^{-1}.

In the test, microbial strains were strains that could degrade the polyethylene screened above. *Trichoderma* were inoculated on a potato glucose solid medium for cultivation, and a spore suspension was prepared. Then, the spore suspension was inoculated and proceeded to liquid fermentation. Finally, the microbial strains were harvested and dried at a low temperature to obtain *Trichoderma* powder and stored at a low temperature for future use.

2.2.2. Mineralization of Soil Organic Carbon

Treatments with different amounts of microbial strains were set; the mass ratios of the microbial agents to soil were 1:100 (B1), 1:200 (B2), 1:300 (B3) and 1:400 (B4), respectively; a control (B0) without microbial agents was also utilized. The indoor experiment was conducted for a month, and static alkali solution absorption methods were used to measure the soil CO_2 emission [23]. Indeed, 100 g of air-dried soil was weighed and placed in a 1000 mL culture flask, and the soil water content was adjusted to 60% of the water-holding capacity. At the same time, a centrifuge tube containing 5 mL of 1 mol L^{-1} NaOH (Sinopharm Group Co., Ltd., Shanghai, China) solution was placed in the culture jar, and then the sealed culture flask was covered for culture at 25 °C in the dark. The culture tube containing NaOH solution was taken out on days 1, 3, 5, 9, 16, 23, 30, 45 and 60. Then, 5 mL of 1 mol L^{-1} $BaCl_2$ (Sinopharm Group Co., Ltd., Shanghai, China) and 2 drops of phenolphthalein indicator were added and titrated with a 0.5 mol L^{-1} standard HCl (Sinopharm Group Co., Ltd., Shanghai, China) solution until the red color disappeared, and the HCl consumption was recorded. During the incubation period, the soil water content was supplemented by weighing the jars. At the same time, a blank control without soil samples was set. The CO_2 emissions were calculated using the following equation:

$$CO_2\text{-}C = 1/2cHCl \times (V0 - V) \times 12 \div m \times 1000 \quad (1)$$

where CO_2-C is the mineralization of soil organic carbon (mg kg^{-1}), V0 and V are the amounts of HCl used by the control and samples (mL), cHCl is the concentration of HCl (mol L^{-1}), and m is the weight of the air-dried soil (g).

The mineralization rate of the soil organic carbon was calculated using the accumulative mineralization divided by the incubation time. The dynamics of soil organic carbon mineralization were fitted by using the first-order kinetic equation [23]. The first-order kinetic equation is listed below:

$$Cp = C0(1 - e^{-kt}) \quad (2)$$

where Cp is the accumulated amount of soil organic carbon (SOC) mineralized at time t, k is the mineralization rate constant, and C0 is the potentially mineralizable SOC.

The soil organic carbon mineralization intensity was calculated with via the mineralization of the soil organic carbon and total organic carbon in soil, calculated with C0/SOC [23].

2.2.3. Soil Organic Carbon and Its Composition

The content and components of soil organic carbon in different treatments were determined after indoor cultivation. The content of soil organic carbon (SOC) was determined using the potassium dichromate oxidation external heating method [24]. Soil microbial biomass carbon (MBC) was determined using the chloroform fumigation abstraction method [25], applying the following equation:

$$MBC = \Delta EC/K_{EC} \quad (3)$$

where ΔEC is the difference between the organic carbon extracted from non-fumigated soil and fumigated soil, and K_{EC} is the conversion factor (0.38).

To determine water-soluble organic carbon (WSOC), the soil was mixed well with water and centrifuged with a 0.45 μm filter membrane for suction filtration, and the carbon concentration of the solution was measured with a total organic C analyzer-Multi N/C 3100 (Analytik Jena, Jena, Germany) [25]. The content of rapidly oxidized organic carbon (ROC) was determined using the potassium permanganate oxidation method. Indeed, 10 g air-dried soil samples were mixed with 20 mL of 333 mmol L^{-1} $KMnO_4$ for 1.5 h. After the centrifugation of the suspension, the supernatant was diluted with distilled water, and the optical density was measured at 565 nm. The ROC content of each sample was calculated on basis of decrements in the $KMnO_4$ content, given that 1 mmol L^{-1} of MnO_4^- could consume 9 mg of C [25]. For each sample, the difference in the total SOC and ROC contents was regarded as the non-readily oxidizable organic carbon (NROC) content. The soil carbon pool active degree (CA), carbon pool active index (CAI), carbon pool index (CPI) and carbon pool management index (CPMI) were calculated with the long-term abandoned farmland as the control soil according to formulas given in previous studies [25,26], applying the following equations:

$$CA = SOC/(SOC - EOC) \quad (4)$$

$$CAI = CA \text{ in the sample}/CA \text{ in the control} \quad (5)$$

$$CPI = SOC \text{ of samples}/SOC \text{ of control} \quad (6)$$

$$CPMI = CAI \times CPI \times 100 \quad (7)$$

2.3. Statistical Analysis

The data were statistically analyzed using SPSS 19.0 (IBM, Armonk, NY, USA), where one-way analysis of variance (ANOVA) was used to assess the significance of different treatments following least significant difference tests at $p < 0.05$. The figures were designed with SigmaPlot 12.5 (Systat Software Inc., London, UK).

3. Results

3.1. Isolation and Identification of Polyethylene-Degrading Fungi

The adapted fungal colonies were transferred to potato dextrose agar medium using the drilling method for repeated isolation and purification, yielding six pure fungal cultures named PF1, PF2, PF3, PF4, PF5 and PF6. These strains were individually inoculated into a liquid inorganic salt medium supplemented with 1% polyethylene film and incubated at 28 °C with agitation at 150 rpm. After 30 days of degradation, the PF2 strain induced the highest weight loss rate of 4.15% in the polyethylene film (Table 1). Consequently, PF2 was chosen as the focal strain for further polyethylene-degradation experiments.

The resulting DNA fragment from PF2 was 613 base pairs in length. BLAST analysis revealed over 98% sequence homology between strain PF2 and various *Trichoderma* strains. Phylogenetic analysis placed strain PF2 in the same clade as *Trichoderma asperellum* (MH215548.1, MH215555.1 and MK841024.1) with a bootstrap value of 99, showing high confidence in the classification (Figure 1). Based on its morphological and molecular characteristics, the strain was provisionally identified as *Trichoderma asperellum* PF2.

Table 1. The weight loss of the polyethene film after 4 weeks of incubation.

Strain	Weight Loss Rate (%)
PF1	0.12 ± 0.012 d
PF2	4.15 ± 0.036 a
PF3	0.23 ± 0.010 c
PF4	0.47 ± 0.018 b
PF5	0.24 ± 0.013 c
PF6	0.12 ± 0.011 d

Note: The data are presented as the means ± standard deviations of three replicates. Different lowercase letters indicate significant differences between strains ($p < 0.05$).

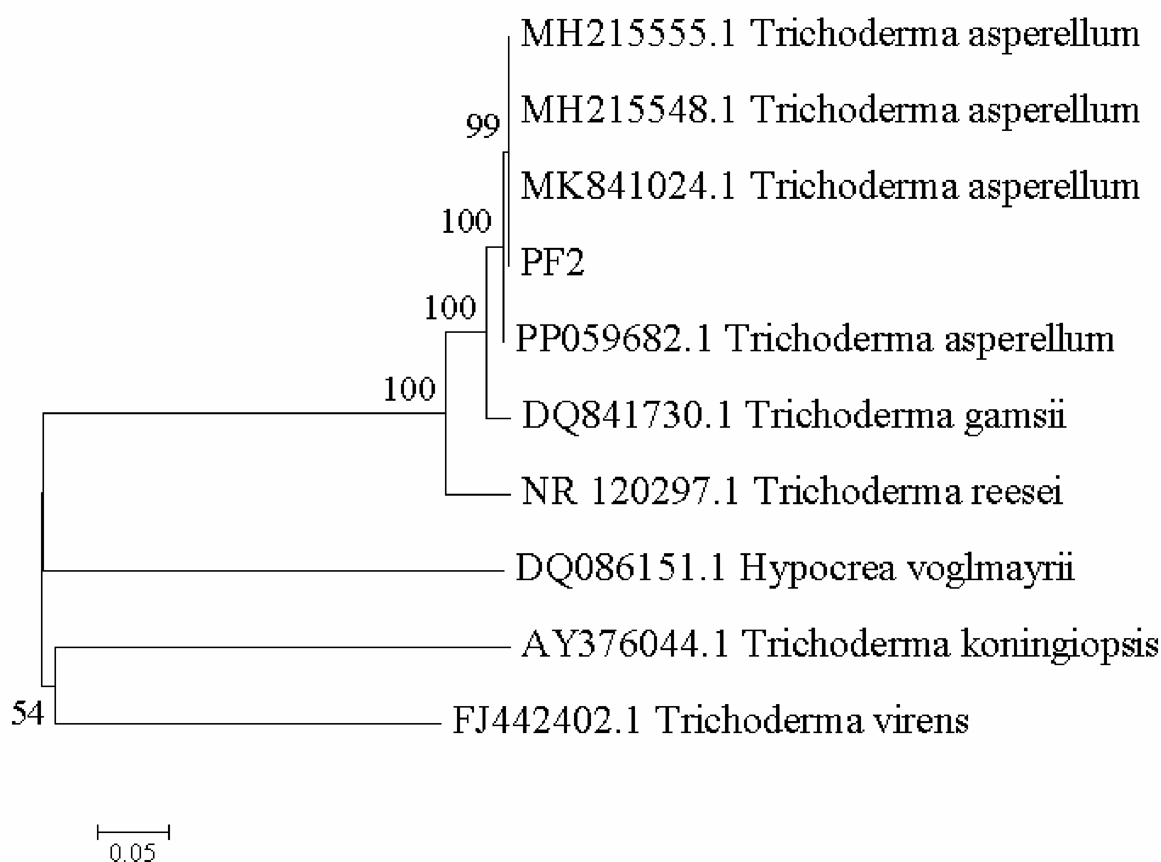

Figure 1. The phylogenetic tree concluding strain PF2.

3.2. Degradation and Enzyme Production Characteristics of Strain PF2

Scanning electron microscope analysis revealed that the uninoculated polyethylene film had a smooth surface, while the polyethylene film exposed to strain PF2 had a rough texture with pits and cracks (Figure 2a). As shown in Figure 2b, the polyethylene film inoculated with strain PF2 exhibited distinct absorption peaks within the ranges of 1160–1300 cm^{-1}, 1550–1610 cm^{-1}, 1631–1707 cm^{-1} and ~2350 cm^{-1}. These peaks corresponded to the presence of ether bonds (-C-O-C-), carboxyl groups (-COOH), ketone bonds (-C(=O)-) and carbon-carbon triple bonds (-C≡C-), respectively.

The laccase activity produced by strain PF2 during coculture with polyethylene was determined (Figure 2c). The laccase activity significantly increased in line with the incubation time, with the lowest value being recorded on day 7, and the highest value of laccase activity was 41.30 U L^{-1}, which was recorded at the end of the culture period.

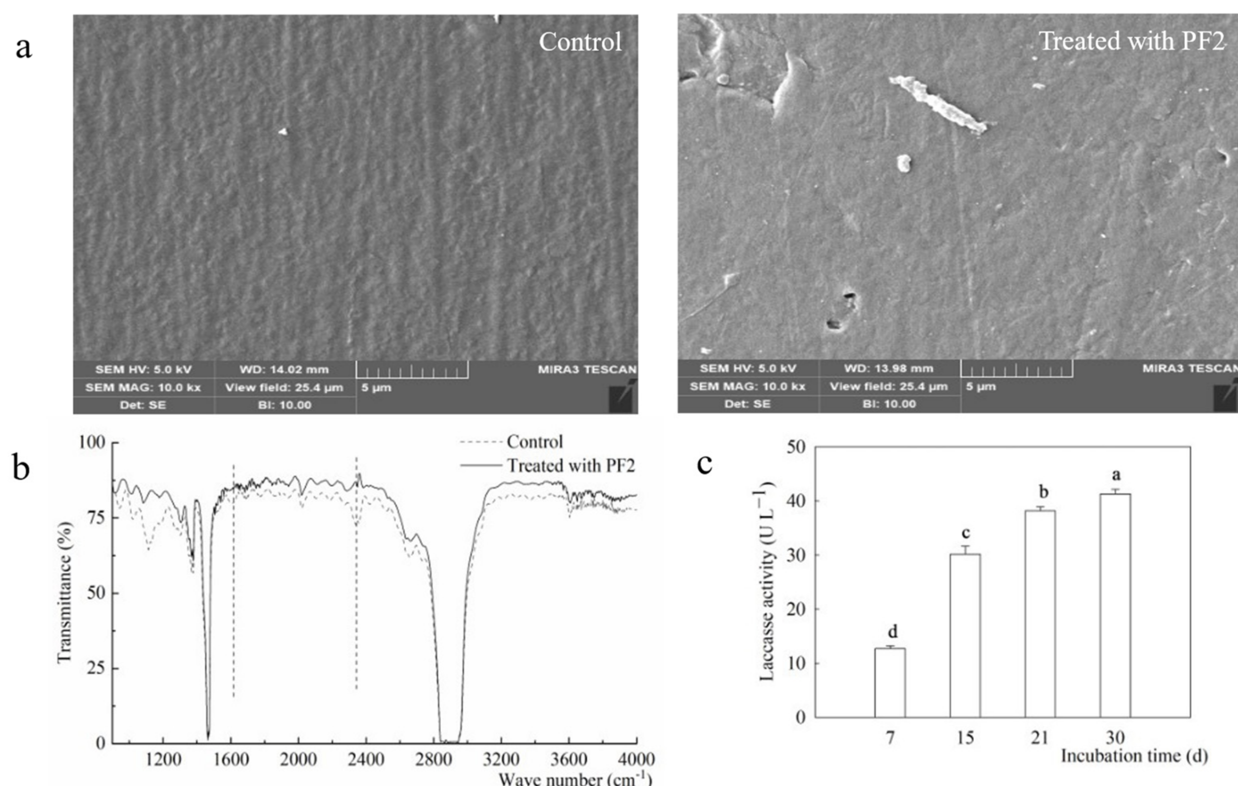

Figure 2. The degradation effect and laccase activity of strain PF2 on the polyethylene film after a 30-day incubation period. (**a**) The surface morphology of the degraded polyethylene with (right) or without (left) strain PF2; (**b**) the Fourier transform infrared spectrum of the degraded polyethene after biodegradation with strain PF2; (**c**) the laccase activity in the culture supernatant. Different letters on the error bars represent significant changes ($p < 0.05$).

3.3. Effects of *Trichoderma* on the Mineralization of Soil Organic Carbon

According to Figure 3a, the cumulative CO_2 emissions of different treatments increased with the incubation time but slowed down over the incubation period of the period. By the end, treatments B1, B2, B3 and B4 had significantly higher SOC mineralization rates than the B0 treatment, with increases of 132.53%, 101.09%, 55.41% and 31.62%, respectively. The rate of SOC mineralization decreased with the incubation time (Figure 3b), falling into three stages. Initially (1–16 days), the CO_2 emission rates dropped sharply from their peak. B4 showed no significant change for the first 7 days, while B2, B3 and B4 had lower rates than B0. In the middle stage (16–30 days), the rate generally decreased to a stable level, with the rates on days 16 and 30 being 19.85–51.68% and 16.31–50.69% of those on day one, respectively. In the final stage (30–60 days), the B1, B2 and B3 rates became similar, while B4 remained lower and B0 had the lowest rate, which, by day 60, was reduced to just 4.77–25.35% of the initial rate.

The contents of SOC in different treatments ranged from 8.87 to 14.04 g kg^{-1}. Significant differences in the SOC content among treatments were observed (Figure 3c). The SOC content increased by 27.87–58.38% for treatment with *Trichoderma* relative to B0, with the B1 treatment showing the most significant enhancement. According to Figure 3d, after 60 days of incubation, the B1 and B2 treatments significantly increased the SOC mineralization intensity compared to B0. The B3 and B4 treatments did not show significant differences from B0, although they were still distinct numerically, with the B3 and B4 treatments showing decreases of 3.43% and 5.02%, respectively, compared to B0.

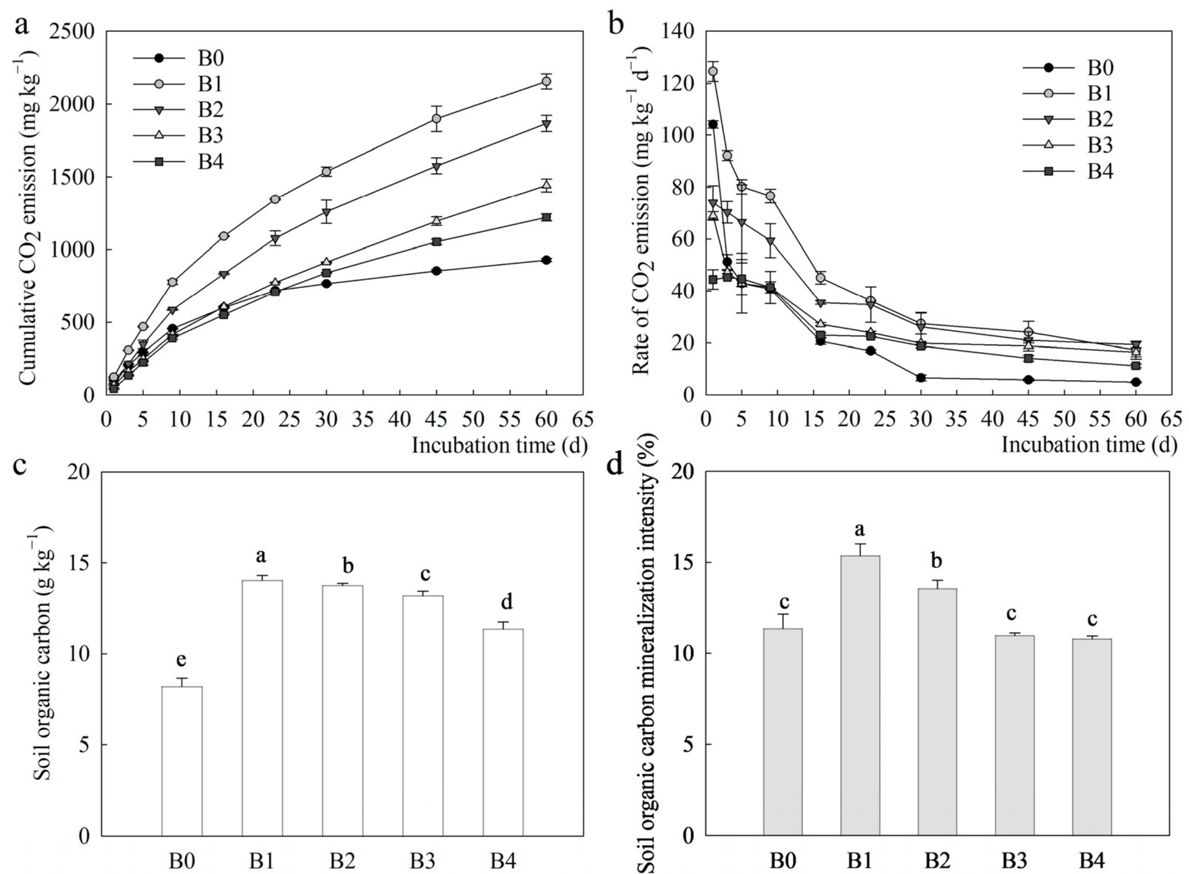

Figure 3. The soil organic carbon and mineralization dynamic in different treatments. (**a**) Cumulative CO_2 emissions, (**b**) CO_2 emissions rate, (**c**) soil organic carbon content and (**d**) mineralization intensity. B0: control, soil without *Trichoderma asperellum*; B1, B2, B3 and B4: *Trichoderma asperellum* applied at ratios of 1:100, 1:200, 1:300 and 1:400, respectively. The values in the figures are means ± standard deviations of the three replicates. The lowercase letters above the bars in (**c**,**d**) indicate significant difference at $p < 0.05$.

The soil organic carbon mineralization data were fitted using a kinetic model (Table 2). The high coefficient of determination (R^2) of the equation indicated that the first-order kinetic equation well described the dynamics of SOC mineralization. The Cp values of the SOC across treatments followed an order of B1 > B2 > B3 > B4 > B0. Compared with B0, the Cp of B1, B2, B3 and B4 treatments increased significantly by 157.58%, 141.56%, 103.04% and 58.78%, respectively. The k value ranged from 0.0251 to 0.0739 d^{-1} across treatments, with B0 treatment being the largest, followed by B1, B2 and B4, while the B3 treatment had the lowest values.

Table 2. The parameters of the first-order kinetics values for SOC mineralization.

Treatment	Cp (mg kg^{-1})	k (d^{-1})	R^2
B0	1.57 ± 0.09 c	6.63 ± 0.42 c	42.88 ± 3.06 c
B1	2.53 ± 0.19 a	11.51 ± 0.10 a	58.45 ± 0.58 a
B2	2.42 ± 0.17 a	11.34 ± 0.31 a	56.14 ± 0.66 ab
B3	1.88 ± 0.04 b	11.28 ± 0.14 a	55.61 ± 1.16 ab
B4	1.84 ± 0.02 b	9.50 ± 0.36 b	53.10 ± 1.21 b

Note: The data are presented as the means ± standard deviations of three replicates. Different lowercase letters indicate significance at $p < 0.05$.

3.4. Effects of *Trichoderma* on Soil Organic Carbon Components and the CPMI

Significant differences were observed in the contents of different organic carbon fractions among different treatments (Table 3). Compared with B0, the ROC in treatments B1, B2, B3 and B4 increased by 61.49%, 54.26%, 20.00% and 17.45%, respectively, with no significant difference between B1 and B2 or between B3 and B4. *Trichoderma* notably enhanced the NROC, with increases ranging from 43.18% to 73.56% compared to B0. After incubation, the WSOC increased significantly with the addition of *Trichoderma*, with the B1, B2, B3 and B4 treatments showing increases of 36.31%, 30.90%, 29.67% and 23.83%, respectively, compared to B0. The MBC varied from 130.46 to 279.28 mg kg^{-1} across treatments. Compared with B0, the MBC in treatments B1, B2, B3 and B4 increased by 114.07%, 88.23%, 67.41% and 54.41% respectively, showing an increasing trend with the increase in the amount of *Trichoderma* added. Consistent with MBC, the qMBC of the B1 treatment was the highest (1.99%) and that of B0 was the lowest (1.60%). There was no significant difference between the B3 and B4 treatments compared to B0.

Table 3. Soil organic carbon components in different treatments.

Treatment	ROC (g kg^{-1})	NROC (g kg^{-1})	WSOC (mg kg^{-1})	MBC (mg kg^{-1})	qMBC (%)
B0	1.57 ± 0.09 c	6.63 ± 0.42 c	42.88 ± 3.06 c	130.46 ± 2.54 e	1.60 ± 0.00 d
B1	2.53 ± 0.19 a	11.51 ± 0.10 a	58.45 ± 0.58 a	279.28 ± 4.01 a	1.99 ± 0.06 a
B2	2.42 ± 0.17 a	11.34 ± 0.31 a	56.14 ± 0.66 ab	245.56 ± 1.35 b	1.78 ± 0.02 b
B3	1.88 ± 0.04 b	11.28 ± 0.14 a	55.61 ± 1.16 ab	218.41 ± 5.79 c	1.66 ± 0.05 c
B4	1.84 ± 0.02 b	9.50 ± 0.36 b	53.10 ± 1.21 b	184.78 ± 10.91 d	1.63 ± 0.09 cd

Note: The data are presented as the means ± standard deviations of three replicates. Different lowercase letters within the same column indicate significance at $p < 0.05$.

Trichoderma has a significant impact on the soil carbon pool index (Figure 4). Compared with B0, the B1 and B2 treatments had no significant effect on soil CPA and CPAI, while the B3 and B4 treatments led to a significant decrease, with B3 showing the most significant decrease in both CPA and CPAI. Compared with B0, the CPI was significantly increased by 71.26%, 67.77%, 60.50% and 38.26% in B1, B2, B3 and B4 treatments, respectively. The soil CPMI is an index to evaluate the quality of soil organic carbon. Compared with the B0 treatment, the CPMI of the B1, B2, B3 and B4 treatments was increased by 59.42%, 51.48%, 13.25% and 13.41%, with no significant difference between B1 and B2 or between B3 and B4.

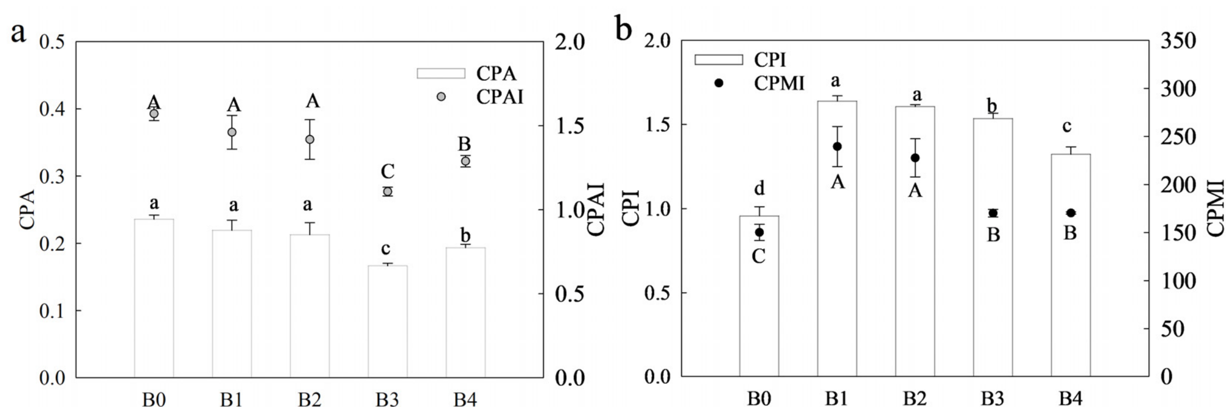

Figure 4. Soil carbon management pool indexes in different treatments. (**a**) Different lowercase letters indicate significant differences in the CPA, and different uppercase letters indicate significance in the CPAI; (**b**) different lowercase letters indicate significant differences in the CPI, and uppercase letters indicate significant differences in the CPMI. B0: control, soil without Trichoderma asperellum; B1, B2, B3 and B4: Trichoderma asperellum applied at ratios of 1:100, 1:200, 1:300 and 1:400, respectively. Values in the figures are means ± standard deviations of three replicates.

4. Discussion

4.1. Degradation of Polyethylene by Degrading Microorganisms

In this study, strain PF2, with its high polyethylene degradation efficiency, was successfully screened from soil. Strain PF2 was preliminarily identified as *Trichoderma asperellum*. *Trichoderma asperellum* is a saprophytic living fungus capable of degrading bio-organic components, and it is classified within the *Hemifungi* class, *Filopodia* order, *Trichoderma* genus [27]. It was found that *Trichoderma asperellum* could break down cellulose, secrete growth-promoting factors and degrade polycyclic aromatic hydrocarbons [28]. The fungi known to degrade polyethylene belong to four classes under the phyla *Ascomycota* and *Basidiomycota*, including *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium*, with *Aspergillus* being the most widely studied [16]. At present, there have been no reports on the degradation of polyethylene by *Trichoderma asperellum* [16,18]. Therefore, this study enriched the strain resources of polyethylene-degrading microorganisms and laid important foundations for further research on polyethylene degradation.

The microbial degradation of mulch film is a multi-step process typically involving attachment, hyphal growth and reproduction and enzyme degradation [14]. In the present study, the degradation of polyethylene by strain PF2 was carried out under oligotrophic conditions from initial attachment to the film surface through to its degradation. After 30 days, a weight loss rate of 4.15% was observed. During this period, hyphae effectively invaded and perforated the polyethylene film, resulting in significant damage and the formation of holes. Strain PF2 catalyzed a chemical transformation within the polyethylene, introducing active functional groups such as ether bonds, carboxyl group, ketone bonds and carbon-carbon triple bonds. Thus, strain PF2 could oxidize the hydrocarbon chain of polyethylene, thereby generating these functional groups. Typically, microorganisms struggle to utilize polyethylene as a carbon source. However, under oligotrophic conditions, strain PF2 adapted by shifting its carbon utilization preference, employing polyethylene for growth and metabolic activities [16]. The laccase activity in the culture medium was detected using the guaiacol method, and it was found that laccase was produced in the culture medium. The laccase activity gradually increased along with the incubation time, and it was undetectable on day 1, indicating that the laccase was an inducible enzyme crucial for polyethylene degradation [17,29]. The enzyme activity of strain PF2 observed in this study was lower compared to the strain reported in previous research, which may be attributed to strain differences and the varying conditions of polyethylene during incubation [29].

Ruan et al. [30] cocultured *Trichoderma harzianum* with polyethylene film for 30 days, and the weight loss rate of polyethylene was 3.39%, which was relatively consistent with the results of this study. In contrast, Sowmya et al. [31] employed pretreatments such as high-pressure sterilization, surface disinfection and UV radiation on the polyethylene film before cocultivation with *Trichoderma harzianum*, leading to significantly higher weight loss rates of 23%, 13% and 40%, respectively, after three months. UV radiation has been shown to enhance microbial degradation sensitivity, biofilm formation and hydrophilicity [32], thereby facilitating polyethylene degradation. Das and Kumar [33] demonstrated that the pretreatment of polyethylene powder and film with xylene or ethanol resulted in degradation rates of 11% and 16%, respectively, surpassing the degradation observed in untreated samples [34]. Additionally, Khan et al. [35] found that the addition of 2% glucose to the basic inorganic salt medium significantly improved the degradation ability of *Aspergillus pennis* on plastic film. These studies collectively indicate that modifying experimental conditions, such as medium composition, irradiation and polyethylene properties [16], could enhance the degradation rate.

4.2. Effects of Trichoderma on Soil Organic Carbon Mineralization

Minimizing soil CO_2 emissions was an effective strategy for improving soil fertility and mitigating climate change. The incorporation of amendments, such as *Trichoderma*, could alter soil's physical, chemical and biological properties, thereby affecting SOC min-

eralization. The study of SOC mineralization dynamics post-*Trichoderma* addition was, thus, crucial. Our results indicated that treatments with *Trichoderma* exhibited significantly higher cumulative CO_2 emissions and emission rates compared to the control (B0) at the end of the culture period. This increase might be attributed to the fact that *Trichoderma* could secrete laccase, a type of polyphenol oxidase capable of degrading organic matter in the soil [21]. In the present study, the B1 and B2 treatments significantly increased the mineralization intensity of SOC relative to B0, while B3 and B4 tended to reduce it. This indicated that lower doses of *Trichoderma* may result in a smaller proportion of substrate being available for microbial decomposition [23]. This dual effect of laccase on organic matter decomposition and stable carbon accumulation may have been modulated by the varying amounts of *Trichoderma* added [21,29]. In contrast, Huang et al. [36] found that the addition of laccase-producing fungi, including *Trichoderma* species, accelerated SOC mineralization and organic matter decomposition. These findings suggested that the impact of *Trichoderma* on SOC dynamics was dose dependent and might vary with the soil type.

The CO_2 emission rate across all treatments exhibited a comparable decline, which is consistent with [22]. This trend could be attributed to the fact that at the initial stage of mineralization, the large amounts of active organic carbon and available nutrients in the soil stimulated the microbial activity and promoted CO_2 emissions. As incubation progressed, the SOC predominantly comprised recalcitrant carbon fractions resistant to degradation, leading to a reduction in the microbial activity and constraining further mineralization of soil organic carbon [23].

The content of organic carbon available for mineralization in soil was governed by the equilibrium between the input and output of soil organic carbon. This study found that the B1 treatment exhibited the highest cumulative CO_2 emissions and emission rates, whereas the B0 treatment displayed the lowest ones. The addition of *Trichoderma* altered the soil microbial community structure, proliferating other functional microorganisms [21], and enhanced microbial participation in organic matter synthesis and degradation [27]. With *Trichoderma* application, SOC was gradually converted into labile organic carbon, intensifying the SOC mineralization intensity. Increased *Trichoderma* addition continually enhanced the mineralization rate, indicating the significant influence of higher *Trichoderma* concentrations on SOC mineralization. Generally, a higher abundance of soil fungi correlated with a greater number of functional species [27], and fungi played a vital role in soil C loss through priming effects [37]. However, microbial competition, synergy and substrate limitations may have led to a threshold effect, as beyond a certain microbial concentration, further increases did not significantly enhance the organic carbon mineralization rates [38].

The potential mineralizable organic carbon (Cp) was an indicator of the soil microorganisms' capacity to exploit carbon sources. Our results showed that the Cp levels increased across different various *Trichoderma* treatments compared with the control, with the exception of the lack of a significant difference between B4 and the control. The addition of *Trichoderma* augmented the microbial population and activity, thereby enhancing the microbial utilization of the soil carbon pool [28], in turn facilitating an increase in the potential mineralizable soil organic carbon content. Conversely, *Trichoderma* significantly decreased the turnover rate (k) of SOC, extending the retention time of organic carbon in the soil, with the most pronounced effect being observed for the B3 treatment. This reduction in the turnover rate might be attributed to the elevated C/N ratio associated with *Trichoderma*, which, upon application, could increase the soil C/N ratio, thereby limiting microbial activity and carbon mineralization. Additionally, *Trichoderma* played a vital role in enhancing soil aggregation and contributed to carbon protection [22,37]. Notably, the impact of *Trichoderma* on SOC turnover might be dose dependent, since the lowest k value was observed for the B3 treatment.

4.3. Effects of Trichoderma on Soil Organic Carbon Fractions

Correlation analysis revealed a strong inter-relationship among the components of the SOC pool, which is consistent with the findings reported by Suárez et al. [39]. The formation

and transformation of SOC and its active fractions were intricate and dynamic, being influenced by the synergy of the soil organic matter, introduced microorganisms and other environmental factors, which altered the SOC turnover dynamics and ultimately impacted the SOC content and its active constituents. SOC and its fractions are crucial for maintaining ecological stability and soil balance and promoting sustainable soil recycling. Fu et al. [28] demonstrated a significant increase in SOC content following *Trichoderma* application in black soil subjected to long-term corn cultivation. Similarly, our results showed that *Trichoderma* significantly increased SOC and its fractions, with the SOC content increasing in proportion to the amount of *Trichoderma* added. Evidently, *Trichoderma* enhanced the self-regulation ability of soil. The secretions of *Trichoderma* effectively dissolved soil minerals and activated soil nutrients, thereby enriching the soil with active nutrients and improving the soil ecological environment [21,27].

As a labile fraction within SOC, the ROC was characterized by high availability and susceptibility to microbial degradation [26]. Our results revealed a significant increase in both the ROC and WSOC following *Trichoderma* addition. *Trichoderma* could modify the soil microbial community structure, improve the microbial activity and accelerate the decomposition and mineralization of SOC [21]. Microorganisms are important carbon pools in soil, regulating SOC dynamics through "extracellular modification" and "intracellular turnover", thereby playing an important role in the soil carbon cycle. In this experiment, *Trichoderma* addition significantly increased soil MBC, indicative of increased microbial activity. The qMBC for the B1, B2 and B3 treatments was significantly higher than that for the control, indicating enhanced soil microbial activity and a tendency toward SOC mineralization [40], which aligned with the above-mentioned CO_2 emissions data. Correlation analysis confirmed significant positive relationships between SOC mineralization and SOC, MBC, ROC and qMBC, indicating the importance of labile organic C pools in determining soil CO_2 emissions [23]. Obviously, *Trichoderma* addition improved the soil's ecological environment, promoted microbial activity and increased the soil microbial biomass [21,22,27], thereby increasing the soil qMBC. However, no difference in the qMBC was observed between B4 and the control, possibly due to the limited effect of low *Trichoderma* concentrations on SOC transformation. Notably, the trend of qMBC did not reflect those of the WSOC, MBC and ROC, highlighting the necessity of integrating qMBC with other indicators to assess SOC quality.

The soil CPMI is an indicator that represents the change in the soil carbon pool. An elevated CPMI was associated with higher SOC and ROC contents, indicating higher soil fertility. In this experiment, *Trichoderma* addition significantly increased the SOC, ROC and NROC contents, indicating that *Trichoderma* not only improved the quality and quantity of SOC but also enhanced the carbon sequestration capacity of soil [26]. Both the soil CPI and CPMI increased significantly after *Trichoderma* addition, with the CPMI values for the B1 and B2 treatments significantly surpassing those for the B3 and B4 treatments, indicating the dose-dependent impact of *Trichoderma* on soil carbon pool quality. A significant correlation between the CPMI and ROC was observed, with the strongest coefficient among SOC fractions (Figure 5), which is consistent with a previous study [26]. However, Wang and Huang [41] showed that the ratio of DOC to SOC was a more effective indicator for evaluating the CPMI in rice fields. The increase in SOC was closely related to increases in other active organic carbon components, and the CPMI showed significant correlation with these fractions and SOC. Therefore, the CPMI might serve as a sensitive indicator of soil quality changes due to the addition of *Trichoderma*.

The addition of *Trichoderma* into soil had a beneficial impact on the soil organic carbon pool. However, the application rate of *Trichoderma* must be carefully managed. Insufficient quantities might fail to effectively facilitate organic carbon transformation and soil quality enhancement, whereas excessive amounts can accelerate SOC mineralization, compromising carbon sequestration and potentially increasing costs. Thus, the application of *Trichoderma* should be tailored to the soil characteristics and types present to balance ecological enhancement with economic efficiency. Notably, our research was conducted

for scientific purposes to verify the positive effect of *Trichoderma* on soil carbon turnover. The *Trichoderma*-to-soil-weight ratio was not suitable for agricultural practice. We will try to improve the method of *Trichoderma* application to make it economically bearable for farmers in the future.

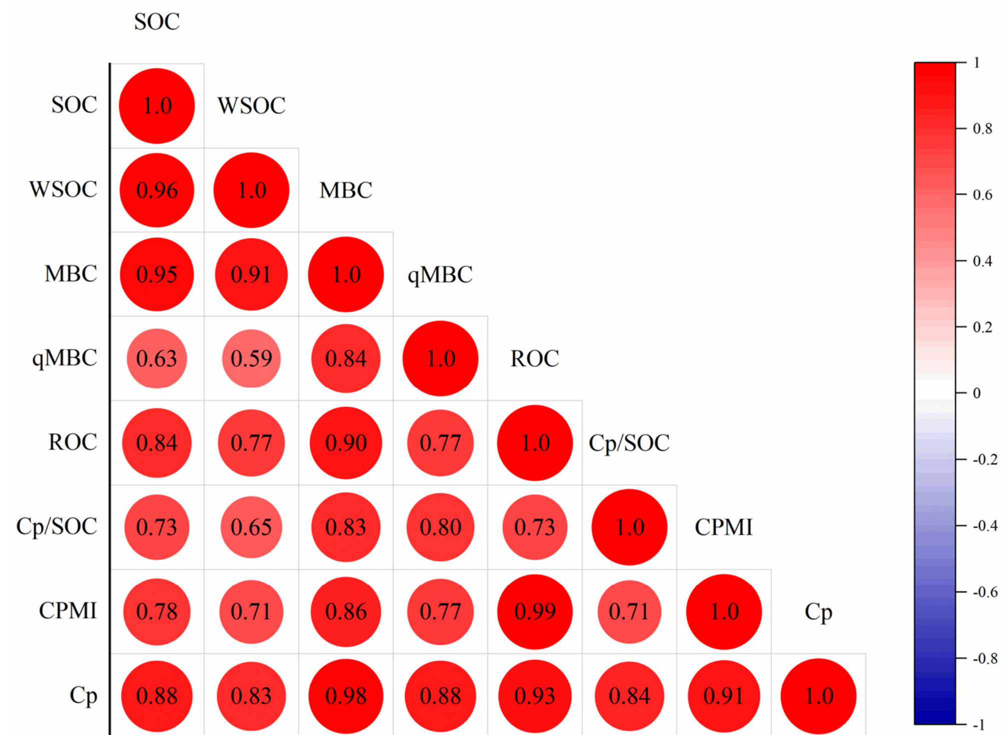

Figure 5. The correlation analysis between CO_2 emission and soil organic carbon.

5. Conclusions

Our study demonstrated the promising biodegradation potential of *Trichoderma asperellum* isolated from long-term cultivated soil on polyethylene, with a degradation rate of 4.15% over a 30-day period. The introduction of active functional groups into the polyethylene structure, which was facilitated by the laccase enzyme secreted by the fungus, highlighted the mechanism behind this biodegradation process. Furthermore, the application of *Trichoderma asperellum* to soil had a dose-dependent effect on the SOC dynamics. While higher doses of the fungus significantly increased the total and active organic carbon components, they also promoted SOC mineralization. The B3 treatment, with a *Trichoderma*-to-soil mass ratio of 1:300, was the most effective way of improving SOC quality and quantity, thereby contributing to enhanced soil fertility. This finding suggested that the optimal use of *Trichoderma asperellum* can be a strategic approach for both plastic waste management and soil health improvement.

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