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Biochar

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Enhancing oxytetracycline degradation and reducing its transfer to lettuce using biochar combined with carbohydrate carbon sources

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Abstract

Biochar has been widely recognized for its ability to adsorb soil antibiotics (like oxytetracycline, OTC) and inhibit their translocation to plants, but there remain limitations in further reducing OTC toxicity. In this study, lettuce was used as the test crop, with treatments including biochar alone (BC), biochar combined with carbohydrate carbon sources (CCS), and a control treatment without biochar (CK). Specifically, the treatments that combined biochar and CCS included biochar-glucose (BCG), biochar-sucrose (BCSU), and biochar-starch (BCST). The objectives were to improve the soil degradation efficiency of OTC, lower its toxicity, reduce its transfer to lettuce roots and leaves, and promote lettuce growth. In addition, the study explored the mechanisms of soil improvement and OTC degradation when biochar was combined with CCS. The results showed that BC increased the soil OTC degradation efficiency by 22–67% and reduced the translocation of OTC to lettuce leaves by approximately 0.51–1.23 mg kg^{-1}, while slightly increasing microbial activity. By activating redox enzyme activity without reducing soil mineralization, the BCG, BCSU, and BCST treatments significantly enhanced OTC degradation efficiency to 87%, 86%, and 92%, respectively. However, because low-molecular-weight sugars metabolize quickly, the BCG and BCSU treatments did not maintain a prolonged enhancement of soil enzyme activity and OTC degradation efficiency. In contrast, the BCST treatment exhibited superior performance, characterized by a gradual increase in soil enzyme activity, a 55% increase in microbial biomass carbon (MBC) throughout the experimental period, and a reduction in the OTC half-life to approximately 8 days. Furthermore, there were no significant differences in lettuce biomass among the treatments, but the BCST significantly improved nitrogen uptake and Soil and Plant Analyzer Development (SPAD) values in lettuce. These findings provide an effective strategy for promoting the degradation of non-prescription drugs and offer valuable insight for further eliminating antibiotics from soil.

Highlights

1. Biochar accelerated soil OTC degradation by 22–67% and reduced OTC transfer to lettuce leaves.
2. Carbohydrate carbon sources promoted soil OTC degradation by activating oxidoreductase activity.
3. Compared to biochar with glucose and sucrose, biochar combined with starch was more effective in degrading OTC.

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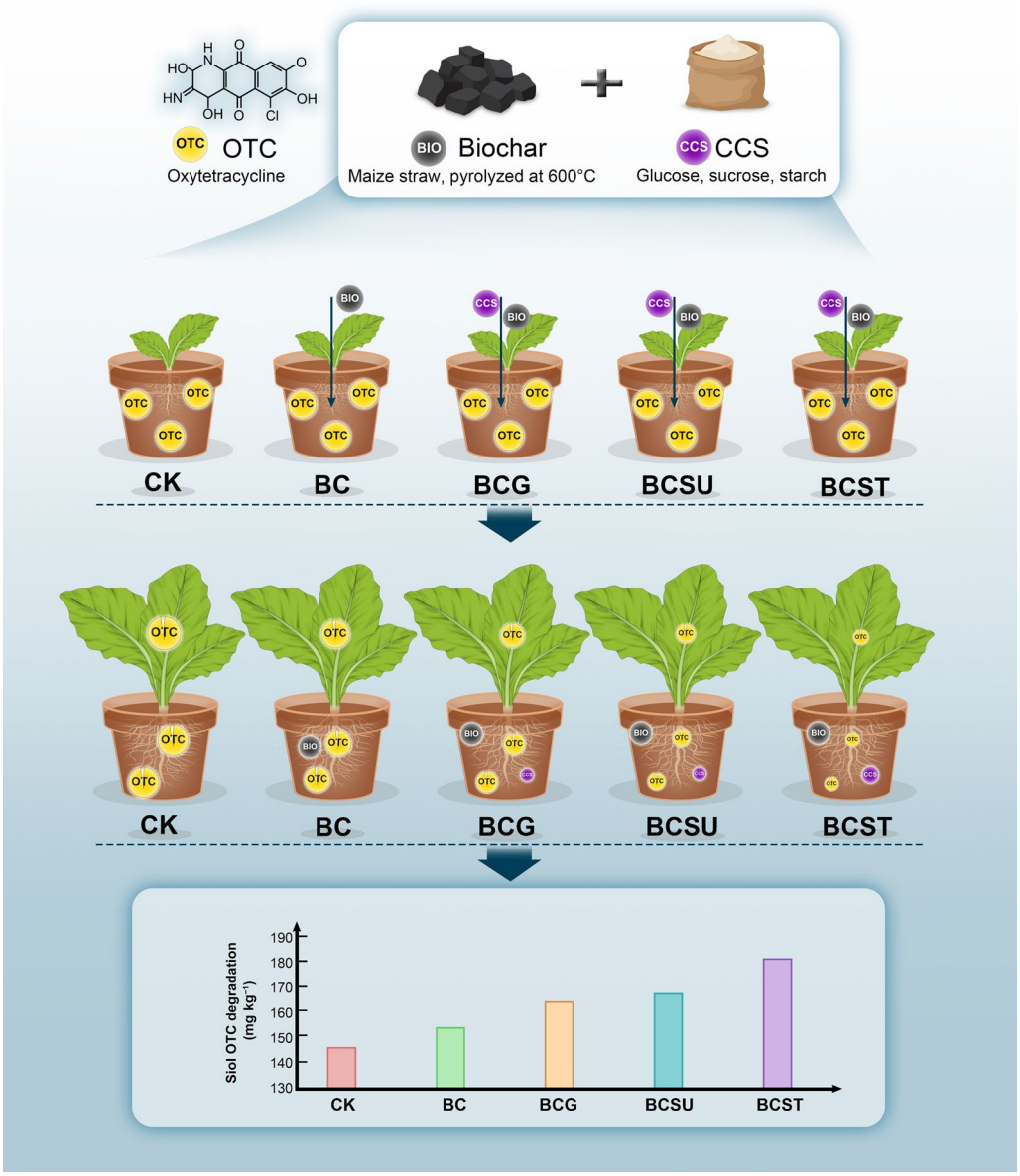
Keywords Biochar mix CCS, OTC translocation, Antibiotic pollution, Soil enzyme, Soil improvement

Graphical Abstract



1 Introduction

Antibiotics are the most commonly used medicine for human and animal health in the world. As of the most recent reports, global antibiotic consumption has increased significantly. Between 2000 and 2018, the global consumption rate of antibiotics rose by 46%, from 9.8 to 14.3 defined daily doses (DDD) per 1,000 inhabitants per day (Browne et al. 2021). These trends indicate that the overuse and misuse of antibiotics are becoming increasingly severe worldwide, with growing global concern about antibiotic pollution (Rukyaa et al. 2024). Each year, a large amount of antibiotics enters the soil and water bodies through animal waste (Barola et al. 2024). Additionally, due to the current limited sewage treatment capacity, residual antibiotics that are not effectively removed are also discharged into the environment,

leading to a series of environmental problems in ecosystems (Ren et al. 2021). Numerous studies have shown that antibiotics are frequently detected in soil, surface water, and groundwater (Li et al. 2021b; Tadic et al. 2020).

Oxytetracycline (OTC, $C_{22}H_{24}N_2O_8$) is a typical Tetracycline Antibiotics (TCs). A study found that OTC was detected in 90% of 1183 soil samples (from garden lands, vegetable fields, grain lands, etc.) (Zeng et al. 2018). The soil matrix strongly adsorbed OTC, causing it to rapidly accumulate in the topsoil of farmland, with concentrations ranging from 0 to 8400 μg kg^{-1} (Rahman et al. 2018). OTC can persistently accumulate in agricultural topsoil and subsequently be continuously taken up by crops. Residual OTC in soil can adversely affect microbial activity, inhibit crop growth, induce antibiotic resistance genes (ARGs), and pose genetic safety risks through plant uptake and subsequent transfer along the food chain (Wang et al. 2022). Moreover, OTC residues in edible crops, such as leafy vegetables, raise food safety concerns due to potential human exposure, thereby posing significant threats to public health. OTC and its photolysis products can alter the structural framework of soil enzymes, thereby enhancing the toxicity and side effects of OTC on soil microbial activity (Zhu 2020). Additionally, the inhibition of soil hydrolase activity by OTC directly affected the absorption of nutrients by plants, with lettuce growth being impacted when the OTC concentration reached 1.0 mg L^{-1} (Qi et al. 2025; Yao et al. 2010). OTC can induce oxidative stress, apoptosis, and reproductive toxicity, and reduce chloroplast synthase activity in plants, thereby inhibiting photosynthesis (Wright 2010). Moreover, residual OTC in the soil can induce the production of resistance genes, which are enriched in plants through bacterial vectors. Human consumption of crops enriched with resistance genes may pose potential genetic risks (Duan et al. 2017; Tadic et al. 2021). In conclusion, the residual OTC in the soil presents significant risks to ecological safety and human health. Therefore, it is necessary to implement technical measures to prevent OTC accumulation in the soil. This study proposes using biochar and other organic carbons to improve soil conditions and accelerate OTC degradation.

In recent years, the application of organic carbon to improve soil has emerged as a new technology, capable of enhancing soil fertility and reducing OTC contamination (Pan 2020; Zhao et al. 2021). Biochar, as a common form of organic carbon, possesses a large specific surface area, a well-developed microporous structure, a high degree of aromatization, and abundant oxygen-containing functional groups. These properties enable biochar to enhance the adsorption of organic pollutants through mechanisms such as ion exchange, electrostatic adsorption, and hydrogen bonding (Hurtado et al. 2017). Biochar primarily adsorbs OTC via its π-π electron interaction, thereby reducing its presence in soil (Cheng et al. 2020). Due to the strong electron-withdrawing nature of the ketone group (C=O), the aromatic rings on biochar can interact with OTC through specific π–π electron donor–acceptor (EDA) interactions (Luo et al. 2018). Although biochar demonstrates a certain adsorption effect on OTC, the parent structure of OTC remains intact, meaning that adsorbed OTC could still pose environmental risks (García-Delgado et al. 2018).

Many studies have found that biochar has certain limitations in the adsorption and degradation of OTC. Firstly, OTC adsorbed on biochar inhibits the abundance of soil bacteria and fungi, limiting the functional activity of soil microbes and enzyme activity (Jiang et al. 2018). Additionally, OTC can undergo desorption after being adsorbed by biochar (Duan et al. 2017), which allows resistance genes to develop in the soil years after biochar application (Rahman et al. 2018). One study showed that using water as a desorption agent could achieve a 21.54% desorption rate after antibiotics were adsorbed (Zhi et al. 2020). Another study revealed that biochar promotes the desorption capacity of antibiotics, with the degree of desorption positively correlated with the amount of biochar used (Xuan et al. 2017). Furthermore, aging of biochar increases the carboxyl group (–COOH) on its surface, weakening the π–π EDA interaction between biochar and pollutants (Huff and Lee 2016). Aging also reduces biochar's surface pores, decreasing its adsorption capacity for sulfonamide antibiotics (Zhou et al. 2018). Lastly, agricultural irrigation can reduce the adsorption performance of biochar due to ion exchange and hydrogen bond breakage (Yu et al. 2016). In conclusion, using biochar alone is insufficient to completely degrade OTC. The application of carbohydrates as carbon sources in conjunction with biochar soil improvement can alleviate the aforementioned issues.

Carbohydrate carbon sources (CCS) can provide essential energy and carbon skeletons for microbial proliferation, promoting rapid microbial metabolism and absorption (Zhalnina et al. 2018). Based on the co-metabolism of soil microorganisms, CCS facilitate OTC degradation. In the coexistence of pollutants and organic matter, soil microorganisms utilize one or several organic compounds as carbon sources or energy, thereby achieving the degradation of organic pollutants (Bilal et al. 2019). When agricultural irrigation or biochar aging causes desorbed OTC to accumulate in the topsoil, microorganisms secrete large amounts of oxidoreductases (e.g., laccase, manganese peroxidase) to degrade OTC. Simultaneously, CCS promote the growth of microorganisms, enhancing the secretion of

oxidoreductases (Shao 2019). The application of CCS ensures the efficient degradation of antibiotics like OTC in the soil and alleviates the toxicity of antibiotics on soil hydrolase activities (e.g., urease, alkaline phosphatase, sucrase) (Lang et al. 2019). Common CCS include glucose, sucrose, lactose, fructose, maltose, and starch (Xu et al. 2014). Numerous studies have demonstrated that CCS can effectively promote the degradation of OTC. Fast-acting carbon sources, such as glucose and sucrose, can be rapidly metabolized by soil microorganisms, leading to a short-term but significant stimulation of microbial proliferation and oxidoreductase activity (Nair and Sarma 2021). In contrast, slow-release carbon sources like starch decompose gradually in soil, providing a sustained carbon supply that supports prolonged microbial activity and enzyme secretion (Salimi et al. 2023). Therefore, the degradation efficiency of OTC varies depending on the type of carbon source applied. For example: 1) Using glucose as a substrate for OTC removal resulted in a 90% removal rate after 50 h (Zeng et al. 2018). 2) Sucrose demonstrated better OTC removal efficiency compared to starch (Wang and Sun 2020). 3) The degradation efficiency of sucrose for oxytetracycline was 65.78%, higher than that of glucose (47.73%) and lactose (60.36%) (Zhao et al. 2016). 4) As a co-metabolism substrate for microorganisms, sucrose had a better domestication effect on oxytetracycline-degrading bacteria, thereby enhancing the degradation of complex compounds with benzene ring structures (Khan et al. 2014). Additionally, CCS play a crucial role in soil nutrient cycling and crop growth. Glucose enhances soil enzyme activity(Qin et al. 2023), increases soil microbial biomass (Lang et al. 2019; Li et al. 2021a), improves the water-stable aggregate structure for carbon and nitrogen cycling in soil (Deng et al. 2023), and regulates taproot growth (Yuan et al. 2014). Sucrose and starch also showed similar effects in improving soil conditions (Lu et al. 2016).In summary, biochar effectively improves soil properties and adsorbs OTC but is insufficient to fully degrade OTC. Residual OTC still poses risks to soil and plants. Therefore, this study proposes using a combination of biochar and CCS to improve the soil and accelerate OTC degradation. To quantify the effects of this combination on the OTC degradation mechanism in the soil, soil microbial functional activity (oxidoreductase and hydrolase activity), nutrient accumulation, and plant growth, and to determine optimal soil improvement and planting strategies, three CCS (glucose, sucrose, and starch) were applied with biochar. A control group was established, and each treatment was spiked with 200 mg kg^{-1} of OTC. The five treatments were biochar (BC), biochar-glucose (BCG), biochar-sucrose (BCSU), and biochar-starch (BCST), along with a control group (CK) without biochar or CCS.

2 Materials and methods

2.1 Biochar, OTC, soil, and plant samples

The biochar was produced by Golden Future (Liaoning, China) using maize straw as the raw material and pyrolyzed at 600 °C. The biochar was crushed and sieved to a particle size of around 5 mm. The biochar had a BET surface area of 324 m^2 g^{-1}, a pore volume of 0.11 cm^3 g^{-1}, a pH of 8.86, a total carbon content of 657 g kg^{-1}, a total nitrogen (N) content of 9 g kg^{-1}, an available potassium content of 16 g kg^{-1}, an available phosphorus content of 0.8 g kg^{-1}, and a density of 0.297 g cm^{-3}. Technical grade OTC (purity 98%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). BET surface area and pore volume (micropores) were determined using the *Trustar II* 3020 microanalyzer (Micromeritics Instruments, USA), pH values were measured with a pH electrode (Thermo Orion, 420A Plus, USA), CEC (cation exchange capacity) was determined by the sodium acetate-flame photometric method, and Eh (redox potential) was measured with a redox potentiometer (HM-QX6530, China).

Soil and plant samples were collected from the 0th to the 80th day after lettuce planting (DAP), labeled as DAP0 to DAP80. The collected soil samples were divided into two parts: one part was freeze-dried and sieved through a 0.5 mm mesh for determining residual OTC content, soil enzymatic activities, and microbial biomass, while the other part of the soil samples was air-dried for measuring soil pH and cationic exchange capacity (CEC). The collected lettuce samples were washed with distilled water and divided into two parts: one part was freeze-dried for measuring OTC content in the leaves and roots, while the other part was dried for measuring total nitrogen (TN) content and biomass of leaves and roots (BioL and BioR).

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined following chloroform fumigation (Li et al. 2016). Soil microbial biomass phosphorus (MBP) was measured using the method for inorganic phosphate determination (Brookes et al. 1982; Capek et al. 2024). Urease activities (mg NH_4^+–N g^{-1} 24 h^{-1}) and alkali phosphatase (mg Phenol g^{-1} 24 h^{-1}) were determined using the phenol–sodium hypochlorite colorimetric method and the phenyl phosphate disodium colorimetric method, respectively (Boldt-Burisch and Naeth 2017). Sucrase activity was measured using the 3,5-dinitrosalicylic acid colorimetric method (Chen et al. 2016). Catalase activity was determined using the potassium permanganate method (Cai et al. 2017). Laccase activity was measured using the ABTS method (García-Delgado et al. 2018). Manganese peroxidase activity was determined using the ultraviolet spectrophotometer method (Bilal et al. 2016). Aromatic

dioxygenase activity was measured according to a previous study (Li 2020). Briefly, the activity was defined as the amount of enzyme required to catalyze the production of 1 μmol of muconic acid or 2-hydroxyconic semialdehyde within 1 min.

The determination of OTC was based on a previous study (Srinivasan et al. 2012). Briefly, the extraction process of OTC from soil involved adding 1 g of soil to an EDTA-McIlvaine buffer solution (10 ml, 0.1 mol L^{-1}). The mixture was vortexed for 1 min, followed by ultrasonic extraction for 10 min, and centrifuged at 4500 r min^{-1} for 10 min at 4 °C. The precipitate was extracted twice more, and the supernatant was collected after each extraction. Under vacuum conditions, all soil extracts were passed through an SPE column (activated with 6 ml methanol and 6 ml secondary distilled water) at a flow rate of 1 ml min^{-1}. The column was washed with 6 ml of ultrapure water and vacuum dried for 10 min. The column was then eluted with 6 ml of acetone (containing 20% 0.01 mol L^{-1} methanol oxalate). The collected eluate was dried with nitrogen (N_2) at 40 ℃ and then dissolved in a 0.01 mol L^{-1} oxalic acid solution (oxalic acid: acetonitrile: methanol = 76: 16: 8, v/v). OTC was determined by high-performance liquid chromatography (HPLC, WATER 600) with ultraviolet detection.

The process for extracting OTC from plant samples is as follows: 1 g of lettuce leaf or root sample was mixed with 0.25 g sodium sulfate, 0.05 g sodium acetate, 0.025 g sodium EDTA, 0.0375 g calcium chloride, and 7.5 ml acetonitrile. The mixture was vortexed for 1 min, then subjected to ultrasonic extraction for 10 min, and centrifuged at 4500 r min^{-1} for 10 min at 4 °C. The precipitate was extracted twice more, and the supernatant was collected after each extraction. The lettuce sample was separated with n-hexane to remove lipids, and the supernatant is evaporated to 5 ml in a nitrogen (N_2) environment, then diluted with pure water to 50 ml. The subsequent extraction and analysis process followed the same method as that used for determination of OTC in soil.

2.2 Experimental design

The experiment was conducted in a greenhouse at the Tongzhou Experimental Station of China Agricultural University, located in Beijing, China (latitude: 39°42′07.8″N, longitude: 116°41′48.0″E). The greenhouse temperature ranged from 22 to 25 ℃ during the day and from 15 to 18 ℃ at night. The experimental units consisted of cylindrical plastic columns (diameter = 25 cm, height = 20 cm) with five bottom drainage outlets (diameter = 5 cm). The experimental setup included 20 replicate columns; and each was arranged randomly, and filled with 8 kg of soil. The columns were filled with air-dried loam soil that had been sieved to 2 mm. The loam soil (9.6% clay, 52.6% silt, and 37.8% sand) had a pH of 7.78, soil organic carbon (SOC) of 29.71 g kg^{-1}, soil organic nitrogen (SON) of 2.3, NH_4^+–N of 6.48 mg kg^{-1}, NO_3^-–N of 28.00 mg kg^{-1}, available potassium (A-K) of 38.14 g kg^{-1}, and available phosphorous(A-P) of 1.70 g kg^{-1}. Studies have shown that background concentrations of OTC in typical agricultural soils are around 1 mg kg^{-1}, while in heavily polluted areas they can reach several hundred mg kg^{-1} (Chen et al. 2013). In fields irrigated with domestic wastewater, OTC residues of 119–307 mg kg^{-1} have been observed (Xie et al. 2011). Therefore, an OTC concentration of 200 mg kg^{-1} OTC can represent a heavily polluted scenario for evaluating degradation potential and remediation efficacy under extreme conditions.

To evaluate the effects of adding biochar and carbohydrate carbon sources(CCS) on OTC translocation in soil and lettuce uptake, the following treatments were established: CK, unamended soil (control); BC, soil amended with 5% w/w biochar; BCG, soil amended with 5% w/w biochar and 3% w/w glucose as a carbohydrate carbon source ($C_6H_{12}O_6$); BCSU, soil amended with 5% w/w biochar and 3% w/w sucrose as a carbohydrate carbon source ($C_{12}H_{22}O_{11}$); BCST, soil amended with 5% w/w biochar and 3% w/w soluble starch as a carbohydrate carbon source ($(C_6H_{10}O_5)n$). For the biochar treatments (BC, BCG, BCSU, and BCST), the biochar was mixed with air-dried soil and evenly packed into the columns before planting lettuce. After lettuce planting, each treatment received three applications of 200 mg kg^{-1} OTC, and in the BCG, BCSU, and BCST treatments, CCS were added along with OTC. Deionized water was added to the columns to maintain soil moisture. OTC and carbohydrates carbon sources were added within 10 days. One lettuce seedling (*Lactuca sativa* L. var. ramosa Hort.) was planted in each experimental column. During the experiment, foliar fertilizer was applied regularly according to agronomic requirements to maintain soil moisture at 70% of the water holding capacity.

2.3 Analysis of data and statistical

The biological cumulative factor (BCF) and transfer factor (TF) were used to characterize the ability of OTC to transfer from soil to lettuce roots (or leaves) (Du et al. 2014), with the calculation method as follows:

$$BCF_{L/S} = \frac{C_L}{C_S} \quad (1)$$

$$BCF_{R/S} = \frac{C_R}{C_S} \quad (2)$$

$$TF = \frac{C_L}{C_R} \tag{3}$$

where C_L/C_R is the concentration of OTC in leaves (or roots) (ug kg^{-1}). C_S is the OTC concentration in soil (mg kg^{-1}); $BCF < 1$ indicates that the plant has a low intensity of pollutant absorption; the greater the TF value, the more OTC is transported from the roots to the lettuce leaves.

The calculation method for the soil OTC degradation efficiency (η) is as follows:

$$\eta = \frac{C_0 - C_t}{C_0} * 100\% \tag{4}$$

where C_0 is the initial concentration in the soil OTC (mg kg^{-1}); C_t is the concentration of OTC in the soil at time t (mg kg^{-1}). The first-order kinetic equation was used to fit the soil OTC content in different periods.

$$C_t = C_0 * e^{-kt} \tag{5}$$

$$T_{1/2} = ln2/K \tag{6}$$

where K is the degradation rate constant, and the higher the K, the faster the degradation rate; $T_{1/2}$ is the half-life of OTC in soil (days).

Data analysis was performed using *SPSS*22.0 software, with Analysis of Variance (ANOVA) conducted through the General Linear Model Univariate procedure. Tukey's range test was used to analyze significant differences between treatments ($p < 0.05$). Figures were prepared using Origin Pro 2022.

3 Results

3.1 Effects of biochar treatments on the OTC degradation and translocation in soil

Compared with CK treatment, the OTC accumulation in lettuce leaves under BC, BCG, BCSU, and BCST treatments decreased by 39.24, 46.83, 19.56, and 29.10%, respectively, at DAP80 (Fig. 1A). However, there was no significant difference in OTC accumulation in the leaves under different treatments during the growth period.

Although some OTC in the soil can still degrade without the application of carbon sources, the detection levels of OTC are lower after the application of carbon sources. Therefore, the application of carbon sources can accelerate the degradation efficiency of OTC. Especially, the application of biochar significantly inhibited the enrichment of OTC in lettuce roots. Compared with CK treatment, the accumulation of OTC in lettuce roots under BC, BCG, BCSU, and BCST treatments decreased by 27.94%, 39.49%, 22.04%, and 49.56%, respectively, at DAP80 (Fig. 1B) with the BCST treatment showing the best inhibitory effect. Meanwhile, the accumulation of OTC in the soil under CK, BC, BCG, BCSU, and BCST treatments decreased by 145.63, 153.81, 163.59, 167.38, and 181.30 mg kg^{-1}, respectively (Fig. 1C). The application of carbon sources enhanced the soil's ability to degrade OTC, with degradation efficiency gradually increasing over time. Among the treatments, biochar combined with starch (BCST) had the best effect on soil OTC degradation.

We further measured the bioconcentration and transfer factors of OTC in lettuce, as well as the kinetic parameters of OTC degradation in soil, as shown in Table 1. The results showed that the leaf-soil bioconcentration factor ($BCF_{L/S}$) of biochar treatments was lower than that of the control without biochar application (CK). However, there was no significant difference among the various biochar treatments (BC, BCG, BCSU, and BCST). The root-soil

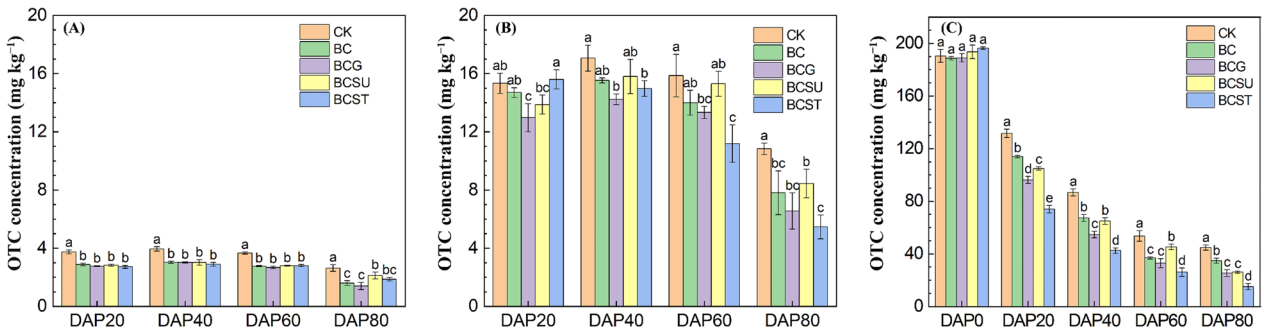


Fig. 1 Effect of biochar treatments on the accumulation of OTC in lettuce leaves, roots and soil at different periods. Effects of biochar treatments on **A** the OTC accumulation in leaves, **B** the OTC accumulation in roots, **C** the OTC accumulation in soil. DAP: from the 0th to the 80th day after lettuce planting. CK: the control treatment any biochar addition. BC: biochar alone. BCG: biochar-glucose. BCSU: biochar-sucrose, BCST: biochar-starch. Data are means ± standard deviation (n = 3), with different letters indicating significant ($P < 0.05$) differences between treatments

Table 1 The $BCF_{L/S}$, $BCF_{R/S}$, TF, and kinetic parameters of OTC translocation and degradation

Treatment	$BCF_{L/S}$	$BCF_{R/S}$	TF	C_0 (mg kg^{-1})	K (d^{-1})	R^2	$t_{1/2}$ (d^{-1})
CK	0.018	0.074	0.243	190.44	0.019	0.996	36.48
BC	0.010	0.051	0.217	188.63	0.025	0.993	27.73
BCG	0.009	0.040	0.217	188.97	0.030	0.993	23.10
BCSU	0.013	0.051	0.254	193.53	0.027	0.993	25.67
BCST	0.010	0.030	0.352	196.37	0.041	0.985	16.91

$BCF_{L/S}$, *$BCF_{R/S}$* the OTC transfer from the soil to the roots (or leaves) of lettuce, *TF* the ability of OTC transfer from the roots to the leaves, *C_0* the initial OTC concentration, *K* the degradation rate constant, *$t_{1/2}$* the half-life of OTC in soil

Table 2 Dynamic changes of soil pH and CEC under different biochar treatments

	Treatment	DAP0	DAP20	DAP40	DAP60	DAP80
pH	CK	7.81b ± 0.14	7.75b ± 0.19	7.69b ± 0.17	7.77a ± 0.15	7.74a ± 0.11
	BC	8.36a ± 0.07	8.24a ± 0.10	8.13a ± 0.09	8.03a ± 0.13	8.01a ± 0.11
	BCG	8.20a ± 0.01	8.26a ± 0.07	8.16a ± 0.08	7.99a ± 0.13	7.91a ± 0.17
	BCSU	8.21a ± 0.03	8.23a ± 0.10	8.14a ± 0.11	8.04a ± 0.13	7.96a ± 0.08
	BCST	8.21a ± 0.06	8.03a ± 0.11	7.89ab ± 0.12	8.00a ± 0.09	7.91a ± 0.02
CEC (cmol kg^{-1})	CK	16.03b ± 0.92	16.55b ± 0.79	15.43b ± 0.66	13.87b ± 0.55	13.82b ± 1.22
	BC	33.72a ± 1.55	32.63a ± 2.24	31.32a ± 2.10	30.29a ± 1.36	29.70a ± 1.62
	BCG	32.13a ± 1.53	29.56a ± 1.02	28.34a ± 1.40	30.71a ± 0.55	29.41a ± 1.03
	BCSU	32.66a ± 0.97	29.52a ± 1.00	28.08a ± 1.40	30.72a ± 0.59	29.19a ± 0.63
	BCST	34.13a ± 0.73	29.56a ± 1.01	27.87a ± 1.30	30.69a ± 0.56	29.18a ± 0.73

Values in the same column with different letters are significantly different at the 0.05 level, while values with the same letter are not significantly different at the 0.05 level. ± represents the standard deviation. The letter labels "a" and "b" indicate significant differences

bioconcentration factor ($BCF_{R/S}$) of the BCST treatment was significantly lower than that of the BC, BCG, and BCSU treatments. This indicates that the primary factor inhibiting OTC accumulation in lettuce leaves was biochar, rather than the carbohydrate carbon source.

The kinetic parameters ($R^2 > 0.98$) indicated that the degradation of OTC in each treatment followed a quasi-first-order reaction kinetic equation (Table 1). The degradation rates of the treatments were BCST > BCG > BCSU > BC > CK. The application of biochar shortened the half-life of OTC by nearly 10 days. The application of the carbohydrate carbon source accelerated the OTC degradation rate, reducing the half-life of soil OTC by approximately 2.06–10.82 days.

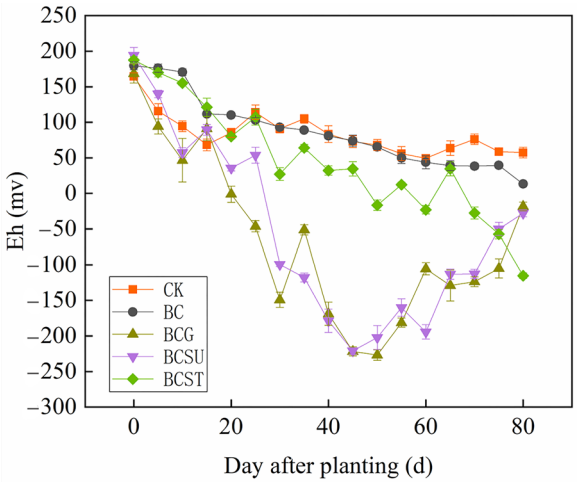


Fig. 2 Effect of biochar treatments on the dynamic changes of soil Eh. Eh: The soil redox potential. CK: the control treatment any biochar addition. BC: biochar alone. BCG: biochar-glucose. BCSU: biochar-sucrose, BCST: biochar-starch

3.2 Changes in soil Eh, pH and CEC

The soil redox potential (Eh) in CK and BC treatments decreased from 164.4 to 57.4 mv and from 179.4 to 13.3 mv, respectively, maintaining an oxidative state throughout the entire growth period of lettuce (Table 2). From DAP0 to DAP20, the soil in BCG and BCSU treatments exhibited an oxidative state, but it shifted to a strongly reductive state from DAP20 to DAP80. The Eh value in the BCST treatment was similar to that in the BC treatment, decreasing from 187.6 mv at DAP0 to −115.2 mv at DAP80 (Fig. 2).

During the period from DAP0 to DAP80, the soil pH values in BC, BCG, BCSU, and BCST treatments were

higher than those in CK treatment, but the differences among the biochar treatments (BC, BCG, BCSU, and BCST) were not obvious. Similar results were observed for soil cation exchange capacity (CEC): from DAP0 to DAP80, compared with CK treatment, the CEC of BC, BCG, BCSU, and BCST treatments increased by 100–113%, with no significant differences in CEC among the biochar treatments. Therefore, carbohydrate carbon sources (CCS) did not affect soil pH and CEC.

3.3 Soil hydrolase activity

At the early growth stage of lettuce (DAP20), the urease activity in CK, BC, and BCST treatments was relatively low, but it gradually increased over time. In contrast, the urease activity in BCG and BCSU treatments showed the opposite trend, gradually decreasing over time. Moreover, at DAP80, the soil urease activity in the BCST treatment was significantly higher than that in other treatments by approximately 54.89–83.27%; at DAP20, the alkaline phosphatase and sucrase activities in BC and BCST treatments showed a similar trend to urease activity: initially at a low level, and then gradually increasing. BCG and BCSU significantly promoted soil alkaline phosphatase and sucrase activities in the early stage, but as glucose and sucrose were consumed, the enzyme activities gradually decreased; the catalase activity in CK and BC treatments was significantly higher than in other treatments. As the lettuce grew, the differences in enzyme activity among the treatments gradually diminished. The soil catalase activity in BCG, BCSU, and BCST treatments was 23.49%, 25.86%, and 17.78% lower than that in BC treatment, respectively (Fig. 3).

Although OTC biotoxicity gradually weakened over time, the application of biochar and CCS still alleviated OTC biotoxicity. Among them, biochar combined with glucose and sucrose (BCG, BCSU) most significantly promoted the activity of hydrolase.

3.4 Soil oxidoreductase activity

Oxidoreductases can catalyze the oxidative cleavage of OTC's tetracyclic backbone, generating a series of phenolic and quinone intermediates that are subsequently

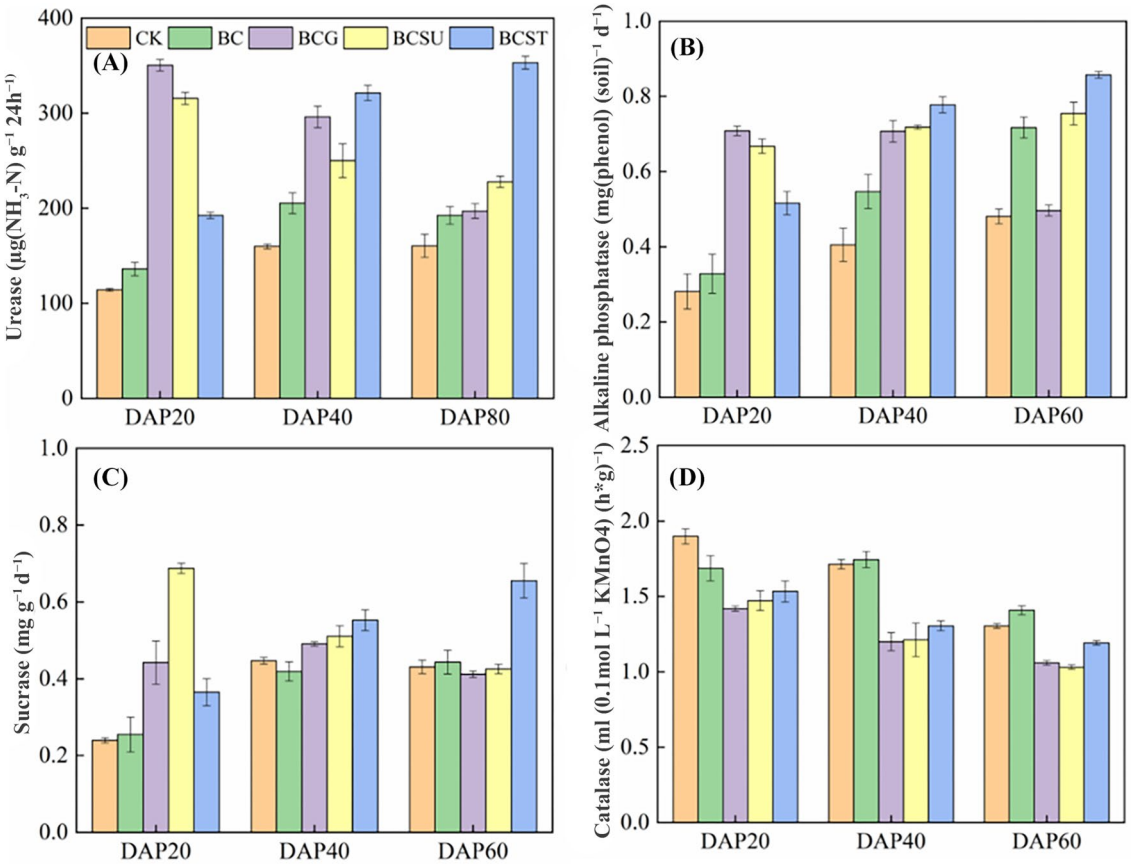


Fig. 3 Dynamic changes of soil hydrolase activities at different periods. Dynamic changes of soil hydrolase: **A** urease, **B** alkaline phosphatase, **C** sucrase, **D** catalase. DAP: from the 0th to the 80th day after lettuce planting. CK: the control treatment any biochar addition. BC: biochar alone. BCG: biochar-glucose. BCSU: biochar-sucrose, BCST: biochar-starch

mineralized by soil microorganisms, thereby enhancing antibiotic degradation efficiency (Xu et al. 2024). Moreover, the activities of soil oxidoreductases—laccase, aromatic dioxygenase, and manganese peroxidase—are strongly positively correlated with OTC removal rates (Ma et al. 2016). Therefore, the extent of OTC degradation can be inferred from the changes in oxidoreductase activity during the lettuce growth period from DAP10 to DAP50. The soil laccase activity in CK treatment fluctuated between 5.98 and 7.66 U g^{-1}. In BC, BCG, and BCSU treatments, the laccase activity was 6.55–9.05 U g^{-1} at DAP10 and 9.10–10.89 U g^{-1} at DAP40, indicating that their laccase activity first increased and then decreased over time. The activity peaks occurred between DAP30 and DAP40, reaching 9.75, 12.69, and 14.36 U g^{-1}, respectively. In BCST treatment, the soil laccase activity was 7.75 U g^{-1} at DAP10 and reached a peak of 11.48 U g^{-1} at DAP50, indicating a gradual increase in laccase activity over time; the aromatic dioxygenase activity in CK treatment was 2.87–3.72 U g^{-1}, and manganese peroxidase activity was 3.95–5.43 U g^{-1}, which were significantly lower than those in other treatments. BC treatment promoted the activities of aromatic dioxygenase and manganese peroxidase, with better results than CK treatment but not as effective as the biochar and carbohydrate carbon source treatments. In BCG treatment, aromatic dioxygenase activity was 6.34–11.69 U g^{-1}, and manganese peroxidase activity was 6.49–8.46 U g^{-1}, while in BCSU treatment, aromatic dioxygenase activity was 6.99–9.26 U g^{-1}, and manganese peroxidase activity was 6.86–7.24 U g^{-1}. Their activity patterns were similar to laccase, showing an initial increase followed by a decrease, with peaks occurring between DAP30 and DAP40. In BCST treatment, aromatic dioxygenase activity gradually increased from 6.06 to 10.89 U g^{-1}, and manganese peroxidase activity increased from 6.77 to 10.20 U g^{-1} (Fig. 4).

Among the three biochar treatments, although the activities of the three oxidoreductases decreased in the later stages after the application of glucose (BCG) and sucrose (BCSU), the overall promotion of oxidoreductase activity was better than in the treatment with starch (BCST).

3.5 Soil microbial biomass

In CK, BC, and BCST treatments, soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP) increased over time. From DAP20 to DAP60, MBC increased by 30.54%, 19.33%, and 54.88%, respectively; MBN increased by 118, 51.97, and 84.16%, respectively; and MBP increased by 95.95%, 69.81%, and 72.49%, respectively (Table 3). In BCG and BCSU treatments, soil MBC, MBN, and MBP decreased over time. At DAP60, the MBC, MBN, and MBP in BCST treatment were significantly higher than those in other treatments, while there was no significant difference in MBN and MBP among BC, BCG, and BCSU treatments.

The type of carbon source applied affected the accumulation of soil microbial biomass. Biochar promoted the increase of soil microbial biomass, while glucose and sucrose were quickly metabolized and utilized by microorganisms, thereby promoting the immobilization of soil C, N, and P in a short time. However, as glucose (BCG) and sucrose (BCSU) were rapidly consumed, microbial biomass decreased, indicating that glucose and sucrose could not continuously increase soil microbial biomass. In contrast, the effect of starch (BCST) on microbial biomass was opposite to that of glucose and sucrose, showing a continuous promotion of microbial biomass C, N, and P.

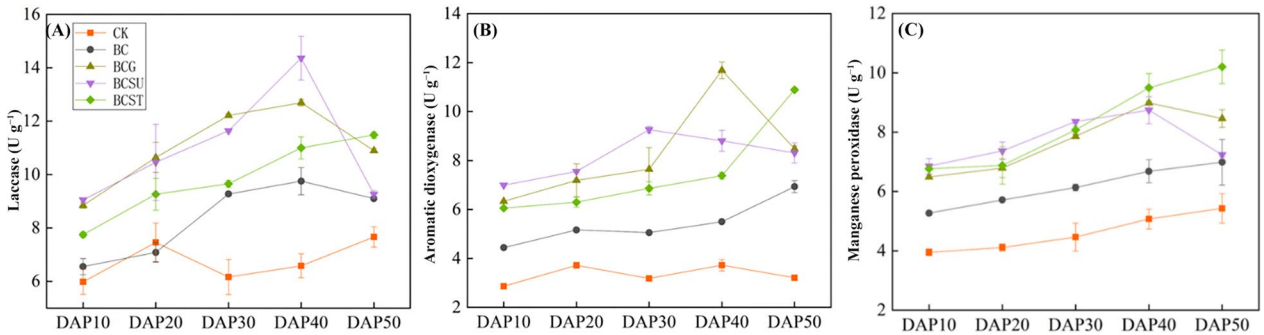


Fig. 4 Dynamic changes of soil oxidoreductase activities at different periods. Dynamic changes of soil oxidoreductase: **A** laccase, **B** aromatic dioxygenase, **C** manganese peroxidase. DAP: from the 0th to the 80th day after lettuce planting. CK: the control treatment any biochar addition. BC: biochar alone. BCG: biochar-glucose. BCSU: biochar-sucrose, BCST: biochar-starch

Table 3 Dynamic changes of microbial biomass under different biochar treatments

	MBC (mg kg^{-1})		
	DAP20	**DAP40**	**DAP60**
CK	63.83c ± 2.64	70.82c ± 2.42	83.33c ± 1.73
BC	81.40b ± 1.85	94.37b ± 3.78	97.13b ± 3.31
BCG	126.98a ± 1.74	97.37b ± 1.12	89.61c ± 1.49
BCSU	122.11a ± 4.33	108.85a ± 2.56	90.06c ± 1.77
BCST	84.49b ± 2.43	95.39b ± 2.18	130.86a ± 2.88
	MBN (mg kg^{-1})		
	DAP20	**DAP40**	**DAP60**
CK	13.97c ± 1.43	20.87c ± 1.51	30.47c ± 0.20
BC	21.55b ± 3.17	21.42c ± 0.52	32.75b ± 1.63
BCG	53.82a ± 1.42	50.47a ± 1.45	34.74b ± 1.53
BCSU	51.29a ± 1.00	35.43b ± 1.51	24.05b ± 2.54
BCST	25.75b ± 1.75	33.30b ± 0.89	47.43a ± 2.22
	MBP (mg kg^{-1})		
	DAP20	**DAP40**	**DAP60**
CK	6.17d ± 0.74	7.71b ± 0 .88	12.08b ± 1.60
BC	8.92d ± 0.64	11.36b ± 1.28	15.15b ± 1.17
BCG	29.31a ± 2.74	22.35a ± 2.13	16.71b ± 0.97
BCSU	24.66b ± 1.04	19.91a ± 1.54	12.79b ± 2.62
BCST	16.44c ± 1.91	22.06a ± 0.57	28.35a ± 1.22

MBC Soil microbial biomass carbon. MBN: microbial biomass nitrogen. *MBP* Soil microbial biomass phosphorus. ± represents the standard deviation. The letter labels "a" and "b" indicate significant differences

3.6 Lettuce growth index

The application of biochar for soil improvement can significantly increase lettuce leaf Soil and Plant Analyzer Development (SPAD) values. Compared with CK treatment, BC, BCG, BCSU, and BCST treatments increased SPAD by 5.24–40.65% at DAP80, and leaf total nitrogen (TN) increased by 17.39%, 18.12%, 30.58%, and 36.67%, respectively (Table 4). However, there was no significant difference in leaf SPAD and TN among the four biochar treatments.

Similar to the changes in leaf SPAD and TN, the biomass accumulation of lettuce in the BC treatment was significantly higher than in the CK treatment at DAP80, with leaf biomass increasing by approximately 26.29% and root biomass by 122.06%. However, there was no significant difference in leaf or root biomass between

Table 4 Dynamic changes of SPAD and total nitrogen in leaves

		DAP0	DAP20	DAP40	DAP60	DAP80
SPAD	CK	16.85c ± 2.87	28.775c ± 2.41	31.55b ± 2.95	34.05b ± 3.17	33.95c ± 1.98
	BC	22.83b ± 1.95	30.45bc ± 2.72	36.48ab ± 1.35	37.25b ± 1.84	35.73c ± 2.53
	BCG	30.70a ± 2.10	37.58a ± 2.25	39.85ab ± 6.64	44.35a ± 2.11	42.4b ± 2.49
	BCSU	31.65a ± 2.88	35.73ab ± 3.60	44.38a ± 2.35	45.58a ± 2.55	45.05ab ± 2.93
	BCST	28.18a ± 3.83	33.05abc ± 2.78	43.38a ± 5.08	49.45a ± 2.38	47.75a ± 2.16
TN (mg kg^{-1})	CK	12.28b ± 1.61	13.92b ± 1.32	14.73b ± 0.58	16.83b ± 1.63	17.25b ± 1.32
	BC	13.65b ± 0.79	15.21b ± 1.88	16.18ab ± 2.65	19.01ab ± 1.18	20.25ab ± 0.71
	BCG	13.13b ± 1.78	16.44ab ± 2.05	19.00ab ± 1.68	19.70ab ± 1.57	20.38ab ± 1.60
	BCSU	17.28a ± 1.13	18.87ab ± 3.28	21.25a ± 3.43	20.93ab ± 2.24	22.53a ± 3.69
	BCST	19.20a ± 1.07	20.90a ± 2.05	21.73a ± 2.14	23.03a ± 1.97	23.58a ± 0.74

SPAD Soil and Plant Analysis Development, *TN* Total Nitrogen. ± represents the standard deviation. The letter labels "a", "b" and "c" indicate significant differences

BC and the three treatments, BCG, BCSU, and BCST. Compared to BC treatment, the leaf biomass in BCG, BCSU, and BCST treatments increased by approximately 3.63%, 0.3%, and 2.10%, respectively, and root biomass increased by 10.48%, 4.58%, and 15.94%, respectively (Fig. 5). This indicates that biochar soil improvement measures promoted chlorophyll synthesis and nitrogen accumulation in leaves, and the combined application of biochar and CCS further enhanced the effect.

The OTC content in lettuce leaves (L_{OTC}) and the OTC content in lettuce roots (R_{OTC}) were significantly positively correlated with the OTC content in the soil (S_{OTC}), indicating that the OTC in lettuce plants was significantly affected by soil pollution (Fig. 6). The more severe the soil pollution, the more OTC accumulated in lettuce tissues.

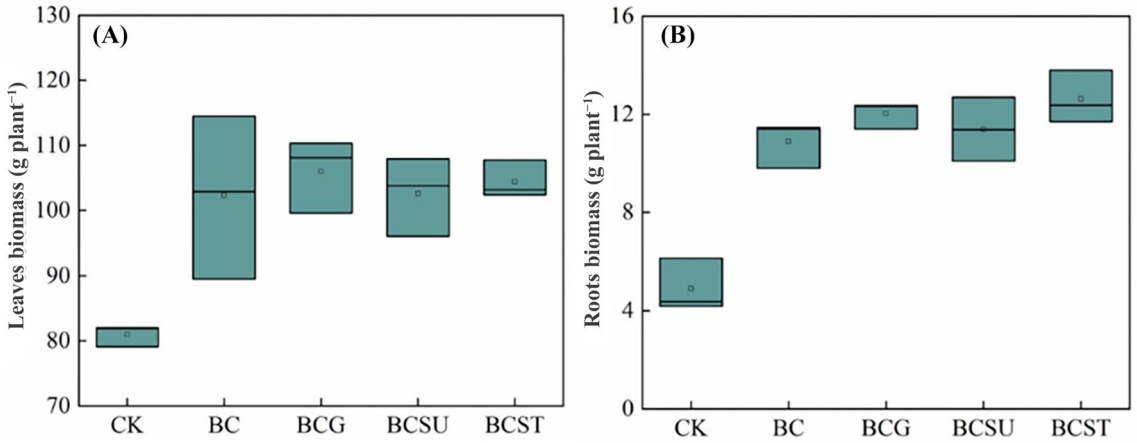


Fig. 5 The biomass accumulation different biochar treatments on the 80th day after lettuce planting. The biomass accumulation in **A** leaves, **B** roots. CK: the control treatment any biochar addition. BC: biochar alone. BCG: biochar-glucose. BCSU: biochar-sucrose, BCST: biochar-starch

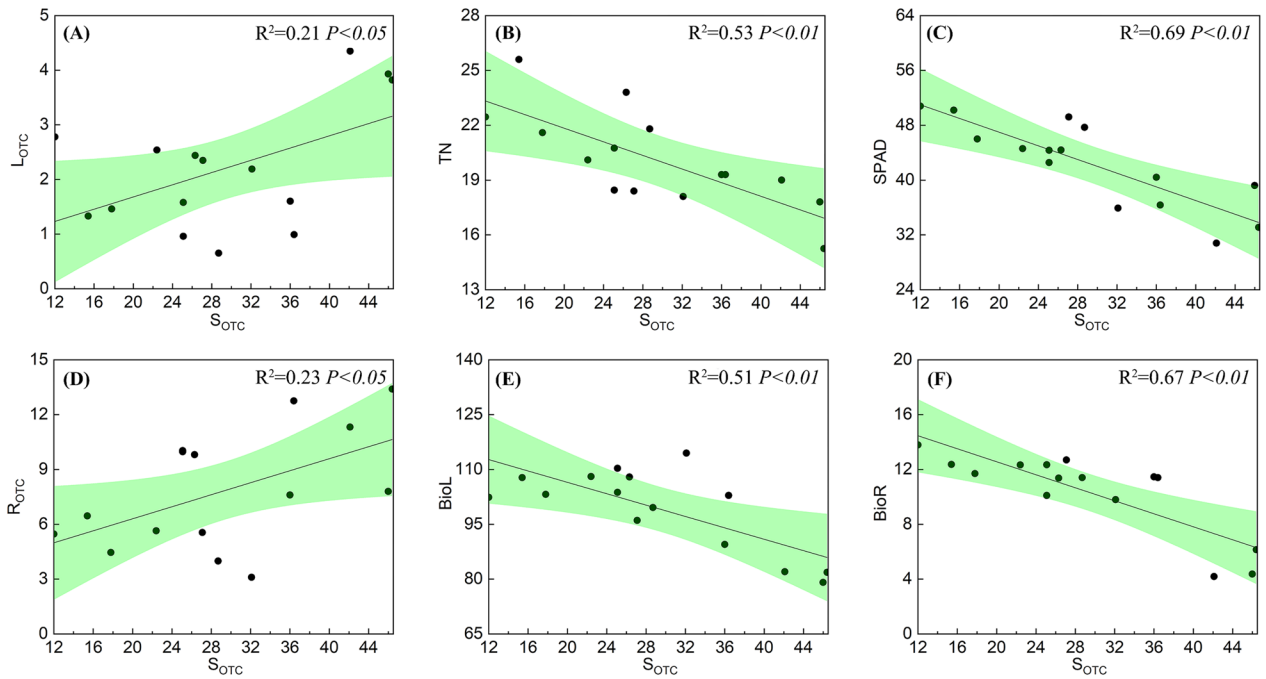


Fig. 6 The correlation between soil OTC and lettuce growth within the lettuce-soil system. The correlation between soil OTC concentration and **A** L_{OTC}, **B** R_{OTC}, **C** SAPD, **D** TN, **E** BioL, **F** BioR. L_{OTC}: the OTC content in lettuce leaves. R_{OTC}: the OTC content in lettuce roots. S_{OTC}: the OTC content in the soil. BioL: the biomass of leaves. BioR: the biomass of roots

SOTC was negatively correlated with SPAD, TN, biomass of leaves (BioL) and biomass of roots (BioR), indicating that OTC in contaminated soil indeed inhibited biomass accumulation and chlorophyll synthesis in lettuce.

4 Discussion

4.1 Effect of biochar on OTC degradation and translocation

The biochar treatments effectively inhibited the translocation of soil OTC to lettuce leaves and its accumulation in the roots (Fig. 1). Table 1 shows that treatments with biochar had lower $BCF_{L/S}$ and $BCF_{R/S}$ compared to CK treatment, which supported the above phenomenon. Many studies have reported similar results (Han et al. 2019; Pan 2020). Other research has shown that the inhibitory factors of biochar on the translocation of soil OTC can be achieved through adsorption mechanisms, such as electrostatic force, π–π electron transfer, and hydrogen bonding (Luo et al. 2018). After carbonization, biochar becomes highly aromatic and contains a large number of π-electron acceptors, which can form π-π bonds with the π-electron donors in OTC (Ji et al. 2009). The biochar surface also has many functional groups, such as carboxyl (–COOH) and hydroxyl (–OH) groups, allowing OTC to form hydrogen bonds with the hydroxyl and carboxyl groups on biochar (Zhang et al. 2012). During the period from DAP0 to DAP80, the soil OTC content in biochar-treated plots was significantly lower than in the CK treatment ($P<0.05$), indicating that biochar did not increase the accumulation of OTC in the soil (Fig. 1C). This phenomenon is consistent with the principle of OTC adsorption on biochar: the stronger the adsorption capacity of biochar, the less residual OTC remains in the soil (Xuan et al. 2017). Biochar increases the adsorption sites in the soil (Luo et al. 2018), and π–π electron transfer prevents instability in adsorption (Schreiter et al. 2018). The ecological benefit of biochar's inhibition of OTC translocation is the reduction of OTC toxicity and the promotion of lettuce growth. Figure 5 shows that biochar improved the soil and significantly promoted the biomass accumulation of lettuce leaves and roots. However (Table 4), biochar did not have a significant effect on leaf SPAD values or total nitrogen content, indicating that biochar did not promote the effective utilization of soil nutrients.

Many studies have shown that the application of biochar can promote the efficient degradation of soil OTC, mainly due to the following reasons: 1) After biochar application, soil moisture content increases, which promotes OTC hydrolysis. The water-holding capacity of the soil is significantly enhanced after biochar amendment, which is believed to accelerate the hydrolysis of residual organic pollutants (Xuan et al. 2010; Zhang et al. 2013). 2) The application of biochar increases soil pH. A higher soil pH may accelerate the hydrolysis of antibiotics, which may also contribute to OTC degradation, as carbon rings are more likely to break and form inactive lactone isomers under alkaline conditions (Li et al. 2024b). One study has shown that when the pH ranges from 3.09 to 10.54, the hydrolysis rate of OTC is highest under neutral conditions, with higher rates observed under alkaline conditions compared to acidic ones (Xuan et al. 2010). In this study, the soil pH in the biochar treatments ranged from 8.01 to 8.36. Based on linear interpolation, the estimated OTC hydrolysis rate under these conditions was approximately 49.2–54.8%. Therefore, the neutral to alkaline soil environment in the experiment likely promoted partial hydrolysis of OTC. 3) Biochar application enhances oxidoreductase activity (Table 2). In the short term, there is a significant negative correlation between enzyme activity and OTC supplementation (Yao et al. 2010). The promotion of oxidoreductase activity by biochar is due to its influence on soil microbial activity and soil conditions.

OTC reduces the activity of oxidoreductases in the soil, such as laccase, aromatic dioxygenase, and manganese peroxidase (Yao et al. 2010). However, the BC treatment with biochar significantly alleviated the inhibitory effect of OTC on these enzyme activities (Fig. 3). The application of biochar also mitigated the ecological toxicity of OTC, creating more space for microbial growth and improving soil conditions (such as moisture, aeration, and organic matter content), thereby stimulating the secretion of oxidoreductases and promoting the degradation of OTC in the soil (Du et al. 2014). This phenomenon was verified by the positive correlation between soil hydrolase activity, microbial biomass, and oxidoreductase activity (Fig. 7). Additionally, the soil hydrolase activity and microbial biomass in biochar treatments were significantly higher than in CK treatments (Table 3 and Fig. 3), indicating that enhanced microbial activity leads to stronger OTC degradation in the soil, which is consistent with other research findings (Bailey et al. 2011; Wang et al. 2015). Furthermore, the application of biochar also promotes the immobilization of laccase, improving its acid–base stability. Biochar binds with laccase through intermolecular forces (such as van der Waals forces, hydrogen bonds, and electrostatic attraction) to form immobilized laccase, which has better tolerance to acidic and alkaline environments than free laccase (García-Delgado et al. 2018).

This study demonstrated that biochar enhances OTC degradation and inhibits its translocation through a synergistic biochar–carbohydrate strategy, which integrates microbial, physicochemical, and agronomic mechanisms. The combined treatment effectively promoted OTC

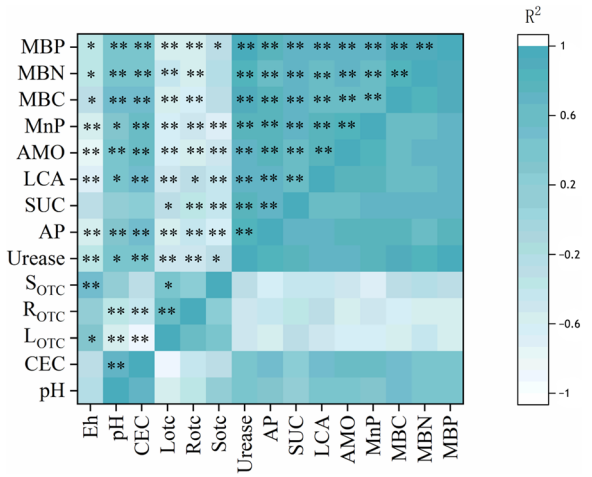


Fig. 7 The correlation in biochemical reactions within the lettuce-soil system. Eh: The soil redox potential. pH: potential of Hydrogen. CEC: cationic exchange capacity. L_{OTC}: the OTC content in lettuce leaves. R_{OTC}: the OTC content in lettuce roots. S_{OTC}: the OTC content in the soil. MBP: microbial biomass phosphorus. MBN: microbial biomass nitrogen. MBC: microbial biomass carbon. MBP: manganese peroxidase. AMO: ammonia monooxygenase. LCA: laccase. SUC: sucrase. AP: alkaline phosphatase

mineralization while significantly reducing its accumulation in both plant roots and leaves. Leveraging the oxidative stress-alleviating capacity of biochar, local microbial communities were continuously activated over an 80-day period. Among all treatments, the BCST treatment showed the most pronounced effects, elevating microbial biomass nitrogen (MBN) by 84.16% and synchronizing with plant nitrogen remobilization, as reflected by a 36.67% increase in leaf total nitrogen. The carbonyl groups on the biochar surface formed charge-transfer complexes with the phenolic rings of OTC, while aged biochar developed nanopore occlusion structures that impeded OTC leaching. Compared with photocatalytic radical-based degradation approaches, BCST exhibits superior long-term preservation of soil functionality. Furthermore, BCST indirectly enhances OTC degradation by reshaping microbial community composition. Studies have shown that this strategy alters the fungal-to-bacterial ratio, suppressing the abundance of pathogenic fungi such as *Fusarium*, while enriching saprotrophic Ascomycota and OTC-mineralizing Actinobacteria. This restructuring of microbial co-occurrence networks improves soil C/N cycling and contributes to greater ecosystem stability (Tao et al. 2025; Zheng et al. 2025).

4.2 Effect of CCS on OTC degradation

The treatments with different carbohydrate carbon sources (CCS) showed significant differences in soil OTC degradation. The OTC removal capacity of biochar-starch (BCST) (92.32%) was superior to that of biochar-glucose (BCG) (86.58%) and biochar-sucrose (BCSU) (86.47%) (Fig. 1C). Studies have shown that microbial degradation of antibiotics mainly relies on non-specific oxidoreductases, such as laccase and manganese peroxidase (Ni et al. 2020). However, compared to BCG and BCSU, the higher OTC removal rate in BCST is based on lower key enzyme activity. During the DAP0 to DAP40 period of lettuce growth, the oxidoreductase activity in BCG and BCSU was significantly higher than that in BCST (Fig. 4). Additionally, the soil Eh value reflects the intensity of redox reactions in the soil. A high-intensity oxidation reaction can rapidly consume oxygen in the soil, leading to strong reducing properties (Cai et al. 2017). Under strong oxidation conditions, the soil Eh value was typically negatively correlated with oxidoreductase activity (Fig. 7). During DAP20 to DAP80, the Eh values in BCG and BCSU were below 0 mv, with the lowest reaching −220 mv, indicating that a vigorous oxidation reaction occurred in the soil after the application of glucose and sucrose. Therefore, although glucose and sucrose promote the secretion of laccase, manganese peroxidase, and aromatic dioxygenase, they do not achieve efficient OTC degradation. The microbial biomass and hydrolase activity in BCG and BCSU were significantly higher than in BCST during DAP0 to DAP40, indicating that glucose and sucrose were not entirely used for OTC metabolism (Table 3 and Fig. 3).

Based on the above analysis, the reasons for the lower OTC degradation efficiency in BCG and BCSU compared to BCST are as follows: 1) Glucose and sucrose are more easily absorbed by microorganisms directly rather than being degraded through co-metabolism. As low-molecular-weight carbon sources, glucose and sucrose are considered the most suitable for microbial absorption and utilization (Tao et al. 2023). Glucose and sucrose not only provide energy for microbial reproduction but also serve as carbon skeletons (Wang and Sun 2020). Starch, as a polysaccharide, has a more complex molecular structure compared to the monosaccharide structure of glucose and the disaccharide structure of sucrose, making it more difficult for microorganisms to metabolize (Tagami 2024). The glucose and sucrose promoted microbial biomass accumulation more effectively than starch (Table 3). And related studies have reached similar conclusions, showing that glucose is best suited for microbial proliferation (Raiesi 2021; Zhalnina et al. 2018). 2) In co-metabolism, OTC competes less for oxidoreductases compared to glucose and sucrose, leading microorganisms to preferentially degrade glucose and sucrose rather than OTC. At the same time, glucose and sucrose stimulate the secretion of oxidoreductases by functional bacteria during co-metabolism, but these oxidoreductases are more

inclined to break down glucose and sucrose rather than organic pollutants (Soja 2016). Some studies have shown that as co-metabolic substrates for microorganisms, glucose and sucrose positively affect the degradation of complex compounds with benzene ring structures (Khan et al. 2014; Wang et al. 2020). 3) The long-term, sustained degradation of OTC in soil is jointly influenced by biochar aging and the degradability of CCS. Over time, the accumulation of humic substances in the nanopores of biochar can gradually block its adsorption sites, resulting in decreased OTC sorption efficiency (Rahim et al. 2024). To address this, we propose the combined application of biochar with a slow-release carbon source, specifically starch, to ensure efficient and stable OTC degradation even during biochar aging. Owing to its slower decomposition rate, starch can continuously supply carbon to soil microorganisms and sustain oxidoreductase activity. This approach effectively compensates for the reduced adsorption capacity of aged biochar and minimizes soil organic carbon (SOC) loss. In contrast, although glucose and sucrose can rapidly stimulate enzyme activity in the short term, they are more likely to trigger dissolved organic carbon (DOC) leaching and SOC priming effects, potentially reducing their long-term remediation effectiveness. In summary, although glucose and sucrose can transiently stimulate oxidoreductase activity, their rapid consumption leads to carbon source depletion, resulting in microbial metabolic stagnation and limiting the extent of OTC degradation; by contrast, the slow-release hydrolysis of starch can continuously supply carbon to microorganisms, sustaining and enhancing oxidoreductase activity, thereby enabling sustained OTC degradation. The difference in OTC degradation efficiency with CCS is related to the type of organic pollutant, and the choice of carbon source type directly determines the co-metabolic efficiency of organic pollutants. Therefore, compared to BCST, BCG and BCSU did not achieve higher OTC degradation efficiency under conditions of high oxidoreductase activity (Fig. 4), indicating that the application of biochar and starch (BCST) is the most effective for OTC degradation.

In addition, biochar mitigates the competition between carbon sources and OTC for enzyme activity through the following mechanisms: (1) Electron shuttling: the surface carbonyl groups (C=O) on biochar facilitate electron transfer from carbohydrates to OTC, generating hydroxyl radicals (·OH) that enhance non-enzymatic degradation (Zhang et al. 2024); (2) Enzyme shielding: the aromatic domains of biochar adsorb proteases, reducing their degradation of laccase and preserving enzyme activity during microbial turnover (He et al. 2024). Therefore, monosaccharides should be avoided in OTC-contaminated soils, as their substrate-level inhibition undermines long-term degradation efficiency. In contrast, the starch–biochar synergy aligns the slow release of carbon with microbial growth cycles, maximizing the synchrony between urease activity and OTC degradation.

4.3 Effect of microbial community on OTC degradation

The differential accumulation patterns of microbial biomass (MBC, MBN, MBP) across treatments reflect not only variations in carbon source availability but also functional specialization of microbial communities in OTC detoxification. In the BCST treatment, microbial biomass increased steadily, with MBC rising by 54.88%, MBN by 84.16%, and MBP by 72.49%, which was closely associated with the dominance of specialized OTC-degrading Actinobacteria (e.g., Streptomycetaceae) (van der Heul et al. 2018). These microbes utilize direct metabolic pathways, including tetracycline oxidoreductases and cytochrome P450 enzyme systems, to cleave OTC's aromatic ring structures, resulting in a high soil OTC degradation (−181.30 mg kg^{-1}) and a relatively fast degradation rate (kinetic rate, $k = 0.041\ d^{-1}$). The enzymatic pathways involved are stabilized by starch's slow-release carbon supply, enhancing consistent expression of laccase and monooxygenase genes critical to OTC decomposition. Conversely, transient spikes in microbial biomass observed in BCG and BCSU treatments initially stimulate opportunistic r-strategists (e.g., Pseudomonadaceae) (Kukurugya et al. 2019). These microbes primarily assimilate labile carbon substrates, co-metabolizing OTC as a secondary substrate. Although this results in rapid initial OTC degradation (BCG: $k = 0.030\ d^{-1}$, BCSU: $k = 0.027\ d^{-1}$), the rapid depletion of glucose or sucrose leads to carbon starvation, causing microbial biomass and enzyme activities to collapse. Consequently, root OTC inhibition was 22% lower compared to BCST. Notably, the BC treatment alone enriches oligotrophic extracellular polymeric substance (EPS)-producing microbes, enhancing OTC adsorption and reducing its accumulation in roots; However, their capacity for biological degradation is limited, with soil OTC reduced by only 153.81 mg kg^{-1} (Tables 1, 3 and Fig. 1).

Glucose and sucrose rapidly deplete dissolved oxygen through accelerated microbial respiration, reducing soil redox potential to Eh < −150 mV. This hypoxic condition inhibits obligate aerobic Actinobacteria (e.g., *Streptomyces* spp.), whose cytochrome P450 systems require molecular oxygen to cleave the aromatic rings of OTC. Previous studies have shown that under low-oxygen conditions, P450 gene expression in *Streptomyces* can be downregulated by up to 78%. Meanwhile, these labile carbon sources enrich facultative anaerobic Proteobacteria (r-strategists), which preferentially express hydrolytic enzymes rather than xenobiotic-detoxifying

oxidoreductases (Mao et al. 2023). These microbes exhibit low affinity for OTC, diverting over 70% of their catalytic activity toward carbohydrate metabolism, which partially explains the limited degradation efficiency observed in this study. In contrast, the slow-release nature of starch maintains a redox potential of Eh >+50 mV, creating a favorable environment for K-strategist Actinobacteria, whose enzymatic systems are highly optimized for tetracycline degradation. Notably, the electron-shuttling capacity of biochar is redox-sensitive: it functions effectively under the aerobic conditions of BCST treatments but is suppressed under the low-Eh environments of BCG and BCSU, forcing the system into inefficient reductive pathways that accumulate persistent epi-OTC isomers (Li et al. 2024a). Therefore, the starch–biochar synergy enables sustained OTC mineralization by simultaneously promoting Actinobacteria enrichment, maintaining redox homeostasis, and enhancing enzymatic degradation.

4.4 Effect of BCST on plant health and its applications in agricultural production

Previous studies have demonstrated that the accumulation of soil OTC induces dual phytotoxic effects: (1) inhibition of chlorophyll biosynthesis through suppression of Mg-chelatase activity, resulting in reduced SPAD values (Yang et al. 2020) (2) competition with NO_3^- and NH_4^+ for root transporter proteins, consequently impairing nitrogen uptake and lowering total nitrogen (TN) content (Tang et al. 2020). Biochar mitigates OTC stress primarily by reinforcing the rhizosphere barrier, significantly reducing OTC accumulation in roots. In the present study, BCST notably decreased OTC accumulation in lettuce roots by 49.56%, exhibiting the lowest root-to-soil bioconcentration factor ($BCF_{R/S}$) among treatments, thus effectively limiting OTC upward translocation. Furthermore, BCST treatment synergistically promoted plant recovery via two mechanisms: (1) sustaining elevated microbial biomass, accelerating OTC biodegradation in soil, and alleviating competition between OTC and nitrogen absorption; (2) Microbial necromass mineralization releases plant-available NH_4^+, leading to a 36.67% increase in total nitrogen content in plant leaves and reactivation of chlorophyll synthesis, with SPAD values increasing by up to 40.65%. The superior performance of BCST originates from the slow-release carbon provided by starch, which synchronizes OTC degradation with peak nutrient demand periods (DAP60–80) and maintaining stable microbial biomass to sustain enzymatic activities (e.g., tetracycline oxidoreductases) (Fig. 1B, Table 4). In summary, the BCST markedly improved plant health by alleviating antibiotic-induced phytotoxicity and enhancing nutrient uptake efficiency. Specifically, BCST strengthened the rhizosphere barrier, significantly reducing OTC accumulation in plant roots and leaves, thereby restricting its upward transport. This alleviation of toxic stress enhanced chlorophyll biosynthesis and nitrogen assimilation, ultimately resulting in increased plant biomass and leaf nitrogen content.

In agricultural applications, the combined use of biochar and CCS (particularly starch) effectively promotes OTC degradation and enhances plant health. BCST represents a practical and sustainable approach to mitigating antibiotic pollution in agricultural soils, while simultaneously increasing crop yield and enhancing food safety. However, when scaling up this strategy to actual field conditions, further evaluations of the economic aspects, including costs associated with biochar production and transportation, frequency and methods of carbon source application, as well as corresponding benefits such as crop yield improvement and reductions in antibiotic residues, are required. Additionally, assessments of the applicability and stability of this approach across different soil types, climatic conditions, and crop species should be conducted to support its broader adoption in agricultural practices.

Although the combined application of biochar and CCS has shown promising results in antibiotic degradation and soil remediation, its prolonged or excessive application may pose ecological risks, particularly by disrupting soil microbial community balance and nutrient cycling. Studies have indicated that when biochar additions exceed 20 t ha^{-1}, excessive carbon inputs may disrupt the microbial carbon-to-nitrogen (C:N) balance, thereby inhibiting microbial abundance and enzyme activities, and potentially reducing crop yields (Hu et al. 2025). Furthermore, while CCS such as glucose, sucrose, and starch can temporarily enhance microbial activity, continuous application at high concentrations may increase dissolved organic carbon (DOC) leaching risks, disturb nutrient cycling, and reduce soil aggregate stability. Simple sugars like glucose and sucrose, in particular, are more likely to induce positive priming effects, accelerating the decomposition of native soil organic matter and potentially depleting soil carbon reserves. Therefore, future field studies should emphasize evaluating the environmental impacts of this strategy under practical agricultural conditions and optimizing biochar and CCS application rates to balance soil health and agricultural sustainability.

5 Conclusions

Biochar, through its strong sorption capacity, can reduce the translocation of OTC in lettuce leaves from 2.63 mg kg^{-1} in the CK treatment to 1.60 mg kg^{-1} at DAP80. After biochar application, the soil's cation exchange capacity (CEC) and pH increased, enhancing

its ability to capture OTC, thereby reducing the OTC residue in the soil and preventing its absorption by lettuce roots. However, the application of biochar alone prolongs the accumulation time of OTC in the soil, posing a threat to soil microbial activity. Therefore, the addition of carbohydrate carbon sources (CCS) such as glucose, sucrose, and starch to the soil is necessary. The results showed that their application significantly promoted soil microbial activity and increased oxidoreductase activity (e.g., laccase, aromatic oxygenase, and manganese peroxidase), thereby degrading the residual OTC in the soil.

During the DAP0 to DAP80 growth period of lettuce, the soil oxidoreductase activity in the biochar-starch treatment (BCST) showed a continuous increase. However, in the biochar-glucose treatment (BCG) and biochar-sucrose treatment (BCSU), the CCS were rapidly consumed by microorganisms, leading to a gradual decline in soil oxidoreductase activity. Additionally, compared to the CK treatment, the application of biochar and CCS improved soil hydrolase activity and microbial biomass, which enhanced the total nitrogen (TN) content and biomass of lettuce. Based on soil ecological safety and lettuce growth, it is recommended to apply a combination of biochar-starch (BCST) to increase OTC degradation in the soil-lettuce system. In this study examining the efficiency of biochar in degrading soil OTC, we only screened biochar and carbohydrate carbon source combinations that are suitable for lettuce growth. Moreover, these findings were obtained using a specific biochar type (maize straw, pyrolyzed at 600 °C), and the results may vary with other biochar feedstocks and preparation conditions, which appropriately limits the generalizability of these conclusions. Further research is needed to explore biochar combinations with better versatility, cost-effectiveness, and degradation efficiency for more crops. Additionally, to determine a more precise biochar improvement strategy, further studies are required on the application amount, timing, ratio, and frequency of the biochar and starch combination.

Author contributions

Jiefeng Zeng: Formal analysis, Methodology, Data curation, Software, Writing-original draft. Xiao Wang: Methodology, Data curation, Software, Writing-original draft. Xin He: Formal analysis, Supervision, Writing-review & editing. Zhanyi Gao: Resources, Supervision, Validation. Feiyang Zeng: Investigation, Conceptualization, Software. Qiang Zheng: Formal analysis, Visualization, Investigation. All authors contributed to the study conception and design. Peiling Yang: Project administration, Supervision, Funding acquisition, Resources, Writing-review & editing. All authors read and approved the final manuscript.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare no competing financial interest.

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