

Effects of amendment of different biochars on soil carbon mineralisation and sequestration

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Abstract. The aim of this study was to determine the impact of addition of different biochars on soil carbon mineralisation and sequestration. Different biochars were produced from two types of feedstock, fresh dairy manure and pine tree woodchip, each of which was pyrolysed at 300, 500, and 700°C. Each biochar was mixed at 5% (w/w) with a forest loamy soil and the mixture was incubated at 25°C for 180 days, during which soil physicochemical properties and soil carbon mineralisation were measured. Results showed that the biochar addition increased soil carbon mineralisation at the early stage (within the first 15 days) because biochar brought available organic carbon to the soil and changed associated soil properties, such as increasing soil pH and microbial activity. The largest increase in soil carbon mineralisation at the beginning of incubation was induced by the dairy manure biochar pyrolysed at 300°C. Soil carbon mineralisation was enhanced more significantly by the dairy manure biochars than by the woodchip biochars, and the enhancement effect decreased with increasing pyrolysis temperature. Although the biochar addition induced increased soil carbon mineralisation at the beginning of the incubation, soil carbon mineralisation rates decreased sharply within a short time (within 15 days) and then remained very low afterwards. Carbon mineralisation kinetic modelling indicated that the stable organic matter in biochars could be sequestered in soil for a long time and resulted in high levels of carbon sequestration, especially for the woodchip biochars pyrolysed from higher temperatures.

Additional keywords: biochar, carbon sequestration, feedstock, pyrolysis temperature, soil carbon mineralisation, soil physicochemical properties.

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Introduction

Soil plays a key role in the global carbon (C) cycle. Soil is the most important terrestrial C sink that releases carbon dioxide (CO₂) into the atmosphere. There is much interest in biochar applications to soils as a means of improving soil quality and sequestering C in soils (Lehmann *et al.* 2006; Lehmann 2007; Laird 2008). Biochar is the product of biomass through pyrolysis. Several studies have shown the potential role of biochar in climate change mitigation by offsetting C emission associated with the burning of fossil fuels (Lehmann 2007; Laird 2008).

Soil C mineralisation is the CO₂ efflux produced from soil metabolic processes, which are influenced by many soil properties. The amount and availability of soil organic C has a large impact on the atmospheric CO₂ concentration because soil organic C can be decomposed and eventually returned to the atmosphere (Lal *et al.* 2007). The decomposition rate of soil organic C is determined by various soil physicochemical factors. For instance, Blagodatskaya and Kuzyakov (2008) reported that the amount of primed CO₂ emission increased with increasing soil pH. Warnock *et al.* (2007) proposed that alteration of soil properties, including surface area, pH, and C/N ratio, was partly responsible for changes of soil microbial biomass and activity, and could consequently influence soil carbon mineralisation.

Biochar addition has been shown to have important effects on soil physicochemical properties, including improvement of soil structure, increase in soil pH, and enhancement of soil aeration and water retention capacity (Wardle *et al.* 2008; Downie *et al.* 2009; Laird *et al.* 2010). Nonetheless, results about C mineralisation in soils following biochar addition are not consistent in the literature. Novak *et al.* (2010) reported that biochar addition did not significantly change soil C mineralisation, possibly attributable to the highly condensed aromatic structure that was physically resistant to degradation. Zimmerman *et al.* (2011) observed that biochar produced at low temperatures stimulated C mineralisation due to decomposition of labile components of biochar over a short term, whereas biochar produced at high temperatures suppressed C mineralisation. Luo *et al.* (2011) found that biochars produced with different pyrolysis temperatures consistently promoted soil C mineralisation. Besides the differences in soils and biochars used in these studies, the diverse results should also be related to the accompanying changes in soil physicochemical properties following biochar applications (Sohi *et al.* 2010).

The importance of biochar as a C sequestration agent requires full understanding of its properties and the mechanisms controlling its activity in soils (Abdullah *et al.* 2010; Spokas

2010). For example, the volatile matter content in biochar should be a simple indicator to evaluate the stability of biochar in soils (Zimmerman 2010). The C/N ratio in biochar can change the ratio of fungi to bacteria in soils and hence affect the turnover of soil organic matter (Helfrich *et al.* 2008). All of these biochar properties are largely dependent on the feedstock and pyrolysis condition for biochar production. As shown in the literature, biochars made from wood have higher C/N values than those made from grass (Krull *et al.* 2009). Higher pyrolysis temperatures result in lower volatile matter content and higher C/N ratio in biochar (Braadbaart *et al.* 2004; Downie *et al.* 2009; Krull *et al.* 2009).

To better understand the change in soil C mineralisation and sequestration following biochar additions, it is necessary to quantify the characteristics of different biochars and the effects of biochars on soil physicochemical properties associated with soil C cycling. The objectives of this study were to investigate the characteristics of biochars made with different feedstocks and pyrolysis temperatures, and to study effects of the different biochars on soil physicochemical properties (e.g. soil pH, soil organic C, soil dissolved C) related to soil C mineralisation and sequestration. It was hypothesised that treatments with biochars made with different feedstocks and pyrolysis temperatures affected soil physicochemical properties as well as soil C mineralisation and sequestration differently.

Materials and methods

Production of biochar

Two different types of biomass feedstock (fresh dairy manure and pine tree woodchip) were used for biochar production following the procedure of Yu *et al.* (2013). Briefly, after air-drying and crushing, each of the feedstocks was filled into crucibles sealed with lids to prevent oxygen from entering, and then pyrolysed in a muffle furnace, heating at a rate of $10^{\circ}\text{C min}^{-1}$ and holding for 1 h at 300, 500, or 700°C . Biochars produced from dairy manure at the low (300°C), medium (500°C), and high (700°C) temperatures were denoted DL, DM, and DH, respectively, whereas biochars produced from woodchip were denoted WL, WM, and WH, respectively. After cooling, each biochar was passed through a 2-mm sieve. Biochar characteristics were examined as follows. Structural characteristics of biochars (e.g. distributions of pores and particles) were observed from scanning electron microscopy (SEM) (Peng *et al.* 2011). Surface organic functional groups presented on the biochars were determined by Fourier transform infrared spectroscopy (FTIR) (Nicolet iS10; Thermo Fisher Scientific Inc., Waltham, MA, USA) (Luo *et al.* 2011). Biochar ash content (w/w in %) was determined by dry combustion at 760°C in air for 6 h using a laboratory muffle furnace (Novak *et al.* 2009). Volatile matter was estimated from weight loss of biochar after combustion at 900°C for 6 min in a ceramic crucible (Zimmerman *et al.* 2011). Specific surface area (SSA) of biochar was evaluated using the Brunauer, Emmett, and Teller (BET) nitrogen (N) adsorption technique (Brunauer *et al.* 1938). Concentrations of elemental C, hydrogen (H), and N were determined with an elemental analyser (Vario EL; Elementar Analysensysteme GmbH, Hanau, Germany) (Baneschi *et al.* 2013), from which C/N ratios were

estimated. Biochar pH was measured using a pH probe with a 1 : 2.5 (w/w) suspension of biochar in deionised water (Luo *et al.* 2011). Water-extractable concentrations of NO_3^- were measured with an ion chromatograph (DX-600; Dionex Corp., Sunnyvale, CA, USA) following Yu *et al.* (2013). The initial dissolved organic C (DOC) of biochars was determined with the method of Bruun *et al.* (2012). Briefly, 5 g of biochar was suspended in 25 mL 0.01 M CaCl_2 , which was shaken on a low speed reciprocal shaker for 1 h. The supernatant was filtered through a micro-filter (0.45 μm). The extract was analysed for DOC on a total organic C (TOC) analyser (TOC-V CSH; Shimadzu Corp., Kyoto, Japan).

Soil collection and experimental design

Soil samples (0–10 cm depth) were collected in the Dinghushan Nature Reserve ($23^{\circ}09'21''$ – $23^{\circ}11'30''\text{N}$, $112^{\circ}30'39''$ – $112^{\circ}33'41''\text{E}$), Guangdong Province in southern China. After air-drying, the soil samples were sieved with a 2-mm sieve and thoroughly homogenised. The soil was a forest loamy soil with 40% sand, 35% silt, and 25% clay, and with a bulk density of 1.22 g cm^{-3} . Total C, H, and N, and initial DOC of the soil were measured using the methods mentioned above.

An incubation experiment was conducted at 25°C for 180 days as follows. Each of the six types of biochar was mixed into 120 g of the air-dried soil sample based on a rate of 5% (w : w on an air-dried weight basis), and soil without biochar addition was used as the control. The seven treatments were denoted with S (for soil) followed by the biochar treatment acronym, and CK (the control). The initial C contents for CK, SDL, SDM, SDH, SWL, SWM, and SWH were 10.7, 30.3, 20.2, 24.7, 36.3, 32.3, and 26.3 mg C g^{-1} soil, respectively. The bulk density of the mixture of soil and biochar was calculated as follows (Adams 1973):

$$\rho_b = 100 / [(x/\rho_1) + (100 - x)/\rho_2] \quad (1)$$

where ρ_b is the bulk density of the mixture of soil and biochar (g cm^{-3}), x is the percentage by weight of biochar, ρ_1 is the bulk density of biochar (g cm^{-3}), and ρ_2 is the bulk density of soil (g cm^{-3}). A glass cylinder was filled with biochar particles. The filled volume and mass of biochar in the cylinder were used to calculate the biochar bulk density (Pastor-Villegas *et al.* 2006). Bulk densities were 1.22, 0.60, 0.60, 0.59, 0.29, 0.41, and 0.38 g cm^{-3} for the soil and biochars DL, DM, DH, WL, WM, and WH, respectively. Based on Eqn 1, the initial bulk densities of the soil–biochar mixtures were 1.16, 1.16, 1.16, 1.05, 1.11, and 1.10 g cm^{-3} for SDL, SDM, SDH, SWL, SWM, and SWH, respectively. Each of the mixtures of soil and biochar or the soil alone was packed into a 500-mL pot based on the bulk densities above. The pot was covered with a piece of plastic sheet to prevent moisture loss. A few small holes were pricked on the plastic sheet to keep atmosphere pressure inside the bottle. For each pot, deionised water was added to retain a volumetric water content of 80% of field water capacity (the water content at suction of 330 cm) determined based on the soil–water retention curve (Ouyang and Zhang 2013). During the incubation period, the soil water content was adjusted weekly based on weight loss. Twenty-four replicates (pots) were prepared for each treatment.

Chemical analyses

Soil organic C (SOC), DOC, microbial biomass C (MBC), pH, and C/N ratio were measured on days 0, 5, 15, 30, 50, 80, 120, and 180 of the incubation experiment. These measurements were conducted using three destructive samples from the replicates on each sampling date. The SOC of the mixtures was measured by oxidation with potassium dichromate (Peng *et al.* 2011), DOC was measured using the method of Bruun *et al.* (2012), and MBC was determined using the fumigation–extraction method, in which soil K₂SO₄ extract was analysed using the TOC analyser (Brookes *et al.* 1985). Sample pH and C/N ratio were measured using the methods mentioned above.

Soil C mineralisation measurements were conducted every day from days 0 to 15, every 3 days from days 16 to 60, and every 10 days from day 61 to the end of incubation, using the method of Tang *et al.* (2011). At each measurement day, the initial concentration of CO₂ within the headspace of each incubation pot was measured with an infrared ray gas analyzer (IRGA). Then the pot was airproofed. After 2 hours, the concentration of CO₂ within the headspace of the pot was measured with IRGA. The CO₂ emission rate was calculated using the following relationship:

$$F_{\text{CO}_2} = [(\Delta C/\Delta t)(VM_C/22.4)(T_1/T_2)(P_2/P_1)]/m_s \quad (2)$$

where F_{CO_2} is the CO₂ emission rate ($\mu\text{g C s}^{-1} \text{ g}^{-1} \text{ soil}$), $\Delta C/\Delta t$ is the CO₂ concentration gradient calculated with the two measurements at each measurement day ($\mu\text{g g}^{-1} \text{ soil s}^{-1}$), V is the headspace volume (L), M_C is the C molecule weight (g mol^{-1}), T_1 is the temperature in the normal state (273 K), T_2 is the air temperature in the headspace (K), P_1 is the air pressure in the normal state ($1.013 \times 10^5 \text{ Pa}$), P_2 is the air pressure in the headspace (Pa), and m_s is the dry mass of total incubated soil in the pot (g). Carbon mineralisation kinetics was fitted with a two-pool model as follows (Cayuela *et al.* 2010):

$$C_r = f \exp(-k_1 t) + (100 - f) \exp(-k_2 t) \quad (3)$$

where C_r is the C fraction remained in the soil (%), f is the C fraction of the active or fast turnover pool (%), k_1 is the decomposition rate constant for the active pool (day^{-1}), k_2 is

the rate constant for the slow turnover pool (day^{-1}), and t is the incubation time (days). The humification coefficient (h) is defined as the fraction of organic matter remaining in the soil after 1 year and is calculated with Eqn 3 by setting $t = 365$ days (Bradbury *et al.* 1993). The humification coefficient gives an estimation of the stable organic matter remaining in the soil and it is used as an indicator of C sequestration potential (Cayuela *et al.* 2010).

Statistical analyses

All data were analysed using the SPSS software package (SPSS Inc. 2003). Significant differences among different treatments and sampling dates were tested by the one-way analysis of variance (ANOVA), in which the post-hoc test of least significant difference (l.s.d.) was used. The two-way ANOVA was used to identify the primary factors (feedstock and pyrolysis temperature) on C mineralisation. Differences between the values were considered to be statistically significant at $P = 0.05$. The coefficient of determination (R^2) was used to determine the accuracy of regressions.

Results

Biochar properties

The measured physicochemical properties of the biochars and soil are listed in Table 1. The pH values of biochars were significantly higher than that of the soil ($P < 0.05$). The woodchip biochars had higher C/N ratio and specific surface area than the dairy manure biochars. The volatile component of the biochars decreased with increasing pyrolysis temperature, and the dairy manure biochars contained a higher volatile component than the woodchip biochars. Under the same pyrolysis temperature, the concentrations of available N were significantly higher in the dairy manure biochars than in the woodchip biochars ($P < 0.05$). Most of the initial DOC values were significantly higher in the biochars, especially in the dairy biochars produced at lower temperatures, than in the soil ($P < 0.05$). Higher pyrolysis temperatures resulted in greater surface areas, higher pH values and ash contents, and lower available N contents (Table 1). Scanning electron microscopy of the biochars showed that biochar particles became smaller as the pyrolysis temperatures increased (Ouyang and Zhang 2013).

Table 1. Selected physicochemical characteristics of biochars and soil

DL, DM, and DH: Biochars produced with dairy manure at the low (300°C), medium (500°C), and high (700°C) temperatures, respectively; WL, WM, and WH: biochars produced with woodchip at the low, medium, and high temperatures; SSA, specific surface area of biochar, DOC, dissolved organic carbon. Results for pH, ash content, volatile matter, NO₃⁻-N, and DOC were calculated based on three replicates and are presented as mean \pm standard deviation; values of C, H, N, and SSA were obtained from one measurement. –, Data not determined

Property	Soil	DL	DM	DH	WL	WM	WH
pH (1:5 w/v)	4.25 \pm 0.03	7.46 \pm 0.01	9.28 \pm 0.09	9.70 \pm 0.03	7.16 \pm 0.04	7.96 \pm 0.04	10.1 \pm 0.12
C (%)	1.07	39.3	19.0	27.9	51.2	43.2	31.1
H (%)	0.67	3.79	1.13	0.97	4.47	2.03	1.01
N (%)	0.08	2.68	1.16	1.09	0.97	1.11	0.61
C/N ratio	13.4	14.6	16.4	25.7	52.9	38.9	51.4
Ash content (%)		50.3 \pm 1.62	63.7 \pm 2.33	68.7 \pm 0.78	40.1 \pm 2.12	55.6 \pm 1.54	59.2 \pm 2.43
Volatile matter (%)		38.6 \pm 2.01	13.7 \pm 0.60	12.1 \pm 1.13	30.1 \pm 1.28	6.50 \pm 0.47	1.31 \pm 0.32
SSA ($\text{m}^2 \text{ g}^{-1}$)	11.5	14.3	44.1	83.4	24.04	67.3	124
NO ₃ ⁻ -N (mg kg^{-1})	–	13.1 \pm 0.70	10.5 \pm 0.75	9.93 \pm 1.01	12.5 \pm 0.51	8.76 \pm 0.65	8.39 \pm 0.53
DOC (mg kg^{-1})	69.1 \pm 8.2	339 \pm 14	172 \pm 8.3	97.8 \pm 10	110 \pm 8.9	63.4 \pm 4.1	58.9 \pm 6.6

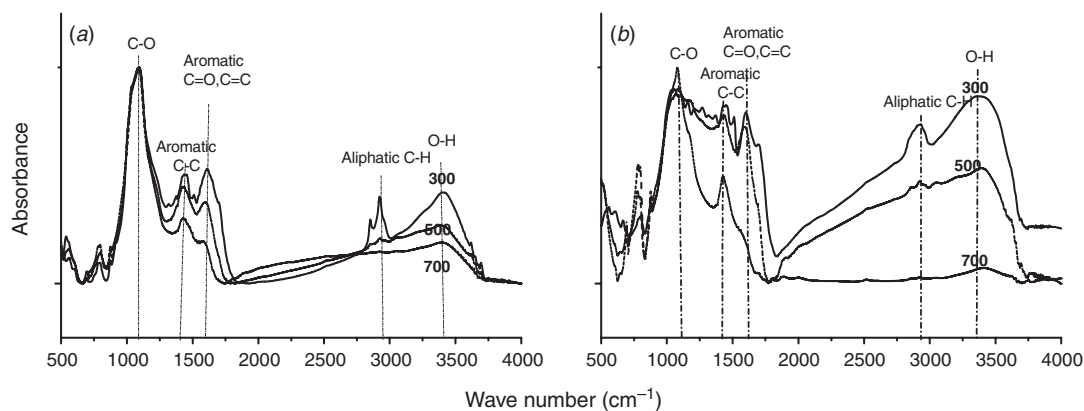


Fig. 1. Fourier transform infrared spectroscopy (FTIR) of (a) dairy manure biochars and (b) woodchip biochars produced under temperatures from 300 to 700°C.

Graphs of FTIR are presented for the dairy manure biochars (Fig. 1a) and the woodchip biochars (Fig. 1b) produced under temperatures from 300 to 700°C. The spectra included several adsorption bands. The band around 800–1600 cm^{-1} was associated with aromatic C-H, C=C, C=O stretching, 2900 cm^{-1} with aliphatic C-H stretching, and 3400 cm^{-1} with O-H stretching, respectively. As the charring temperature increased, adsorption intensities of biochar at the bands 3400 cm^{-1} and 2900 cm^{-1} decreased, indicating a reduction of O-H and aliphatic C-H bonds, whereas the adsorption at the band 1400 cm^{-1} was intensified, indicating an increase of aromatic C, especially for the woodchip biochars (Peng *et al.* 2011).

Carbon mineralisation

Throughout the incubation, maximum mineralisation rates were observed for all seven treatments on day 1, with the SDL treatment showing the highest mineralisation rate (Fig. 2). For the same feedstock, the biochar-induced increases in soil mineralisation at the early incubation stage were $\text{SDL} > \text{SDM} > \text{SDH}$ (increase by 5.41, 2.69, and 1.92 times over CK, respectively), and $\text{SWL} > \text{SWM} > \text{SWH}$ (increase by 2.58, 1.78, and 1.61 times over CK, respectively). At the later incubation stage (after about day 15), except for SDL, the differences of soil C mineralisation rates among the six treatments were not significant ($P > 0.05$). Soil cumulative CO_2 emissions were significantly different among the seven treatments, ranging from 41.31 to 224.4 $\text{mg CO}_2\text{-C kg}^{-1}$ soil day^{-1} at the end of the incubation ($P < 0.05$) (Fig. 3). Soil cumulative CO_2 emissions were significantly different between treatments with the dairy manure biochars and the woodchip biochars produced with the same pyrolysis temperature ($P < 0.05$). In all biochar treatments, the cumulative C losses in the soil were positively correlated with the volatile matter contents in the biochars ($R^2 = 0.75$). The two-way ANOVA analysis showed that partial η^2 values for feedstock *v.* C mineralisation and pyrolysis temperature *v.* C mineralisation were 0.985 and 0.993, respectively, indicating that the pyrolysis temperature and the feedstock were equally important in affecting C mineralisation. The temporal distributions of C mineralisation rates in all treatments were similar (Figs 2 and 3).

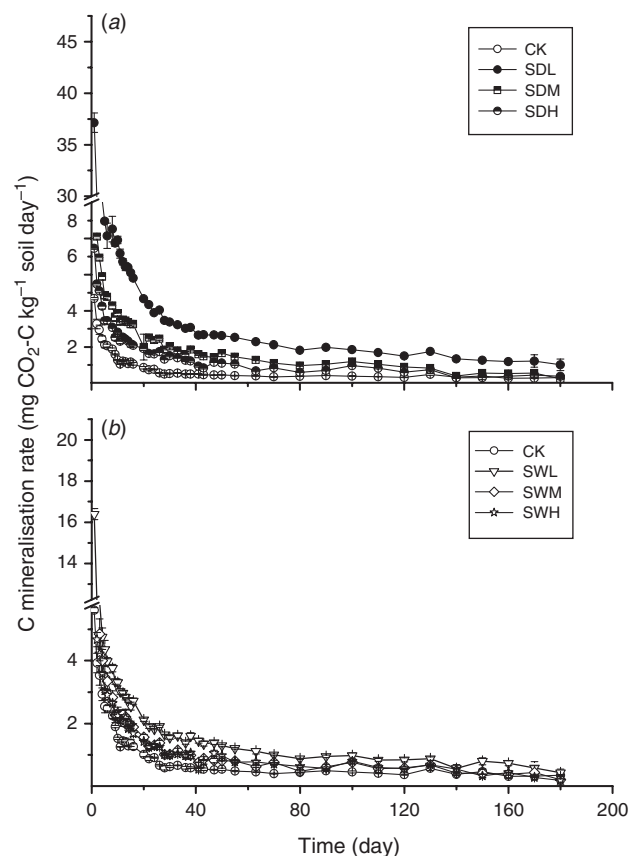


Fig. 2. Soil carbon (C) mineralisation rates (mean \pm standard deviation) of (a) the control (CK, soil only) and treatments with dairy manure biochars (SDL, SDM, SDH: soil + dairy manure pyrolysed at 300, 500, and 700°C, respectively); and (b) CK and treatments with woodchip biochars (SWL, SWM, SWH: soil + woodchip pyrolysed at 300, 500, and 700°C) ($n = 3$).

The C decay patterns in soil after the application of different biochars were fitted with the two-pool model. As shown in Table 2, values of the C fraction of the active or fast turnover pool (f) for the seven treatments ranged from 0.18% to 0.61%, whereas values of the humification coefficient (h) were $>99\%$. Values of the decomposition rate constant for the active pool (k_1)

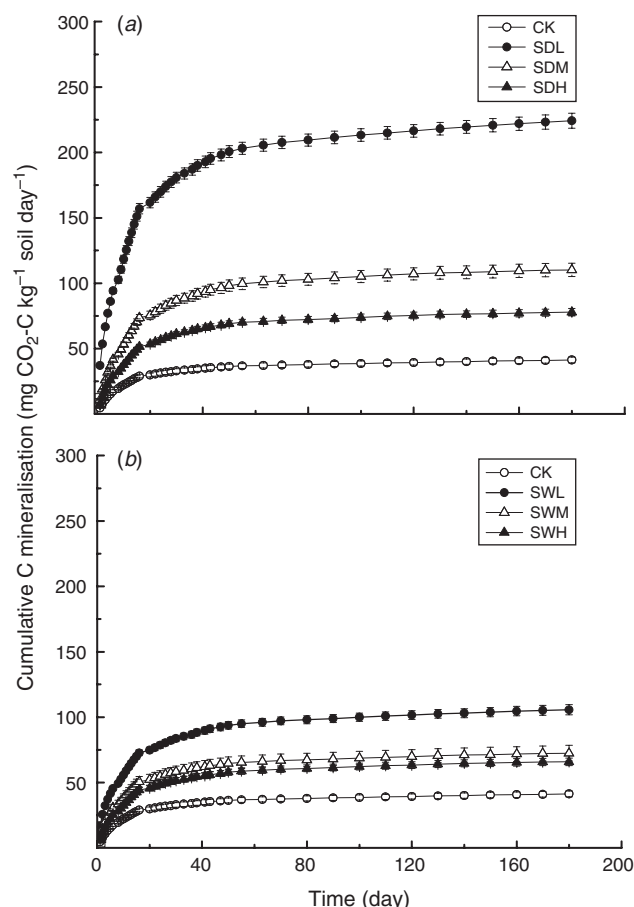


Fig. 3. Soil cumulative carbon (C) emissions (mean \pm standard deviation) of (a) the control (CK: soil only) and treatments with dairy manure biochars (SDL, SDM, SDH: soil + dairy manure pyrolysed at 300, 500, and 700°C, respectively); and (b) CK and treatments with woodchip biochars (SWL, SWM, SWH: soil + woodchip pyrolysed at 300, 500, and 700°C) ($n = 3$).

Table 2. Parameters obtained by fitting the two-pool model to measured data

CK, Soil only; SDL, SDM, SDH: soil amended with biochars produced with dairy manure at the low (300°C), medium (500°C), and high (700°C) temperatures, respectively; SWL, SWM, SWH: soil amended with biochars produced with woodchip at the low, medium, and high temperatures; f , the fraction of organic matter in the active pool; k_1 , the rate constant of the active pool; k_2 , the rate constant of the slow pool; h , the humification coefficient

Treatment	f (%)	k_1 (day ⁻¹)	k_2 (day ⁻¹)	R^2	h (%)
CK	0.31	0.111	4.6×10^{-6}	0.996	99.4
SDL	0.61	0.107	8.4×10^{-6}	0.990	99.1
SDM	0.45	0.087	5.8×10^{-6}	0.996	99.3
SDH	0.26	0.087	3.4×10^{-6}	0.996	99.6
SWL	0.23	0.112	3.7×10^{-6}	0.989	99.6
SWM	0.18	0.105	2.5×10^{-6}	0.994	99.7
SWH	0.20	0.095	3.0×10^{-6}	0.996	99.7

in all treatments were ~ 0.1 day⁻¹, whereas values of the rate constant for the slow turnover pool (k_2) were as low as 2.5×10^{-6} to 8.4×10^{-6} day⁻¹. The values of f and k_2 in all biochar-treated soils, except SDL and SDM, were lower than

Table 3. Remaining fraction of total C (mg C g⁻¹ soil) in soil over time calculated with the two-pool model

CK, Soil only; SDL, SDM, SDH: soil amended with biochars produced with dairy manure at the low (300°C), medium (500°C), and high (700°C) temperatures, respectively; SWL, SWM, SWH: soil amended with biochars produced with woodchip at the low, medium, and high temperatures

Treatment	Day 0	Day 5	Day 180	1 year	5 years	10 years
CK	10.70	10.68	10.66	10.65	10.58	10.49
SDL	30.34	30.26	30.11	30.26	29.70	29.24
SDM	20.21	20.18	20.10	20.08	19.91	19.70
SDH	24.65	24.63	24.57	24.56	24.43	24.28
SWL	36.32	36.28	36.21	36.19	35.99	35.75
SWM	32.29	32.27	32.22	32.20	32.09	31.94
SWH	26.27	26.25	26.20	26.19	26.07	25.93

those of CK. After the 180-day incubation, all biochar treatments led to >99% of C sequestered in the mixtures, and the h values and the remaining C amount were higher in the treatments with the woodchip biochars than with dairy manure biochars (Table 2). Based on the two-pool model simulation results (Table 3), the amounts of sequestered C in biochar-treated soils will increase by 1.88–3.28 times compared with the sole soil in 10 years.

Soil organic C, and dissolved organic C

The biochar application systematically increased SOC, although the increases varied with the different biochars (Table 4). Biochars produced under the lower pyrolysis temperature (300°C) increased the SOC more markedly than biochars produced at the higher pyrolysis temperatures (500 and 700°C). The SOC values of the treatments with the woodchip biochars were significantly higher than those with the dairy manure biochars produced with the same pyrolysis temperature ($P < 0.05$).

As shown in Fig. 4, the DOC values were significantly higher ($P < 0.05$) in most of biochar-treated soils than in the control. The DOC values in the biochar treatments decreased as pyrolysis temperatures increased. With the same pyrolysis temperature, the dairy manure biochars led to a larger increase in soil DOC than the woodchip biochars. The temporal changes of DOC, which decreased with time within the whole incubation, were similar for all treatments.

For the different sampling dates, the C mineralisation rates of the seven treatments were positively correlated with the corresponding DOC values, with R^2 values ranging from 0.707 to 0.953. The slopes of the linear relationships decreased from 0.089 on day 0 to 0.0024 on day 180. For the samplings on days 0, 5, and 30, the C mineralisation rates and the MBC values of the seven treatments also showed a positive relationship, with R^2 values of 0.936, 0.726, and 0.775, respectively. However, R^2 values of the linear regressions between the C mineralisation rates and the MBC values for days 80, 120, and 180 were 0.428, 0.107, and 0.034, respectively.

Soil pH and C/N

Soil pH values significantly increased with the addition of biochar (Fig. 5). On average, the different biochar treatments

Table 4. Values of soil organic carbon (mg g⁻¹ soil) with different treatments

CK, Soil only; SDL, SDM, SDH: soil amended with biochars produced with dairy manure at the low (300°C), medium (500°C), and high (700°C) temperatures, respectively; SWL, SWM, SWH: soil amended with biochars produced with woodchip at the low, medium, and high temperatures. Values presented in columns are mean \pm standard deviation ($n=3$). Within columns, means followed by the same lower case letter are not significantly different ($P>0.05$). Within rows, means followed by the same upper case letter are not significantly different ($P>0.05$)

Treatment	Day 5	Day 15	Day 30	Day 50	Day 80	Day 120	Day 180
CK	8.43 \pm 0.29aD ^c	8.79 \pm 0.10aD	9.00 \pm 0.34aD	6.75 \pm 0.15aC	6.56 \pm 0.53aBC	6.06 \pm 0.10aB	4.78 \pm 0.26aA
SDL	28.4 \pm 0.05eC	29.1 \pm 0.79eC	27.0 \pm 0.74eC	25.8 \pm 0.82eB	20.3 \pm 0.70eB	20.3 \pm 0.61dB	20.1 \pm 1.55dA
SDM	25.7 \pm 0.82cdC	26.7 \pm 0.16cC	25.8 \pm 0.70deC	22.1 \pm 0.65bcB	20.3 \pm 0.80cdAB	19.2 \pm 1.21bcA	18.7 \pm 0.64cdA
SDH	25.3 \pm 1.55cD	22.7 \pm 0.81bCD	23.5 \pm 1.05cD	20.4 \pm 0.81bBC	19.5 \pm 0.67bcB	17.6 \pm 0.68cB	15.8 \pm 0.67bAA
SWL	35.4 \pm 0.88fC	32.5 \pm 0.50dB	27.0 \pm 0.67eA	26.3 \pm 1.46dA	26.2 \pm 0.84fA	25.8 \pm 1.23eA	24.7 \pm 0.37eA
SWM	28.1 \pm 0.41deD	26.7 \pm 1.26cCD	24.6 \pm 1.01cdC	22.1 \pm 1.05bcB	21.0 \pm 0.42dB	20.4 \pm 0.08cAB	18.4 \pm 1.32cA
SWH	21.3 \pm 0.14bCD	22.0 \pm 0.08bD	19.7 \pm 0.96bBC	20.7 \pm 0.17bBC	18.9 \pm 0.39bABC	17.9 \pm 1.43bAB	17.3 \pm 1.03bcA

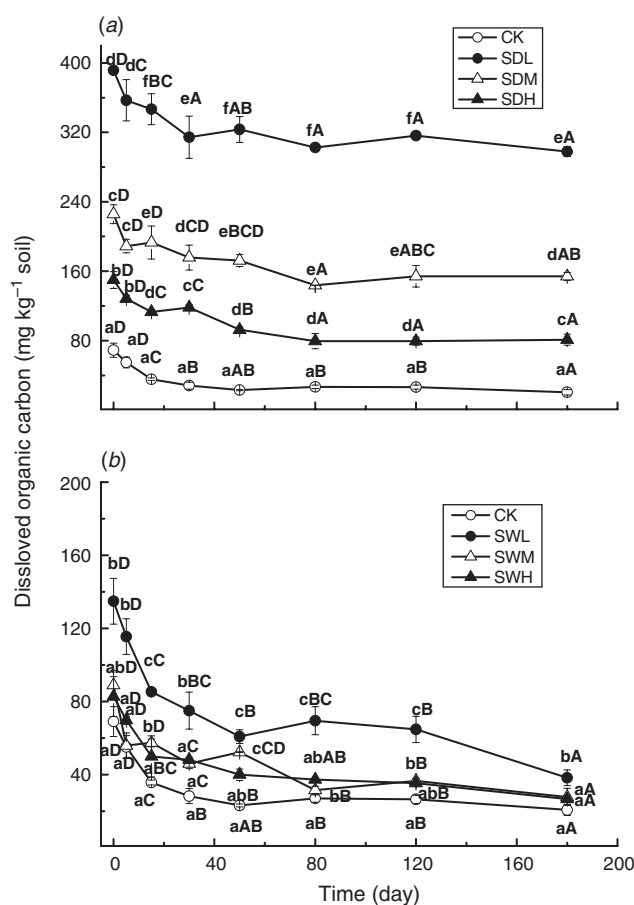


Fig. 4. Soil dissolved organic carbon values (mean \pm standard deviation) of (a) the control (CK: soil only) and treatments with dairy manure biochars (SDL, SDM, SDH: soil + dairy manure pyrolysed at 300, 500, and 700°C, respectively); and (b) CK and treatments with woodchip biochars (SWL, SWM, SWH: soil + woodchip pyrolysed at 300, 500, and 700°C) ($n=3$).

resulted in an increase of 0.79–2.21 pH units. On the sampling dates, SDM and SWH resulted in the largest increases in pH among the dairy manure biochars and the woodchip biochars, respectively. The dairy manure biochars had greater effect in increasing soil pH than the woodchip biochars. After 120 days of incubation, pH values decreased significantly with time in all treatments relative to the start of the experiment.

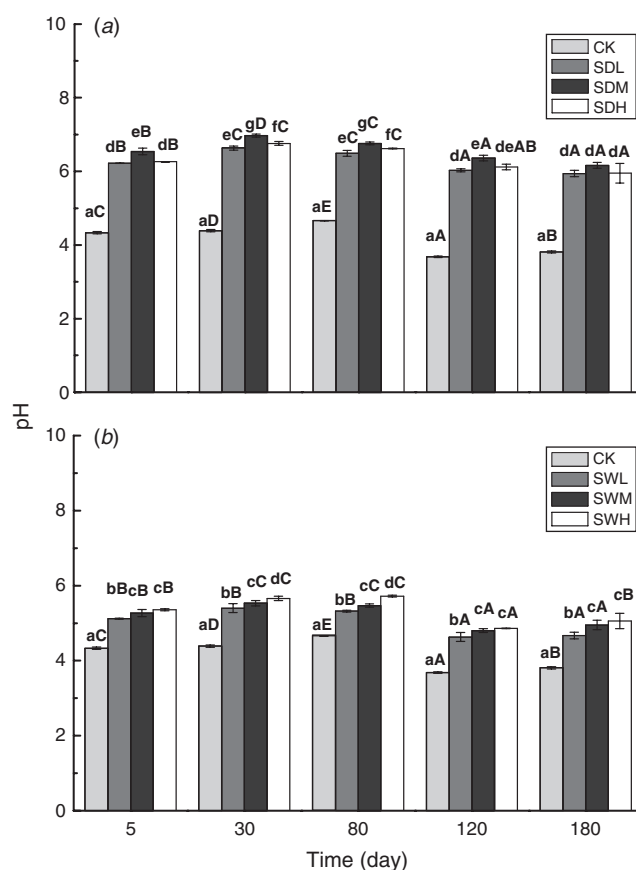


Fig. 5. Soil pH values (mean \pm standard deviation) of (a) the control (CK: soil only) and treatments with dairy manure biochars (SDL, SDM, SDH: soil + dairy manure pyrolysed at 300, 500, and 700°C, respectively); and (b) CK and treatments with woodchip biochars (SWL, SWM, SWH: soil + woodchip pyrolysed at 300, 500, and 700°C). Among the seven treatments for a single day, the same lower case letter indicates no significant difference ($P>0.05$). Among the six sampling days (days 5, 30, 80, 120, and 180) for a single treatment, the same upper case letter indicates no significant difference ($P>0.05$) ($n=3$).

Most of the biochar treatments increased the C/N ratio of the mixtures, with the extent of increase related to the feedstock and the pyrolysis temperature for biochars (Table 5). The C/N ratios were significantly higher in the woodchip biochar treatments

Table 5. Values of the C/N ratio in different treatments

CK, Soil only; SDL, SDM, SDH: soil amended with biochars produced with dairy manure at the low (300°C), medium (500°C), and high (700°C) temperatures, respectively; SWL, SWM, SWH: soil amended with biochars produced with woodchip at the low, medium, and high temperatures. Values presented are mean \pm standard deviation ($n=3$). Within columns, means followed by the same lower case letter are not significantly different ($P>0.05$). Within rows, means followed by the same upper case letter are not significantly different ($P>0.05$)

Treatment	Day 5	Day 80	Day180
CK	13.72 \pm 1.257aB ^c	13.83 \pm 0.492aB	10.83 \pm 0.351aA
SDL	13.50 \pm 0.377aA	13.27 \pm 0.061aA	13.15 \pm 0.073bA
SDM	14.47 \pm 0.318aA	14.73 \pm 0.160aA	13.65 \pm 0.914bA
SDH	18.88 \pm 1.182bA	18.48 \pm 0.083bA	17.21 \pm 0.981cA
SWL	30.68 \pm 1.617eB	29.08 \pm 0.988dB	21.22 \pm 0.963dA
SWM	25.74 \pm 0.079cA	25.51 \pm 0.024cA	27.35 \pm 0.202fB
SWH	28.01 \pm 0.604dA	26.99 \pm 1.369cA	25.28 \pm 0.958eA

than in the dairy manure biochar treatments ($P<0.05$). The C/N values in the dairy manure biochar treatments increased with the pyrolysis temperature, whereas in the woodchip biochar treatments, C/N ratio in SWL (300°C) was the highest at the early incubation stage.

Discussion

Similar to other studies (Hamer *et al.* 2004; Hilscher *et al.* 2009), all of the biochars examined here stimulated soil C mineralisation, especially at the early incubation stage. The increase in soil C mineralisation was a result of stimulation of microbial activity through the addition of labile C and nutrients within biochars (Hamer *et al.* 2004). The volatile matter and available nutrients in the biochars (Table 1) could be a good source for microorganisms, resulting in an increase in SOC and DOC in all biochar-treated soils, and consequently enhancing the microbial biomass. In this study, biochar application increased the SOC content, possibly because biochar contained high C content and could protect organic C from utilisation (Glaser *et al.* 2002; Cayuela *et al.* 2010). In addition, the woodchip biochars were mostly composed of highly condensed, aromatic structures that were physically resistant to degradation. Although the application of woodchip biochars resulted in higher SOC values, the dairy manure biochar treatments contained higher DOC content, which could provide a more labile source of energy and nutrients for soil microorganisms (Bowen 2006). The linear relationships between the C mineralisation rates and DOC values of the seven treatments indicated that the enhanced C mineralisation was partly attributed to increased DOC values following biochar additions. This may explain the higher C mineralisation rates in the soil treated with the dairy manure biochars. The different contents of labile C in the seven treatments were also reflected from their distinct microbial responses. As reported by Ouyang and Zhang (2013), the MBC was significantly higher in the biochar treatments than in the control, which showed the stimulating effects of biochars on soil microbial activities. Similar results have been reported in the literature, mainly related to the availability of an easily decomposable, non-aromatic fraction of biochar (Steiner *et al.*

2008; Kolb *et al.* 2009; Kuzyakov *et al.* 2009; Novak *et al.* 2010). The biochars pyrolysed at lower temperatures induced a greater increase in C mineralisation than the biochars pyrolysed at higher temperatures, primarily because the biochars produced at lower temperatures contained more of the incompletely pyrolysed fraction of the feedstock material (Bruun *et al.* 2011). For instance, biochars pyrolysed at higher temperatures have less impact on soil MBC and DOC during the incubation. Further, the different chemical properties and physical structures of biochars, which were largely determined by feedstock and pyrolysis temperatures, significantly influenced short-term biochar-C loss. As shown in Fig. 1, more aromatic C-C bonds in biochars pyrolysed at higher temperatures indicated greater chemical recalcitrance and stability of the biochars, whereas more aliphatic C-H bonds in biochars pyrolysed at lower temperatures indicated more useable substrate in the biochars (Bruun *et al.* 2008; Yuan *et al.* 2011). The dairy manure biochars decayed faster than the woodchip biochars, which might be due to the lower C/N ratios and higher available-N contents in the dairy manure biochars (Helfrich *et al.* 2008).

The increase in soil pH with biochar application also affected C mineralisation. Increases in soil pH can stimulate CO₂ emission, and the effect is more pronounced in low pH soils (Blagodatskaya and Kuzyakov 2008; Luo *et al.* 2011). With relatively low pH in our soil (4.25), the significantly increased pH values in the mixtures after applying biochar should partially contribute to the increased CO₂ emission. Another factor contributing to the increased CO₂ emission in the biochar-amended soil might be that the biochar enhanced mineralisation of native organic matter in the soil (Wardle *et al.* 2008). Soil C/N ratio and pH can change the microbial population and activity (Helfrich *et al.* 2008). The short-term microbial responses might also be related to the increased solubility of organic matter with the increase in soil pH (Curtin *et al.* 1998). The increased pH values in the original low pH soil could also enhance the availability of nutrients and consequently increase soil microbial biomass (Atkinson *et al.* 2010; Warnock *et al.* 2010). At the early stage of incubation, the increase in soil pH after biochar addition might benefit r-strategists, which are more dominant in higher pH soils (Bååth and Anderson 2003) and can rapidly utilise labile components (Fig. 5). At the later stage of incubation, rates of soil C mineralisation were not significantly different among the treatments. A possible reason for the result is that the labile composition of biochars was almost mineralised and the soil organic matter might be adsorbed to biochars, either within biochar pores or onto external biochar surfaces (Zimmerman *et al.* 2011). Sobek *et al.* (2009) showed that biochars could adsorb organic matter and protect it from being utilised. In this study, the lower soil mineralisation rates in the treatments with biochars produced at higher temperatures than at lower temperatures might be partly attributable to their higher surface areas with greater adsorption affinity for natural organic matter (Kasozzi *et al.* 2010).

Although biochar addition induced significant short-term CO₂ emission from the soil, rates of soil C mineralisation decreased sharply within a short period and then remained at stably low values with time. Most of the C (>99%) from the

biochar addition remained in the soil for a long time, indicating that biochar has high C-sequestration potential. The low degradability of C from biochar has been reported in the literature (Kuzyakov *et al.* 2009; Major *et al.* 2010) and is strongly promoted for C-sequestration aims (Lehmann 2007; Laird *et al.* 2009). The small rate constant values for the slow turnover pool (in the order of 10^{-6} day⁻¹) indicate that the increased C resulting from biochar application will stay in the soil for long time. The higher refractory C in biochar should also account for the C-sequestration potential of biochar. As suggested by Cayuela *et al.* (2010), biochar with more refractory C and higher C/N ratio had higher C sequestration potential. Compared with the dairy manure biochar treatments, the woodchip biochar treatments resulted in lower rates of C mineralisation, and higher values of SOC, C/N ratio, and humification coefficient, suggesting that the woodchip biochars should have higher C-sequestration potential. Although the increase of pyrolysis temperature resulted in a decrease in C content in biochar, the treatments with biochars produced at higher temperatures showed higher C-sequestration ability, as indicated by the lower rate constant for the slow turnover pool and higher humification coefficient.

Conclusions

This study investigated the effects of biochars produced from dairy manure and woodchip under different temperatures on soil C turnover. The biochar application induced a rapid increase in soil C mineralisation during the early incubation period (within the first 15 days), primarily because of the labile C fraction in the biochars. However, rates of soil C mineralisation decreased sharply after a short period and then remained at stably low values with time. Soil physicochemical changes with the biochar addition, including increases in SOC, DOC, MBC, and pH values, also affected soil C mineralisation and sequestration. The feedstock and pyrolysis temperature largely determined the physicochemical properties of biochars and, hence, had a significant impact on effects of the biochars on soil C turnover and associated soil properties. The dairy manure biochars produced at lower temperature, which contained more labile fraction and relatively easily degradable structure, resulted in the largest increase in soil C mineralisation and the highest microbial biomass. Although significant C losses from soils treated with biochars were observed, the incorporation of biochar increased total SOC and compensated for the C losses in the soil. The high humification coefficients and low rate constants for the slow turnover pool in the biochar-treated soils indicated the biochar's C-sequestration potential. Meanwhile, >99% of biochar-induced C remained as stable organic C in the soil after 180 days, further demonstrating the ability of biochar in sequestering C. Biochar produced from woodchip and at higher pyrolysis temperature showed higher C sequestration potential. The information from this study should be valuable for the utilisation of biochar for soil C sequestration.

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