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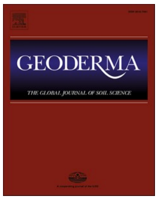

Applying biochar under topsoil facilitates soil carbon sequestration: A case study in a dryland agricultural system on the Loess Plateau

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ABSTRACT

The remarkable soil carbon sequestration and greenhouse gas mitigation effects of biochar have spurred great interest in exploring ways to maximize its benefits. However, it remains unclear how biochar application depth impacts soil carbon dioxide (CO_2) emissions and methane (CH_4) uptake in upland soil. Therefore, we carried out a 16-month field experiment in a dryland agricultural system to answer the above questions. Woody biochar (20 t ha^{-1}) was mixed into three soil layers: 0–10 cm ($BC_{0\text{-}10cm}$), 10–20 cm ($BC_{10\text{-}20cm}$), and 0–20 cm ($BC_{0\text{-}20cm}$). Soil without biochar addition was used as the control (CK). We monitored soil CO_2 and CH_4 fluxes continuously and determined the metabolic quotient (qCO_2) and the sensitivity of soil respiration to temperature (Q_{10}). The results indicated that CO_2 emissions, CH_4 uptake, qCO_2 and Q_{10} were significantly affected by biochar application depth. Overall, compared with CK, $BC_{0\text{-}10cm}$ increased total CO_2 emissions by 10.13%, while $BC_{10\text{-}20cm}$ and $BC_{0\text{-}20cm}$ showed no significant effect. $BC_{0\text{-}10cm}$ and $BC_{0\text{-}20cm}$ exhibited greater soil CH_4 uptake enhancement than $BC_{10\text{-}20cm}$, but the enhanced CH_4 uptake resulted in limited net greenhouse gas mitigation. $BC_{10\text{-}20cm}$ and $BC_{0\text{-}20cm}$ had a lower qCO_2 than the other treatments, which likely increased the carbon use efficiency and decreased the stress on soil microbes, but $BC_{0\text{-}10cm}$ showed the opposite effect. In addition, $BC_{0\text{-}10cm}$ significantly reduced Q_{10} mainly due to the enhanced lability of the native carbon and microbial activities. Changes in environmental factors in the 0–10 cm soil largely explained the variations in CO_2 emissions, CH_4 uptake and Q_{10} (>88%). Nevertheless, the enhanced microbial biomass in the 10–20 cm soil helped lower qCO_2 in the whole 0–20 cm layer. In summary, adding biochar to surface soil (0–10 cm) likely accelerates carbon loss, due to the strong shift in the environment of the surface soil caused by complex interactions among hydrothermal conditions, nutrient levels (i.e., N, NH_4^+, NO_3^- and available P) and labile carbon. However, adding biochar to subsurface soil (10–20 cm) can effectively avoid severe disturbance of the surface soil environment and thus benefit soil carbon sequestration in the long term.

1. Introduction

Evidence suggests that agricultural production contributes 10–14% of biogenic greenhouse gases, mainly via emissions from soil (Paustian et al., 2016; Xu et al., 2019). Additionally, intensive agricultural production has caused considerable soil carbon loss to the atmosphere (Lal, 2004). In the context of global warming and soil degradation, adding biochar, the solid product of organic matter pyrolysis (Lehmann et al., 2011), to soil has been considered a promising strategy to enhance long-term soil carbon sequestration and greenhouse gas mitigation (Bamminger et al., 2018). Numerous studies have shown that the potential of biochar for greenhouse gas mitigation is affected by many factors, such as the type of biochar and soil (Hawthorne et al., 2017; Zhou et al., 2017b), the biochar addition rate and experiment duration (Li et al., 2020; Liu et al., 2019), and soil moisture and temperature (Hawthorne et al., 2017; Karhu et al., 2011). However, most of the studies have been based on a homogeneous mixing system of biochar and soil (He et al., 2016), and biochar application methods, such as application depth, as a potentially important factor affecting greenhouse gas emissions have not received due attention.

Recent studies have shown that biochar surface application and biochar incorporation at different soil depths can change the soil's

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hydraulic properties and the processes controlling inorganic nitrogen (N) and dissolved organic carbon (DOC) leaching; these changes are mainly driven by different biochar application strategies altering soil layer structures and thereby affecting soil porosity and continuity (Castellini et al., 2015; Li et al., 2016, 2018; Liu et al., 2016). Additionally, incorporating biochar into the 0–20 cm soil generally increases the surface soil sensitivity to air temperature changes because it interferes with bidirectional heat movement (Ding et al., 2019), while adding biochar to deep soil layers may offset this effect (He et al., 2016). Generally, the increased CO_2 emissions observed after biochar application have been attributed to the priming effect of labile carbon attached to the surface and internal pores of biochar on native soil organic carbon (SOC) mineralization (Lu et al., 2019). The decrease in soil CO_2 emissions is attributed to the C-degrading microbial activity that is inhibited by biochar and biochar-mineral interactions (Grunwald et al., 2017). However, the impact of management practices and soil environmental variables on CO_2 production also vary depending on the soil layer (Yu et al., 2017). For example, Yu et al. (2017) found that soil CO_2 production was positively correlated with soil moisture in the 0–10 cm layer but negatively correlated with soil moisture in the 10–20 cm layer. Considering that biochar application depth alters environmental factors (e.g., soil temperature, moisture, labile C, etc.) that are vital for soil respiration (Rs) (Grunwald et al., 2017; He et al., 2016; Li et al., 2018; Liu et al., 2016), we expect that biochar application depth may have a significant effect on soil CO_2 fluxes.

The efficiency of microbial carbon use can be taken as a key indicator of microbial health, and it is generally measured by the metabolic quotient (qCO_2). A lower qCO_2 indicates lower environmental stress on microbes and usually represents good environmental management (Zhou et al., 2017b). It has been reported that biochar can reduce qCO_2 values by 6–21% by enhancing microbial growth but does not result in a net increase in Rs (Zhou et al., 2017b), while Rs is generally positively correlated with microbial biomass (Perveen et al., 2019). This seemingly contradictory result is usually interpreted as due to the biochar itself, wherein the resultant improved soil environment provides a suitable habitat for microbes (Lehmann et al., 2011; Zhou et al., 2017a) and thus enhances microbial carbon use efficiency by improving the composition of the soil carbon and microbial community (Chen et al., 2019; Li et al., 2020). Recent studies reported that microbial respiration in the surface soil contributes a large proportion of CO_2 production (Ge et al., 2019b; Min et al., 2020; Wang et al., 2018) and that biochar additions have significant positive effects on microbial biomass at a soil depth of 10–20 cm rather than at a soil depth of 0–10 cm (Yu et al., 2017). One possible explanation was that complex environmental factors (e.g., soil moisture and nutrients) partly counteract the positive effect of biochar on microbial growth in surface soil (Dutta et al., 2017), with recalcitrant substrate immobilization and labile C (e.g., DOC) migrating downward with water but also an ongoing downward movement of microbes in the surface soil (Ge et al., 2019a). Do these results imply that applying biochar to 10–20 cm soil will further reduce both CO_2 emissions and qCO_2? If biochar is directly input to 0–10 cm soil will it obtain the opposite result? These interesting and important questions urgently need to be answered.

The temperature sensitivity of soil respiration (referred to as Q_{10}) is a key parameter in modeling the carbon cycle, but its variations after biochar application are still poorly understood (Chen et al., 2018; He et al., 2016; Pei et al., 2017). For example, according to the “carbon temperature hypothesis”, Q_{10} should increase under the application of biochar because the breakdown of biochemically recalcitrant carbon requires activation energy (Davidson and Janssens, 2006). However, the opposite results have often been reported (Chen et al., 2019); these are generally attributed to enhanced organomineral interactions and enhanced soil carbon protection (Grunwald et al., 2017). As mentioned above, the different application depths of biochar directly affect the carbon composition, physicochemical properties and microbial communities of different soil layers and thereby affect the process of CO_2 emission from the soil surface, and changes in these parameters are likely to affect Q_{10}. Nevertheless, how the depth of biochar application affects Q_{10} remains unclear.

CH_4 is an important greenhouse gas whose global warming potential is 25 times that of CO_2 (Paustian et al., 2016). A recent *meta*-analysis of biochar’s effect on CH_4 emissions and uptake found that biochar may decrease the CH_4 sink in upland agricultural systems (Jeffery et al., 2016; Ramlow and Cotrufo, 2018). Although global CH_4 fluxes are net positive, as rice cultivation is a much larger source of CH_4 than the sink contribution of upland soils, well-aerated upland soils are important biological sinks for atmospheric CH_4 and are believed to contribute to approximately 15% of global CH_4 oxidation (Jeffery et al., 2016). However, few studies have examined the potential of biochar to simultaneously reduce CO_2 emissions and enhance CH_4 uptake in well-aerated upland soils, which are generally biological sources of CO_2 via microbial respiration and sinks for CH_4 via oxidation. Given that the key determinants of soil CH_4 fluxes are generally aeration, substrate availability, and the microbial community (Karhu et al., 2011), all of which may be affected by the biochar application depth, the response of CH_4 flux to the depth of biochar application is a topic worth studying.

To address the above issues, we carried out a 16-month field experiment with biochar application in a dryland agricultural system on the Loess Plateau. We hypothesized the following: (1) adding biochar at 20 t ha^{-1} to soil would increase soil CO_2 emissions according to our previous incubation experiments (Li et al., 2017) and decrease CH_4 uptake, and these results would be affected by the depth of biochar application (i.e., 0–10 cm, 10–20 cm, or 0–20 cm); (2) adding biochar to the 10–20 cm soil would result in a lower qCO_2 than the other treatments and (3) adding biochar to the soil would not increase Q_{10}, but whether it would decrease Q_{10} would also depend on the application depth. Specifically, the objectives of this study were (1) to investigate the effects of biochar addition to different soil depths on CO_2 emissions and CH_4 uptake, as well as qCO_2 and Q_{10}; (2) to understand the mechanism by which biochar application depth affects Rs characteristics and CH_4 flux; and (3) to identify promising application strategies that can maximize biochar’s potential to enhance soil carbon sequestration.

2. Materials and methods

2.1. The experimental site, soil and biochar

The biochar field trial was carried out from January 2017 to May 2018 in Yangling, Shaanxi, China (34°18′15″ N, 108°02′30″E), and was located on the southern Loess Plateau (Fig. S1). The climate in this study area is semihumid, with a mean annual air temperature of 13 °C and mean annual precipitation of 632 mm. The daily precipitation and mean air temperature data during the experimental period were obtained from the Yangling weather station (Yangling Meteorological Bureau, 2018; Fig. S2). Historically, the field site implemented a corn and wheat rotation system, but three years (i.e., 2014–2016) before the experiment, only winter wheat (Triticum aestivum L. cv., Xiaoyan No. 22) was cultivated at a seeding rate of 150 kg ha^{-1}. Before planting, a small rotary tiller was used to perform rotary tillage on the plow layer soil (0–20 cm) in late September every year. The basal application of urea (120 kg N ha^{-1}) and calcium superphosphate (80 kg P_2O_5 ha^{-1}) to the plow layer was performed each year before sowing in early October of 2014–2015. No tillage occurred during the growth stage, and weeds were regularly removed by hand. Wheat was harvested manually at maturity in late May each year by cutting the aboveground biomass and removing it from the plots. Additionally, all the vegetation was manually removed by hand, and the soil had been kept bare since June 2016.

The soil type in the study area is a Eum-Orthic Anthrosol (local name: Lou soil). As described by Li et al. (2019), the soil contains 25% clay, 68% silt and 7% sand, and it is classified as a silty clay according to the USDA system. The soil pH was 7.99, the bulk density (BD) was 1.32 g cm^{-3}, the total organic carbon was 6.15 g kg^{-1}, and the total N was 0.71

g kg^{-1}. The biochar was made from apple (*Malus pumila* Mill.) branches pyrolyzed at 450 °C for 8 h as described in detail by Li et al. (2017). The biochar pH was 9.67, and its specific surface area was 14.22 m^2 g^{-1}. Other physicochemical properties of the biochar are shown in Table S1, and the determination methods were reported in Li et al. (2019). The biochar for the field trials was sieved ($<$2 mm) after oven-drying at 60 °C.

2.2. Experimental design

This study used a randomized block design with four treatments, and each treatment was conducted in triplicate. The three application methods mixed 20 t ha^{-1} biochar into different depths of soil, namely, 0–10 cm ($BC_{0\text{-}10\ cm}$), 10–20 cm ($BC_{10\text{-}20\ cm}$), and 0–20 cm ($BC_{0\text{-}20\ cm}$). Soil without biochar was included as a control (CK). Each plot had an area of 1.5 m^2 (1 m × 1.5 m). The plots were separated by rectangular polyvinyl chloride frames (PVCs, 1 m × 1.5 m × 0.45 m) inserted vertically into the soil to 40 cm depth, 5 cm above the soil surface. The biochar was mixed with the soil as follows. First, 0–10 cm and 10–20 cm of soil were transferred from the plot to the polyethylene film on the side with a shovel, and visible roots and stones were removed. Then, the biochar was homogeneously mixed into the corresponding depth of the soil layer according to the experimental design. Finally, the 0–10 cm and 10–20 cm soils were returned to their original locations. To ensure the comparability of the CK and biochar treatments, the soil of the CK treatment underwent the same process of shifting out and backfilling. To keep the soil BD close to the actual field soil and reduce soil moisture loss, a cylindrical iron roller (diameter: 0.3 m, length: 1.0 m, and weight: 85 kg) was rolled back and forth over the soil 5 times to moderately compact the soil; this was performed based on the local traditional practice after rotary tillage. Considering that the objectives of this work are to preliminarily explore the effect of biochar application depth on fluxes of CO_2 and CH_4 from the soil while limiting the respiration and transport of target gases by plants, the experimental plots were kept bare through weeding by hand, and no crop was planted (Min et al., 2020). Additionally, no fertilization, no irrigation and no other tillage activities occurred during the experiment.

2.3. Soil CO_2 emissions, CH_4 uptake fluxes, temperature and moisture

Three days after biochar application, the soil CO_2 and CH_4 fluxes began to be monitored regularly with an ultraportable gas analyzer equipped with a chamber that was 20 cm in diameter (SF-3000 & SC-21, Los Gatos Research, USA). The SC-21 chamber uses a separate micro air pump to pneumatically drive the opening and closing, and the gas analyzer system adopts a steady-state measurement method. Briefly, driven by the gas chamber (SC-21) and the internal gas pump of the SF-3000 analyzer, the gas in the chamber enters the analyzer for concentration analysis and returns to the gas chamber to form a closed loop. The rate of increase in the measured gas in the chamber was used to estimate the rate at which the measured gas entered the free air outside the gas chamber. The gas analyzer parameters were as follows: balance time of 90 s and measurement time of 120 s. More detailed measurement principles of the SF-3000 & SC-21 soil gas flux system are shown in supplementary text 1 and Fig. S3. Before the measurements, any visible living organisms were removed without disturbance to the soil, e.g., insects were expelled. Measurements were carried out at least every two weeks during the 16-month experimental period, and each measurement was taken from 10:00 am to 11:00 am to represent the mean value for the day (Sun et al., 2018). The cumulative CO_2 emissions and CH_4 uptake were calculated by linear interpolation between two successive measurements (Bamminger et al., 2018) and expressed per square meter (g CO_2-C m^{-2} and g CH_4-C m^{-2}, respectively). Simultaneously with the gas flux measurement, the soil temperature (5 cm depth) was determined by a temperature probe equipped with a gas measuring system. In addition, the moisture in the 0–10 cm and 10–20 cm soil layers in each treatment was determined monthly by the drying method at 105 °C and then converted into soil volumetric water content (%).

2.4. Soil sampling and analysis of soil physicochemical properties

A total of 492 days after initial biochar application in late May 2018, to determine the changes in soil properties, we used a soil drilling sampler (4 cm inner diameter) to randomly sample the soil at a depth of 0–20 cm at 10-cm intervals in each plot. All soil samples were sieved through 2-mm sieves to remove debris. Then, the soil samples were divided into two portions. One portion was stored in a cool box and transferred to the laboratory to determine soil microbial biomass carbon (MBC) and inorganic N (i.e., NH_4^+ and NO_3^-). The other portion was dried at room temperature and used to determine the other physiochemical soil properties. In addition, the soil BD was measured using a 100-cm^3 cylinder.

The MBC was estimated by the chloroform fumigation-extraction method, and the conversion factor was 0.45 (Vance et al., 1987; Wu et al., 1990). NH_4^+ and NO_3^- were extracted with a 2 mol L^{-1} KCl solution and measured with flow injection analysis (TRAACS 2000, Bran and Luebbe, Germany). The SOC was determined according to the dichromate oxidation method (Nelson et al., 1982). The DOC was extracted with water (1:2, soil:water) for 1 h, filtered through 0.45-μm membranes, and determined with a total organic carbon analyzer (Shimadzu, TOC Vwp, Japan). The total N was measured by acid digestion according to the Kjeldahl method (Bremner and Mulvaney, 1982) and determined with an automatic nitrogen analyzer (FOSS Kjeltec 8400, Denmark). The soil pH was measured with a pH meter (Mettler-Toledo FE 20; Switzerland). The soil available phosphorus (AP) was extracted in 0.5 M $NaHCO_3$ and determined by the colorimetric method (Bao, 2001).

2.5. Calculation of qCO_2 and Q_{10}

qCO_2 is the ratio between the emitted CO_2-C and MBC and can be expressed as mg CO_2-C g^{-1} MBC d^{-1}. The area-related CO_2 flux was converted to CO_2-C per g plow layer soil by applying the specific BD values at depths of 0–10 and 10–20 cm (Li et al., 2020). To assess the microbial response to the experimental treatment as accurately as possible, we used the CO_2 flux in the final monitoring stage (March 1 to May 16, 2018) and the MBC measured at the end of the experiment (May 16, 2018) to estimate the qCO_2 of each treatment. Additionally, fitting the CO_2 flux (i.e., Rs, μmolm^{-2} s^{-1}) and temperature data obtained throughout the experiment to an exponential function yielded the Q10 value (Fang and Moncrieff, 2001).

$$Rs = \beta_0 e^{\beta 1 T} \tag{1}$$

$$Q_{10} = e^{10\beta 1} \tag{2}$$

where β0 and β1 are fitted parameters, and T is the soil temperature (°C) at a depth of 5 cm.

2.6. Statistical analyses

The repeatedly measured data (i.e., CO_2 and CH_4 fluxes, soil temperature and moisture) were analyzed via repeated-measures ANOVA. The differences in the cumulative CO_2 and CH_4 fluxes, qCO_2, Q_{10}, MBC and soil physicochemical properties were tested by one-way ANOVA. Multiple comparisons were performed with Duncan's multiple range test. Statistical test results with $P \leq 0.05$ were considered significant. Pearson correlation analysis was used to explore the relationship between soil physicochemical factors and the cumulative fluxes of CO_2 and CH_4, qCO_2 and Q_{10}. These statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). In addition, redundancy analysis (RDA) and variance partitioning analysis (VPA) were performed to determine the soil environmental factors related to the variations in CO_2

and CH_4 fluxes, qCO_2 and Q_{10}. To estimate the significance of environmental factors, “envfit” was performed based on 999 permutations. Soil environmental factors were divided into three categories for the VPA: physical properties (i.e., soil temperature, moisture and BD), labile carbon and microbial biomass (MBC), and nutrients (i.e., N, NH_4^+, NO_3^-, AP and pH). The RDA and VPA were conducted using the “vegan” package in R-3.6.1.

3. Results

3.1. *Responses of Rs and cumulative CO_2 emissions to biochar application depth*

The Rs of all treatments showed strong temporal variability and was significantly affected by biochar application depth (Fig. 1A and Table 1). Sixteen of the 32 sampling events after the application of biochar showed significant treatment effects (Fig. 1B). In detail, three days after initial biochar application, all three biochar application depths (i.e., BC$_{0\text{-}10cm}$, BC$_{10\text{-}20cm}$ and BC$_{0\text{-}20cm}$) showed increased Rs to a level higher than that of CK by 33%-54% ($P < 0.05$). Rs was not significantly affected by the biochar application depth in the subsequent two months of winter 2017, but in the following spring of 2017, the biochar treatments, especially BC$_{10\text{-}20cm}$ and BC$_{0\text{-}20cm}$ showed a tendency to promote Rs (Fig. 1B). Interestingly, the seasonal and mean Rs of BC$_{0\text{-}10cm}$ were persistently higher than that of CK from summer 2017 to spring 2018 (143–492 days after biochar application), and each measurement of Rs consistently showed an increasing trend (Fig. 1B and Table 1). In addition, BC$_{0\text{-}10cm}$ showed a continuous positive effect on seasonal CO_2 emissions during this period, and the total CO_2 emission of BC$_{0\text{-}10cm}$ was significantly higher than that of CK by 10.13% ($P < 0.05$). However, from the spring of 2017 to the end of the experiment (50 days after biochar application), there were no significant differences among the seasonal Rs of CK, BC$_{10\text{-}20cm}$ and BC$_{0\text{-}20cm}$ overall, except that BC$_{0\text{-}20cm}$ saw decreased Rs in Autumn 2017 (Table 1). In addition, there were no significant differences among the seasonal and total CO_2 emissions of CK, BC$_{10\text{-}20cm}$ and BC$_{0\text{-}20cm}$ ($P > 0.05$).

3.2. *Responses of soil CH_4 uptake to biochar application depth*

The flux and cumulative uptake of CH_4 also showed strong temporal variability and were significantly affected by biochar application depth (Fig. 2 and Table 2): A total of 24 of the 32 sampling events after the application of biochar showed significant treatment effects on CH_4 flux, 19 sampling events showed that biochar reduced CH_4 flux, and 5 sampling events showed that biochar would increase CH_4 flux (Fig. 2B). Compared with CK, BC$_{0\text{-}10cm}$, BC$_{10\text{-}20cm}$ and BC$_{0\text{-}20cm}$ significantly increased the total CH_4 uptake by 21.70%, 10.75% and 18.78% ($P < 0.05$), respectively. Overall, BC$_{0\text{-}10cm}$, BC$_{10\text{-}20cm}$ and BC$_{0\text{-}20cm}$ all reduced seasonal CH_4 flux and increased the cumulative uptake of CH_4 from winter 2017 to winter 2018 (Table 2). In particular, at the end of May 2017 (summer), the CH_4 fluxes under the biochar treatments were significantly higher than those of CK by 28.89%–43.79% (Fig. 2). In the winter of 2018, only BC$_{0\text{-}10cm}$ showed a significant effect of reducing seasonal CH_4 flux, while BC$_{10\text{-}20cm}$ showed a promotion effect overall, but it significantly reduced the CH_4 flux on March 1, 2018 (Fig. 2). In the following spring of 2018, although there was no significant difference between the seasonal CH_4 flux of CK and that of the biochar treatments (Table 2), both BC$_{0\text{-}10cm}$ and BC$_{10\text{-}20cm}$ saw significantly increased CH_4 flux in early May 2018.

3.3. *Changes in soil temperature under different biochar application depths*

The dynamics of soil temperature at 5 cm depth were consistent

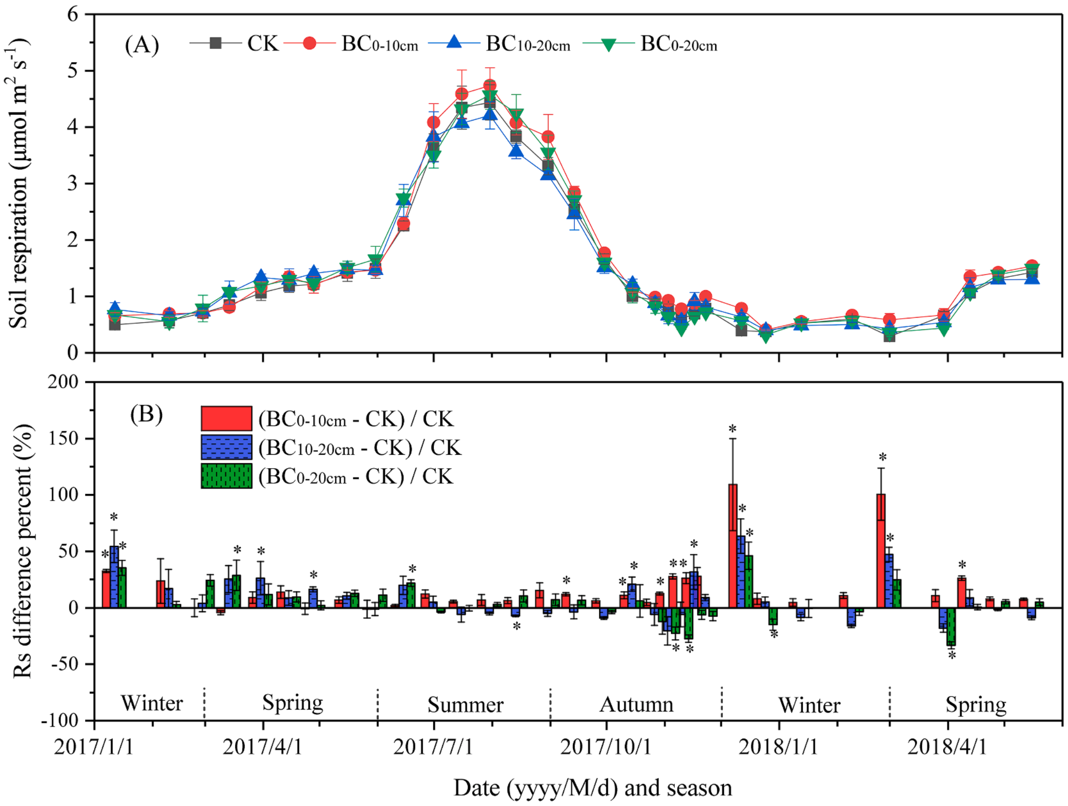

Fig. 1. Dynamics of soil respiration (A) and the differences between biochar application treatments and CK (B). CK refers to the treatment without biochar addition. BC$_{0\text{-}10cm}$, BC$_{10\text{-}20cm}$, and BC$_{0\text{-}20cm}$ refer to the treatments mixing biochar with the soil at depths of 0–10 cm, 10–20 cm and 0–20 cm, respectively. The “*” above bars indicates a significant difference between the Rs of CK and of the treatment ($P < 0.05$).

Table 1
Effect of biochar application depth on seasonal soil respiration and cumulative CO_2 emission.

Variables	Treatment	Winter (3–50)	Spring (51–142)	Summer (143–234)	Autumn (235–325)	Winter (326–415)	Spring (416–492)	Mean Rs/total CO_2 (3–492)
Rs (μmol $m^2 s^{-1}$)	CK	0.59 ± 0.06a	1.20 ± 0.07a	3.63 ± 0.13a	1.13 ± 0.02b	0.47 ± 0.01a	0.95 ± 0.05a	1.45 ± 0.04a
	$BC_{0\text{-}10cm}$	0.68 ± 0.02b	1.24 ± 0.06a	3.93 ± 0.12b	1.28 ± 0.01c	0.61 ± 0.01b	1.12 ± 0.06b	1.61 ± 0.04b
	$BC_{10\text{-}20cm}$	0.72 ± 0.04b	1.34 ± 0.10a	3.59 ± 0.13a	1.13 ± 0.02b	0.50 ± 0.02a	0.95 ± 0.06a	1.49 ± 0.05a
	$BC_{0\text{-}20cm}$	0.71 ± 0.05b	1.33 ± 0.08a	3.82 ± 0.13ab	1.08 ± 0.03a	0.50 ± 0.02a	0.95 ± 0.03a	1.51 ± 0.03a
	F	5.03	2.38	4.88	51.47	43.76	9.07	7.75
	P	*	ns	*	***	***	**	**
CO_2 emission (g CO_2-C m^{-2})	CK	27.95 ± 3.32a	108.91 ± 5.21a	331.20 ± 12.20a	136.29 ± 3.14a	37.16 ± 0.95a	70.98 ± 3.75a	712.49 ± 18.48a
	$BC_{0\text{-}10cm}$	33.30 ± 1.35b	112.12 ± 5.18ab	355.72 ± 8.59b	153.72 ± 4.30b	45.77 ± 0.94b	84.03 ± 4.43b	784.66 ± 20.13b
	$BC_{10\text{-}20cm}$	34.38 ± 2.25b	122.75 ± 8.94b	327.87 ± 11.68a	135.05 ± 4.42a	38.60 ± 1.20a	70.35 ± 4.61a	729.01 ± 23.88a
	$BC_{0\text{-}20cm}$	32.54 ± 2.86ab	120.87 ± 5.20ab	348.68 ± 11.56ab	136.12 ± 4.08a	37.79 ± 1.66a	69.09 ± 2.77a	745.07 ± 17.22a
	F	3.69	3.34	4.41	14.95	32.21	9.36	7.11
	P	ns	ns	*	***	***	**	*

Note: The number in parentheses below the season represents the days after biochar application. CK refers to the treatment without the biochar addition. $BC_{0\text{-}10cm}$, $BC_{10\text{-}20cm}$, and $BC_{0\text{-}20cm}$ refer to the treatments mixing biochar with the soil at a depth of 0–10 cm, 10–20 cm and 0–20 cm, respectively.
Data are presented as means (±standard errors) of three replicate plots. Different letters in the same column indicate significant differences ($P < 0.05$).
Among treatments. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, $P > 0.05$.

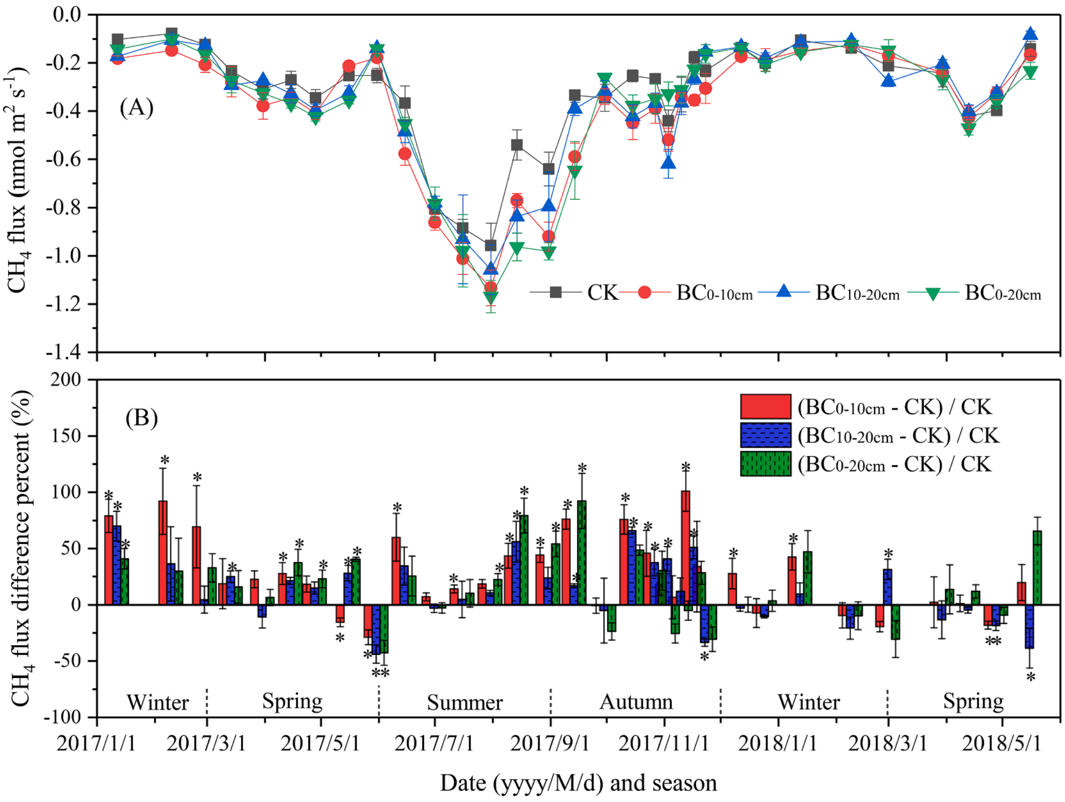

Fig. 2. Dynamics of soil CH_4 flux (A) and the differences between biochar application treatments and CK (B). CK refers to the treatment without biochar addition. $BC_{0\text{-}10cm}$, $BC_{10\text{-}20cm}$, and $BC_{0\text{-}20cm}$ refer to the treatments mixing biochar with the soil at depths of 0–10 cm, 10–20 cm and 0–20 cm, respectively. The "*" above bars indicates a significant difference between the CH_4 flux of CK and of the treatment ($P < 0.05$).

among all treatments, and only 4 of 32 sampling events showed the treatment effect, which frequently appeared in autumn 2018 (Fig. 3A). The results of repeated variance analysis indicated that the biochar application depth had no significant effect on the seasonal and mean soil temperature (data not shown). Although the soil temperature in treatment $BC_{0\text{-}10cm}$ appears to be higher than that of CK in the July-October 2017 and March-May 2018 time periods, the warming effect was not significant ($P < 0.05$) except for the measurement on March 1, 2018. In addition, compared with CK, a higher soil temperature appeared in late autumn of 2017 under the $BC_{0\text{-}10cm}$, $BC_{10\text{-}20cm}$ and $BC_{0\text{-}20cm}$ treatments (Fig. 3A).

3.4. *Changes in soil moisture under different biochar application depths*

The soil moisture (soil volumetric water content) at depths of 0–10 cm and 10–20 cm showed strong temporal variability, but all treatments

Table 2
Effect of biochar application depth on seasonal flux and cumulative uptake of CH_4.

Variables	Treatment	Winter (3–50)	Spring (51–142)	Summer (143–234)	Autumn (235–325)	Winter (326–415)	Spring (416–492)	Mean flux/total uptake (3–492)
CH_4 flux (nmol m^2s^{-1})	CK	−0.10 ± 0.01a	−0.28 ± 0.02a	−0.70 ± 0.03a	−0.30 ± 0.01a	−0.15 ± 0.00b	−0.28 ± 0.01ab	−0.33 ± 0.00a
	BC_{0-10cm}	−0.18 ± 0.00c	−0.30 ± 0.02ab	−0.88 ± 0.02c	−0.41 ± 0.01c	−0.16 ± 0.01b	−0.27 ± 0.02ab	−0.40 ± 0.00d
	$BC_{10-20cm}$	−0.14 ± 0.01b	−0.29 ± 0.00ab	−0.81 ± 0.02b	−0.36 ± 0.02b	−0.13 ± 0.00a	−0.26 ± 0.01a	−0.37 ± 0.01b
	BC_{0-20cm}	−0.14 ± 0.01b	−0.32 ± 0.01b	−0.89 ± 0.03c	−0.33 ± 0.02ab	−0.16 ± 0.01b	−0.30 ± 0.02b	−0.39 ± 0.01c
	F	84.59	5.18	38.22	24.80	7.30	3.18	131.80
	P	***	*	***	***	*	ns	***
CH_4 uptake (g CH_4-C m^{-2})	CK	0.46 ± 0.02a	2.56 ± 0.10a	6.33 ± 0.21a	3.06 ± 0.06a	1.10 ± 0.01b	2.31 ± 0.15a	15.81 ± 0.17a
	BC_{0-10cm}	0.83 ± 0.03c	2.89 ± 0.18bc	7.78 ± 0.19c	4.34 ± 0.11c	1.26 ± 0.10c	2.15 ± 0.16a	19.24 ± 0.11d
	$BC_{10-20cm}$	0.64 ± 0.06b	2.79 ± 0.01b	7.23 ± 0.13b	3.74 ± 0.26b	0.99 ± 0.04a	2.11 ± 0.12a	17.51 ± 0.21b
	BC_{0-20cm}	0.61 ± 0.05b	3.04 ± 0.07c	7.79 ± 0.23c	3.82 ± 0.20b	1.16 ± 0.02bc	2.37 ± 0.20a	18.78 ± 0.19c
	F	35.31	10.78	37.68	27.83	11.80	1.76	237.79
	P	***	**	***	***	**	ns	***

Note: The number in parentheses below the season represents the days after biochar application. CK refers to the treatment without biochar addition. BC_{0-10cm}, $BC_{10-20cm}$, and BC_{0-20cm} refer to the treatments mixing biochar with soil at a depth of 0–10 cm, 10–20 cm and 0–20 cm, respectively.
Data are presented as means (±standard errors) of three replicate plots. Different letters in the same column indicate significant differences ($P < 0.05$).
Among treatments. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, $P > 0.05$.

showed consistent fluctuations (Fig. 3B and C). The results of a repeated-measures ANOVA showed that biochar application depth had a significant effect on the soil moisture at 10–20 cm but not at 0–10 cm (Table 3). Overall, $BC_{10-20cm}$ saw decreased soil moisture at 10–20 cm by 8.68% ($P < 0.05$), but there were no significant differences among the soil moisture levels at 10–20 cm for BC_{0-10cm}, BC_{0-20cm} or CK ($P > 0.05$). Furthermore, the biochar application depth significantly affected soil moisture at depths of 0–10 cm and 10–20 cm in winter of 2017 and 2018, and the soil moisture of 10–20 cm was also affected by the biochar application depth in the spring of 2018 (Table 3).

3.5. Soil physicochemical properties affected by biochar application depth

The physicochemical properties of the soil at depths of 0–10 cm and 10–20 cm were largely dependent on the biochar application depth, except the NH_4^+ of the soil at depths of 0–10 cm (Table 4). Compared with CK, BC_{0-10cm} significantly increased the SOC, TN, C:N ratio and DOC in the 0–10 cm soil. $BC_{10-20cm}$ increased these variables in the 10–20 cm soil. In addition, BC_{0-20cm} increased these variables in both the 0–10 cm and 10–20 cm soil, but the extent of the increase in a single soil layer was smaller than the corresponding increase in treatment BC_{0-10cm} or $BC_{10-20cm}$. Additionally, BC_{0-10cm} and $BC_{10-20cm}$ significantly decreased the BD of the soil at depths of 0–10 cm and 10–20 cm, respectively, while BC_{0-20cm} decreased the BD of soil at depths of both 0–10 cm and 10–20 cm (Table 4). BC_{0-10cm} substantially increased the soil NH_4^+ content, and all treatments increased the AP content of soil at 10–20 cm depth.

3.6. MBC, qCO_2 and Q_{10} affected by the different biochar application depths

Biochar application depth significantly affected the MBC, qCO_2 and Q_{10} (Fig. 4). Compared with CK, all biochar treatments showed the potential to enhance the MBC, but only BC_{0-20cm} significantly increased the MBC in soil at depths of 0–10 cm and 10–20 cm. In addition, $BC_{10-20cm}$ prominently enhanced the MBC of the soil at depths of 10–20 cm (Fig. 4A and B). Moreover, BC_{0-20cm} and $BC_{10-20cm}$ significantly reduced qCO_2, but BC_{0-10cm} had a higher qCO_2 value than CK did (Fig. 4C). BC_{0-10cm} and $BC_{10-20cm}$ saw declined Q_{10} ($P < 0.05$), while the difference in Q_{10} between CK and BC_{0-20cm} was not significant ($P > 0.05$; Fig. 4D).

3.7. Key environmental factors related to CO_2 emissions, Q_{10}, qCO_2, and CH_4 uptake

CO_2 emissions were positively correlated with various environmental factors of soil at depths of 0–10 cm, e.g., soil temperature, C, DOC, N and C:N ratio, and negatively correlated with BD and AP (Fig. 5A). In contrast, there was no significant correlation between CO_2 emissions and the environmental factors of the 10–20 cm soil layer (Fig. 5B). Q_{10} was significantly negatively correlated with the NH_4^+ of soil at depths of 0–10 cm (Fig. 5A), and qCO_2 was negatively correlated with the N, AP and pH of the soil at 0–10 cm (Fig. 5A) and the moisture and DOC of the soil at 10–20 cm (Fig. 5B). CH_4 uptake was negatively correlated with soil temperature, C, DOC, N and the C:N ratio of the soil at a depth of 0–10 cm and with C and NO_3^- for the 10–20 cm soil; it was positively correlated with BD and AP for the 0–10 cm soil and pH for the 10–20 cm soil (Fig. 5).

The RDA diagram visually shows the relationships among samples, soil environmental factors and CO_2 emissions, qCO_2, Q_{10} and CH_4 uptake (Fig. 6). Overall, the environmental factors of the 0–10 cm soil explained 88.10% of the variation in CO_2 emissions, CH_4 uptake, qCO_2 and Q_{10}, whereas subsoil environmental factors accounted for 43.24% of the variation (Fig. 6). The VPA results showed that environmental factors of the 0–10 cm soil explained more of the variation in CO_2 emissions, CH_4 uptake and Q_{10} than those of the 10–20 cm soil (Fig. 7). The variations in CO_2 emissions and CH_4 uptake were mainly explained by changes in the physical properties and nutrient levels of 0–10 cm soil as well as by their interactions with the MBC and DOC. However, the explanatory power of MBC or DOC alone was very low (Fig. 7A and B). In addition, MBC and DOC changes in the 0–10 cm soil explained 64% of the variation in Q_{10} (Fig. 7D), and those in the 10–20 cm soil explained 17% and 42% of the variations in qCO_2 and CH_4 uptake, respectively (Fig. 7B and C).

4. Discussion

4.1. Effect of biochar application depth on soil CO_2 emissions

Of the three biochar application depths, only BC_{0-10cm} significantly increased the Rs and cumulative CO_2 emissions, while $BC_{10-20cm}$ and BC_{0-20cm} showed no significant effect (Table 1), which was not

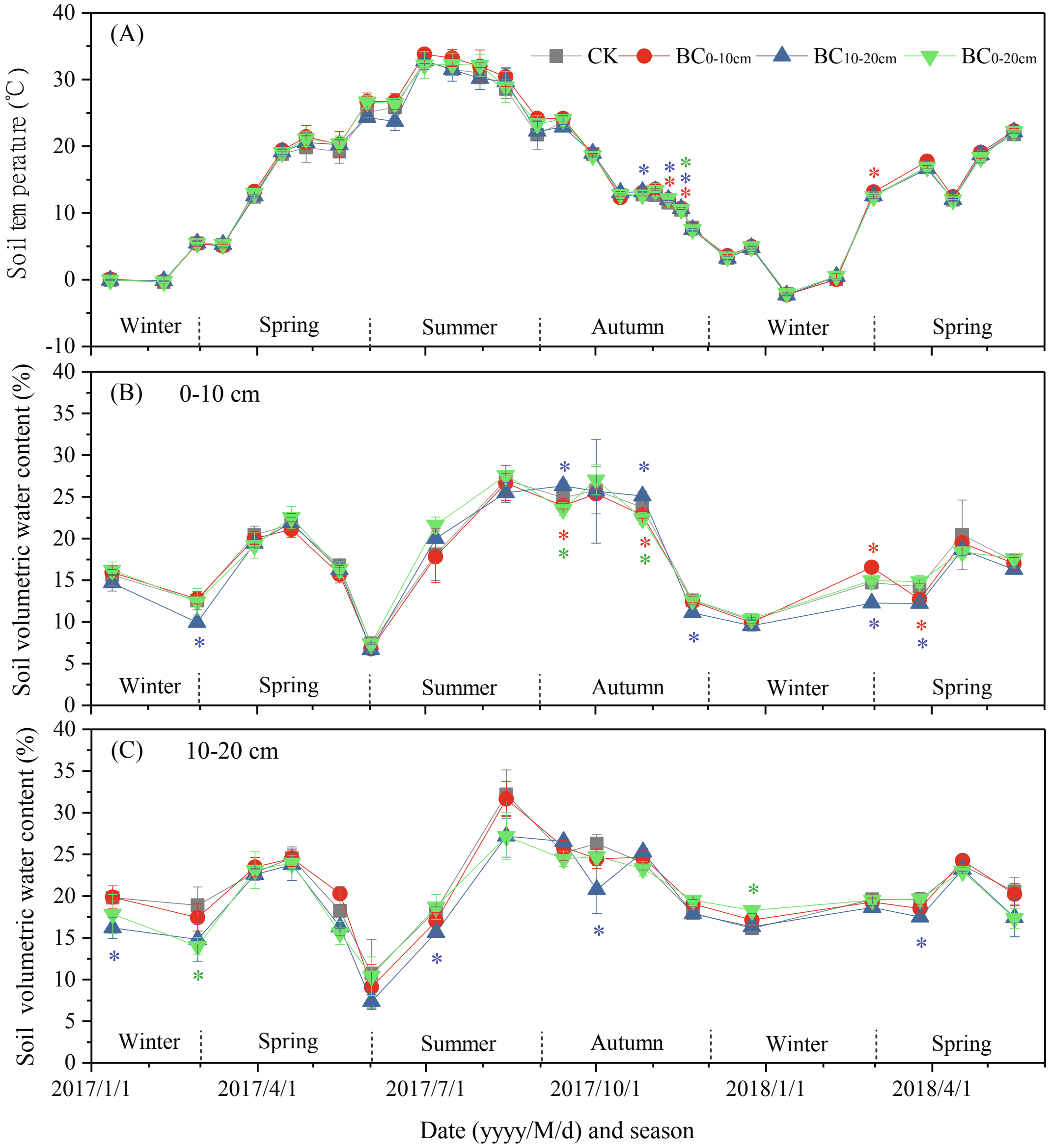

Fig. 3. Variations in soil temperature (°C) and moisture (%). Subgraph A shows the dynamics of soil temperature at a depth of 5 cm. Subgraphs B and C show the soil volumetric water content at depths of 0–10 cm and 10–20 cm, respectively. CK refers to the treatment without biochar addition. $BC_{0\text{-}10cm}$, $BC_{10\text{-}20cm}$, and $BC_{0\text{-}20cm}$ refer to the treatments mixing biochar with the soil at depths of 0–10 cm, 10–20 cm and 0–20 cm, respectively. The "*" above or below the scatter indicates that the soil volumetric water content of the treatment was significantly higher or lower than that of the CK ($P < 0.05$). The color of the "*" corresponds to the color of each treatment legend.

completely consistent with our hypothesis. Generally, soil temperature and moisture, DOC and MBC, nutrient availability and BD are all key soil environmental parameters influencing soil CO_2 emissions (He et al., 2016; Li et al., 2020; Pei et al., 2017). Our results suggested that treatments $BC_{0\text{-}10cm}$ and $BC_{10\text{-}20cm}$ significantly enhanced C and N-related indicators (i.e., SOC, TN, C:N ratio, DOC, MBC) and BD in the 0–10 cm soil and 10–20 cm soil, respectively. Nevertheless, the effect of treatment $BC_{0\text{-}20cm}$ on these variables was slightly weaker than that of $BC_{0\text{-}10cm}$ for 0–10 cm soil and $BC_{10\text{-}20cm}$ for 10–20 cm soil (Table 4 and Fig. 6), because the same amount of biochar was distributed evenly in 0–20 cm soil under the $BC_{0\text{-}20cm}$ treatment. This result agreed with previous studies showing that the effect of biochar on the physiochemical properties of the soil is very dependent on the application rate (Li and Shangguan, 2018; Lu et al., 2019). The results of the linear correlation and RDA both show that there was a positive correlation between CO_2 emissions and labile C (i.e., DOC and MBC) in the 0–10 cm soil, but they separately explained only 4% of the variation in CO_2 emissions (Figs. 5A, 6A and 7A), while the interactions among soil physical properties, nutrients and labile carbon explained most of such variance (Fig. 7A). Therefore, we suggest that the increased CO_2 emissions were mainly caused by the complex interactions among the surface soil environmental factors influenced by biochar rather than exclusively by the increase in labile carbon.

Our results were also concordant with the hypothesis that microorganisms prefer the dissolved or volatile carbon provided by biochar (Cheng et al., 2017; Liu et al., 2019). All biochar application treatments increased Rs in the first week after biochar addition, but in the next months, this unified priming effect seemed to disappear while showing the effect of biochar application depth on Rs (Fig. 1 and Table 1). This implied that the stimulating effect of the labile biochar-C pool (approximately 3% of biochar-C) on Rs lasted only a few weeks (Wang et al., 2016), but the effect of biochar application depth was persistent and showed strong temporal variability. Rs may be partly dependent on the effect of biochar application depth on the coordinated variation in

Table 3
Results of repeated-measures ANOVAs showing the effect of biochar application depth on the volumetric water content (%) of the soil at different layers.

Soil depth	Treatment	Winter (3–50)	Spring (51–142)	Summer (143–234)	Autumn (235–325)	Winter (326–415)	Spring (416–492)	Mean (3–492)
0–10 cm	CK	14.08 ± 0.17b	19.64 ± 0.26a	17.51 ± 1.15a	21.75 ± 0.80a	12.45 ± 0.38b	17.32 ± 1.46a	17.85 ± 0.56a
	$BC_{0\text{-}10cm}$	14.35 ± 0.14b	18.93 ± 0.21a	17.08 ± 1.64a	21.14 ± 0.34a	13.25 ± 0.35c	16.39 ± 0.42a	17.47 ± 0.46a
	$BC_{10\text{-}20cm}$	12.32 ± 0.22a	19.19 ± 0.18a	17.39 ± 0.58a	22.05 ± 1.38a	10.91 ± 0.29a	15.72 ± 0.32a	17.15 ± 0.22a
	$BC_{0\text{-}20cm}$	14.33 ± 1.26b	19.33 ± 1.06a	20.77 ± 3.24a	21.39 ± 0.83a	12.65 ± 0.24bc	16.94 ± 0.32a	18.16 ± 0.70a
	F	6.74	0.839	1.62	0.57	29.01	2.32	2.13
	P	*	ns	ns	ns	***	ns	ns
10–20 cm	CK	19.35 ± 0.91b	21.87 ± 1.12a	20.41 ± 2.20b	23.36 ± 0.86a	17.87 ± 0.44a	21.27 ± 0.49c	21.09 ± 0.89b
	$BC_{0\text{-}10cm}$	18.66 ± 1.25b	22.77 ± 0.73a	19.92 ± 2.76ab	23.53 ± 0.77a	18.21 ± 0.64ab	21.00 ± 0.49bc	21.08 ± 0.64b
	$BC_{10\text{-}20cm}$	15.51 ± 1.64a	20.87 ± 0.52a	16.73 ± 0.88a	22.62 ± 0.89a	17.48 ± 0.50a	19.36 ± 0.70a	19.26 ± 0.11a
	$BC_{0\text{-}20cm}$	15.93 ± 0.78a	20.90 ± 1.29a	18.76 ± 0.99ab	23.01 ± 0.71a	18.89 ± 0.26b	19.99 ± 0.61ab	20.04 ± 0.60ab
	F	7.80	2.64	3.01	0.74	4.65	7.07	5.89
	P	**	ns	ns	ns	*	*	*

Note: The number in parentheses below the season represents the days after biochar application. CK refers to the treatment without biochar addition. $BC_{0\text{-}10cm}$, $BC_{10\text{-}20cm}$, and $BC_{0\text{-}20cm}$ refer to the treatments mixing biochar with the soil at a depth of 0–10 cm, 10–20 cm and 0–20 cm, respectively. Data are presented as means (±standard errors) of three replicate plots. Different letters in the same column indicate significant differences ($P < 0.05$) among treatments. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, $P > 0.05$.

Table 4
The effect of biochar application depth on soil physicochemical properties.

Depth	Treatment	BD	pH	SOC	TN	C:N	DOC	NH_4^+	NO_3^-	AP
0–10 cm	CK	1.31 ± 0.01b	7.89 ± 0.02b	6.73 ± 0.19a	0.78 ± 0.01a	8.50 ± 0.30a	83.15 ± 4.30a	1.14 ± 0.06a	0.43 ± 0.03c	15.13 ± 0.30b
	$BC_{0\text{-}10cm}$	1.21 ± 0.03a	7.83 ± 0.03a	13.13 ± 0.42c	0.98 ± 0.05c	13.37 ± 0.53b	119.09 ± 1.72b	1.49 ± 0.08b	0.32 ± 0.02b	12.43 ± 0.77a
	$BC_{10\text{-}20cm}$	1.28 ± 0.02b	7.91 ± 0.02b	7.14 ± 0.32a	0.79 ± 0.01a	9.13 ± 0.38a	88.42 ± 4.06a	1.24 ± 0.08ab	0.23 ± 0.01a	15.18 ± 0.99b
	$BC_{0\text{-}20cm}$	1.23 ± 0.02a	7.88 ± 0.02b	11.02 ± 0.71b	0.86 ± 0.03b	12.82 ± 1.28b	120.32 ± 3.10b	1.14 ± 0.26a	0.40 ± 0.01c	14.00 ± 1.64ab
	F	12.74	4.94	141.58	29.06	34.72	98.06	3.64	60.88	6.12
	P	**	*	***	***	***	***	ns	***	**
10–20 cm	CK	1.32 ± 0.02c	8.01 ± 0.02b	6.09 ± 0.23a	0.73 ± 0.04a	8.32 ± 0.66a	75.77 ± 1.61a	0.99 ± 0.02a	0.25 ± 0.01a	9.77 ± 1.30a
	$BC_{0\text{-}10cm}$	1.31 ± 0.03bc	7.98 ± 0.02b	7.57 ± 0.26b	0.71 ± 0.02a	10.67 ± 0.66b	97.76 ± 2.76b	1.22 ± 0.07b	0.29 ± 0.03ab	11.83 ± 1.01bc
	$BC_{10\text{-}20cm}$	1.22 ± 0.01b	7.94 ± 0.03a	14.31 ± 0.86d	0.95 ± 0.04c	15.12 ± 1.41c	140.21 ± 1.63d	0.93 ± 0.08a	0.53 ± 0.07c	12.43 ± 0.43c
	$BC_{0\text{-}20cm}$	1.26 ± 0.03ab	7.98 ± 0.03b	10.08 ± 0.76c	0.84 ± 0.08b	11.99 ± 0.67b	108.17 ± 8.47c	0.90 ± 0.05a	0.35 ± 0.01b	10.78 ± 0.85bc
	F	9.52	5.75	108.42	15.40	32.04	101.84	16.54	29.03	6.06
	P	**	*	***	***	***	***	***	***	**

Note: Data are presented as means (±standard errors) of three replicate plots. CK refers to the treatment without biochar addition. $BC_{0\text{-}10cm}$, $BC_{10\text{-}20cm}$, and $BC_{0\text{-}20cm}$ refer to the treatments mixing biochar with the soil at a depth of 0–10 cm, 10–20 cm and 0–20 cm, respectively. BD, SOC, TN, DOC and AP are the bulk density, soil organic carbon, total nitrogen, dissolved organic carbon and available phosphorus, respectively. The unit of BD is grams per cubic centimeter. The units of SOC and TN are grams per kilogram. The units of DOC, NH_4^+, NO_3^- and AP are milligrams per kilogram. Different letters in the same column indicate significant differences among treatments at the same soil depth ($P < 0.05$). ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, $P > 0.05$.

soil temperature and moisture (Ge et al., 2019a; Min et al., 2020), although overall, there was no significant correlation between soil moisture and CO_2 emissions. For example, in autumn, when the temperature started to drop but precipitation was abundant (Fig. S1), $BC_{10\text{-}20cm}$ significantly enhanced the soil temperature and moisture at 0–10 cm, which likely stimulated microbial activity and thus increased Rs (Bai et al., 2019), while both $BC_{0\text{-}10cm}$ and $BC_{0\text{-}20cm}$ reduced the soil moisture and increased the soil temperature but showed the opposite effect on Rs. In addition, in the early winter and spring of 2018, which saw a short-term rise in the temperature and moisture of the 0–10 cm soil layer (Fig. 3A and B), the treatments at the three biochar application depths all showed the potential to enhance the Rs, ordered as follows from the strongest to weakest effect: $BC_{0\text{-}10cm}$, $BC_{10\text{-}20cm}$ and $BC_{0\text{-}20cm}$ (Fig. 1). These results imply that the biochar application depth may influence the sensitivity of Rs to soil temperature and moisture at 0–10 cm through complicated cascade reactions, e.g., the migration of labile C and nutrients (Li et al., 2018), microbial reproduction and C metabolism-related enzyme secretion, and microbial community composition and function (Ge et al., 2019a; Watzinger et al., 2014). In addition, biochar weathering under field conditions can strongly change surface chemical functional groups and hence their effects on CO_2 emissions over time through both abiotic and biotic processes (Ribas et al., 2019). Hence, the long-term monitoring of changes in the characteristics of biochar and the underlying biochemical processes under different biochar application depths is necessary.

4.2. *Effect of biochar application depth on qCO_2*

qCO_2 is an indicator of environmental stresses on soil microbes and their energy consumption for carbon use (Anderson, 2003). The lower qCO_2 observed in $BC_{10\text{-}20cm}$ and $BC_{0\text{-}20cm}$ during the final monitoring stage (March 1 to May 16, 2018) indicated that microbial mass-specific respiration was reduced and that microbial carbon use efficiency was enhanced (Li et al., 2020; Pei et al., 2017), which reflected lower stresses in this ecosystem with good management practices (Anderson and Domsch, 2010; Zhou et al., 2017b). In contrast, the higher qCO_2 observed in $BC_{0\text{-}10cm}$ could be interpreted as a positive effect on the decomposition of the soil labile carbon pool (Kuzyakov et al., 2000),

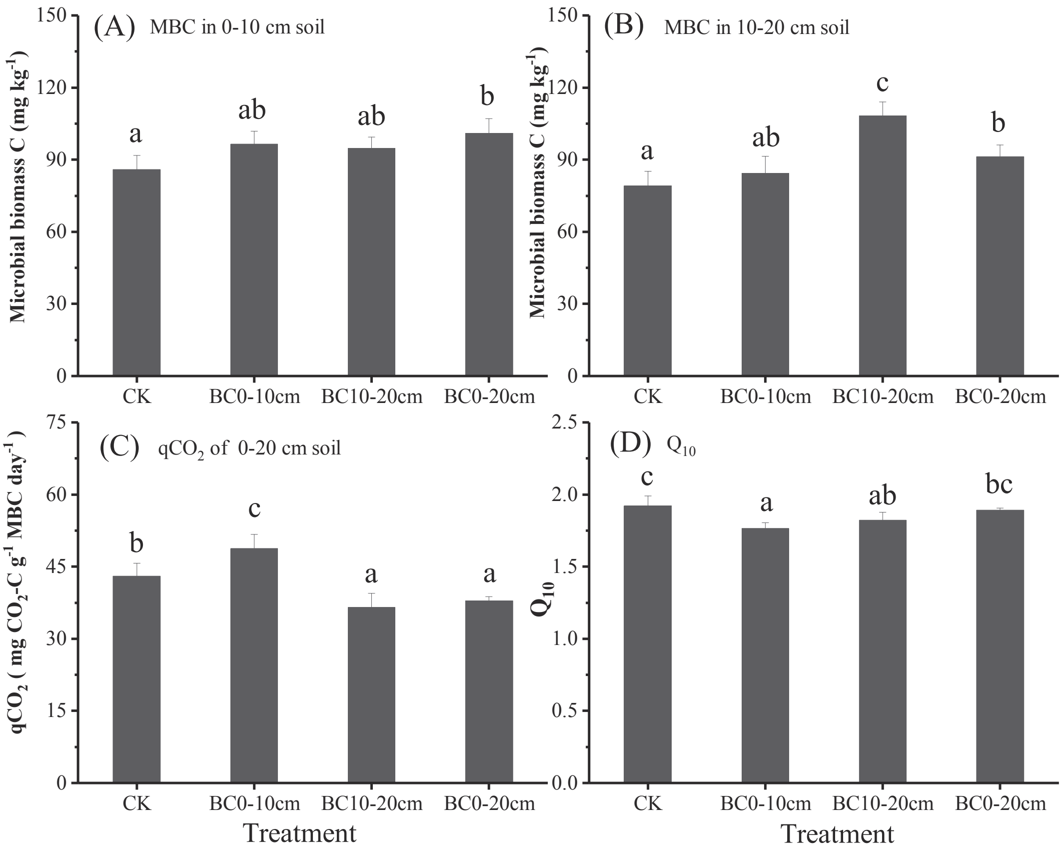

Fig. 4. Effects of biochar application depth on the microbial biomass carbon (MBC) in the 0–10 cm soil (A) and 10–20 cm soil (B), the microbial quotient (qCO_2, C), and the temperature sensitivity of soil respiration (Q_{10}, D). CK refers to the treatment without biochar addition. $BC_{0\text{-}10cm}$, $BC_{10\text{-}20cm}$, and $BC_{0\text{-}20cm}$ refer to the treatments mixing biochar with the soil at depths of 0–10 cm, 10–20 cm and 0–20 cm, respectively. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

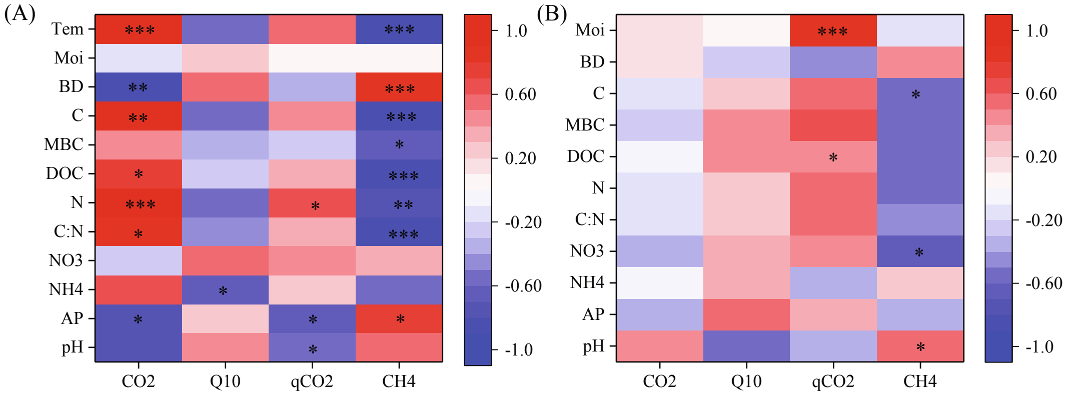

Fig. 5. Correlation heat map between soil physicochemical properties at depths of 0–10 cm (A) and 10–20 cm (B) and the soil respiration characteristics or CH_4 flux. Tem, Moi and BD are the soil temperature, soil moisture and bulk density, respectively. NO3, NH4 and AP are the NO_3^--N, NH_4^+-N and available phosphorus, respectively. MBC and DOC are the microbial biomass carbon and soil water dissolved organic carbon, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

which generally implies soil degradation due to intensive land use (Zhou et al., 2017b).

$BC_{0\text{-}10cm}$ and $BC_{10\text{-}20cm}$ showed positive effects on the soil microbial biomass, especially at the corresponding application depth, and on soil surface CO_2 emissions largely from the 0–10 cm soil (Ge et al., 2019a; Wang et al., 2018). This may partly contribute to the lower qCO_2 in $BC_{10\text{-}20cm}$ and the higher qCO_2 in $BC_{0\text{-}10cm}$, which is consistent with our hypothesis. However, a lower qCO_2 was also observed in $BC_{0\text{-}20cm}$, which significantly promoted the growth of microorganisms in the 0–10 cm and 10–20 cm soil layers (Fig. 4). One widely accepted possibility is that the high adsorption capacity of biochar results in the colocation of substrates, nutrients and microorganisms, thus enhancing carbon use efficiency and reducing qCO_2 (Pei et al., 2017). In our study, qCO_2 was negatively correlated with AP in the 0–10 cm soil (Fig. 5A) and positively correlated with DOC in the 10–20 cm soil (Fig. 5B). These results suggest that the decrease in labile carbon in the 10–20 cm soil benefited the carbon use efficiency of microorganisms in the whole plow layer and thereby likely protected the soil carbon from decomposition. Treatment $BC_{0\text{-}10cm}$ had lower AP in the 0–10 cm soil and lower DOC in the 10–20 cm soil than the other biochar treatments, while the opposite conditions

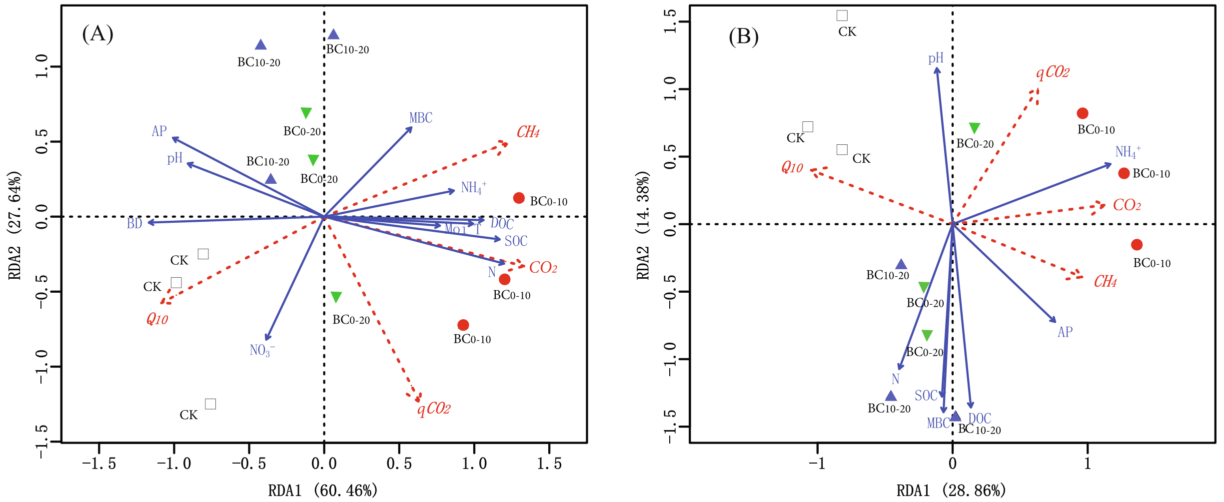

Fig. 6. Redundancy analysis (RDA) of soil respiration characteristics and CH_4 uptake changes with environmental factors in the 0–10 cm soil (A) and 10–20 cm soil (B). CK refers to the treatment without biochar addition. $BC_{0\text{-}10}$, $BC_{10\text{-}20}$, and $BC_{0\text{-}20}$ refer to the treatments mixing biochar with the soil at depths of 0–10 cm, 10–20 cm and 0–20 cm, respectively. Blue arrows represent environmental variables that are significantly correlated with the response variables represented by red dashed arrows. Solid black triangles and the text below them represent the soil samples in each treatment. AP and BD are the available phosphorus content and the bulk density, respectively. DOC and MBC are the soil water dissolved organic carbon and the microbial biomass carbon, respectively. Moi and T are the soil moisture and soil temperature, respectively.

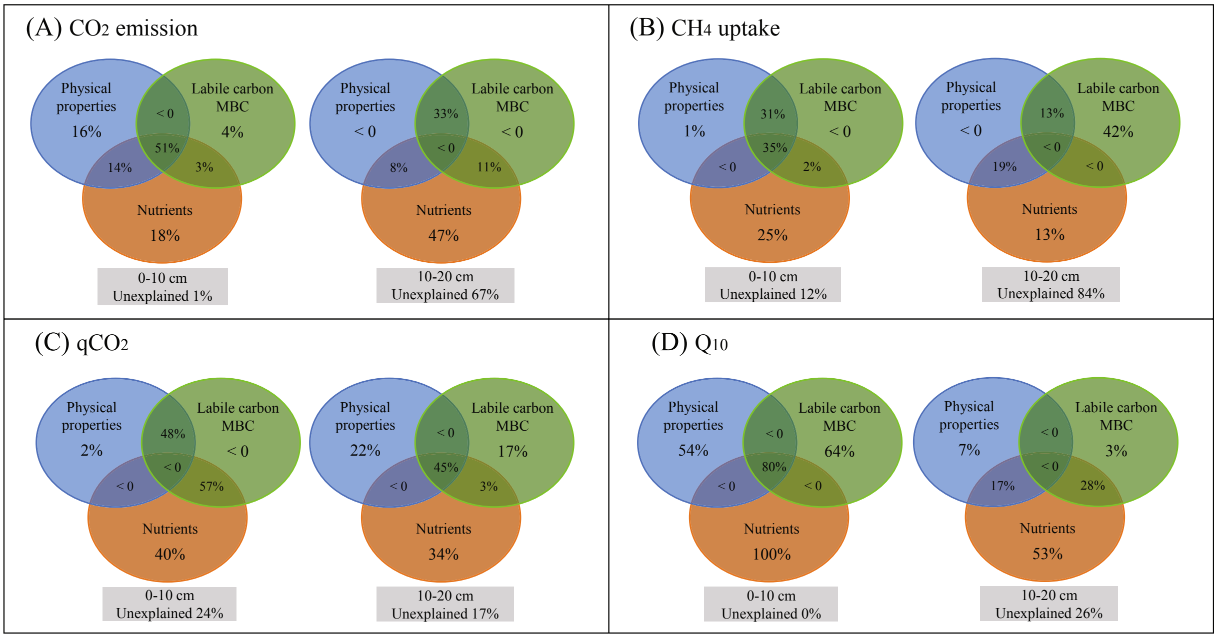

Fig. 7. Variation partitioning analysis (VPA) showing the effects of soil environmental factors at depths of 0–10 cm and 10–20 cm on CO_2 emissions (A), CH_4 uptake (B), qCO_2 (C), and Q_{10} (D). Soil environmental factors were divided into three categories: physical properties (i.e., soil temperature, moisture and bulk density), labile carbon (DOC) and microbial biomass (MBC), and nutrients (i.e., N, NH_4^+, NO_3^-, available P and pH).

were observed for $BC_{10\text{-}20cm}$ (Table 4 and Fig. 6). From these facts, we deduced that microbial community adaptation and/or changes in community composition at $BC_{10\text{-}20cm}$ and $BC_{0\text{-}20cm}$ were more conducive to improving soil carbon use efficiency than those at $BC_{0\text{-}10cm}$ (Pei et al., 2017); this finding needs further study. It is worth mentioning that the increased soil temperature at $BC_{0\text{-}10cm}$ in spring 2018 may have also contributed to its higher qCO_2 (Fig. 3A) because the utilization efficiency of microorganisms for soil carbon, including recalcitrant biochar carbon, generally decreased with increasing soil temperature (Anderson and Domsch, 2010; Frey et al., 2013).

4.3. *Effect of biochar application depth on Q_{10}*

In our work, biochar showed the potential to reduce Q_{10}, which is consistent with a few previous studies (Chen et al., 2018; Pei et al., 2017). We also found that Q_{10} was affected by the biochar application depth, which is consistent with our hypothesis (Fig. 4D). The sensitivity of SOC decomposition to temperature is usually linked to carbon protection by the soil matrix, which limits substrate availability (Conant et al., 2011). In addition, numerous studies have proposed that the role of biochar in protecting SOC from decomposition by microorganisms is an important underlying mechanism for the reduction in Q_{10} (Fang et al., 2017, 2014; Pei et al., 2017). Nevertheless, our results indicated that biochar likely enhanced rather than restricted the SOC availability to microorganisms, as evidenced by the increased Rs in the treatments compared to that in CK (Fig. 1 and Table 1). Furthermore, the lower qCO_2 may have helped attenuate Q_{10}, as a low qCO_2 is generally accompanied by less temperature-sensitive SOC decomposition (Bradford et al., 2010). Indeed, $BC_{10\text{-}20cm}$ had lower qCO_2 and Q_{10} values than CK (Fig. 4C and D). However, considering that $BC_{0\text{-}10cm}$ had a higher Rs and qCO_2 but a lower Q_{10} than the other treatments (Figs. 1A, 4C and D), the hypothesis that either the soil carbon stabilization or the lowering of qCO_2 by biochar reduced Q_{10} was not supported by the results for $BC_{0\text{-}10cm}$.

The reduction in Q_{10} was closely related to the increased MBC, DOC and NH_4^+ of soil at depths of 0–10 cm rather than 10–20 cm (Figs. 6 and 7D). Biochar contains very low amounts of labile carbon (3%), which is most likely rapidly consumed by microorganisms (Bamminger et al., 2018). In addition, soils with high clay content (as in this study) have a greater ability to stabilize biochar-C via cation bridging, van der Waals interactions and ligand exchange than other soil types (Fang et al., 2015; Wang et al., 2016). Therefore, the increased DOC at 16 months after biochar addition may have been the result of the microbial decomposition of native SOC, which can be captured by porous biochar and efficiently utilized by microorganisms (Lehmann et al., 2011; Pei et al., 2017). These results suggest that the enhanced native SOC lability and microbial activity induced by biochar likely reduced Q_{10}, which is in line with Pei et al. (2017). Moreover, if extremely recalcitrant biochar is excluded from the carbon pool that is accessible to microbes (Wang et al., 2016), this inference is supported by kinetic theory as well as evidence that labile carbon has a lower temperature sensitivity than resistant carbon (Conant et al., 2011; Davidson and Janssens, 2006). Furthermore, the increased NH_4^+ content and soil temperature in $BC_{0\text{-}10cm}$ may have further promoted lower Q_{10} values (Fig. 5A) because the interactions between labile carbon and N may have increased microbial activity (Ge et al., 2019a; Li et al., 2020).

4.4. *Effect of biochar application depth on soil CH_4 uptake*

Biochar application generally increased CH_4 uptake, and the extent of the increase was affected by the biochar application depth (Fig. 2); these results are not in line with our hypothesis or with those of a previous *meta*-analysis study, which suggested that biochar decreased the CH_4 sink in upland agricultural systems (Jeffery et al., 2016). This may be because the method applied in their analysis does not allow the inclusion of negative fluxes (e.g., CH_4 uptake in the present study) and thus was restricted in the conclusions that could be drawn. However, our result agreed with that of Ramlow and Cotrufo (2018), who found that woody biochar increased the CH_4 sink by approximately 48.6% in upland soil, although the increased NH_4^+ in $BC_{0\text{-}10cm}$ may have limited CH_4 oxidation due to competition with CH_4 at binding sites (Jeffery et al., 2016; Nazaries et al., 2013). The sorption of CH_4 to the biochar surface is one of the mechanisms of enhanced soil CH_4 uptake (Karhu et al., 2011). In addition, improved soil aeration may lead to CH_4 utilization by methanotrophs that outpaces CH_4 production (Feng et al., 2012). Hence, all three biochar addition treatments decreased diffusive CH_4 flux, in all probability by improving soil aeration through the soil layer structure and the reduced BD (Jeffery et al., 2016; Castellini et al., 2015; Li et al., 2018). However, in contrast with previous studies suggesting that labile carbon from biochar stimulates CH_4 production (Ramlow and Cotrufo, 2018), we found that the content of labile carbon and the size of the microbial population (i.e., DOC and MBC) in the 0–10 cm soil were negatively correlated with CH_4 flux (Fig. 5A). This implies that labile carbon may promote the growth of topsoil methanotrophs because soil CH_4 uptake is driven by methanotrophs microbially oxidizing CH_4 (Jeffery et al., 2016). Moreover, CH_4 uptake was mainly affected by the environmental factors in the 0–10 cm soil, which explained 88% of the variation in CH_4 uptake, whereas the environmental factors in the 10–20 cm soil explained only 16% of the variation (Fig. 7B). Thus, the lower labile C content and microbial activity in the 0–10 cm soil in $BC_{10\text{-}20cm}$ may contribute to the higher CH_4 flux. $BC_{10\text{-}20cm}$ presented a finer texture layer overlying a coarser texture layer, which is characterized by the production of fast-flowing wetting fingers on the interface, thus promoting water infiltration (Li et al., 2018) and decreasing gas phase pores in the soil below 10 cm, which is conducive to methanogen activity (Wang et al., 2018).

4.5. *Implications for field application*

This study was the first to explore the effect of biochar application depth on Rs characteristics and CH_4 fluxes in upland agricultural soil systems, offering certain significant insights for further optimizing the potential of biochar for carbon sequestration and greenhouse gas emission reduction. Overall, $BC_{10\text{-}20cm}$ and $BC_{0\text{-}20cm}$ were more beneficial to the stability of soil organic C than $BC_{0\text{-}10cm}$, while $BC_{0\text{-}10cm}$ had a higher ability to increase the CH_4 sink. However, the net CH_4 uptake due to biochar application in all three treatments was far less than their net CO_2 emissions (Tables 1 and 2), even if CH_4-C was converted into CO_2-C according to their global warming potential (data not shown). Finally, $BC_{10\text{-}20cm}$ enhanced soil CH_4 uptake without increasing CO_2 emissions, improved carbon utilization efficiency (qCO_2) and reduced Q_{10}, and it can be considered the optimal strategy for enhancing soil carbon sequestration in this study. Additionally, it is worth noting that although $BC_{10\text{-}20cm}$ showed a negative effect on soil moisture in 10–20 cm soil (Fig. 3B and Table 3), it did not indicate a reduced water supply capacity. First, the soil volumetric water content was used to represent the soil moisture condition in this study, while $BC_{10\text{-}20cm}$ had no significant effect on the soil mass water content (data not shown). Second, our previous study suggested that $BC_{10\text{-}20cm}$ could effectively improve hydraulic conductivity, which implies that the water had been stored in a deeper layer of soil (Li et al., 2018).

To maximize the benefits of biochar while minimizing costs, combining biochar application with other farming measures that can synchronously bury biochar in the subsurface soil, such as deep tillage, furrow dressing and deep fertilizer placement (Li et al., 2018), is recommended. This may strengthen the beneficial effects of biochar or even create benefits in addition to carbon sequestration, including reducing fertilizer N losses, improving soil hydraulic characteristics (Li et al., 2016; Yao et al., 2018), facilitating legume N transfer and arbuscular mycorrhizal inoculation (Liu et al., 2018, 2017), breaking down root-restricting soil layers, and promoting root growth (Baumhardt et al., 2008; Schneider et al., 2017), thus improving soil quality and plant productivity. However, because this study focused on soil CO_2 and CH_4 dynamics due to microbial respiration and diffusion while limiting the respiration and transport of CO_2 and CH_4 by plants, it conducted a field study without the presence of crops (Min et al., 2020); therefore, the system does not represent completely realistic conditions. Given that the interaction between biochar application depth and plant growth (e.g., rhizosphere deposition) could be a key determinant for carbon cycling processes (Keiluweit et al., 2015; Philippot et al., 2013; Zhalnina et al., 2018), it is necessary to carry out biochar application experiments in actual crop production systems in the future.

5. Conclusions

Soil CO_2 emissions and CH_4 uptake showed strong temporal patterns and were notably affected by the biochar application depth. Overall, $BC_{10\text{-}20cm}$ and $BC_{0\text{-}20cm}$ did not significantly increase CO_2 emissions but did increase CH_4 uptake and reduce qCO_2; in addition, $BC_{10\text{-}20cm}$ showed a negative effect on Q_{10}. However, $BC_{0\text{-}10cm}$ increased CO_2 emissions and enhanced CH_4 uptake, increased qCO_2 and decreased Q_{10}. The variations in CO_2 emissions, CH_4 uptake and Q_{10} were mainly caused by the complex interactions among hydrothermal conditions and porosity (i.e., temperature, moisture and BD), nutrients (i.e., N, NH_4^+, AP) and labile carbon content (MBC and DOC) in the 0–10 cm soil layer. However, the enhanced microbial biomass and DOC in the 10–20 cm soil was beneficial for the microbial carbon use efficiency of the whole 0–20 cm soil layer. Additionally, the increases in carbon lability and microbial activity in the surface soil, rather than the enhanced soil carbon protection, contributed to the reduction in Q_{10}. Taken together, such results indicate that $BC_{10\text{-}20cm}$ is a promising way to improve carbon utilization efficiency and stabilize soil organic carbon in the long term. Nevertheless, considering that biochar application depth critically influences edaphic factors and carbon cycling, the carbon sequestration potential of biochar under different application depths in plant cultivation systems remains to be explored.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115186.

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