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Biochar reduces soil heterotrophic respiration in a subtropical plantation through increasing soil organic carbon recalcitrancy and decreasing carbon-degrading microbial activity

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ABSTRACT

Carbon (C) storage in forest soils can be enhanced through increasing organic C input and decreasing soil heterotrophic respiration (R_H). The inhibitory effect of biochar on R_H has been extensively studied in agricultural soils, while such an effect and the mechanisms involved remain unknown in forest soils. Here, we examine the response of soil physicochemical and microbial properties to biochar application and how these factors mediate the biochar-induced change in soil R_H in a subtropical bamboo plantation. Our results showed that biochar application significantly reduced R_H, and markedly altered most of the studied soil properties important for R_H in the bamboo plantation. Biochar application did not affect soil temperature and no relationship between soil R_H and either soil moisture or labile organic C content was observed, excluding the possibility that biochar reduced the R_H through changing soil temperature, moisture or labile organic C content, factors commonly considered to control R_H. As compared to the control, biochar application significantly increased the aromatic C content and RubisCO enzyme activity, while decreased β-glucosidase and cellobiohydrolase (CBH) activities. In addition, the soil R_H was positively ($P < 0.01$) correlated with β-glucosidase and CBH activities, while negatively ($P < 0.05$) correlated with RubisCO enzyme activity. Further, using structural equation modelling, we revealed that bicohar reduced R_H through increasing the proportion of soil recalcitrant C fraction and decreasing the β-glucosidase and CBH activities in relation to the decomposition of carbohydrates and celluloses in the soil. This is the first report that increased soil organic C recalcitrancy and decreased activities of C-degrading enzymes are responsible for biochar to reduce R_H in the subtropical plantation, which may be key to regulating R_H in subtropical plantations through forest management.

1. Introduction

The global soil organic carbon (SOC) stock is larger than the sum of atmospheric C and plant biomass C (Lehmann and Kleber, 2015), and the SOC stored in forests accounts for about 70% of the global SOC (Jandl et al., 2007), suggesting that changes in the SOC stock in forests could considerably influence the atmospheric CO_2 concentration (Peng et al., 2008). Soil respiration (R_S) is the main pathway of C efflux from the soil to the atmosphere that has an annual rate of 68–79 Pg CO_2-C globally (Raich and Potter, 1995; Xu and Shang, 2016). The R_S consists of autotrophic respiration (R_A) and heterotrophic respiration (R_H), with R_A originating from root and rhizosphere respiration, and R_H from microbial decomposition of soil organic matter (SOM) (Baggs, 2006; Hopkins et al., 2013). The change in R_A has little effect on the variation in the SOC stock (Kuzyakov, 2006), while the change in R_H could substantially alter the pool size of SOC and the atmospheric CO_2

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concentration (Moinet et al., 2016). Thus, it is critically important to regulate the rate of R_H for forecasting future changes in SOC stock and mitigation of global climate change (Wang et al., 2010; Moinet et al., 2016).

Elucidating the mechanisms that affect soil R_H is critical for developing methods to manage R_H. The rate of soil R_H greatly depends on soil microbial activity and substrate availability, since it is derived from the microbial decomposition of SOM and plant residue (Zhou et al., 2012; Whitaker et al., 2014; Ding et al., 2016). Soil temperature and moisture have been considered as vital factors that control R_H through directly affecting soil microbial activity and indirectly changing the substrate availability (Davidson et al., 2006; Wan et al., 2007). Although soil temperature and moisture content can account for a considerable proportion of the variation in R_H (Li et al., 2010; Moyano et al., 2013; Matteucci et al., 2015), a large proportion of the variation has not been fully understood.

Soil microorganisms generally play a vital role in the decomposition of SOM. The microbial mechanisms involved in regulating R_H, however, has rarely been thoroughly investigated (Cleveland et al., 2007; Schmidt et al., 2011; Chen et al., 2017b). Neither soil microbial biomass nor the microbial community composition determined by phospholipid fatty acids (PLFAs) accounted for the variation of R_H across four subtropical forests (Wei et al., 2015). Changes in R_H have been linked to soil bacterial community composition using PCR-based methods (Cleveland et al., 2007; Fierer et al., 2007). The decomposition or transformation of SOM is also affected by the activities of extracellular enzymes such as β-glucosidase (Chen et al., 2013; Ge et al., 2017), cellobiohydrolase (CBH) (Zhang et al., 2017b) and RubisCO enzyme (Guo et al., 2015). While field evidence on the relationship between R_H and C-cycling enzyme activities is still scarce (Zhou et al., 2012; Xue et al., 2016). In addition, the positive relationship between substrate availability and R_H has mostly been studied in laboratory experiments (Wild et al., 2014), with the relationship seldom tested in field studies. Moreover, substrate availability may also indirectly affect the R_H through altering the soil microbial community composition (de Graaff et al., 2010; Thiessen et al., 2013; Ma et al., 2016). However, there has been no field study to comprehensively evaluate relationships among R_H and soil substrate availability, microbial community composition, and the activities of C-cycling enzymes.

Increasing organic C input to the soil or decreasing SOM decomposition are effective ways to increase soil C stock (Paustian et al., 2016). Addition of biochar derived from plant biomass not only can reduce pollutant (heavy metals and organic pollutants) concentrations in soils (Inyang et al., 2016; Igalavithana et al., 2017; Thangarajan et al., 2018), but can also increase soil C sequestration due to their high resistance to decomposition (Baldock and Smernik, 2002; Lehmann and Joseph, 2015; Li et al., 2018), and therefore has been used as a management strategy to mitigate global climate change (Cox et al., 2000; Zimmerman et al., 2011; Zhou et al., 2017; Zhang et al., 2017a; Bamminger et al., 2018). Biochar application strongly influences both the composition of and substrate availability to soil microbial communities (Steinbeiss et al., 2009; Zimmerman, 2010; Khodadad et al., 2011; Luo et al., 2013), which jointly control the decomposition of native SOC and ultimately affect R_H (Zimmerman et al., 2011; Lu et al., 2014; Zhou et al., 2017). Owning to the broad impact on various soil properties by biochar application (Igalavithana et al., 2017), significant gaps remain in our current understanding of how soil R_H respond to biochar-induced changes in SOC bioavailability, microbial community composition and function, and their interactions. Such gaps would cause large uncertainties regarding how to develop policy-relevant quantitative measures in relation to biochar addition and expected C sequestration (Schmidt et al., 2011).

Forest plantations in China account for about one third of the global area of plantation and contributed about 80% of the total forest C sink increment in China (FAO, 2010; Fang et al., 2014). Recently, some studies have revealed that management practices such as fertilization and understory removal would markedly decrease SOC stock and increase R_S (Liu et al., 2011; Li et al., 2013, 2014; Vogel et al., 2015), which negatively affects C sequestration in forest soils. Therefore, it is of great significance to develop forest management practices that can increase SOC stock but decrease R_H. Application of biochar produced by forest-origin residues has been regarded as an economical and environmentally sustainable strategy for C sequestration (Jeffery et al., 2015). The inhibitory effect of biochar on SOM decomposition or R_H in agricultural soils has been tested through laboratory incubation experiments (Lu et al., 2014; Chen et al., 2017a), while such an effect and the mechanisms involved are poorly understood in forest soils (Li et al., 2018).

Bamboo is widely distributed in subtropical and tropical regions, accounting for approximately 0.8% of the global total forest area in 2010 (FAO, 2010). Among the bamboo species, the Moso bamboo (*Phyllostachys edulis*) has a global area of 4.2 million ha and is the most abundant bamboo species (Yan et al., 2015; Yuen et al., 2017). Moso bamboo is a fast-growing forest species with a great potential of fixing CO_2 from the atmosphere and provides substantial economic and ecological benefits in the Asia-Pacific region (Yuen et al., 2017; Hu et al., 2018). Here, we investigate the response of soil physicochemical and microbial properties to biochar application and how these factors mediate biochar-induced changes in soil R_H in a subtropical Moso bamboo plantation. The specific objectives of this study were (i) to investigate the effects of biochar application on the soil R_H and the soil physicochemical and microbial properties important for R_H in the bamboo plantation and (ii) to elucidate the mechanisms for the biochar-induced changes in soil R_H in the bamboo plantation.

2. Materials and methods

2.1. Experimental site

The research was carried out at an experimental site in Shankou Township (30°14′N, 119°42′E), Hangzhou City, China. The site is in a hilly area with an elevation of ~150 m. The climate is subtropical with a mean annual temperature of 15.8 °C, and1946 h of sunshine and 239 frost-free days per year. The monthly average air temperature and monthly cumulative precipitation during the study (from September 2015 to September 2016) are presented in Fig. S1. The soil is a Ferralsol according to the FAO soil classification system (WRB, 2006). We selected an 1500 m^2 area to establish the experiment in December, 2014. The Moso bamboo plantation in the study site was converted from a natural evergreen broadleaf forest by planting bamboo after harvesting the broadleaf forest. The Moso bamboo plantation in this study was 16 years old in 2014. The mean diameter at breast height of the studied bamboo plantation was 9.9 cm, with a stocking density of 2880 culm ha^{-1} when measured at the beginning of this study in 2014. In June of each year, fertilizers including urea (200 kg N ha^{-1}), super phosphate (60 kg P ha^{-1}), and potassium chloride (70 kg K ha^{-1}) were broadcast applied, followed by deep tillage to 30–35 cm depth. During the experimental period, no fertilizer was applied in the plots. The ground vegetation in the study plots was manually removed annually. The experimental area was divided into 3 blocks, and soil samples were taken from the 0–20 cm depth from five randomly selected points in each block with a corer. The five soil samples collected from each block were mixed to form a composite sample for each block. The average values for the selective physicochemical properties (see methods described below) in this site were: pH of 4.48, bulk density of 1.14 g cm^{-3}, organic C of 18.6 g kg^{-1}, total N of 2.14 g kg^{-1}, available P of 9.98 mg kg^{-1}, available K of 99.3 mg kg^{-1}, sand of 354 g kg^{-1}, silt of 382 g kg^{-1}, and clay of 264 g kg^{-1}.

2.2. Experimental design

The experiment included three treatments with four replications.

The plots were arranged in a randomized block design, with each plot 8 m × 12 m in size and a 3 m buffer zone among plots. The three treatments were no biochar application as control (B0), biochar application at 5 t ha^{-1} (B5), and 15 t ha^{-1} (B15), which were equivalent to an application rate of 0.22% and 0.66% (w/w), respectively, when incorporated in the 0–20 cm topsoil. The lower rate is comparable to the addition of charcoal from natural fires to forest soils (Hart and Luckai, 2013). The bamboo leaf biochar was produced through pyrolysis under an oxygen-limited condition at 500 °C by Zhejiang Bulaimeng Science and Technology Corporation. The pyrolysis conditions were as follows: heating rate of 10 °C min^{-1}, N_2 flow of 3.0 L min^{-1} and final holding temperature of 500 °C lasting for two hours. The biochar had C, H, O, S and ash contents of 667, 30, 248, 3 and 267 g kg^{-1}, respectively, with a pH of 9.37 and a specific surface area of 4.07 m^2 g^{-1}.

To separate the total R_S into R_A and R_H, we adopted the trenching technique (Bond-Lamberty et al., 2011). Three polyvinyl chloride (PVC) collars with a coverage area of 314 cm^2 (diameter 20 cm) and a height of 11.5 cm were installed 2–2.5 cm below the soil surface at random locations in each plot and used to measure the R_S. The trenched subplots (1 m × 1 m) were established near the above-mentioned three collars by digging a trench 15 cm wide and 70 cm deep, to below the main rooting zone of bamboo plants. PVC panels of 1 × 1 m in size was inserted into the trench to stop root growth in the trenched subplots, and the soil taken out through digging the trench was back filled to the trench. Three PVC collars with the same size were installed in the same way in the center of each trenched subplot to measure R_H. To minimize the trenching practice effects on soil disturbance and effects of dead roots decomposition in the trenched subplots, we began to measure R_S in September, 2015, 9 months after trenching. In the trenched subplots, ground vegetation was periodically removed manually throughout the experiment to eliminate root growth.

In September 2015, the biochar was added to the B5 and B15 plots and tilled into the 0–20 cm soil layer. Biochar was weighed and applied to each trenched subplot to ensure accurate application rates. Prior to the application, the biochar was over-dried, ground and sieved (< 2 mm).

2.3. Measurement of R_S

R_S was measured at 1, 4, 7, 14, 21, 28, 42, 56, 70 and 84 days after the applications of biochar, and once a month thereafter. The R_S was measured on rainless mornings (between 09:00 a.m. and 11:00 a.m.). The CO_2 efflux and soil temperature (at 5 cm depth) data were determined using a LI-8100 soil CO_2 flux system (LI-COR Inc., Lincoln, Nebraska) that was equipped with a temperature probe. For each collar and at each sampling, soil CO_2 efflux was determined twice. The time for each determination was 90 s, separated by 30 s between the two measurements.

2.4. Measurement of biochar properties and soil physicochemical properties

The properties of biochar were determined as follows. The pH was determined on a 1:20 (w/v) water suspension of the biochar samples after stirring for 1 h. The ash content of biochar was measured according to the ASTM D1762-84 method. The total C, H, O and S contents of biochar were determined using an elemental analyzer (Flash EA1112, Thermo Finnigan, Italy). The Brunauer-Emmett-Teller (BET) surface area of the biochar was measured by N_2 sorption analysis at 77 K using a surface analyzer (TristarII3020, Micromeritica Instrument Corporation, USA) after degassing.

The physicochemical properties of soil samples taken from the experimental site prior to biochar application in this study were determined as follows. Soil pH was measured in a 1:2.5 (w:v) mixture of soil and distilled water. The SOC and total N (TN) concentrations were determined with an elemental analyzer (CHN-O-RAPID, Heraeus, Germany). Available P concentration was determined by the Bray procedure (Bray, 1945). Available K (extracted by 1 mol L^{-1} NH_4OAc) concentration was determined by the flame photometric method (Lu, 1999). The soil's particle-size distribution was analyzed using the pipette method after the sample was pretreated with H_2O_2 (15%) and $Na_4P_2O_7$ (0.1 mol L^{-1}) (Lu, 1999). At each Rs measurement time, soil samples in the 0–20 cm layer were taken from three randomly-selected locations near each collar and thoroughly mixed for determining soil moisture content and concentrations of water soluble organic C (WSOC) and microbial biomass C (MBC). Soil moisture content was determined by the difference in mass after drying at 105 °C until constant weight. The concentration of soil WSOC was measured following Wu et al. (2010), and soil MBC concentration was measured by the chloroform fumigation-extraction method (Vance et al., 1987), following the detailed procedure described in Li et al. (2013).

2.5. Determination of SOC chemical composition

Before the analysis described below, the samples were pre-treated with 10% (v/v) hydrofluoric acid (HF) solution to remove Fe^{3+} and Mn^{2+} ions in soils as detailed in Li et al. (2013). The ^{13}C cross polarization/total sideband suppression (CP/TOSS) analysis with magic angle spinning (MAS) was performed using a NMR spectrometer (AVANCE 400, Bruker, Germany). The spectrometer frequency for ^{13}C was 100 MHz, the spinning speed was 5 kHz, the ^1H 90° pulse-length was 4 μs and the recycle delay time was 0.8 s. Four-pulse TOSS was performed before detection, and two-pulse phase-modulated (TPPM) decoupling was employed in order to obtain an optimum resolution during detection (Mao et al., 2008; Li et al., 2017). As described previously (Huang et al., 2008; Li et al., 2013), each spectrum obtained from the NMR analysis was divided into the following four regions: 0–46 ppm, 46–114 ppm, 114–164 ppm and 164–220 ppm. These four regions represent the alkyl C, O-alkyl C, aromatic C and carbonyl C, respectively. The relative contents of the aforementioned four C fractions were calculated according to the area under the curve in each region.

2.6. Measurement of soil enzyme activities

The soil invertase (EC 3.2.1.26) activity was determined according to the method of Frankenberger and Johanson (1983). The soil β-glucosidase (EC 3.2.1.21) activity was determined using the method of Alef and Nannipieri (1995). The soil cellobiohydrolase (CBH; EC 3.2.1.91) activity was determined following the method of Zhang et al. (2017b). The RubisCO enzyme (EC 4.1.1.39) activity was measured by the method of Guo et al. (2015). The soil dehydrogenase activity was measured following the method described in Casida et al. (1964). The urease (EC 3.5.1.5) activity was measured using the method described in Kandeler and Gerber (1988). The detailed procedures for the determination of the aforementioned six soil enzyme activities can be found in the Supplementary Information.

2.7. DNA extraction and real-time PCR

The PowerSoil DNA Isolation Kit (MoBio Labs, Solana Beach, CA) was used to extract the total microbial DNA of soil samples according to the manufacturer's instructions. DNA concentration and quality were determined using a NanoDrop spectrophotometer, and the DNA samples were stored at −40 °C until further analysis. The gene copy numbers of phylogenetic and functional marker genes (bacterial 16S rRNA and *cbbL*, fungal 18S rRNA and *cbh*I) were determined in triplicates using the CFX96™ Real-Time System (Bio-Rad Laboratories, USA). The primes and PCR conditions are described in Table S1. The melt curve analysis at the end of the PCR runs and visualization by agarose gel electrophoresis were used to check the specificity of PCR products. Copy numbers of the target gene in soil samples were based on a

standard curve generated using purified template plasmid DNA with a log-linear effect of target concentration. The efficiencies of the real-time PCR of four genes were 98.2–105%, with an R^2 value greater than 0.993. The results regarding gene copy numbers were expressed on the basis of dry soil weight.

2.8. Analysis of soil bacterial and fungal community compositions

The genotypic fingerprinting approach using the terminal restriction fragment length polymorphism (T-RFLP) technique was used to determine the bacterial and fungal community compositions. The PCR amplification of bacterial 16S rRNA gene and fungal internal transcribed spacer (ITS) region was conducted in triplicate for each soil sample using primers 8F/926R (Liu et al., 1997) and ITS1/ITS4 (White et al., 1990; Gardes and Bruns, 1993), respectively. The forward primers 8F and ITS1 were fluorescently labeled with 6-carboxyfluorescein (6-FAM) at the 5' end, as the method used in previous studies (Liu et al., 1997; Kasel et al., 2008). PCR conditions are described in Supplementary Table 6. The PCR products were purified with the SanPrep PCR Purification Kit (Sangon Biotech, Shanghai, China), and the concentration of purified PCR products was measured with a NanoDrop spectrophotometer. Approximately 100 ng of purified PCR products were digested using the restriction enzymes *Msp*I (16S rRNA) and *Taq*I (ITS region) at 37 °C and 65 °C, respectively, for 4 h. A fragment analysis was employed by capillary electrophoresis (3730 Genetic Analyzer; Applied Biosystems, CA) with a GeneScan ROX-labeled GS500 internal size standard. The relative abundance of true terminal restriction fragments (T-RFs) within a given T-RFLP pattern was generated as a ratio of the respective peak area. To avoid detecting primers and uncertainties in size determination, T-RFs were filtered with the T-REX online software (http://trex.biohpc.org/), and T-RF sizes were aligned to the nearest integer (Culman et al., 2009). We used the T-RFs with a relative abundance above 1% for the ordination analysis.

2.9. Statistical analysis

The R_S components were calculated as follows.

R_S = Measured values from soil collars in non-trenched plots
R_H = Measured values from soil collars in trenched plots
$R_A = R_S - R_H$

Annual cumulative soil CO_2 emissions were calculated as

$$M = \Sigma\,(R_{i+1}+R_i)\,/2 \times (t_{i+1}-t_i) \times 3600 \times 24 \times 44 \times 10^{-8} \quad (1)$$

where M is the cumulative value of soil CO_2 emissions (t CO_2 $ha^{-1}\,yr^{-1}$), R is the soil respiration rate (μmol $m^{-2}\,s^{-1}$), i is the sampling number, and t is the sampling time based on the Julian day.

An exponential model was used to present the relationship between R_S components and soil temperature:

$$y = a \times e^{k \times t} \quad (2)$$

where y is the soil CO_2 efflux rate of soil respiration components (μmol $m^{-2}\,s^{-1}$), t is soil temperature, and a and k are constants.

The temperature sensitivity of the soil CO_2 efflux (Q_{10}) was calculated by

$$Q_{10} = e^{10 \times k} \quad (3)$$

A repeated-measures analysis of variance (ANOVA) was used to assess the significance of the impacts of biochar application, season of sampling, and their interaction on R_S components, soil temperature, moisture content, WSOC and MBC concentrations, the content of different organic C fractions, bacterial and fungal abundances, bacterial and fungal diversities, *cbbL* and *cbh*I gene copy numbers, and different soil enzyme activities. A one-way ANOVA and the least significant difference (LSD) test were employed to detect the statistical significance of the biochar application rate on mean annual CO_2 flux, annual cumulative CO_2 flux, and Q_{10}. Before conducting the ANOVA, the normality and homogeneity of the variance were tested, and the data were log-transformed if the assumption of homogeneity was not met. The significance was determined at alpha = 0.05 for all statistical analyses, unless specifically indicated. The statistical analyses were conducted using SPSS version 18.0 (SPSS, Chicago, IL, USA).

The Shannon Index (Shannon, 1948) of bacterial and fungal community compositions was calculated based on the T-RFLP profile. Significance tests of the effects of biochar application on the overall bacterial and fungal community compositions with multi-response permutation procedures (MRPP) and NMDS ordination were carried out using the R package vegan (Oksanen et al., 2007). The Mantel tests were used to determine the relationships between soil enzymes activities and soil microbial (bacterial and fungal) community composition.

A structural equation model (SEM) was established using AMOS 22.0 (SPSS Software, Chicago, IL) to examine how the soil R_H rate was driven by the main soil factors that are altered by biochar application. First, linear regression analyses were performed to determine the relationships between soil R_H rate and different soil enzyme activities. Then, we found that the soil R_H rate was significantly correlated with the activity of three soil enzymes, including β-glucosidase, CBH and RubisCO enzyme, activities. Second, we selected the soil properties that were significantly changed by biochar application according to the results of the repeated-measures ANOVA. Then, we have selected 8 important soil properties, including contents of soil moisture, WSOC, MBC, alkyl C, O-alkyl C, aromatic C, fungal abundance and Shannon Index of bacteria. Third, linear regression analyses were performed to explore the relationships among the three soil enzymes activities selected in the first step and the 8 important properties selected in the second step. In addition, the abundance of *cbbL* and *cbh*I genes were included, since they encode the enzymes of RubisCO and CBH. Fourth, a structural equation model was established according to the results obtained in the previous three steps. Last, the fitness of the model was checked by the maximum likelihood ($\chi 2$) goodness-of-fit test, the goodness-of-fit index (GFI) and the root-mean-square error of approximation (RMSEA).

3. Results

3.1. Soil microclimate and organic C pool

During the one-year field experiment, seasonal variations of soil temperature followed that of air temperature (Fig. 1a; Fig. S1). There was no effect of biochar application on soil temperature (Table S2). The mean soil moisture content across the measurement period was 318.3 mg kg^{-1} in the control (No biochar, B0), 329.3 mg kg^{-1} in the B5 treatment, and 340.7 mg kg^{-1} in the B15 treatment (Fig. 1b). Compared to the control, the B5 and B15 treatments increased WSOC concentration by an average of 13.9% and 27.0%, respectively (Fig. 1c), and MBC concentration by 12.4% and 15.1%, respectively (Fig. 1d). The O-alkyl C (48.3–55.4%) dominated the SOC regardless of the treatment or sampling date, followed by alkyl C (24.2–27.2%) (Fig. 2). The B5 and B15 treatments decreased the O-alkyl C content while increased the aromatic C content, as compared to the control (Fig. 2; Table S3).

3.2. Soil microbial community composition and function

As compared to the control, the B5 and B15 treatments generally increased soil bacterial 16S rRNA gene copy numbers 1 and 3 months after biochar application, but decreased them 6 and 12 months after biochar application ($P < 0.05$) (Fig. 3a). The B5 and B15 treatments increased bacterial Shannon Index 1 and 12 months after biochar application as compared to the control (Fig. 3c), and decreased the soil

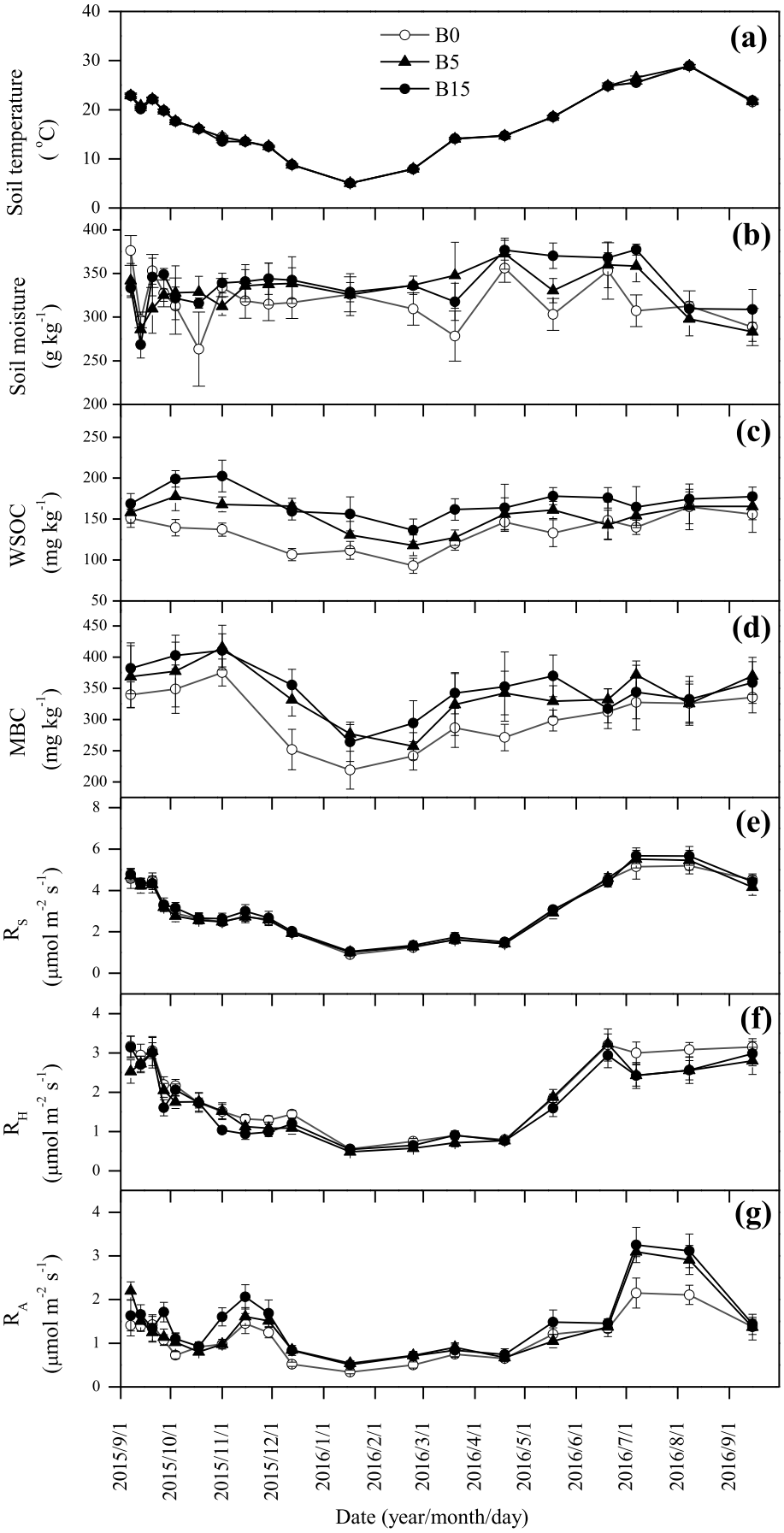

Fig. 1. Temporal changes of (a) soil temperature at the 5 cm depth, (b) soil moisture content in the 0–20 cm soil layer, (c) soil water soluble organic C (WSOC) concentration in the 0–20 cm soil layer, (d) soil microbial biomass C (MBC) concentration in the 0–20 cm soil layer, (e) soil respiration (R_S) rate, (f) autotrophic respiration (R_A) rate, and (g) heterotrophic respiration (R_H) rate in a Moso bamboo plantation from September 2015 to September 2016. The three treatments studied are: B0-no biochar application, B5-biochar application at 5 t ha^{-1}, and B15-biochar application at 15 t ha^{-1}. Vertical bars are standard deviations of the mean (n = 4).

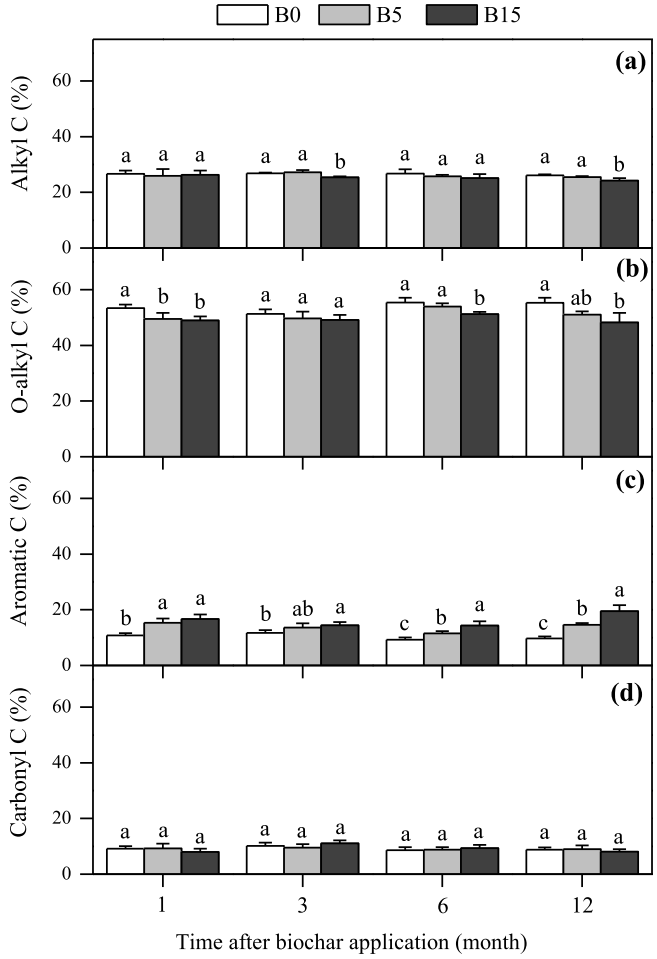

Fig. 2. Temporal changes of (a) alkyl C, (b) O-alkyl C, (c) aromatic C, and (d) carbonyl C contents in the 0–20 cm soil layer in a Moso bamboo plantation under three treatments: B0-no biochar application, B5-biochar application at 5 t ha^{-1}, and B15-biochar application at 15 t ha^{-1}. Vertical bars are standard deviations of the mean (n = 4).

fungal 18S rRNA gene copy numbers 1 and 6 months after biochar application ($P < 0.05$). For both bacterial and fungal community compositions, the nonmetric multidimensional scaling (NMDS) analysis showed that the samples under a given treatment were grouped together regardless of the sampling date (Fig. 4), confirming the significant effect of biochar application on the overall bacterial and fungal community composition ($P < 0.05$) (Table S4). The B5 and B15 treatments increased ($P < 0.05$) bacterial *cbbL* gene copy numbers and RubisCO enzyme activity (Figs. 3e and 5d) but decreased ($P < 0.05$) the fungal *cbh*I gene copy numbers and CBH activity (Figs. 3f and 5c) as compared to the control, regardless of the sampling date. There were positive relationships between *cbbL* gene copy numbers and RubisCO enzyme activity ($R^2 = 0.21$, $P < 0.01$), and between *cbh*I gene copy numbers and CBH enzyme activity ($R^2 = 0.57$, $P < 0.01$). In addition, biochar application decreased the β-glucosidase activity regardless of the sampling date (Fig. 5b), and decreased the dehydrogenase and urease activities 1 and 12 months after application (Fig. 5e and f).

3.3. Soil respiration components

The R_S, R_H and R_A in the B0, B5 and B15 treatments were in the ranges of 0.90–5.68, 0.48–3.23, and 0.3–3.25 μmol CO_2 m^{-2} s^{-1}, respectively (Fig. 1e–g). Regardless of the biochar treatment, the R_S and R_H decreased gradually at the beginning of the experiment, then increased steadily and reached their maximum in June–August (Fig. 1e and f). Compared to the control, the B15 treatment increased the annual cumulative CO_2 flux of R_S, while the B5 treatment did not cause any change on it (Table 1). However, the B5 and B15 treatments significantly decreased the annual cumulative R_H by 11.1% and 12.3%, respectively, as compared to the control. The biochar treatments increased the annual cumulative R_A, with a greater effect at the higher application rate (Table 1).

3.4. Relationships between soil R_H and soil properties

The soil R_H was exponentially related to soil temperature ($P < 0.01$) regardless of the treatment (Fig. 6a–c). The Q_{10} values for R_H among the B0, B5 and B15 treatments were not different, with values of 2.21, 2.30 and 2.24, respectively. No significant relationship was found between soil R_H and soil moisture content or WSOC concentration regardless of the treatment (Fig. 6d–i). Soil R_H and MBC concentration were positively correlated ($P < 0.01$) in the control but not in the B5 and B15 treatments (Fig. 6j–l). The soil R_H was positively ($P < 0.01$) correlated with β-glucosidase and CBH activities, while negatively ($P < 0.05$) correlated with RubisCO enzyme activity (Fig. 7b–d). The SEM revealed that soil R_H was driven by CBH ($P < 0.001$) and β-glucosidase activities ($P = 0.007$), and these two enzymes accounted for 50% and 31%, respectively, of the variation in soil R_H (Fig. 8). Through altering the CBH activity, soil R_H was indirectly enhanced by *cbh*I gene abundance, but indirectly decreased by aromatic C content. Biochar application decreased the abundance of the *cbh*I gene but increased the aromatic C content, and the latter also indirectly decreased soil R_H through decreasing the β-glucosidase activity (Fig. 8).

4. Discussion

Although the effect of biochar on overall soil respiration have been extensively studied (Sackett et al., 2015; Liu et al., 2016; Zhou et al., 2017), its effect on soil respiration components, i.e., R_A and R_H, and the mechanisms involved are still poorly understood. Elucidating the mechanisms for biochar to decrease soil R_H is vital to developing strategies for increasing soil C sequestration (Schmidt et al., 2011). In this study, we demonstrated that biochar application significantly decreased R_H, while increased R_A, in addition to significant changes in soil moisture content, the quantity and quality of SOC, microbial abundance and community composition, and activities of C-cycling enzymes, in the subtropical bamboo plantation (Figs. 1–5; Table 1). This is the first field evidence in subtropical forest ecosystems to reveal the inhibitory effect of biochar on R_H, an important step forward from previous laboratory incubation experiments (Zimmerman et al. 2011; Lu et al., 2014). In combination with findings that biochar application can significantly increase SOC stock and decrease soil N_2O emissions in subtropical plantations (Wang et al., 2014; Xiao et al., 2016), biochar application should be an effective way to increase SOC stock and mitigate greenhouse gas emissions in such plantations.

Soil temperature and moisture are two vital environmental factors determining R_H (Davidson et al., 2006; Wan et al., 2007). Consistent with previous studies (Hinko-Najera et al., 2015; Zhou et al., 2017), we also found that soil temperature was the dominant factor influencing soil R_H in the bamboo plantation (Fig. 6a–c). However, the decreased R_H caused by biochar application could not be explained by changes in soil temperature because biochar application did not significantly affect soil temperature (Fig. 1a; Table S2). In addition, regardless of the biochar treatment, no relationship between soil R_H and soil moisture content was observed in this study (Fig. 6d–f). Therefore, we can exclude the possibility that bicohar reduces R_H through changing soil temperature and moisture content.

Soil R_H is generally increased with the increasing supply of organic substrates (Javed et al., 2009; Mcmullen et al., 2015; Chen et al., 2017b); however, in the studied Moso bamboo plantation, biochar

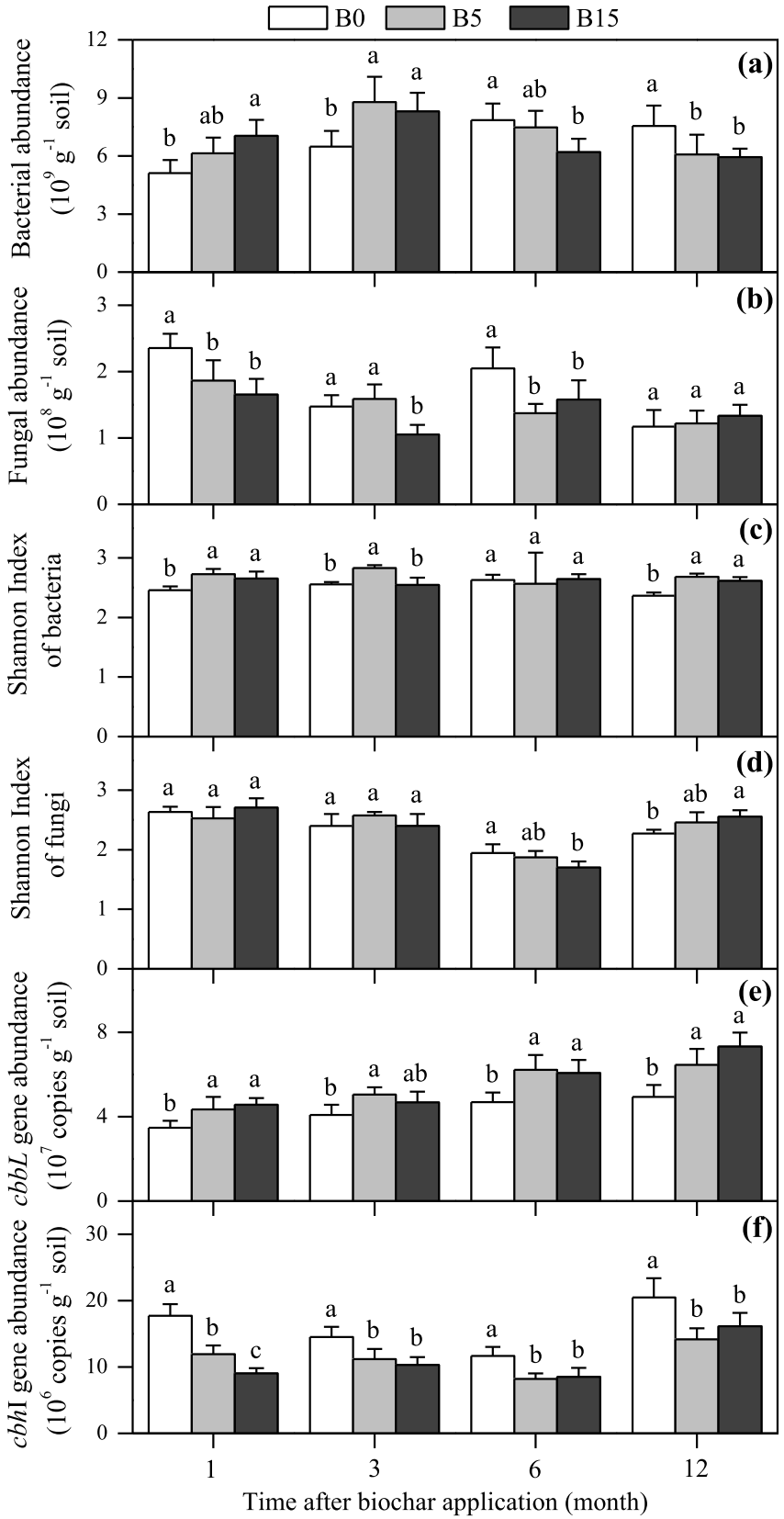

Fig. 3. Temporal changes of (a) bacterial 16S rRNA gene abundance, (b) fungal 18S rRNA gene abundance, (c) Shannon Index of bacteria, (d) Shannon Index of fungi, (e) *cbbL* gene abundance, and (f) *cbh*I gene abundance in the 0–20 cm soil layer in a Moso bamboo plantation under three treatments: B0-no biochar application, B5-biochar application at 5 t ha^{-1}, and B15- biochar application at 15 t ha^{-1}. Vertical bars are standard deviations of the mean (n = 4).

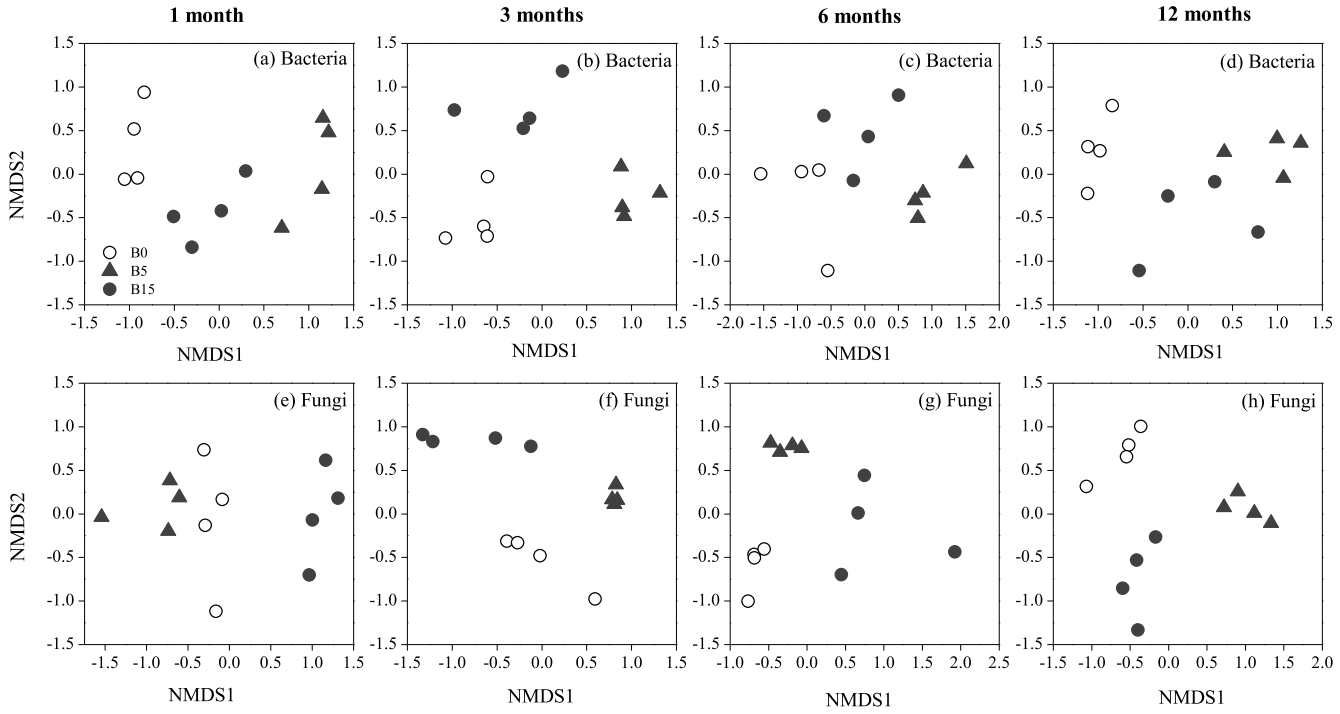

Fig. 4. Nonmetric multidimensional scaling (NMDS) ordination (k = 2, stress = 0.09) of weighted UniFrac distances between (a–d) bacterial and (e–h) fungal communities, showing differences across treatments for a given sampling date.

application decreased soil R_H while increased soil WSOC and MBC concentrations (Fig. 1), with no relationship between R_H and either WSOC or MBC in the biochar treatment (Fig. 6). Therefore, the decreased R_H caused by biochar application could not be attributed to the increased concentrations of WSOC and MBC, excluding the possibility that biochar reduces the R_H through reducing the labile organic C pool size.

In addition to changing the labile organic C pool size, biochar application increased aromatic C but decreased O-alkyl C contents (Fig. 2), indicating that biochar application increased the contents of lignin and aromatic compounds in the soil. Aromatic compounds are abundant in biochar which is resistant to biological degradation (Lehmann et al., 2011; Bamminger et al., 2018) and directly affect soil microbial community composition and activity (Ng et al., 2014; Li et al., 2018). Our SEM analysis also revealed that the increased soil aromatic C content by biochar application indirectly decreased soil R_H in the bamboo plantation (Fig. 8). Therefore, the decrease in R_H by the biochar application in our study was linked to the increased aromatic C content.

The substrate availability plays a vital role in controlling native SOC decomposition (Uchida et al., 2012), and biochar application would markedly influence the native SOC decomposition through altering the substrate availability (Kasozi et al., 2010; Zimmerman, 2010). In some cases, biochar application had a negligible effect on CO_2 emissions due to its slow decomposition rate as revealed by a meta-analysis (Awad et al., 2018), or even reduced CO_2 emissions via reducing decomposition of native SOC (Lu et al., 2014), which is termed a negative priming effect (Kuzyakov et al., 2000). Our field study shows a negative priming effect by biochar application in the subtropical plantation. The decrease in R_H in the initial period in this study might be attributed to, 1) biochar application decreased O-alkyl C content (Fig. 2), suggesting decreased labile C availability for microbial decomposition; and 2) the adsorption of native SOC by biochar prevents microbes and their extracellular enzymes from accessing native SOC (Zimmerman et al., 2011), thus reducing the effect of biochar application on native SOC decomposition or R_H.

Soil R_H is closely associated with soil microbial community composition and activity, since the process of R_H is mainly driven by soil microorganisms (Whitaker et al., 2014; Ding et al., 2016). Our results suggest that the decreased R_H by biochar application could be attributed to the suppression of C mineralization and cellulose degradation, and stimulation of CO_2 assimilation (Figs. 5 and 7). Both short-term laboratory incubation and long-term field studies showed the inhibitory effect of biochar application on the activity of β-glucosidase (Wang et al., 2015; Tian et al., 2016), which is involved in the degradation of carbohydrates in soils (Chen et al., 2013). Through the SEM analysis, we revealed the first possible mechanism for biochar application to decrease R_H was partially from the result of the reduction in C mineralization (β-glucosidase), by altering soil bacterial community composition (Table S5), and was indirectly decreased by increasing aromatic C content (Table S6; Fig. 8).

Soil fungi and their oxidative enzymes play an essential role in the degradation of organic compounds with condensed aromatic ring structure (e.g., lignin) (Talbot et al., 2012; Burns et al., 2013; Xu et al., 2017). Therefore, the fungal community and function would be more significant in the biochar (with aromatic C content) treatment, especially in acidic forest soils (Whitman et al., 2016). In this study, the SEM analysis revealed that R_H was mainly driven by CBH activity (Fig. 8). Our previous results revealed that the shifts in *cbh*I-containing fungal community could be explained by altering O-alkyl content in a subtropical plantation (Li et al., 2017). Moreover, the soil fungal community composition was correlated only with the CBH activity (Table S5). Therefore, the second possible mechanism for biochar reducing R_H was that biochar application decreased the CBH activity through altering the soil fungal community composition, decreasing *cbh*I gene abundance, and increasing the soil aromatic C content.

Soil R_H is the outcome of decomposition of SOC, which is affected by soil CO_2 fixation capacity (He et al., 2010). Therefore, the response of CO_2 assimilation by autotrophic bacteria to the biochar application should not be ignored. In our study, biochar-decreased R_H and increased RubisCO enzyme activity causing the negative relationship between R_H and RubisCO enzyme activity (Figs. 1f, 5d and 7d) implied that increased potential of microbial CO_2 fixation has contributed to the reduced R_H. The close relationship between RubisCO enzyme activity

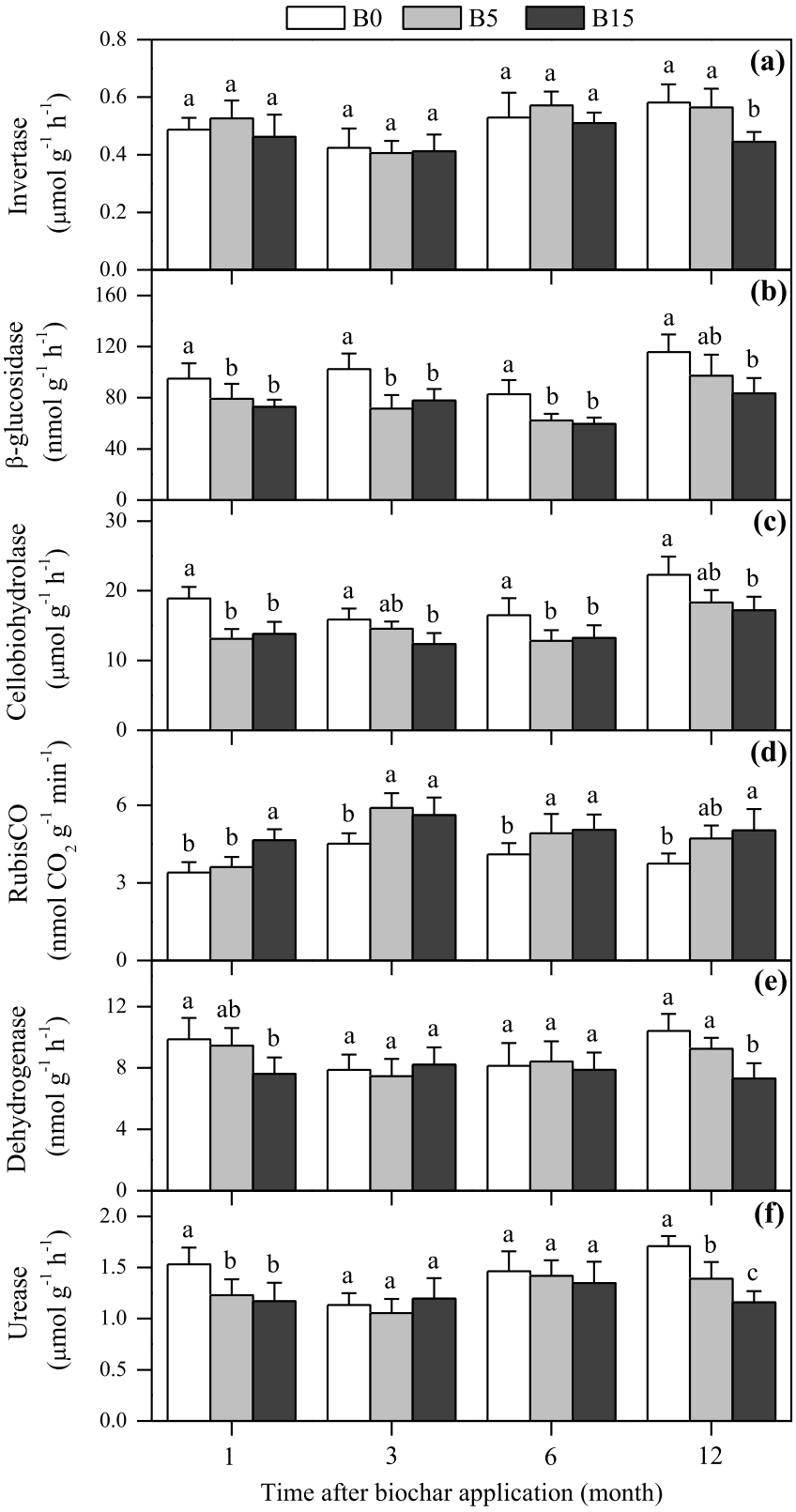

Fig. 5. Temporal changes of (a) invertase, (b) β-glucosidase, (c) cellobiohydrolase, (d) RubisCO enzyme, (e) dehydrogenase, and (f) urease activities in the 0–20 cm soil layer in a Moso bamboo plantation under three treatments: B0-no biochar application, B5-biochar application at 5 t ha^{-1}, and B15-biochar application at 15 t ha^{-1}. Vertical bars are standard deviations of the mean (n = 4).

and *cbbL* gene abundance (Fig. 8) indicates that RubisCO enzyme activity was closely associated with *cbbL* gene abundance (Guo et al., 2015). One possible mechanism for the biochar effects on the soil RubisCO enzyme activity was through altering the soil bacterial community composition, which was confirmed by the evidence that close relationship between the soil RubisCO enzyme activity and bacterial community composition (Table S5). Another possible mechanism was that biochar application increased the *cbbL* gene abundance by

Table 1
Effects of biochar application rate on mean annual and annual cumulative soil respiration (R_S), heterotrophic respiration (R_H), and autotrophic respiration (R_A) in a Moso bamboo plantation.

Treatment	Mean annual CO_2 flux (μmol m^{-2} s^{-1})			Annual cumulative CO_2 flux (t ha^{-1} yr^{-1})		
	R_S	R_H	R_A	R_S	R_H	R_A
B0[a]	3.14(0.23)b[b]	2.00(0.16)a	1.14(0.07)c	41.45(2.94)b	25.88(2.10)a	15.57(0.92)c
B5	3.13(0.18)b	1.79(0.15)b	1.34(0.03)b	41.85(2.88)b	23.02(2.37)b	18.83(0.79)b
B15	3.25(0.23)a	1.78(0.14)b	1.48(0.09)a	43.41(3.16)a	22.69(1.83)b	20.72(1.47)a

[a] B0, B5 and B15 indicate the treatments of no biochar application, biochar application at 5 t ha^{-1}, and biochar application at 15 t ha^{-1}, respectively.
[b] Means with different letters within one column indicate significant differences between different treatments for each parameter at $P = 0.05$, based on the least significant difference (LSD) test.

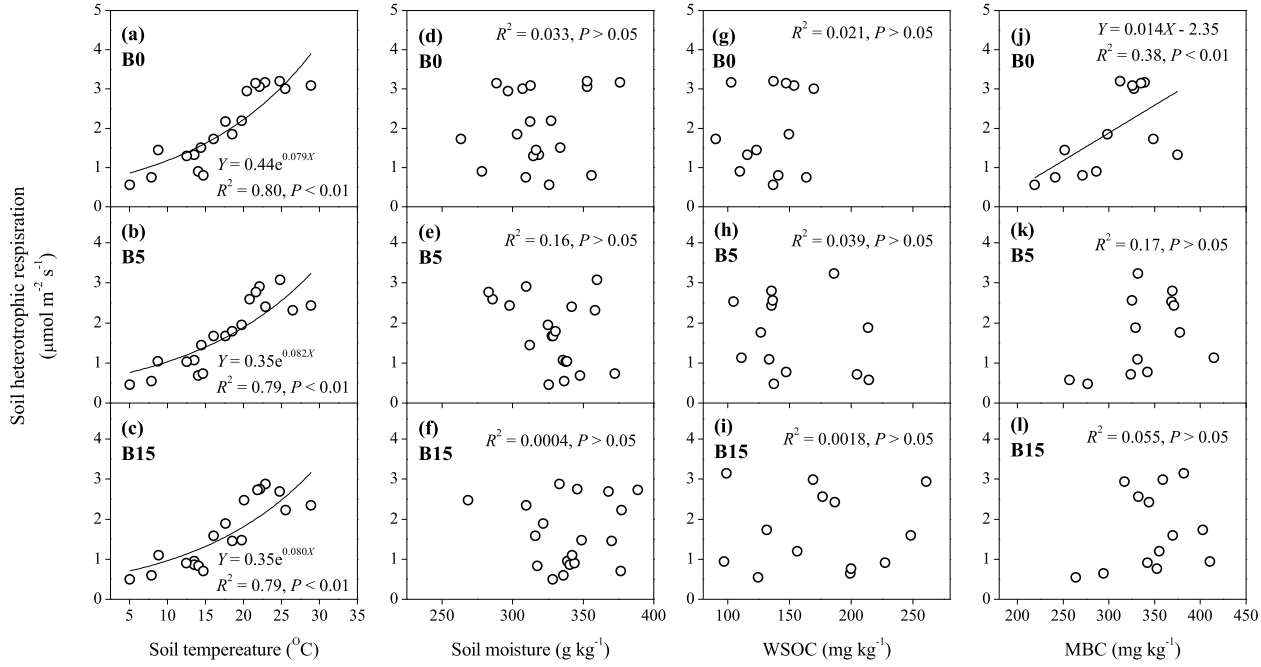

Fig. 6. Relationships between soil respiration components and environmental factors, including soil temperature, soil moisture content, water soluble organic C (WSOC) and microbial organic C (MBC) in the 0–20 cm soil layer in a Moso bamboo plantation under three treatments: B0-no biochar application, B5-biochar application at 5 t ha^{-1}, and B15-biochar application at 15 t ha^{-1}.

increasing soil pH, and consequently increased the soil RubisCO enzyme activity, since bacteria generally exhibit optimal growth within the pH range of 4–8 (Rousk et al., 2010). This assumption was confirmed by the positive relationships between soil pH and *cbbL* gene abundance ($R^2 = 0.17$, $P < 0.01$), and between *cbbL* gene abundance and RubisCO enzyme activity. However, RubisCO enzyme activity failed to exhibit a significant effect on R_H in the SEM, which might be explained by the fact that the abundance of *cbbL* gene only accounted for 0.56–1.23% of the 16S rRNA gene abundance in this setting, while a high density of CO_2-assimilating bacteria is essential for significant C-fixation to occur (Tolli and King, 2005; Selesi et al., 2007). Although the contribution of the increase in both *cbbL* gene abundance and RubisCO enzyme activity to the decreased R_H was not significant in this study, the response of soil microbial CO_2 fixation to forest management practices should be considered to better understand the microbially mediated C process having positive or negative feedback on CO_2 emissions.

5. Conclusions

In conclusion, our data clearly demonstrate that bicohar reduced R_H through increasing the proportion of soil recalcitrant C fraction and decreasing the microbial activity in relation to the decomposition of carbohydrates and celluloses in the soil, rather than via changing soil temperature, moisture or labile organic C contents. The chemical-microbial mechanism for reducing R_H by biochar application revealed in this study may be key to regulating R_H in subtropical plantations through forest management. Further, assuming that 10% of the current subtropical plantation area in China, approximately 4.35 million ha, is managed through biochar application at the rate of 5 t ha^{-1}, the potential for reducing the amount of annual CO_2 emission through R_H by biochar is estimated to be 12.4 Tg CO_2, based on the reduction rate of 2.86 t ha^{-1} yr^{-1} of CO_2 emission through R_H in the Moso bamboo plantation by biochar. In addition, this one-year field study covered a relatively short-time span after the biochar application, future research should address the long-term impacts of different types and application rates of biochar on soil C dynamics and to develop sustainable and economical strategies to increase soil C sequestration through adding exogenous C sources.

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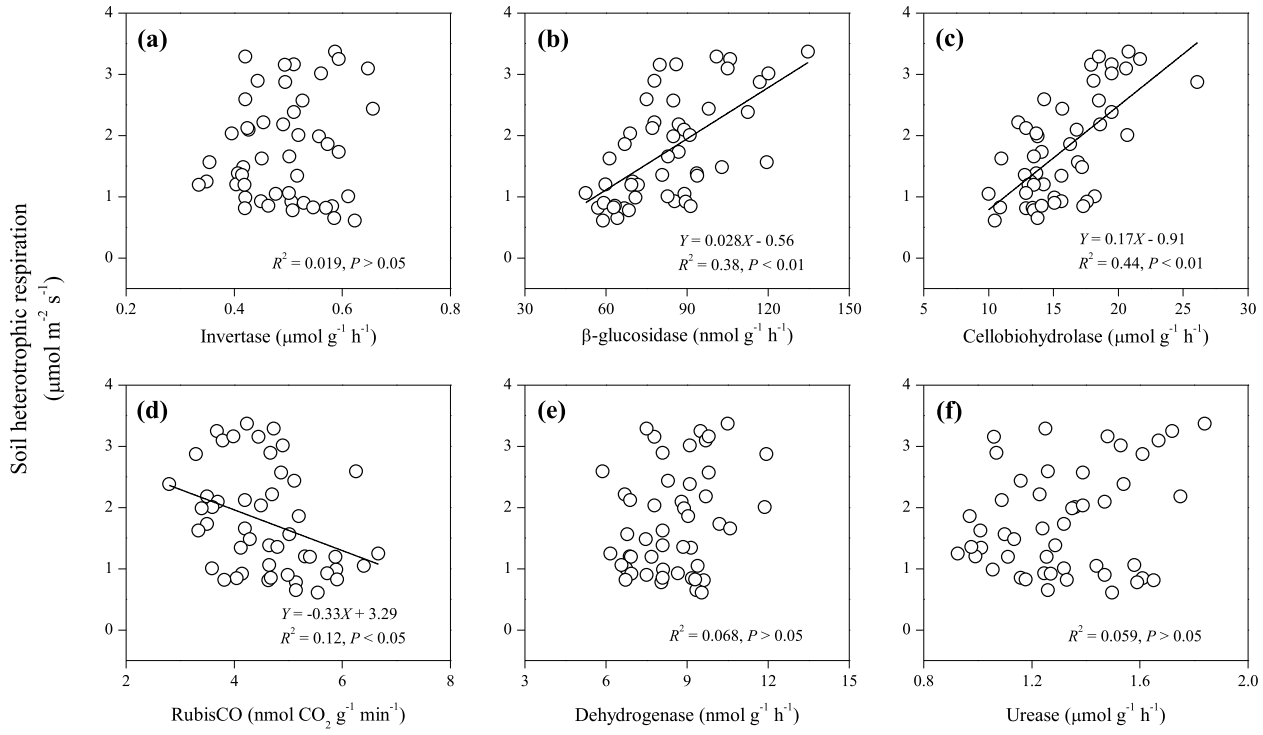

Fig. 7. Relationships between soil heterotrophic respiration and activities of (a) invertase, (b) β-glucosidase, (c) cellobiohydrolase, (d) RubisCO enzyme, (e) dehydrogenase, and (f) urease in the 0–20 cm soil layer in a Moso bamboo plantation.

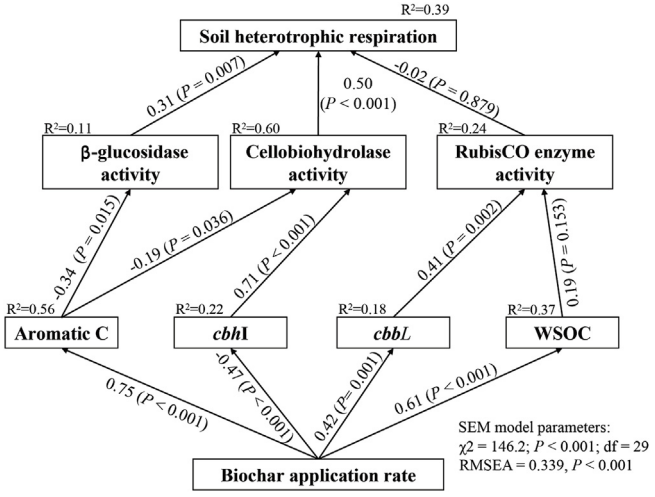

Fig. 8. Structural equation model (SEM) demonstrating the direct and indirect effects of biochar application rate, aromatic C content and WSOC concentration, abundance of *cbh*I and *cbbL* genes, and enzyme activities on soil heterotrophic respiration in a Moso bamboo plantation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.04.019.

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