Biomass Conversion and Biorefinery (2023) 13:1153–1162
https://doi.org/10.1007/s13399-021-01301-7

ORIGINAL ARTICLE

Dynamics of soil organic carbon mineralization and enzyme activities after two months and six years of biochar addition

Zewen Jin[1,2] · Xiaoling Zhang[1] · Xiaomin Chen[1] · Zhenjie Du[3] · Lifeng Ping[2] · Zhaoqiang Han[1] · Pengchuang Tao[1]

Received: 10 September 2020 / Revised: 7 January 2021 / Accepted: 12 January 2021 / Published online: 4 February 2021

Abstract
Applying biochar to agricultural soils has been proposed as a means of sequestering carbon while simultaneously enhancing soil health and agricultural sustainability. While, our understanding of the long-term effects of biochar on soil organic carbon mineralization and enzyme activities under field conditions is limited. In our study, soil samples, which were improved by biochar after two months and six years, were collected from biochar-amended treatments with seven biochar application rates (0, 2.5, 5, 10, 20, 30, and 40 t ha^{-1}, respectively) in upland red soil. The results showed that the soil chemical properties (available P, pH, soil organic carbon, total nitrogen, and C/N ratio) after two months of biochar addition increased more significantly than those after six years of biochar addition. The cumulative soil organic carbon mineralization (C_m) was increased rapidly at first and then decreased gradually and finally flattened out in the late incubation. The C_m/SOC ratios were significantly lower in C1 to C6 treatments than in C0 treatment. High application rates (40 t ha^{-1}) of biochar inhibited the carbon mineralization, while the effects diminished after six years of biochar addition. The multiple linear model and correlation analysis suggested that the effects of invertase activity and total nitrogen on C_m were more significant than other indexes.

Keywords Biochar · SOC mineralization · Enzyme activity · Upland red soil

1 Introduction

Red soils (equivalent to Ultisols in the Soil Taxonomy System of the USA) cover an area of 2.04 million km^2, accounting for 11% of the total land in China. The red soil regions are important for agricultural production due to the abundant rainfall and high temperature [1]. However, long-term irrational utilization and management resulted in severe soil degradation, such as the depletion of soil organic carbon (SOC), soil erosion, and destruction of soil aggregation[2]. Consequently, to meet the demand for creating sustainable agriculture, decreasing the depletion of SOC and increasing soil organic carbon storage would be extremely urgent.

Biochar is a carbon-rich material produced via pyrolysis of biomass in an absence of oxygen at temperatures > 250 °C [3]. Recently, biochar has been advocated as a soil amendment potentially serving the double purpose about improving soil quality and enhancing soil carbon sequestration in fields [4]. Some studies have shown that the improvement of soil quality by biochar addition comprised the increased water retention, nutrient retention, and soil pH[5–7]. Currently, understanding the effect of biochar on SOC cycling and biological reaction has been identified as a research priority. Many studies have shown that biochar has significant influence on SOC mineralization. Lehmann et al. [8] found that biochar can decrease SOC mineralization and restrain CO_2 emissions by permitting a more efficient use of carbon by microbes and the precipitation of CO_2 as carbonates. Spokas and Reicosky [9] found decreases in SOC mineralization after testing 16 different types of biochar. Biochar can also reduce the amount of CO_2 emission by converting soil-borne CO_2 to carbonate by abiotic precipitation stimulation[8, 10, 11]. Meanwhile, little decreases of soil CO_2 fluxes by biochar amendment and no

✉ Xiaomin Chen
xmchen@njau.edu.cn

1 College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, People's Republic of China

2 Key Laboratory of Recycling and Eco-treatment of Waste Biomass of Zhejiang Province, School of Environmental and Natural Resources, Zhejiang University of Science & Technology, Hangzhou 310023, Zhejiang, China

3 Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences, Xinxiang 453003, China

influence in SOC mineralization were observed in other studies [12]. The physical properties of biochar evolve with the incubation time increased, the surface charges and functional groups of biochar converted from positive to negative with biotic and abiotic oxidation, resulting in the change of biochar influence on soil organic carbon mineralization [13–15]. Accordingly, it is necessary to study the different effects of biochar on SOC mineralization.

Soil enzyme activity, which is a sensitive indicator, has been shown in large studies to be quickly sensitive to soil quality in different soil environments under contrasting management practices because of their ease of measurement and rapid response to soil changes [5, 16–18]. Previous works demonstrated that β-glucosidase and urease activities were significantly higher in biochar-amended treatments than control because of the increased microbial biomass [19]. Another study has found that arylsulfatase activity was not significantly different in field plots with biochar amendment as a result of enzyme or microbe stabilizing interactions[20]. Meanwhile, soil enzymes also have important effects on soil C cycle and organic matter degradation. Taylor et al. [21] found that the extracellular enzyme secreted by microorganisms directly affected the soil organic carbon cycle.

The duration of the experiments, which were used to understand the effects of biochar on SOC mineralization and soil enzyme activity, most ranges from several weeks to several years under controlled laboratory conditions [12, 22, 23]. However, field conditions are more complex because of the tillage practices, the application of fertilizer, and the cultivation of crops. In addition, the nature of biochar and biochar-amended soil may be changed after field incorporation [23], as Prost et al. [24] found that the surface area and porosity of biochar decrease during amendment due to soil particle clogging biochar pores. The soil pH, cation exchange capacity (CEC), and nutrient contents also changed over time after biochar addition [25]. Hence, the question remains if and how SOC mineralization and enzyme activities are affected by biochar over time under field conditions.

This study explored an experiment with long time span amended with different dose of biochar. The objectives of this study were (1) to clarify the change of soil chemical properties after biochar applying to upland red soil; (2) to investigate the effects biochar on SOC mineralization and enzyme activities over time under field conditions with different dose of biochar applied; and (3) to analyze the relationship between SOC mineralization and enzyme activities after biochar applying to upland red soil. We hypothesized that biochar-induced changes of physicochemical soil properties continue to influence SOC mineralization and enzyme activities over a long period of time, while the effects of biochar on these indicators may weakened after several years.

2 Material and methods

2.1 Study site

This research was conducted at the Institute of Red Soil, Jinxian County (28°37′ N, 116°26′ E, 26 m above sea level), Jiangxi Province, China. The local climate conditions were governed by a typical subtropical monsoon climate with a mean annual temperature of 17.5 °C and an annual precipitation of 1549 mm. The total number of sunlight hours per year averages 2000, with 282 frost-free days per year. The soil is derived from Quaternary red clay. Basic properties of the studied soil are given in Table 1.

2.2 Biochar amendment

Biochar used in this site experiment was pyrolyzed at 450 °C by wheat straw in a vertical kiln made of refractory bricks at Sanli New Energy Company, Henan Province, China. About 35% of the wheat straw dry matter was turned into biochar during the process [26]. The original biochar was ground to pass through a 2-mm sieve so as to mix uniformly with the soil mass. The properties of biochar are shown in Table 1.

2.3 Field experiment

Seven treatments were established according to different application rates of biochar (0, 2.5, 5, 10, 20, 30, and 40 t ha^{-1}), which were designated as treatment C0, C1, C2, C3, C4, C5, and C6, respectively. A randomized complete block design with three replications was laid out on the similar soil type and with consistent management. Each trial plot covered an area of 20 m^2 (4m × 5 m). Biochar was spread on the surface of the soil and thoroughly mixed into a depth of 15 cm by manual plowing on 18 August 2011. No more biochar was supplemented in the subsequent years.

We adopted the rapeseed-sweet potato rotation tillage system with rapeseed planted in October and harvested in mid-May, and the sweet potato planted in late May and harvested in late September.

2.4 Soil sampling and analysis

Soil samples were collected at the depth of 0–15 cm with gauge auger (30 mm diameter) on 19 October 2011 (3 months after biochar addition) and 17 October 2017 (6 years after biochar addition). Each plot of soil was randomly sampled using the five-point composite method throughout the entire experimental site. The composite samples were sealed in sterile plastic bags and placed on ice for transport to the laboratory within 2 days. The soil samples were air-dried and ground to pass 2-mm and 1-mm sieves after the litter layer, and crop residues were removed. The properties of biochar, soil, and

Table 1 Basic properties of biochar and upland red soil

	pH	SOC (g/kg)	TN (g/kg)	CEC (cmol/kg)	Bulk density (g/cm^3)	Surface area (m^2/g)	Ash content (%)	Textural composition (%)		
								Clay	Silt	Sand
Soil	4.54	9.45	1.06	15.2	1.23	–	–	31.6	39.12	29.28
Biochar	10.35	467.2	5.9	21.7	0.65	8.92	20.8	–	–	–

biochar-amended soil were determined with the methods suggested by Bao [27]. The pH was determined in a 1:5 soil: water suspension using a Thermo Orion pH meter with a combination electrode. SOC and total nitrogen (TN) contents were determined via wet digestion using $K_2Cr_2O_7$ oxidation and the Kjeldahl method, respectively. Soil available P was determined by using 1 M HCl and 1 M NH_4F solutions as an extractant by Bray II method [28].

Three soil enzymes, urease, catalase, and invertase, were measured to determine the effect biochar on soil enzyme activities. Urease activity in the soil was determined by spectrophotometry at 578 nm as the $NH^+{}_4$–N released from 5.0 g of soil after a 24-h incubation at 37 °C with 10% (w/v) urea solution, in 20 ml 1 M citrate buffer at pH 6.7 [29]. Catalase activity in the soil was measured by back titration residual H_2O_2 with 0.1 M $KMnO_4$. For invertase, 5 g of soil; 15 ml 8% sucrose solution; 5 ml phosphate buffer, pH 5.5; and 0.25 ml toluene were incubated in 50-mL Erlenmeyer flasks at 37 °C for 24 h. The mixture was filtered by filter paper. After stopping the reaction with 3 ml 3, 5-dinitrosalicylic acid, the activity was measured at a wavelength of 508 nm [30] .

2.5 Laboratory incubation

Aliquots of each soil (equivalent to 100 g) were wetted with distilled water to obtain 60% of water holding capacity. There were three replicates per treatment. A control treatment (without soil samples) in triplicate was also included. The soil samples were placed in hermetically sealed canning jars and put in an incubation cabinet at 25 °C in the dark. The emitted CO_2 was trapped in 20 ml 0.5 M NaOH. At days 1, 3, 7, 12, 17, 23, 31, 38, 45, 53, 61, 67, 78, and 89, the vials with NaOH were removed and titrated with 0.4 M HCl after prior precipitation (with 20 ml of 1 M $BaCl_2$) of the carbonate formed [31]. The water content of each soil was adjusted weekly to maintain an initial status.

2.6 Statistical analysis

One-way analysis of variance (ANOVA) was performed by SPSS 20.0. The differences in the group means were examined using the Duncan significant difference test with a significance level of $p < 0.05$. Pearson's correlation coefficients were calculated to determine the relationship between the cumulative SOC mineralization and soil enzymes activity. Prior to the linear regression analysis, the normal distribution of C_m data was examined with a Kolmogorov-Smirnov test, which tests for normality by examining the observed and theoretical distributions and determining if the difference between them is significant[32]. A $\log(x + 1)$ transformation was performed for the C_m data to meet the assumptions of normality. A simple linear regression model between SOC mineralization parameters were evaluated by using Origin software version 9.4.

3 Results

3.1 Soil chemical properties response to biochar addition

Soil chemical properties increased with biochar addition compared to C0 treatment (Table 2). The available P, pH value, SOC, total N, and C/N ratio significantly increased after 2 months of biochar addition, and the highest value of available P, pH, SOC, total N, and C/N ratio was all observed in C6 treatment, where those parameters increased by 201.80%, 8.96%, 57.79%, 24.49%, and 26.78%, respectively, compared with C0 treatment. However, the influence of biochar on soil chemical properties weakened after 6 years. Only C6 treatment significantly increased available P, SOC, pH, and C/N ratio compared with the relative C0 treatment in 2017, while there was no difference in TN content between C0 and biochar treatments after 6 years of cultivation.

3.2 Dynamics of soil organic carbon mineralization

The dynamics of SOC mineralization in 2011 and 2017 were presented in Figs. 1 and 2. The emitted CO_2 varied from 20.71 to 65.29 mg kg^{-1} d^{-1} for 2011 on the first day of the incubation. In addition, the SOC mineralization rates were increased rapidly in the early incubation and then flattened to small constant value in the late incubation among all treatments. Moreover, the highest initial mineralization rates in 2011 and 2017 were all measured in C0 treatment (65.29 mg kg^{-1} d^{-1} for 2011 and 40.88 mg kg^{-1} d^{-1} for 2017) (Figs. 1 and 2).

Generally, the total amount of CO_2 released by organic carbon mineralization in a certain period of time is regarded

Table 2 The chemical properties of biochar-amended soil in 2011 and 2017

	Treatments	Available P (mg/kg)	pH	SOC (g/kg)	Total N (g/kg)	C/N
2011	C0	21.08±1.60d	4.80±0.08c	8.67±0.25 g	0.98±0.03d	8.85±0.27f
	C1	39.56±3.00c	4.97±0.06b	9.36±0.31f	1.02±0.01c	9.18±0.09e
	C2	42.13±3.19c	4.97±0.14b	10.32±0.25e	1.02±0.02c	10.12±0.20c
	C3	48.13±3.65bc	5.12±0.18ab	10.91±0.30d	1.13±0.02b	9.66±0.17d
	C4	52.24±3.96b	5.04±0.11b	12.85±0.14c	1.15±0.03b	11.18±0.29a
	C5	61.07±4.63ab	5.21±0.17ab	12.95±0.08b	1.2±0.01a	10.79±0.09b
	C6	63.62±4.82a	5.23±0.06a	13.68±0.28a	1.22±0.03a	11.22±0.28a
2017	C0	22.24±1.69b	4.81±0.04b	9.13±0.05b	1.04±0.01a	9.16±0.22b
	C1	23.89±2.34b	4.74±0.07b	9.29±0.07b	1.02±0.03a	9.11±0.09b
	C2	22.25±2.37b	4.81±0.12b	9.78±0.38b	1.07±0.02a	9.22±0.26b
	C3	24.21±3.05b	4.87±0.11b	9.62±0.31b	1.05±0.02a	9.08±0.16b
	C4	23.25±3.28b	4.91±0.07b	10.18±0.34ab	1.07±0.02a	9.32±0.18b
	C5	26.64±3.61ab	4.87±0.09b	9.94±0.29b	1.03±0.01a	9.28±0.09b
	C6	28.36±3.82a	5.13±0.14a	11.05±0.06a	1.00±0.02a	10.11±0.17a

Means ± standard errors followed by the same letter within a column are not significantly different at $p < 0.05$

as the cumulative SOC mineralization (C_m). The value of C_m ranged from 293.44 to 425.98 mg kg^{-1} in 2011 and 481.19 to 610.26 mg kg^{-1} in 2017 among all treatments. Interestingly, we found that low-medium biochar application rates (5 to 20 t ha^{-1}) considerably increased the value of C_m, and the highest value of C_m was all found in C4 treatment in 2011 and 2017. However, high application rates (30 to 40 t ha^{-1}) of biochar inhibited the carbon mineralization. The C_m in C5 and C6 were lower than that in C0 treatment. Similarly, the ratios of C_m/SOC were significantly lower in biochar treatments than that in C0 in both 2011 and 2017, and C6 treatment distinctly decreased the ratio of C_m/SOC by 2.05 compared to C0 treatment in 2011. In 2017, the C_m/SOC ratio was significantly decreased by 2.11 in C6 treatment compared with C0 treatment. Overall, the C_m/SOC ratios in 2017 were higher than those in 2011. In order to clearly assess the dynamics of SOC mineralization in different treatments, nonlinear regression models were fitted to obtain the appropriate equations (Table 3). The R^2 were never below 0.90 and usually better than 0.95.

3.3 The activities of urease, catalase, and invertase in response to biochar addition

As shown in Fig. 3, urease activities generally increased with the increasing doses of biochar during these two periods. Particularly, urease activity in C6 treatment increased by 85.90% compared with C0 treatment after 2 months of biochar addition. However, the effectiveness of biochar on urease activity weakened after 6 years. The urease activities in 2017 were generally lower than that in 2011 under the same biochar addition.

Catalase is an important enzyme for reflecting the potential of biological oxidation in soil [33]. Compared with C0

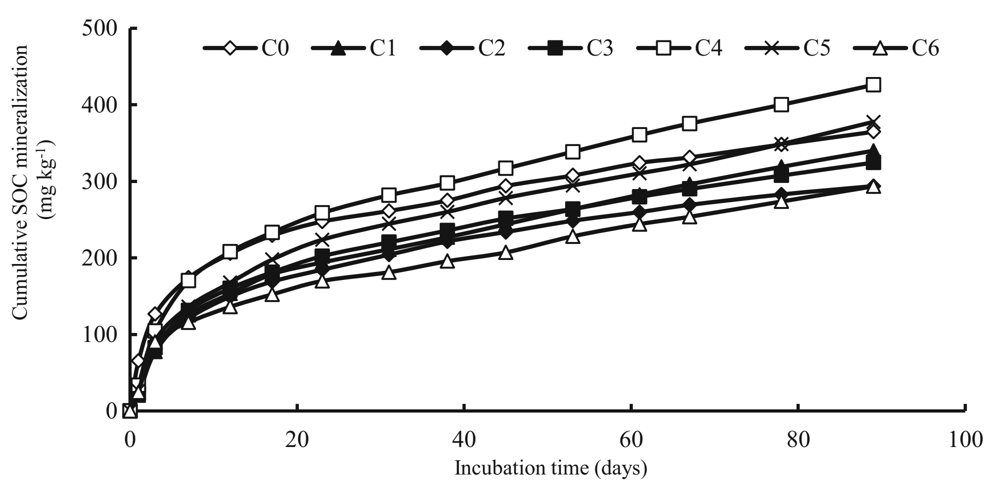

Fig. 1 Dynamics of SOC mineralization among treatments in 2011

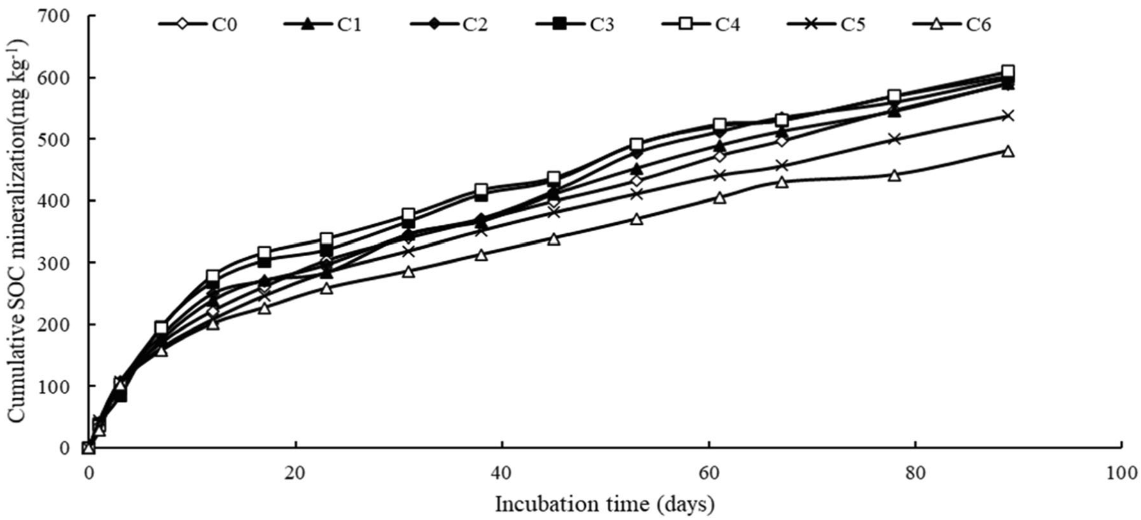

Fig. 2 Dynamics of SOC mineralization among treatments in 2017

treatment, the catalase activity significantly increased with fresh biochar, while, there were no obvious rules among all treatments in 2011. The highest catalase activity (1.59 mg·g^{-1}·h^{-1}) was found in C5 treatment, that value was 1.7 times greater than the C0 treatment. Similarly, catalase activities in 2017 were generally lower than that in 2011 under the same biochar addition (Fig. 4). As shown in Fig. 5**,** biochar application decreased soil invertase activity, except for C4 treatment. The greatest activities of soil invertase about 8.50 mg g^{-1} and 8.90 mg g^{-1} were found in C4 treatment in both 2011 and 2017. Besides, invertase activity in 2017 was generally lower than that in 2011 with the same biochar addition.

3.4 The SOC mineralization (C_m) multiple linear model and correlation coefficient analysis

The SOC mineralization (C_m) multiple linear regression model coefficients were shown in Table 4, with their predictors in order of importance based on their absolute β scores. The overall model fit was $R^2 = 0.852$.

Equation (1) shows the regression equation derived:

$$\begin{aligned} \mathrm{Cm} = {} & 709.14 + 34.57\mathrm{IA} \\ & + 640.07\mathrm{TN} - 243.40\mathrm{pH} - 340.42\mathrm{CA} - 11.98\mathrm{SOC} \\ & + 19.59\mathrm{C/N} - 92.545\mathrm{UA} \end{aligned} \tag{1}$$

Table 3 Model fitting of a nonlinear regression model in 2011 and 2017:$C_m = a\,(1 - e^{-bt})$

Year	Treatments	C_m (mg/kg)	C_m/SOC (%)	*a*	*b*	R^2
2011	C0	364.78±3.05c	4.20	319.39	0.085	0.923
	C1	339.73±6.66d	3.63	307.80	0.046	0.948
	C2	293.69±12.04f	2.85	268.93	0.059	0.959
	C3	324.55±5.89e	2.97	292.37	0.057	0.957
	C4	425.98±3.87a	3.32	379.24	0.055	0.949
	C5	377.49±5.95b	2.91	336.62	0.050	0.955
	C6	293.44±3.97f	2.15	259.54	0.051	0.917
2017	C0	589.49±4.57b	6.46	571.58	0.032	0.965
	C1	591.53±2.58 g	6.37	280.75	0.056	0.938
	C2	598.17±16.65f	6.12	375.00	0.033	0.974
	C3	601.75±2.54e	6.26	434.44	0.034	0.975
	C4	610.26±11.68a	5.99	663.39	0.024	0.981
	C5	538.27±12.52d	5.42	467.84	0.036	0.952
	C6	481.19±4.09c	4.35	502.23	0.037	0.974

C_m is the cumulative SOC mineralization at incubation time *t*

Means ± standard errors followed by the same letter within a column are not significantly different at $p < 0.05$ (the same as below)

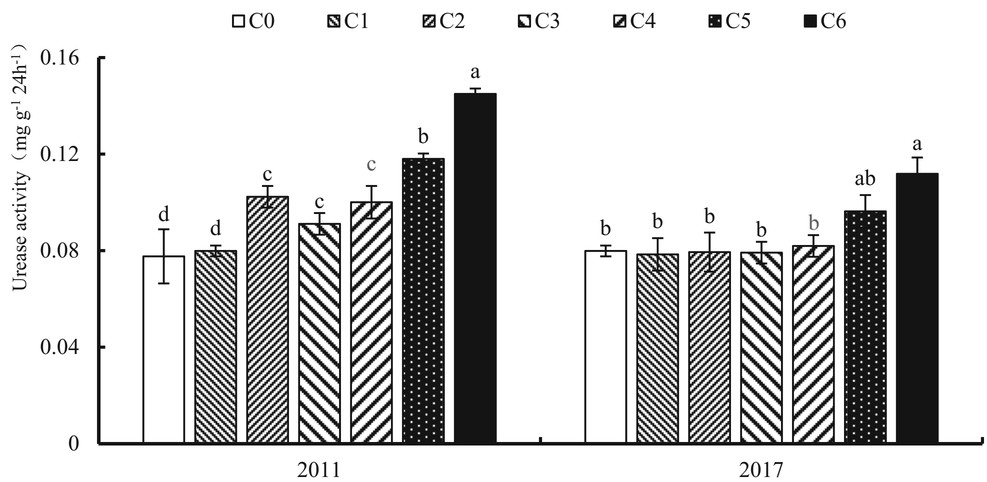

Fig. 3 Soil urease activities in the consecutive two years

The model and the absolute β scores showed that the effects of invertase activity and total nitrogen on C_m were more significant than other indexes.

Table 5 presented the correlation coefficients between soil enzymes activity and cumulative SOC mineralization (C_m) in 2011 and 2017. The urease activity showed a significantly positive correlation with biochar application rate both in 2011 and 2017 ($r_1 = 0.914$, $r_2 = 0.849$, $p < 0.01$). Besides, a significantly positive correlation between the C_m and invertase activity was also found both in 2011 and 2017 ($r_1 = 0.727$, $r_2 = 0.829$, $p<0.01$), while the C_m had low correlations with urease and catalase activity.

4 Discussion

4.1 Effect of biochar application on dynamics of soil organic carbon mineralization

The experiment showed that low-medium biochar addition (5 to 20 t ha^{-1}) to soil might benefit the organic carbon sequestration, but an excessive application of biochar (30 to 40 t ha^{-1}) leads to reduced cumulative organic carbon mineralization. Meanwhile, the ratios of C_m/SOC in biochar treatments were significantly lower than that in C0 treatment. This result would be closely linked with the complex effects of biochar on SOC decomposition[34, 35]. Previous studies have shown that the addition of fresh and easily degradable carbon to soil enhances the preexisting soil organic matter decomposition[36, 37]. Biochar comprises of a myriad of small molecules such as hydroxy and acetoxy acids, n-alkanoic acids, diols, benzoic acids, triols, and phenols, which are degraded rapidly[38, 39]. The increase in cumulative organic carbon mineralization could be attributed to the labile carbon from biochar. Moreover, the higher cumulative SOC mineralization in low-medium biochar-amended soil may be related to the improved effects of soil structure[40]. The soil used in this experiment had a high clay content of more than 30%. The aeration of the soil was not optimal, and microbes had limited access to oxygen. Low-medium application rates of biochar can loosen the soil and allow oxygen demanding processes like carbon mineralization in deeper soil layers.

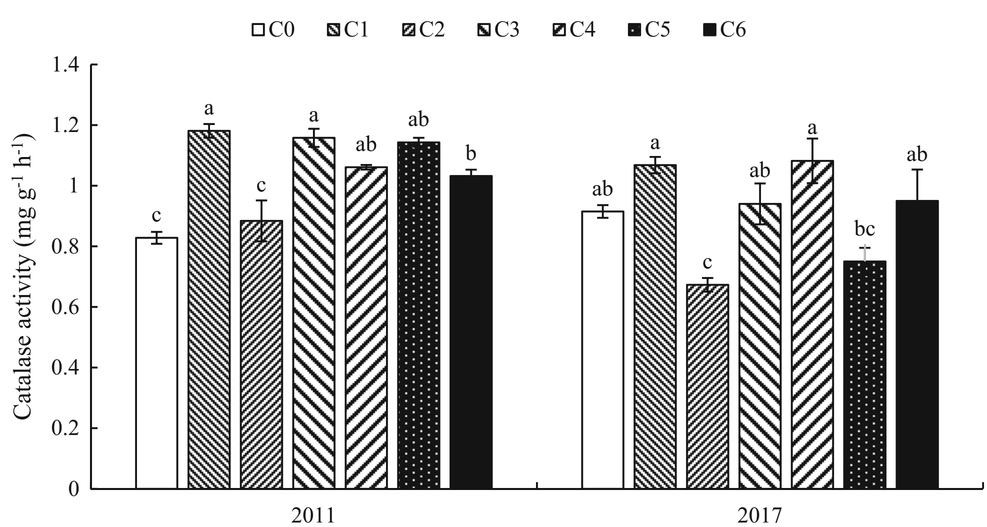

Fig. 4 Soil catalase activities in the consecutive two years

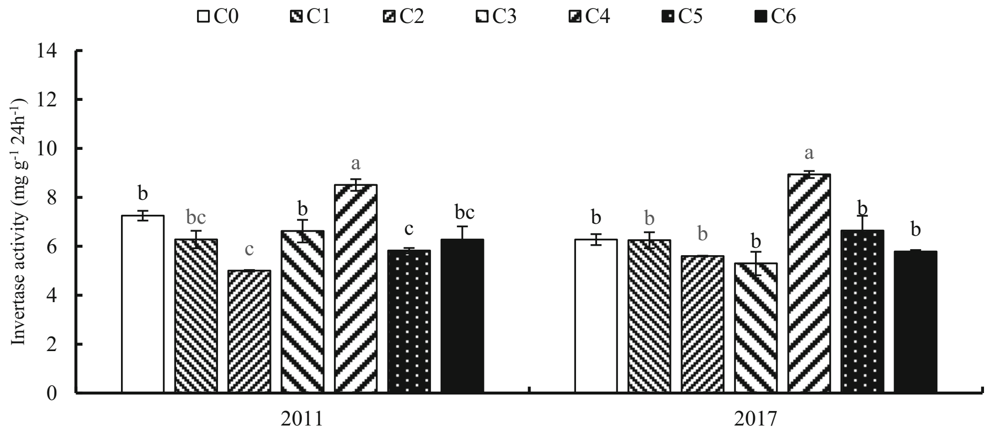

Fig. 5 Soil invertase activities in the consecutive two years

Thus, low-medium dosage of biochar supplementation could notably improve the soil organic carbon mineralization[41].

Contrary to the above, high biochar addition inhibits (30 to 40 t ha^{-1}) soil organic carbon mineralization. The C_m in C5 and C6 treatments were lower as compared to C0 treatment (Table 3). Application of biochar at 90 t ha^{-1} reduced carbon mineralization by 2% and reduced carbon mineralization by 25% after 2 weeks of biochar addition at 80 t ha^{-1} was also recorded [4, 42]. The biochar was made from straw that received no additional nutrients as a post-pyrolysis treatment. Fresh biochar often has a deficient nitrogen content but a high capacity to bind nutrients. Therefore, when applying high amounts of fresh biochar to the soil (30 to 40 t ha^{-1}) after 2 months, a deficiency of available nitrogen is caused in the soil, and this prevents the mineralization of carbon, because microbial communities cannot utilize carbon efficiently without access to nitrogen. Moreover, volatile compounds present in biochar have the potential to decrease microbial biomass nitrogen and soil microbial activity when biochar was applied at a high dose [43, 44].

Our results show that the soil organic carbon mineralization is different after 2 months and 6 years of biochar addition based on the values of C_m and C_m/SOC. The C_m and C_m/SOC in 2011 were lower than those in 2017 under the same biochar addition, which reflect that the inhibition effect of biochar on soil organic carbon mineralization weakened over time. First of all, our previous research found that soil microbial communities adapted to soil require some time to metabolize exogenous organic matter substrates[45]. Similarly, the transformation from microaggregation into macroaggregation also needs a specific time. Therefore, the microorganisms that play a key role in promoting soil organic carbon mineralization need a certain period after biochar addition, which caused the C_m in 2011 lower than that in 2017. Secondly, volatile compounds in biocharwill inhibit the carbon mineralization based on our previous study[46]. With the aging of biochar, the content of volatile compounds decomposes gradually; therefore, the inhibition of biochar on soil organic carbon mineralization weakened over time[31] .

4.2 Effect of biochar on activities of urease, catalase, and invertase

Soil enzymes, mostly originated from soil fungi, bacteria, and plant roots, are believed to play a critical role in mediating biochemical transformations involving organic matter decomposition and nutrient cycling in soil [47]. Urease plays a critical role in the process of nitrogen cycling. The consistently higher urease and catalase activities in biochar-amended soil than in control treatment with a previous laboratory study was also conducted by Yang and Demisie [19, 48]. Here, it was found that biochar application significantly increased urease activities 2 months after biochar application, which was mainly ascribed to the promoted physicochemical properties in biochar-amended soil [49]. This study showed that biochar can have a diverse effect on soil chemical properties (such as available P, total N, carbon content, and C/N ratio), which were beneficial to the growth and activity of soil microflora and then increased the source of many soil enzymes. Besides, the increased pH value would also promote the availability of substrates for enzymatic reactions and lead to the increase in

Table 4 The multiple linear regression model coefficients

	Constant	IA	TN	pH	CA	SOC	C/N	UA
B	709.14	34.57	640.07	−243.40	−340.42	−11.98	19.59	−92.55
β		0.826	0.540	−0.354	−0.215	−0.199	0.164	−0.029

IA invertase activity, *TN* total nitrogen, *CA* catalase activity, *UA* urease activity

Table 5 Pearson's correlations matrix for the enzymes activity and soil organic carbon mineralization

Year	Indicator	Biochar	Urease	Catalase	Invertase	C_m
2011	Urease	0.887**	1			
	Catalase	0.391	0.083	1		
	Invertase	−0.022	−0.256	0.045	1	
	C_m	−0.01	−0.297	0.591	0.727**	1
2017	Urease	0.942**	1			
	Catalase	0.391	0.387	1		
	Invertase	0.293	0.233	0.335	1	
	C_m	0.269	0.263	0.011	0.829**	1

**Correlation is significant at the 0.01 level

urease activities[50]. Other studies also reported that additional source of nutrients (such as P, K, and Mg) and the enhanced sorptive capacity of soil due to the application of biochar had a significant stimulating effect on the activity of the enzymes[49]. Moreover, the long-term action of the biochar on the soil would provide a certain stable environment to acclimatization of microorganisms [49].

Invertase activity is involved in the degradation of soil organic compounds and can be used as energy sources for microorganism actions [16]. We assessed that biochar amendment can increase invertase activity in this study, while the result showed that invertase activities were lower in most biochar-amended treatments in comparison to the C0 treatment.The possible explanation for the decreased invertase activity was a significantly lower SOC mineralization rates after biochar amendment. Besides, the general decreased invertase activity may be also ascribed to the increased microbial efficiency as a result of the co-location of carbon and microorganism on biochar surfaces [16].

4.3 The main influencing factors of cumulative SOC mineralization

Soil organic carbon mineralization is an important microbiological process by which carbon is transformed from organic form into inorganic form. The results of multiple linear models of cumulative SOC mineralization showed that the effects of invertase activity and total nitrogen on C_m were more significant than other indexes. Soil enzymes produced by soil microbes could catalyze the decomposition of soil organic matter and make available compounds for plant growth [19]. Soil pH was the dominant factor over microbial activity by controlling microbial enzyme production, ionization-induced conformational changes of enzymes, availability of substrates and enzymatic co-factors, carbon and nutrient availabilities, and the concentration of DOC[51]. In this study, the addition of biochar increased the catalase activity by increasing soils' pH. In addition, the TN content also had a significant effect on C_m based on the higher β value (Table 4). The TN content in soil is positively related to the enzyme activity, which showed positive relationships with SOC decomposition [52]. Jin et al [5] found that biochar can stimulate the internal processes of N assimilation and conversion, as well as increasing the content of $NH_4{}^+$-N and $NO_3{}^-$-N. The nitrogen can be used as energy sources for microbial communities and enhance the enzymatic activities, as well as C_m based on our multiple linear models.

5 Conclusions

Our results showed that the soil chemical properties and the activities of urease and catalase increased after 2 months of biochar addition, while the effectiveness weakened after 6 years of biochar addition. Low-medium biochar application rates (5 to 20 t ha^{-1}) considerably increased the value of C_m, while high application rates (30 to 40 t ha^{-1}) of biochar inhibited the carbon mineralization, and the inhibition of biochar on soil organic carbon mineralization weakened over time. The multiple linear model and correlation analysis suggested that the effects of invertase activity and total nitrogen on C_m were more significant than other indexes.

Acknowledgments The authors thank Grace Billy and Cunliu Tang for their grammatical edits and comments on this paper.

Funding This work was supported by funding from the National Key R & D Program (2016 YFD 0200305), the Natural Science Foundation of Jiangsu Province, China (No. BK 20150909); the Major Projects for Science and Technology Development of Zhejiang Province, China (No. 2019C02061); the National Natural Science Foundation of China (51779260); Henan province Keypoint Research and Invention Program (192102110051); and the Initial Scientific Research Fund of Zhejiang University of science and technology (F701119J07).

References

1. Li ZW, Zeng GM, Zhang H, Yang B, Jiao S (2007) The integrated eco-environment assessment of the red soil hilly region based on GIS—A case study in Changsha City China. Ecol Model 202(3–4): 540–546
2. Jin ZW, Chen XM, Chen C, Tao PC, Han ZQ, Zhang XL (2016) Biochar impact on nitrate leaching in upland red soil China. Environ Earth Sci 75:1109
3. Lehmann J, Joseph S (2009) Biochar for environmental management: an introduction. In: Lehmann J, Joseph S (eds) Biochar for Environmental Management: Science and Technology. Earthscan, London, pp 1–12
4. Munda S, Bhaduri D, Mohanty S, Chatterjee D, Tripathi R, Shahid M, Kumar U, Bhattacharyya P, Kumar A, Adak T, Jangde HK, Nayak AK (2018) Dynamics of soil organic carbon mineralization and C fractions in paddy soil on application of rice husk biochar. Sci Total Environ 115:1–9
5. Jin Z, Chen C, Chen X, Hopkins I, Zhang X, Han Z, Jiang F, Billy G (2019) The crucial factors of soil fertility and rapeseed yield - A

five-year field trial with biochar addition in upland red soil, China. Sci Total Environ 649:1467–1480
6. Chan KY, Van Zwieten L, Meszaros I, Downie A, Joseph S (2008) Using poultry litter biochars as soil amendments. Soil Res 46(5): 437
7. Zhang QQ, Song YF, Wu Z, Yan XY, Gunina A, Kuzyakov Y, Xiong ZQ (2020) Effects of six-year biochar amendment on soil aggregation, crop growth, and nitrogen and phosphorus use efficiencies in a rice-wheat rotation. J Clean Prod 242:118435
8. Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota – A review. Soil Biol Biochem 43(9):1812–1836
9. Spokas KA, Reicosky DC (2009) Impacts of sixteen different biochars on soil greenhouse gas production. Am J Environ Sci 3:179–193
10. Joseph SD, Camps-Arbestain M, Lin Y, Munroe P, Chia CH, Hook J, Van Zwieten L, Kimber S, Cowie A, Singh BP, Lehmann J, Foidl N, Smernik RJ, Amonette JE (2010) An investigation into the reactions of biochar in soil. Soil Res 48(7):501
11. Case SDC, McNamara NP, Reay DS, Whitaker J (2014) Can biochar reduce soil greenhouse gas emissions from a Miscanthus bioenergy crop? GCB Bioenergy 6(1):76–89
12. Kuzyakov Y, Subbotina I, Chen H, Bogomolova I, Xu X (2009) Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biol Biochem 41(2):210–219
13. Cheng CH, Lehmann J (2009) Ageing of black carbon along a temperature gradient. Chemosphere 75(8):1021–1027
14. Liang B, Lehmann J, Solomon D, Kinyangi J, Grossman J, O'Neill B, Skjemstad JO, Thies J, Luizão FJ, Petersen J, Neves EG (2006) Black Carbon Increases Cation Exchange Capacity in Soils. Soil Sci Soc Am J 70(5):1719–1730
15. Liang B, Lehmann J, Solomon D, Sohi S, Thies JE, Skjemstad JO, Luizão FJ, Engelhard MH, Neves EG, Wirick S (2008) Stability of biomass-derived black carbon in soils. Geochim Cosmochim Acta 72(24):6069–6078
16. Paz-Ferreiro J, Fu S, Méndez A, Gascó G (2014) Interactive effects of biochar and the earthworm Pontoscolex corethrurus on plant productivity and soil enzyme activities. J Sol-Gel Sci Technol 14(3):483–494
17. Paz-Ferreiro J, Trasar-Cepeda C, Leirós MC, Seoane S, Gil-Sotres F (2010) Effect of management and climate on biochemical properties of grassland soils from Galicia (NW Spain). Eur J Soil Biol 46(2):136–143
18. Paz-Ferreiro J, Trasar-Cepeda C, Leirós MC, Seoane S, Gil-Sotres F (2011) Intra-annual variation in biochemical properties and the biochemical equilibrium of different grassland soils under contrasting management and climate. Biol Fertil Soils 47(6):633–645
19. Demisie W, Liu Z, Zhang M (2014) Effect of biochar on carbon fractions and enzyme activity of red soil. Catena 121:214–221
20. Sun Z, Bruun EW, Arthur E, Jonge LWD, Moldrup P, Hauggaard-Nielsen H, Elsgaard L (2014) Effect of biochar on aerobic processes, enzyme activity, and crop yields in two sandy loam soils. Biol Fertil Soils 50(7):1087–1097
21. Taylor JP, Wilson B, Mills MS, Burns RG (2002) Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biol Biochem 34(3):387–401
22. Novak JM, Busscher WJ, Watts DW, Laird DA, Ahmedna MA, Niandou MAS (2010) Short-term CO2 mineralization after additions of biochar and switchgrass to a Typic Kandiudult. Geoderma 154(3–4):281–288
23. Cross A, Sohi SP (2011) The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. Soil Biol Biochem 43(10):2127–2134
24. Prost K, Borchard N, Siemens J, Kautz T, Séquaris JM, Möller A, Amelung W (2013) Biochar Affected by Composting with Farmyard Manure. J Environ Qual 42(1):164–172
25. Aller D, Mazur R, Moore K, Hintz R, Laird D, Horton R (2017) Biochar Age and Crop Rotation Impacts on Soil Quality. Soil Sci Soc Am J 81(5):1157–1167
26. Pan G, Lin Z, Zhang A, Zheng J, Zhang X (2011) Perspective on biomass carbon industrialization of organic waste from agriculture and rural areas in China. Agric Sci Technol 13:75–82
27. Bao SD (2000) Soil and agricultural chemistry analysis. China Agricultural Science Technology Press, Beijing
28. Lu RK (2000) Methods of soil and agro-chemical analysis. China Agricultural Science and Technology Press, Beijing
29. Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fertil Soils 6(1):68–72
30. Guan SY (1986) Soil enzyme and rechearch method. Agriculture Press, pp 274–276
31. Ameloot N, Graber ER, Verheijen FGA, De Neve S (2013) Interactions between biochar stability and soil organisms: review and research needs. Eur J Soil Sci 64(4):379–390
32. Olea RA, Pawlowsky-Glahn V (2009) Kolmogorov-Smirnov test for spatially correlated data. Stoch Env Res Risk A 23(6):749–757
33. Cui H, Zhou J, Zhao Q, Si Y, Mao J, Fang G, Liang L (2013) Fractions of Cu, Cd, and enzyme activities in a contaminated soil as affected by applications of micro- and nanohydroxyapatite. J Soils Sediments 13(4):742–752
34. Zimmerman AR, Gao B, Ahn MY (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biol Biochem 43(6):1169–1179
35. Luo Y, Durenkamp M, Nobili MD, Lin Q, Brookes PC (2011) Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biol Biochem 43:2304–2314
36. Derrien D, Plain C, Courty PE, Gelhaye L, Moerdijk-Poortvliet T, Fabien T, Versini A, Zeller B, Koutika LS, Boschker H, Epron D (2014) Does the addition of labile substrate destabilise old soil organic matter? Soil Biol Biochem 76:149–160
37. Kuzyakov Y (2010) Priming effects: Interactions between living and dead organic matter. Soil Biol Biochem 42(9):1363–1371
38. Hernandez-Soriano MC, Kerré B, Kopittke PM, Horemans B, Smolders E (2016) Biochar affects carbon composition and stability in soil: a combined spectroscopy-microscopy study. Sci Rep 6(1): 25127
39. Graber E, Meller Harel Y, Kolton M, Cytryn E, Silber A, Rav David D, Tsechansky L, Borenshtein M, Elad Y (2010) Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. Plant Soil 337(1–2):481–496
40. Chan KY, Van Zwieten L, Meszaros I, Downie A, Joseph S (2007) Agronomic values of greenwaste biochar as a soil amendment. Soil Res 45(8):629
41. Troy SM, Lawlor PG, O'Flynn CJ, Healy MG (2013) Impact of biochar addition to soil on greenhouse gas emissions following pig manure application. Soil Biol Biochem 60:173–181
42. Zimmerman AR (2010) Abiotic and microbial oxidation of laboratory-produced black carbon (Biochar). Environ Sci Technol 44(4):1295–1301
43. Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol Fertil Soils 45(2):115–131
44. Zhang X, Chen C, Chen X, Tao P, Jin Z, Han Z (2018) Persistent effects of biochar on soil organic carbon mineralization and resistant carbon pool in upland red soil China. Environ Earth Sci 77(5): 177

45. Sun J, He F, Zhang Z, Shao H, Xu G (2016) Temperature and moisture responses to carbon mineralization in the biochar-amended saline soil. Sci Total Environ 569–570:390–394
46. Jin Z, Ping L, Wong M (2020) Systematic relationship between soil properties and organic carbon mineralization based on structural equation modeling analysis. J Clean Prod 277:123338
47. McLatchey GP, Reddy KR (1998) Regulation of organic matter decomposition and nutrient release in a wetland soil. Environ Qual 27:1268–1274
48. Yang X, Liu J, McGrouther K, Huang H, Lu K, Guo X, He L, Lin X, Che L, Ye Z, Wang H (2016) Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. Environ Sci Pollut Res 23(2):974–984
49. Oleszczuk P, Jośko I, Futa B, Pasieczna-Patkowska S, Pałys E, Kraska P (2014) Effect of pesticides on microorganisms, enzymatic activity and plant in biochar-amended soil. Geoderma 214–215:10–18
50. Turner BL (2010) Variation in pH Optima of Hydrolytic Enzyme Activities in Tropical Rain Forest Soils. Appl Environ Microbiol 76(19):6485–6493
51. Makoi JH, Ndakidemi PA (2008) Selected soil enzymes: examples of their potential roles in the ecosystem. Afr J Biotechnol 7(3):181–191
52. Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biol Biochem 34(9):1309–1315