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Effects of nitrogen deposition and biochar amendment on soil respiration in a *Torreya grandis* orchard

Junbo Zhang[1], Quan Li[1], Jiasheng Wu*, Xinzhang Song*

State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Hangzhou 311300, China

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ABSTRACT

Torreya grandis is an important economic nut tree species in southeastern China, but there is little information about its CO_2 efflux under increasing atmospheric nitrogen (N) deposition. There are few studies that assess the response of soil respiration to biochar applications in orchard soils under N deposition conditions. We investigated changes in soil respiration rate and other environmental factors under a factorial combination of biochar amendment (BC0: 0 t ha^{-1}, BC1: 20 t ha^{-1}, BC2: 40 t ha^{-1}) and simulated additional N deposition (N0: 0 kg N ha^{-1} yr^{-1}, NL: 30 kg N ha^{-1} yr^{-1} and NH: 60 kg N ha^{-1} yr^{-1}) treatments over three years (2016–2018). Soil respiration rate showed significant seasonal changes, with the highest rates occurring in summer and the lowest occurring in winter. The annual CO_2 emission amount of the control was 3.1 ± 0.03 kg CO_2 m^{-2}. Nitrogen deposition significantly increased soil respiration, but the positive effects of high-N treatment decreased over time. Meanwhile, N deposition significantly decreased both the soil temperature sensitivity ($Q_{10\text{-soil}}$) and air temperature sensitivity ($Q_{10\text{-air}}$) of soil respiration. Biochar amendment significantly increased soil respiration in the first and third years. However, only BC2 reduced $Q_{10\text{-soil}}$ and $Q_{10\text{-air}}$. The effects of biochar amendment on soil respiration varied with the level of N deposition. Three-factor analysis of variance showed that N deposition, biochar amendment, and time all had significant effects on soil respiration. Our results indicate that biochar could not effectively inhibit the promotion effect of N deposition on soil respiration in *T. grandis* orchard but may reduce soil carbon emission caused by future climate warming.

1. Introduction

Soil respiration is the main way for plant-fixed carbon dioxide (CO_2) to be released into the atmosphere (Högberg and Read, 2006; Gaumont-Guay et al., 2009). Approximately 80–98 Pg C is released to the atmosphere from the soil (Bond-Lamberty and Thomson, 2010), which is > 10 times the annual CO_2 emissions from fossil fuel combustion (Reichstein et al., 2003). Therefore, the changes in soil respiration rate have a large impact on atmospheric CO_2 concentration (Schlesinger and Andrews, 2000). Soil respiration is controlled by a variety of factors, including root biomass, soil organic carbon (SOC), soil nutrient content, and microbial populations and activities (Heimann and Reichstein, 2008).

Atmospheric nitrogen (N) deposition, mainly arising from agricultural N fertilization and fossil fuel consumption, recently reaches 21.1 kg N ha^{-1} yr^{-1} in China (Janssens et al., 2010; Liu et al., 2013). Nitrogen deposition can affect soil respiration by regulating root biomass and soil microbial biomass and activity in forest soils (Liu and Greaver, 2009). Previous research has mostly focused on temperate and boreal forests, which are often considered N-limited ecosystems, and have shown that increased N deposition reduces soil respiration (Bond-Lamberty and Thomson, 2010; Janssens et al., 2010; Sun et al., 2014). In subtropical forests, the effects of N deposition on soil respiration vary between forest stands. Nitrogen deposition promoted soil respiration in *Pleioblastus amarus* bamboo plantations (Tu et al., 2013), evergreen forests (Gao et al., 2014) and Moso bamboo (*Phyllostachys edulis*) forests (Li et al., 2019), and inhibited soil respiration in Chinese fir (*Cunninghamia lanceolata*) forests (Fan et al., 2014) and old-growth monsoon evergreen broadleaf forests (Mo et al., 2008). There have only been a few studies of how N deposition affects soil respiration in forests or for nut or fruit orchards in subtropical regions.

Biochar, produced via pyrolysis of biomass under limited oxygen, is a recalcitrant carbon-rich material with nano-sized pore structure (Lehmann and Joseph, 2015). The addition of biochar to soils has been suggested as a strategy to increase soil carbon storage and improve soil fertility and crop productivity. The stability of biochar is fundamental

* Corresponding authors.
E-mail addresses: wujs@zafu.edu.cn (J. Wu), songxinzhang@gmail.com (X. Song).
[1] These authors contributed equally to this work and should be considered as co-first authors.

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to its function as a long-term option for increasing soil carbon storage, reducing soil respiration, and soil amelioration. For example, Yang et al. (2018) found biochar application decreased CO_2 emissions in rice fields. However, biochar amendments have also been found to increase soil respiration in temperate forests but had no effect in subtropical forests (Zhou et al., 2017). Palviainen et al. (2018) suggested that biochar amendment to boreal forest soil does not cause long-term changes in soil CO_2 fluxes. On the contrary, a meta-analysis of 61 studies found that biochar application resulted in an average 19% increase in soil CO_2 emissions (Song et al., 2016a). As a soil amendment, biochar has been used in apple (*Malus domestica*) (Ventura et al., 2014) and Chinese torreya (*Torreya grandis* 'Merrillii') orchards (R. Zhang et al., 2017). However, there are only few studies on the effect of biochar amendment on soil respiration in orchards in subtropical regions.

T. grandis, a conifer of Taxaceae family, produces a rare and unique nut with high nutritional value and is one of the most important native nut tree species in southeastern China. The main cultivated area of *T. grandis* is affected by the increase of N deposition with an average rate of 30.9 kg N ha^{-1} yr^{-1} (Jia et al., 2014). R. Zhang et al. (2017) found that soil available N, P and K decreased when biochar was applied to a *T. grandis* orchard along with N deposition, and this may also cause changes in soil respiration. The effects of N deposition and biochar on soil respiration are still unclear.

Based on three years of different biochar application rates and N deposition levels, the following hypotheses were tested in this study: (i) N deposition increases soil respiration; (ii) biochar amendment decreases soil respiration; and (iii) biochar amendment counteracts the promotion effects of N deposition on soil respiration.

2. Materials and methods

2.1. Study site

The experimental field is situated in Yuqian Town, Lin'an City (30°14′N, 119°42′E), Zhejiang Province, China. It is located in southeastern China, which has a subtropical, monsoonal climate including clear-cut seasons with mean annual precipitation of 1613.9 mm and mean annual temperature of 15.6 °C, ranging from a minimum average monthly temperature of 4.5 °C in January to a maximum average monthly temperature of 28.9 °C in July. The soil belongs to the yellow-red soil class (Chinese system of soil classification), which is equivalent to a Hapludult soil in the soil taxonomy (Zhang et al., 2019). The *T. grandis* orchard was established in the 2000s and fertilizer was applied annually (58.5 kg N ha^{-1}, 58.5 kg P ha^{-1}, and 58.5 kg K ha^{-1}). There was no other vegetation covering plot soil during the experiment. The orchard was plowed annually in late October after harvest. The initial soil fertility characteristics are shown in Table S1.

2.2. Experimental design and measurements

A field experiment was set up in March 2015, when N deposition and/or biochar applications started with nine treatments, with each treatment having three replications. The 27 plots (4 × 4 m) were separated by buffer zones of at least 2 m, with only one *T. grandis* tree in each plot. The nine treatments were: control (N0-BC0) – neither N nor biochar addition; low N addition (NL: 30 kg N ha^{-1} yr^{-1}); high N addition (NH: 60 kg N ha^{-1} yr^{-1}); low biochar amendment (BC1: 20 t biochar ha^{-1}); high biochar amendment (BC2: 40 t biochar ha^{-1}); low N with low biochar addition (NL-BC1); low N with high biochar addition (NL-BC2); high N with low biochar addition (NH-BC1); and high N with high biochar addition (NH-BC2). The N additions level was chosen to represent the local N deposition rates (30 kg N ha^{-1} yr^{-1}) (Jia et al., 2014) and the widely used method to double and triple the local N deposition rate in order to simulate additional N deposition (Song et al., 2016b). N was added at the beginning of each month as NH_4NO_3 and this started in March 2015. The NH_4NO_3 solution was evenly sprayed from the top of the canopy of the *T. grandis* trees with an electric sprayer. Each control plot received an equal amount of N-free water applied in a similar way to the NH_4NO_3 solution. Biochar was produced through pyrolysis of wheat straw at 450 °C in a vertical kiln made of refractory bricks in Sanli New Energy Company in Henan, China (Zhang et al., 2012). The biochar was milled to pass through a 2 mm sieve and then mixed thoroughly to obtain a fine granular consistency before analysis. The basic properties and element composition of the biochar are shown in Table S2. In March 2015, the quantified biochar was added only once and mixed with the top 20 cm of soil by plowing.

2.3. Soil respiration rate measurement

Soil respiration rates were measured using the widely employed static chamber and gas chromatography technique (Wang and Wang, 2003; Tang et al., 2006; Li et al., 2019). The static chambers were constructed with opaque polyvinyl chloride panels comprising a square base box (0.4 × 0.4 × 0.1 m), incorporating a U-shaped groove (50 mm wide and 50 mm deep) on the upper side, to hold a chamber (0.4 × 0.4 × 0.4 m). In each plot, one base box was inserted to a depth of 0.1 m in the soil, 1 month prior to the initial sampling, and they remained in the field for the duration of the study period. Each base box was placed 0.5 m to the east of the *T. grandis* tree (Wang et al., 2015). The chambers were placed into the base boxes during gas sampling, with the grooves filled with water to function as an air seal. A small fan was installed inside each chamber to mix the air in the chamber during sampling. Gas samples were collected once a month. Each gas sampling was completed between 9 am and 10 am. A 60-mL plastic syringe attached to a three-way stopcock was used to collect gas samples at 0, 10, 20, and 30 min following chamber closure. The gas samples were injected into evacuated bags made of polymer film and aluminum foil (Desen Inc., Dalian, China). The concentrations of CO_2 were analyzed using a gas chromatograph (GC-2014; Shimadzu Corporation, Kyoto, Japan) within two days of sample collection.

2.4. Measurement of soil physicochemical parameters

Soil pH was measured using a pH meter (FE20; Mettler Toledo, Switzerland) after preparing a soil:water (1:2.5 w:v) suspension by shaking for 30 min (Bao, 2008). The concentrations of soil organic carbon (SOC) and total nitrogen (TN) in the extracts were determined using an elemental analyzer (Elementar Vario EL III; Germany). Total phosphorus (TP) concentration was determined using an acid-molybdenum antimony anti-colorimetric method, and total potassium (TK) concentrations were measured using a flame photometer. NO_3^- and NH_4^+ concentrations were measured after initially being extracted with 2 M KCl, followed by steam distillation and titration (Mulvaney, 1996). The alkaline-$KMnO_4$ method was used to determine available N (AN) concentrations (Prasad, 1965). The molybdenum blue method was used to determine available P (AP) concentrations (Watanabe and Olsen, 1965). The flame photometric method was used to determine available K (AK) concentrations (extracted by 1 mol·L^{-1} NH_4OAc) (Bao, 2008).

2.5. Data analyses

The soil CO_2 emission rate was calculated according to Eq. (1) (Liu et al., 2011; Li et al., 2019):

$$F = \frac{d_c}{d_t} \times \frac{M}{V_0} \times \frac{273.15}{T} \times \frac{V}{A} \quad (1)$$

where F (mg m^{-2} h^{-1}) is the soil CO_2 emission rate, dc/dt is the slope of the linear regression between change in CO_2 concentration (dc) and time (dt) in the chamber, M and V_0 are the molar mass and molar volume, respectively, of CO_2 under standard conditions, T is the absolute air temperature during sampling, and V (m^3) and A (m^2) are the effective volume and bottom area of the chamber, respectively.

Eq. (2) was used to calculate cumulative soil CO_2 fluxes (Liu et al., 2011; Li et al., 2019):

$$F_d = \sum (R_{i+1} + R_i)/2 \times (t_{i+1} - t_i) \times 24 \quad (2)$$

where, F_d is the cumulative soil CO_2 flux (mg CO_2 m^{-2} d^{-1}), R is the soil CO_2 flux (mg CO_2 m^{-2} h^{-1}) determined at each sampling time, i is the sampling number, and t is the sampling time.

Based on these measurements, an exponential regression model (Eq. (3)) was used to describe the relationship between soil respiration and soil temperature (Song et al., 2013; Li et al., 2019):

$$Y = \alpha \times e^{kT} \quad (3)$$

where, Y is soil respiration, T is soil temperature at 0.05 m depth, and α and k are the model coefficients. The temperature sensitivity parameter, Q_{10}, was calculated as presented in Song et al. (2013) and Li et al. (2019):

$$Q_{10} = \alpha \times e^{k(T+10)}/\alpha \times e^{kT} = e^{10k} \quad (4)$$

All statistical analyses were performed using SPSS 18.0 for Windows (SPSS Inc., Chicago, Illinois). One-way analysis of variance (ANOVA) and least significant differences (LSD) multiple comparisons were used to identify significant differences in soil CO_2 flux and Q_{10} value. Three-way ANOVA was used to test the significance of the interaction between N deposition and biochar amendment and time for the interannual variation in soil CO_2 emissions. All data were tested for homogeneity of variance and normality of distribution prior to conducting the ANOVA. The data satisfied the assumption of homogeneity of variance.

3. Results

3.1. Changes in soil physicochemical property

Compared with the control, both BC1 and BC2 significantly increased soil NO_3^- concentration but reduced NH_4^+ concentration. Meanwhile, BC2 increased soil pH and TP concentration, but reduced soil microbial biomass carbon (MBC) concentration ($P < 0.05$, Table 1). Nitrogen addition significantly increased soil NO_3^- concentration but reduced NH_4^+ concentration (Table 1). Compared with the NL treatment, both NL-BC1 and NL-BC2 significantly increased soil pH but reduced soil MBC and NO_3^- concentration. Compared with the NH treatment, both NH-BC1 and NH-BC2 significantly increased soil AK and pH, but reduced soil MBC and NO_3^- concentration ($P < 0.05$, Table 1). Soil SOC was increased only under the NL-BC2 treatment.

3.2. Changes in soil respiration

Soil respiration showed the same seasonal variations trends under all treatments (Fig. 1). Compared with the control, NL and NH significantly increased soil respiration rate in most months of 2016 ($P < 0.05$), and NH increased soil respiration more strongly than did NL (Fig. 1). Over the next two years, NH and NL still significantly increased the soil respiration rate ($P < 0.05$), but the promotion effect of the NH treatment was weaker than the NL treatment. Biochar amendment increased soil respiration in summer 2016 ($P < 0.05$) (Fig. 1), with increases of 24.2–46.9% and 32.6–43.5% in BC1 and BC2, respectively. However, the promotion effect disappeared in summer in the next two years. For the combined biochar and NL treatment, the soil respiration rate increased significantly only in April–June 2016 ($P < 0.05$) within three years (Fig. 1). For the combined biochar and NH treatment, there was no significant effect on soil respiration rate (Fig. 1).

3.3. Annual CO_2 emissions

In the first year after the application of biochar and N (i.e., 2016), N

Table 1
Soil physicochemical properties in May 2017 (mean ± SD, n = 3).

Treatment	MBC (mg kg^{-1})	TN (g kg^{-1})	AN (mg kg^{-1})	TP (g kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	pH	SOC (g kg^{-1})	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})
Control	539.8 ± 32.5a	1.9 ± 0.4b	25.2 ± 11.6b	0.9 ± 0.0bc	172.3 ± 37.0b	158.7 ± 25.0b	6.0 ± 0.2c	26.7 ± 5.2b	80.1 ± 1.8a	35.0 ± 4.7e
BC1	519.0 ± 6.9ab	3.1 ± 0.4a	28.0 ± 3.5ab	1.1 ± 0.2b	188.3 ± 3.3b	168.7 ± 17.0b	6.6 ± 0.3bc	30.7 ± 3.2b	31.7 ± 2.0e	65.6 ± 3.3b
BC2	466.6 ± 38.0b	2.9 ± 0.1ab	23.1 ± 5.7b	1.3 ± 0.1a	189.9 ± 37.9b	193.7 ± 7.5ab	7.0 ± 0.5b	34.2 ± 2.6b	34.4 ± 0.3de	84.2 ± 5.5a
NL	488.3 ± 18.0ab	2.6 ± 0.3ab	32.9 ± 8.1ab	0.9 ± 0.1bc	176.1 ± 15.7b	139.7 ± 4.9b	6.1 ± 0.4c	29.8 ± 4.1b	35.1 ± 1.6d	58.1 ± 5.4c
NL-BC1	387.6 ± 32.7c	2.4 ± 0.4b	26.8 ± 7.3ab	1.1 ± 0.1ab	251.4 ± 39.3a	151.3 ± 11.7b	7.2 ± 0.8ab	33.8 ± 3.7b	32.8 ± 0.8e	39.4 ± 3.9d
NL-BC2	406.5 ± 36.7c	2.3 ± 0.4b	38.0 ± 3.6a	1.2 ± 0.2ab	221.7 ± 37.1ab	189.3 ± 24.1ab	7.4 ± 0.4ab	42.3 ± 8.2a	48.0 ± 1.0c	32.0 ± 2.2e
NH	527.1 ± 24.8a	2.4 ± 0.1b	29.9 ± 6.1ab	0.8 ± 0.1c	195.2 ± 14.2b	149.3 ± 39.0b	5.5 ± 0.4c	28.0 ± 1.7b	35.9 ± 0.7de	45.3 ± 5.3 cd
NH-BC1	415.0 ± 19.1bc	2.8 ± 0.1ab	32.7 ± 7.3ab	1.0 ± 0.1bc	236.6 ± 13.3ab	220.3 ± 23.3a	7.0 ± 0.3b	36.4 ± 2.4ab	54.5 ± 0.7b	20.2 ± 3.9f
NH-BC2	407.2 ± 47.5c	3.0 ± 0.8ab	38.0 ± 5.7a	1.2 ± 0.1ab	216.9 ± 16.5ab	224.3 ± 34.2a	7.8 ± 0.4a	36.8 ± 5.6ab	33.6 ± 0.8e	27.6 ± 3.5ef

Means followed by the same letter are not significantly different from one another based on LSD test at $P < 0.05$. MBC, soil microbial biomass carbon; TN, total N; AN, available N; TP, total P; AP, available P; AK, available K; SOC, soil organic carbon content.

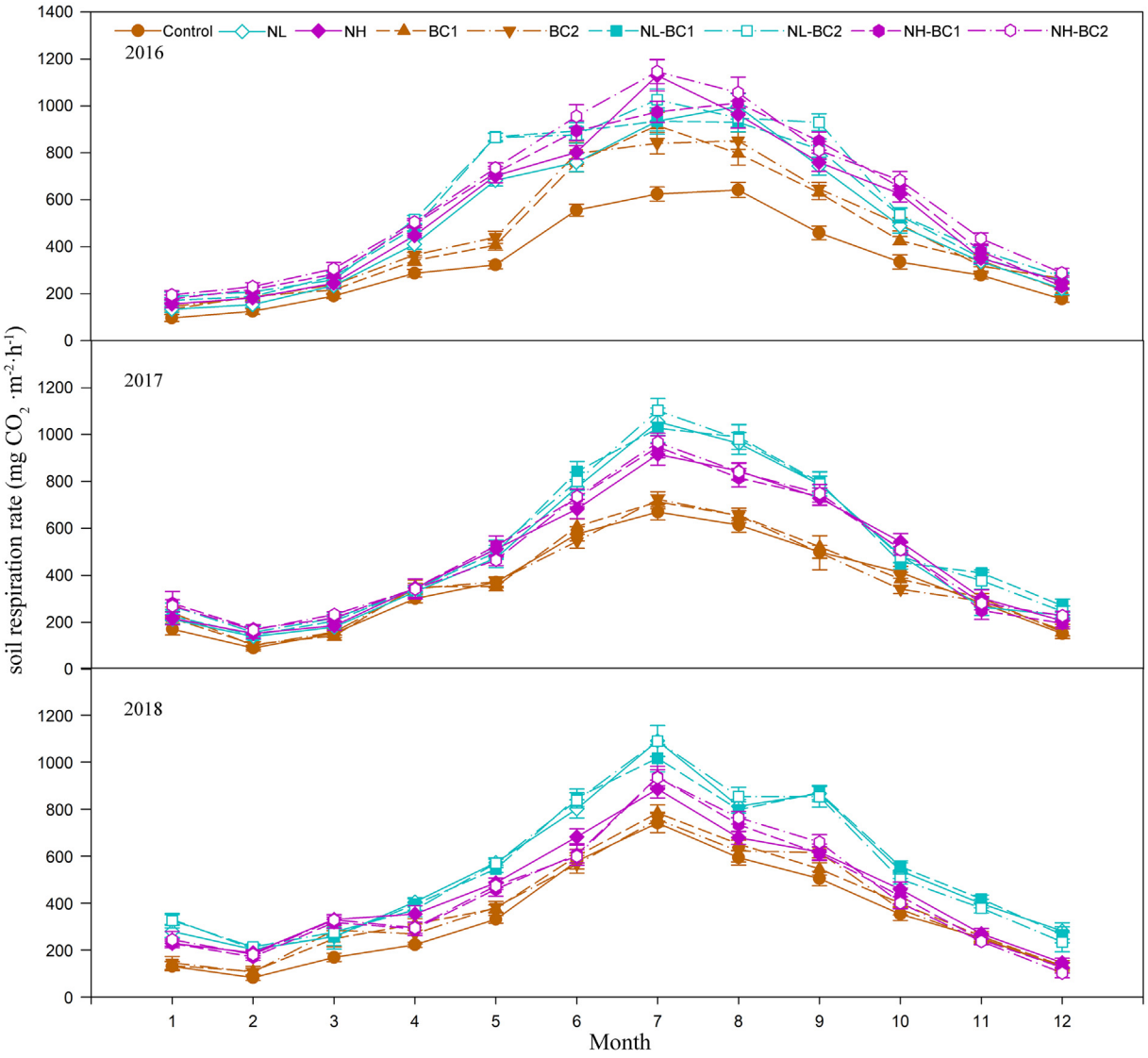

Fig. 1. Soil respiration rates in each month under different nitrogen addition and biochar amendment treatments in *Torreya grandis* orchards from January 2016 to December 2018. n = 3.

deposition significantly stimulated CO_2 emissions; NH induced a higher increase of CO_2 emissions than NL ($P < 0.05$, Fig. 2). The biochar amendments also significantly increased CO_2 emissions; the promotion effects of BC1 and BC2 were similar. At the same time, the addition of biochar also significantly increased CO_2 emissions in NL and NH treatments. Over the next two years (i.e., 2017 and 2018), N deposition still significantly stimulated CO_2 emissions, but the promotion effect of NH was reduced year by year (Fig. 2). Biochar addition had no significant effect on CO_2 emissions in the second year after application of biochar and N (i.e., 2017), but CO_2 emissions were significantly higher under BC2 for the NL treatment ($P < 0.05$, Fig. 2). Biochar amendment significantly increased CO_2 emissions in the third year after application (i.e., 2018) ($P < 0.05$), but not under the NL and NH treatments (Fig. 2). A three-way ANOVA showed that N deposition, biochar amendment, time, either individually or in combination, significantly affected soil CO_2 emissions ($P < 0.001$) (Table S3).

3.4. Temperature sensitivity of soil respiration

There were significant exponential relationships between the soil CO_2 emission rates and the temperatures of both air and soil for all treatments. Nitrogen deposition led to a decrease in temperature sensitivity ($Q_{10\text{-soil}}$ and $Q_{10\text{-air}}$) (Table 2). BC1 had no effect on $Q_{10\text{-soil}}$ and $Q_{10\text{-air}}$, but BC2 reduced $Q_{10\text{-soil}}$ and $Q_{10\text{-air}}$. Compared to N deposition (NL and NH), biochar application (NL-BC1, NL-BC2, NH-BC1 and NH-BC2) had no effect on $Q_{10\text{-soil}}$ and $Q_{10\text{-air}}$. The sensitivities of soil respiration to soil temperature at a depth of 0.05 m ($Q_{10\text{-soil}}$) under all treatments was higher than sensitivity to air temperature ($Q_{10\text{-air}}$).

4. Discussion

4.1. Effect of N deposition on soil respiration

The mean annual soil respiration rate in the control plots (346.4 ± 9.9 mg CO_2 $m^{-2}h^{-1}$) observed in the present study was considerably higher than that previously reported in apple orchards (206.5 ± 27.9 mg CO_2 $m^{-2}h^{-1}$) on the Loess Plateau (Wang et al., 2015), but was lower than that in olive (*Olea europaea*) orchards (493.3 ± 15.4 mg CO_2 $m^{-2}h^{-1}$) of Venturina (Bertolla et al., 2014). The average annual soil temperature (15.7 °C) of the control plots in this study was similar to apple orchards (14.8 °C) and olive orchards (15.1 °C), so the difference in soil respiration between different orchards may be due to different tree species rather than soil temperature. Our research found clear seasonal variations in soil respiration under all

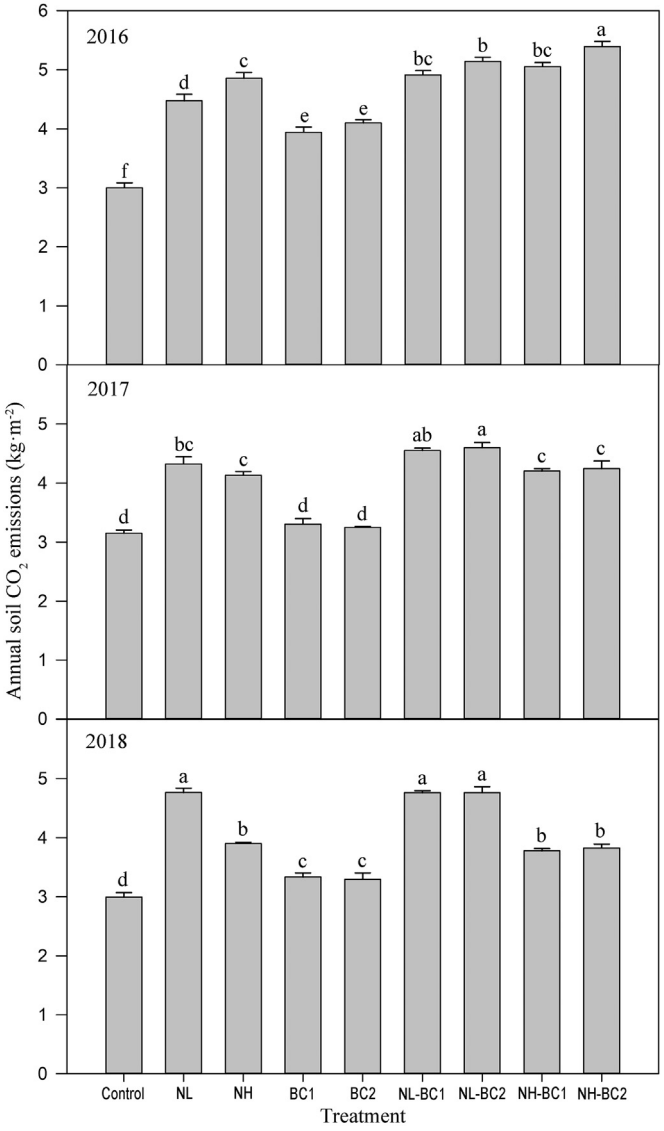

Fig. 2. Mean annual soil CO_2 emissions under different nitrogen addition and biochar amendment treatments in 2016, 2017 and 2018. Lowercase letters indicate differences in CO_2 emissions under different treatments ($P < 0.05$).

Table 2
The sensitivities of soil respiration to soil temperature at a depth of 0.05 m ($Q_{10\text{-soil}}$) and air temperature ($Q_{10\text{-air}}$) under different treatments.

Treatment	$Q_{10\text{-soil}}$	$Q_{10\text{-air}}$
Control	2.3 ± 0.1aA	1.8 ± 0.1aB
BC1	2.3 ± 0.1abA	1.7 ± 0.0abB
BC2	2.2 ± 0.0bA	1.7 ± 0.0bcB
NL	2.2 ± 0.0bA	1.7 ± 0.0bcB
NL-BC1	2.1 ± 0.0bA	1.6 ± 0.0cB
NL-BC2	2.1 ± 0.0bA	1.6 ± 0.0bcB
NH	2.1 ± 0.1bA	1.7 ± 0.0bB
NH-BC1	2.2 ± 0.0bA	1.7 ± 0.0bB
NH-BC2	2.1 ± 0.0bA	1.7 ± 0.0bcB

Different lowercase letters in the same column indicate significant differences ($P < 0.05$) among different treatments. Different capital letters in the same row indicate significant differences between temperature types under the same treatment ($P < 0.05$).

treatments over the three-year study period, with greater values observed in summer and lower values observed in winter (Fig. 1); these results are consistent with the results of numerous studies in forest ecosystems (Deng et al., 2013; Liu et al., 2019a; Liu et al., 2019b). This meant that N deposition did not change the seasonal variations of soil respiration. L. Zhang et al. (2017) also found a similar result in a camphor (*Cinnamomum camphora*) forest. Previous studies have shown that there are regular fluctuations in soil respiration and that these changes are affected by temperature (Zhou et al., 2013; Carey et al., 2016; Lang et al., 2017). The annual trend of soil temperature in this study was consistent with the trend of soil respiration throughout the year (Fig. S1), and the Pearson correlation coefficient of soil respiration and soil temperature under each treatment was > 0.85 ($P < 0.05$).

The results of this study indicated that N addition increased soil respiration rate and annual soil CO_2 emissions (Figs. 1 and 2), which supported our first hypothesis. Our results were contrary to those of Kong et al. (2013), who found that N additions reduced CO_2 emissions from apple orchards. A possible reason for this contradiction is the lower soil pH (4.8) in the apple orchard than that (6.0) in the current study, because the low pH would inhibit soil microbial activity (Kunito et al., 2016) and thus the microbial respiration. It has been shown that N additions increase soil respiration in N-limited temperate and boreal forest ecosystems (Hyvönen et al., 2007) but reduce it in N-rich tropical forest ecosystems (Cusack et al., 2011). Our previous study suggested that there was no N limitation in the current orchards based on *T. grandis* leaf stoichiometry (Zhang et al., 2019), indicating that the effects of N additions on soil CO_2 emissions also depended on vegetation cover, and not only on soil N condition.

Soil respiration comprises both heterotrophic and autotrophic respiration and is mainly regulated by the size of the soil microbial population and amounts of fine roots and litter biomass (Baggs, 2006). It has been reported that heterotrophic respiration could be stimulated after N addition because of high C and N availability of substrate and increasing microbial biomass and activity (Tian et al., 2016). Our study found that N deposition did not increase soil MBC (Table 1), indicating N deposition may have no effect on heterotrophic respiration but likely increased autotrophic respiration. Meta-analysis had found that simulated N deposition increased root biomass and root respiration (Li et al., 2015). Li et al. (2019) suggested that N deposition can enhance bamboo photosynthetic efficiency and result in more carbon distribution to the underground tissues, thereby stimulating root respiration.

In the control treatment, the Q_{10} value of the soil was 2.3 ± 0.07, which was similar to the Q_{10} value in an apple orchard (Q_{10} = 2.25) (Ventura et al., 2014). In our study, N addition significantly reduced $Q_{10\text{-soil}}$. Li et al. (2019) observed that excessive N addition (90 kg N ha^{-1} yr^{-1}) significantly decreased the Q_{10} value in a Moso bamboo forest. Tu et al. (2013) observed that Q_{10} values at 10 cm below the soil surface gradually decreased from 2.9 to 2.1 with increasing N additions (50, 150, and 300 kg N ha^{-1} yr^{-1}) in a *Pleioblastus amarus* bamboo plantation. We also found that the $Q_{10\text{-soil}}$ value was invariably higher than $Q_{10\text{-air}}$ under all treatments; this is similar to the findings of Li et al. (2019). The changes in the Q_{10} value likely reflect shifts in the metabolic pathways and status of plant roots and soil microbes under N-enriched soils (Zhang et al., 2014). This finding ($Q_{10\text{-soil}}$ declined after N addition) has potential importance for models of large-scale C cycling that attempt to predict the effects of atmospheric N deposition combined with global warming.

4.2. Effect of biochar on soil respiration

Our experiment found that the biochar amendment increased soil respiration rate; this does not support the second hypothesis. Song et al. (2016a) found that biochar increased soil CO_2 emissions by an average of 12% in upland fields. BC1 increased soil CO_2 emissions by 15.7% and BC2 increased it by 16.4% over the three-year study period; this was slightly higher than the average of 12% reported in Song et al. (2016a). The increased aeration of the soil induced by biochar amendment could have increased CO_2 emissions (Zhou et al., 2017). Sheng (2017) found that the addition of biochar in acid soil (pH < 7) significantly

increased CO_2 emissions due to carbonate in biochar dissolving in acid soil and releasing CO_2. We found that the biochar amendment increased the soil pH and reduced soil MBC (Table 1). Soil acidity could inhibit microbial activity (Kunito et al., 2016), but the application of biochar could alleviate this effect (Zhao et al., 2015), so even if microbial biomass was reduced, the increase in microbial activity still increased heterotrophic respiration, ultimately contributing to increase soil respiration in biochar amendment treatments. Our results showed that the promotion effect of biochar amendment on soil respiration was significantly higher in June–October 2016 than in the same period of 2017 and 2018, while there was no significant difference in soil temperature over the course of the three-year study period (Fig. S1). The same CO_2 emission pattern was also found in multiple ecosystems (Song et al., 2016a). Because of the specificity of biochar, its delayed release effect on CO_2 (formation and decomposition of bicarbonate) caused this peak to appear (Song et al., 2016a).

In the second year after application, the promoting effect of biochar on soil respiration disappeared. This may be because labile organic carbon in the soil is decomposed by microorganisms (Luo et al., 2011) leaving mostly unavailable carbon behind. Another possible reason was that soil microorganisms may utilize low-molecular weight hydrocarbons from the surface of fresh biochar. When this unstable C pool is exhausted, this effect will soon disappear (Pei et al., 2017). Therefore, there was no difference in CO_2 emissions compared with the control treatment. In the third year after application, biochar amendment increased soil respiration again, probably because biochar application increased plant growth and root biomass (Major et al., 2010; Lehmann et al., 2011), which may promote root respiration and provide additional organic matter for microbial respiration. However, the biochar application possibly had only a slight effect on root biomass and therefore only had a slight effect on soil respiration in the first two years; the effects became stronger over time (Ventura et al., 2014; Song et al., 2016a). The longer-term effects will be investigated in a future continuous experiment.

Our study found that the BC2 significantly reduced Q_{10} value of *T. grandis* orchards. Similar results were also observed in paddy fields (Pei et al., 2017) and organic carbon-poor dry cropland soil (Chen et al., 2018). The apparent temperature sensitivity of soil carbon decomposition can be linked to either intrinsic carbon chemical recalcitrance or carbon protection exerted by the soil matrix; the latter reduces substrate availability at enzymatic reaction sites and hence decreases Q_{10} (Davidson and Janssens, 2006; Conant et al., 2008). Thus, biochar decreased Q_{10} by promoting soil carbon stabilization. Our results suggested that the declined temperature sensitivity of soil respiration under biochar amendment may potentially imply a reduction in soil C loss under future climate warming.

4.3. Interaction between N deposition and biochar amendment and time on soil respiration

The results of three-way ANOVA indicated that the N deposition and biochar amendment significantly influenced soil respiration in the *T. grandis* orchard, alone and in combination. Moreover, the effects depended on the duration of treatment. The combination of N deposition with biochar significantly increased soil respiration in the first year (Fig. 2); this does not support our third hypothesis. A similar result was reported by Senbayram et al. (2019), who found that the combined olive mill biochar and mineral-N significantly increase CO_2 emissions in acid sandy soils (sand 81.8%, silt 14.8%, clay 3.5%) than mineral-N alone. Sorrenti et al. (2017) also found that a synergic effect between compost addition and biochar amendment led to a significantly higher cumulative CO_2 emissions than biochar amendment alone. It has been reported that addition of fertilizers reduces the bioavailability of biochar to enhance microbial abundance (Steiner et al., 2009), but greater nutrient availability could increase microbial abundance due to improved biochar-driven nutrient retention or nutrients released by biochar (Lehmann et al., 2011), eventually leading to the synergy of N deposition and biochar to increase microbial respiration. With the exception of the NL and BC2 treatment in 2017, the interaction of biochar and nitrogen addition had no significant effects on soil respiration in the second and third years after application (Fig. 2), which indicated that the interaction between N deposition and biochar amendment may promote soil respiration but the promotion effect would decline over time until it disappears. Time, as the third factor, also significantly affected soil respiration; this may be due to the delayed release effect of biochar on CO_2 flux (Song et al., 2016a) and the cumulative effect of N deposition (Bowden et al., 2004), which caused soil respiration to vary over time.

5. Conclusions

Based on a three-year field trial of simulated atmospheric N deposition and biochar amendment in a subtropical *T. grandis* orchard, the effects of N deposition and biochar amendment on soil CO_2 emissions were observed. Nitrogen deposition, biochar amendment and duration of treatment all significantly affect soil CO_2 emissions, individually and in combination. Nitrogen addition significantly increased soil respiration, but the positive effects of high-N treatment declined over time. Biochar amendments also increased soil respiration. The effect of biochar amendment on soil respiration depended on the level of N deposition. Individually, N deposition and BC2 could reduce temperature sensitivity of soil respiration but their interaction did not. Results of this study indicate that the biochar amendment did not inhibit the promotion effect of N deposition on soil respiration.

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Appendix A. Supplementary data

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