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Effects of apple branch biochar on soil C mineralization and nutrient cycling under two levels of N

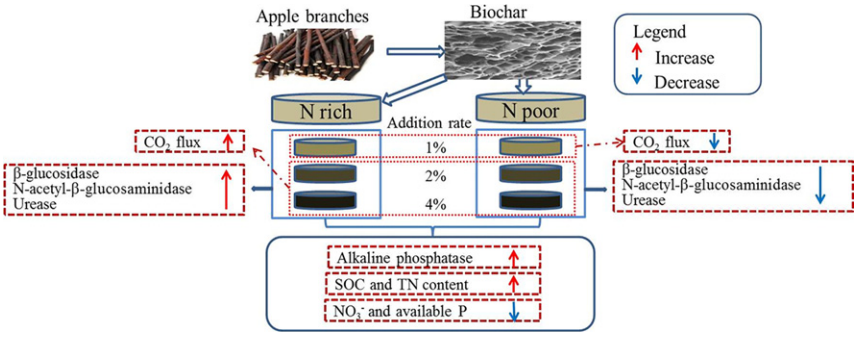
Shuailin Li [a,b], Chutao Liang [a,b], Zhouping Shangguan [a,*]

[a] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Shaanxi 712100, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

HIGHLIGHTS

- A high biochar addition rate increased the CO_2 flux.
- The effects of biochar on enzymes associated C and N cycling depended on N levels.
- Alkaline phosphatase activity increased with the biochar addition rate.
- Biochar increased SOC and total N but decreased NO_3^- and the available P content.

GRAPHICAL ABSTRACT



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ABSTRACT

The incorporation of biochar into soil has been proposed as a strategy for enhancing soil fertility and crop productivity. However, there is limited information regarding the responses of soil respiration and the C, N and P cycles to the addition of apple branch biochar at different rates to soil with different levels of N. A 108-day incubation experiment was conducted to investigate the effects of the rate of biochar addition (0, 1, 2 and 4% by mass) on soil respiration and nutrients and the activities of enzymes involved in C, N and P cycling under two levels of N. Our results showed that the application of apple branch biochar at rates of 2% and 4% increased the C-mineralization rate, while biochar amendment at 1% decreased the C-mineralization rate, regardless of the N level. The soil organic C and microbial biomass C and P contents increased as the rate of biochar addition was increased to 2%. The biochar had negative effects on β-glucosidase, *N*-acetyl-β-glucosaminidase and urease activity in N-poor soil but exerted a positive effect on all of these factors in N-rich soil. Alkaline phosphatase activity increased with an increase in the rate of biochar addition, but the available P contents after all biochar addition treatments were lower than those obtained in the treatments without biochar. Biochar application at rates of 2% and 4% reduced the soil nitrate content, particularly in N-rich soil. Thus, apple branch biochar has the potential to sequester C and improve soil fertility, but the responses of soil C mineralization and nutrient cycling depend on the rate of addition and soil N levels.

1. Introduction

Biochar is a solid product of the pyrolysis of biological matter under anoxic or hypoxic conditions at high temperatures (Sun et al., 2016).

* Corresponding author at: Xinong Rd. 26, Institute of Soil and Water Conservation, Yangling, Shaanxi 712100, China.
E-mail address: shangguan@ms.iswc.ac.cn (Z. Shangguan).

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Amendment of soil with biochar has been evaluated globally as a means to improve soil fertility and mitigate climate change (Lehmann et al., 2011). The persistent nature of biochar carbon (C), along with a reduction in greenhouse gas emissions, will contribute to soil C sequestration (Gul et al., 2015). The very slow decomposition of biochar differentiates it from other soil C pools, but biochar might provide some of the same services as soil organic matter, such as retention of nutrients and stabilization of water and soil (Bruun et al., 2012). However, biochar has also been reported to exert either negative or no effects on soil fertility and C-storage potential (Bruun et al., 2012; Tammeorg et al., 2014). The diverse results of biochar application depend on various factors, including biochar feedstock characteristics, pyrolysis conditions, soil type, application rates and the use of additional fertilizer (Liang et al., 2016; Sui et al., 2016).

Biochar can act as either a sink or source of C (Zimmerman et al., 2011), and recent studies have shown considerable variation in the responses of soil respiration to biochar application and N conditions (Lu et al., 2014; Sui et al., 2016). These inconsistent results are likely due to differences in biochar and soil characteristics and in the experimental conditions in different studies. For example, Lu et al. (2014) applied biochar produced from corn straw to a sandy loam soil under a long-term C_3 crop rotation and performed stable $\delta^{13}C$ isotope analyses to investigate how biochar (0.5% of the soil mass) affects the decomposition of native soil organic C (SOC) under different nitrogen (N) conditions. These researchers found that biochar application reduced the decomposition of native organic C and was a potentially effective measure for C sequestration. Conversely, Sui et al. (2016) performed a consecutive two-year field trial involving the addition of rice straw biochar (0, 1.78, 14.8 and 29.6 t ha^{-1}) in combination with urea (0 kg N ha^{-1} and 210 kg N ha^{-1}) in a rice paddy in northeast China and found that the addition of biochar and N fertilizer enhanced CO_2 emissions. However, crop residue-based biochar, which is rich in nutrients, tends to exhibit a higher pH and a greater surface area than biochar produced from lignocellulosic feedstocks, such as wood (Gul and Whalen, 2016). Apple branches are a widely distributed agricultural wood waste resource, but the characteristics of apple branch biochar and the effects of its incorporation into soil remain unclear. Furthermore, Liu et al. (2016) used the meta-analysis method to examine the responses of soil C to biochar amendment and found that the responses of soil CO_2 fluxes and soil microbial biomass C (SMBC) to biochar addition varied with the changes in soil texture and pH. Therefore, it is necessary to study the characteristics of apple branch biochar and examine its effects on soil C cycling.

As a soil amendment, biochar has the potential to improve N and P cycling in soil-plant systems. The important characteristics of biochar produced via pyrolysis that affect the biochemical cycling of N and P are its large surface area, pH and nutrient content. However, these characteristics vary among different types of biochar depending on their source (feedstock) (Gul and Whalen, 2016), and these diverse physicochemical properties greatly influence soil N and P cycling (Biederman and Harpole, 2012). The extracellular enzymes of microbes are the immediate modifiers of the two most vital soil fertility processes: organic matter decomposition and nutrient cycling (Burns et al., 2013). Thus, enzymatic activities and their responses to the addition of biochar have attracted considerable attention. It has been reported that biochar might generally facilitate the activities of a series of enzymes related to N and P utilization (Bailey et al., 2011) but reduce the activities of C-cycle enzymes (Lehmann et al., 2011), although the results of other studies have been inconsistent (Paz-Ferreiro et al., 2014; Song et al., 2016). The available data have revealed variable effects on enzymatic activities based on the source, type and application rate of biochar as well as the soil type and nutrient contents (Burns et al., 2013; Cusack et al., 2011; Lehmann et al., 2011).

As an intensive management practice in agricultural ecosystems, mineral fertilization may affect soil C and N transformation processes (Lee and Jose, 2003), but the effect of biochar on soil C, N and P cycling remains under debate. It is important to understand the effects of combining biochar with mineral fertilizer on soil physicochemical and biological properties, but there is limited knowledge regarding the effects of apple branch biochar on the dynamic transformation of soil C, N and P under different application rates and N conditions. Therefore, the specific objectives of this study were (1) to characterize soil CO_2 fluxes and the activities of extracellular enzymes associated with C, N and P cycling in response to the addition of apple branch biochar to soils with different levels of N; (2) to explore the effects of apple branch biochar application on the nutrient contents of soils with two levels of N; and (3) to illuminate the effects of the interaction of apple branch biochar and soil N levels on soil C, N and P cycling.

2. Materials and methods

2.1. Biochar characteristics

Biochar derived from apple branches (*Malus pumila* Mill.) was used in this experiment. The furnace temperature was ramped from ambient room temperature to 450 °C at a rate of 30 °C/min and then maintained at 450 °C for approximately 8 h. The resulting biochar was subsequently ground and passed through a 2-mm sieve.

Next, the biochar was added to deionized water, and after the soil-water (1:2.5 w/v) suspension was shaken for 30 min, the pH was measured using a pH meter. Electrical conductivity (EC) was determined in a 1:5 (w/v; g cm^{-3}) biochar-water mixture, and the elemental C, N, hydrogen (H) and oxygen (O) concentrations of the biochar were determined using an elemental analyzer (Flash 2000, Thermo Fisher, USA). The total potassium (K), P, sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and lead (Pb) contents were measured using an inductively coupled plasma (ICP) optical spectrometer (Vista Axial, Varian Medical Systems, USA). After ashing the biochar in a muffle furnace at 500 °C, nutrients were extracted by dissolving the biochar with aqua regia (produced by mixing nitric acid and hydrochloric acid at a volume ratio of 1:3). The physicochemical properties of the biochar are presented in Supplementary Table S1.

Scanning electron microscopy (SEM) images were used to visually observe the variations in the pore surface structure of the biochar (JSM-6360 LV, JEOL, Japan), and the specific surface area of the biochar was assessed using the Brunauer-Emmett-Teller (BET) method, which involves measurement of N adsorption-desorption isotherms at 77 K using an automated gas adsorption analyzer (Micro ASAP2460, Micromeritics, USA). The variability in the functional groups of the biochar was analyzed via solid-state spectroscopy and Fourier transform infrared spectroscopy (FTIR; VERTEX 70 FTIR, Bruker Corporation, Germany) (Bhaduri et al., 2016).

2.2. Soil materials

The soils contained 16.81%, 73.02% and 10.17% clay, silt and sand, respectively, and were classified as silt-clay soils according to the U.S. Department of Agriculture (USDA) system. Bulk soil was collected from the 0-to-20-cm soil layer in Yangling, China (34°17′57″N, 108°04′06″E), then air dried and ground to pass through a 2-mm sieve. The chemical properties of the soil are presented in Supplementary Table S2.

2.3. Soil incubation experiment

An incubation experiment was conducted over 108 days in a dark, enclosed climate chamber (AGC-D001P, Qiushi Corp., China) and included the following eight treatments: no urea (N0), urea (N1) at a concentration of 0.2 g kg^{-1}, no biochar (B0) and biochar amendment at rates of 1%, 2% and 4% by mass (hereafter referred to as B1, B2 and B3, respectively). Specifically, the treatments were formulated by mixing 200 g of soil with 0.25 g of superphosphate, and the appropriate quantities of biochar and urea were subsequently mixed with the samples,

which were then placed in plastic cylindrical containers (with a height and inner diameter of 10 cm). The moisture content of each sample was adjusted to 70–75% of the field water retention capacity and was readjusted by adding deionized water every two days. All of the soil treatments were incubated at 25 °C under 50% air humidity throughout the experiment, and each of the treatments was replicated 15 times. The soil from three replicates was destructively sampled and analyzed for extracellular enzyme activities on days 7, 14, 28, 56 and 108.

2.3.1. Soil respiration

Soil respiration was measured on days 3, 7, 14, 21, 28, 35, 56 and 108 using an infrared gas analyzer (LI-8100A, Lincoln, NE, USA). The survey chamber, which had a volume of 4076.2 cm^3, was placed on the top of the collar (20 cm in diameter and 10 cm in height) and sealed on the bottom with a polyethylene plate. Three replicates of each treatment were assessed in sequence to measure the CO_2 flux over a 2-min period (Hansen et al., 2016). The CO_2 flux was then re-calculated using the true soil area instead of the surface area of the collar.

2.3.2. Extracellular enzyme activity and microbial biomass C, N and P

The four analyzed enzymes were β-glucosidase, *N*-acetyl-β-glucosaminidase, urease and alkaline phosphatase. The activities of β-glucosidase (β-GA) and *N*-acetyl-β-glucosaminidase (β-NA) were quantified according to fluorescence-based protocols, and fluorescence was quantified using a microplate fluorometer (Fluoroskan Ascent FL, Thermo Scientific, USA). Briefly, 1 g of fresh soil was homogenized in 125 ml of 50 mmol L^{-1} acetate acid buffer using a polytron homogenizer, and a magnetic stirrer was employed to maintain a uniform suspension. Sterilized water and the sample suspensions, references (10 μM), and substrates (200 μM) were dispensed into the wells of a black, 96-well microplate, and the microplates were subsequently incubated for 4 h at 25 °C in the dark. After incubation, 10 μl of NaOH solution (1 M) was rapidly added to each well of the microplate to terminate the enzyme reaction, and β-GA and β-NA were expressed in units of nmol MUB h^{-1} g^{-1} (Ai et al., 2015; Ai et al., 2011). Urease activity (UA) was assessed using a 10% urea solution as a substrate, and the sample (2.5 g of fresh soil) was incubated at 37 °C for 24 h. After the addition of a phenol Na hypochlorite solution, 0.5 ml of the extract solution was diluted to 10 ml, and the ammonium content of the diluted solution was measured with a spectrophotometer at 578 nm (Lu et al., 2015). Alkaline phosphatase activity (AlkPA) was measured by adding a 0.5% di-sodium phenol phosphate and borate buffer (pH = 9.8) to 2.5 g of fresh soil, and the mixture was then incubated at 37 °C for 24 h. After the addition of 5 ml of borate buffer, 0.5 ml of 2.5% K ferricyanide solution, 0.5 ml of 5% 4-aminoantipyrine solution and 18.5 ml of distilled water, 0.5 ml of the extracted solution was further diluted to 25 ml, and after 20 min, the diluted samples were measured with a spectrophotometer at 510 nm. The UA and AlkPA references were analyzed at the same time using soil samples without the substrate (Lu et al., 2015).

The SMBC content was determined via the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987), Briefly, fresh soil samples fumigated with chloroform (10.00 g) and non-fumigated samples (10.00 g) were extracted with 50 ml of 0.5 mol L^{-1} K_2SO_4, and the extracts were analyzed with a Phoenix 8000 TOC analyzer (Teledyne Tekmar, Mason, USA). SMBC was calculated as the difference in extractable organic C between the fumigated and non-fumigated soil using a conversion factor of 0.45 (Wu et al., 1990; Zhu et al., 2017). The soil microbial biomass N (SMBN) was extracted from the SMBC extracts through Kjeldahl digestion and measured colorimetrically (Krom, 1980), and SMBN was estimated using a multiple of 0.5 (Joergensen, 1996). Soil microbial biomass P (SMBP) was estimated using the chloroform-fumigation extraction methods reported by (Brookes et al., 1982).

2.3.3. Physicochemical analyses

The SOC content was assayed via dichromate oxidation (Nelson et al., 1982; Zhu et al., 2017), and the total N (TN) content of the soil was assayed using the Kjeldahl method (Bremner and Mulvaney, 1982). The NH_4^+ and NO_3^- contents were extracted by vigorously shaking a sample with 50 ml of 2 mol L^{-1} KCl for 30 min. After the extract was filtered, the NH_4^+ and NO_3^- concentrations were measured using a continuous flow analytical system (Autoanalyzer 3, Bran + Luebbe, Germany) (Zhong et al., 2015). The total P (TP) content of the soil was determined using the molybdenum blue method after digestion with H_2SO_4-$HClO_4$ at 300 °C for 2 h (Qian et al., 2013). Olsen P was extracted with 0.5 M Na bicarbonate and quantified using the molybdenum blue method (Jin et al., 2016).

2.4. Statistical analysis

Two-way ANOVA at the $P < 0.05$ significance level was performed to assess the statistical differences between the rate of biochar addition and soil N levels as well their interaction, and the repeatedly measured data (soil respiration rates and enzymes activity) were analyzed via repeated-measures ANOVA at the $P < 0.05$ significance level. All statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Biochar characterization

SEM images were used to visually inspect the variation in the pore surface structure of apple branch biochar produced at 450 °C. Cross-sections of the biochar samples exhibited an obvious tubular pore structure, with some substances adhering to the walls of the pores and surface (Supplementary Fig. S1).

The variability of the functional groups of the apple branch biochar was investigated via FTIR. As shown in Supplementary Fig. S2, the broad band between 3400 and 3500 cm^{-1} (peak at 3440.30 cm^{-1}) was ascribed to amino and hydroxyl groups (Bhaduri et al., 2016; Song et al., 2016). The spectral peak at 1583.18 cm^{-1} was associated with aromatic C═O stretching, and the peak at 1441.45 cm^{-1} was associated with aromatic rings and phenolic groups (Song et al., 2016). The peaks at 1029.28 cm^{-1} and 874.78 cm^{-1} were likely due to C—O—C stretching associated with —OH-bending cellulose and hemicelluloses and lignin aromatic C—H out-of-plane bands, respectively (Masto et al., 2013).

3.2. Soil respiration

The results of repeated-measures ANOVA are presented in Supplementary Table S3. The soil respiration rate (R_s) was significantly affected by the biochar, N levels and incubation time as well as their interactions (except for the interaction of biochar and N levels). Under the N0 treatments, compared with B0, the B1 treatment significantly decreased mean R_s by 9.52%, while B2 and B3 significantly increased mean R_s by 9.52% and 38.10%, respectively (Table 1). Under the N1 treatments, there was no significant difference between the mean R_s of B1 and B0, but the mean R_s values for B2 and B3 were significantly higher than that of B0 by 10.87% and 30.43%, respectively (Table 1). Because R_s showed clear fluctuations in the N0 treatments at the end of the incubation period, as shown in Fig. 1, the cumulative CO_2 emissions (CO_2-C) in the first 56 days better reflect the actual situation. CO_2-C was significantly affected by biochar addition and N levels as well as their interaction ($P < 0.05$). Compared with the biochar-free treatment (B0), CO_2-C contents in the B1 treatments were significantly lower under N0 and N1 conditions by 21.38% and 23.71%, respectively, in the first 56 days ($P < 0.05$). In contrast, the CO_2-C contents of N0B2 and N0B3 were 30.19% and 66.67% higher, respectively, than that of B0N0 ($P < 0.05$), while the CO_2-C content of N1B3 was 37.67% higher than that of N1B0, in the first 56 days ($P < 0.05$). R_s

Table 1
Effects of biochar (B) and N levels on R_s and enzymes associated with C, N and P cycles.

N level	Biochar	R_s (μmol CO_2 m^2 s^{-1})	β-GA (nmol MUB g^{-1} h^{-1})	β-NA (nmol MUB g^{-1} h^{-1})	UA (mg NH_4^- g^{-1} 24 h^{-1})	AlkPA (mg Phenol g^{-1} 24 h^{-1})
N0	B0	0.42 ± 0.01^{b}	99.39 ± 1.21^{c}	7.97 ± 0.12^{e}	0.194 ± 0.002^{b}	0.54 ± 0.01^{b}
	B1	0.38 ± 0.01^{a}	85.19 ± 2.88^{b}	6.30 ± 0.34^{cd}	0.178 ± 0.008^{a}	0.56 ± 0.02^{b}
	B2	0.46 ± 0.02^{c}	83.31 ± 1.28^{ab}	5.30 ± 0.44^{a}	0.186 ± 0.006^{ab}	0.72 ± 0.01^{d}
	B3	0.58 ± 0.02^{e}	77.23 ± 0.39^{a}	6.46 ± 0.20^{cd}	0.179 ± 0.005^{a}	0.74 ± 0.04^{d}
N1	B0	0.46 ± 0.02^{c}	99.44 ± 2.31^{c}	5.31 ± 0.19^{a}	0.218 ± 0.010^{cd}	0.49 ± 0.01^{a}
	B1	0.45 ± 0.01^{bc}	136.37 ± 6.14^{e}	5.93 ± 0.43^{bc}	0.221 ± 0.005^{cd}	0.61 ± 0.03^{c}
	B2	0.51 ± 0.02^{d}	126.01 ± 4.90^{d}	6.78 ± 0.29^{d}	0.224 ± 0.001^{d}	0.72 ± 0.01^{d}
	B3	0.60 ± 0.03^{e}	131.17 ± 6.10^{de}	5.57 ± 0.26^{ab}	0.209 ± 0.014^{c}	0.64 ± 0.01^{c}

Notes: R_s is the soil respiration rate. β-GA, β-NA, UA and AlkPA are the activities of β-glucosidase, *N*-acetyl-β-glucosaminidase, urease and alkaline phosphatase, respectively. Subscript letters represent the statistical classes among the treatments at $P < 0.05$. Means ± S.E. with the same superscript letters are not significantly different at $P = 0.05$.

fluctuated with the incubation time. Specifically, in N0 soil, the R_s values of N0B2 and N0B3 were generally higher than that of B0N0 at the early incubation stage (first 56 days) but declined suddenly at the end of the incubation period (day 108) (Fig. 1). In the N1 soil, all of the treatments showed the same decreasing trend initially (0–35 days), but the R_s value increased at the end of the incubation period (35–108 days) (Fig. 1).

3.3. Soil extracellular enzyme activities

The results of repeated measures ANOVA showed that β-GA and β-NA were significantly affected by the biochar, N levels and incubation time as well as their interactions (Supplementary Table S3). Furthermore, in N0 soil, the mean β-GAs of B1, B2 and B3 were significantly lower than that of B0 by 14.29%, 16.18% and 22.30% ($P < 0.05$), respectively (Table 1). In N1 soil, the mean β-GAs of B1, B2 and B3 were significantly higher than that of B0 by 37.14%, 26.72% and 31.91%, respectively (Table 1). Additionally, the trends obtained for the variation in β-GA with the incubation time differed between the N0 and N1 soils (Fig. 2). In N0 soil, the β-GAs of the biochar amendment treatments showed a clear decline at day 14 and then gradually recovered to the initial level from day 14 to the end of the incubation period, although that of B0 decreased at day 28 and then presented the same increasing trend. As a result, the β-GAs of N0B1, N0B2 and N0B3 were 47.96%, 63.78% and 90.52% lower, respectively, than that of B0N0 at day 14

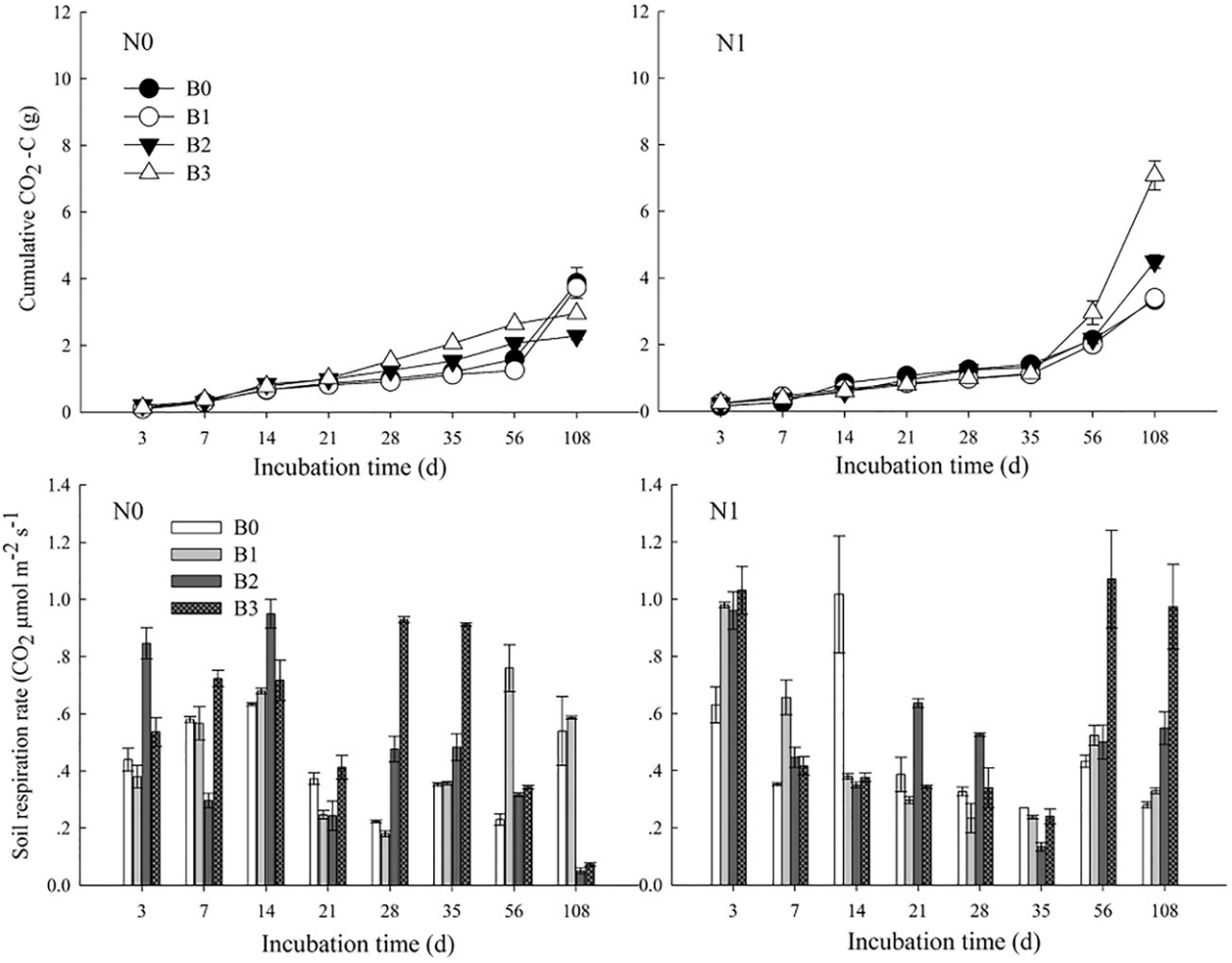

Fig. 1. Dynamic variation in cumulative CO_2 emissions (line chart) and the soil respiration rate (column diagram) observed under the different treatments over 108 days of incubation. The vertical bars in the figures represent the standard errors of the means ($n = 3$). N0 and N1 refer to the no-urea condition and the condition with urea addition at 0.2 g kg^{-1}, respectively. B0, B1, B2 and B3 refer to no biochar input and the incorporation of biochar into the soil at 1%, 2% and 4% by mass, respectively.

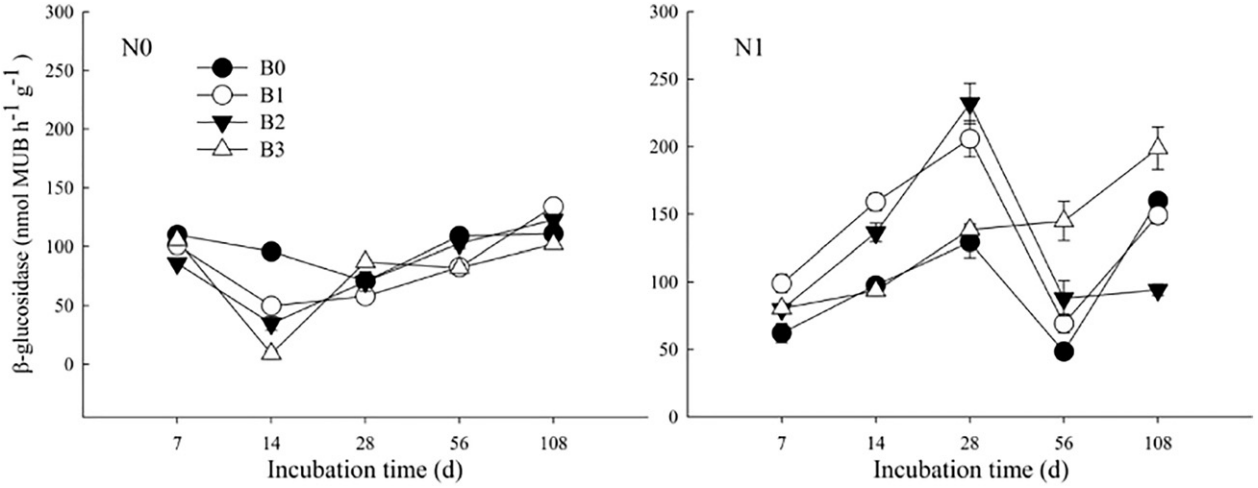

Fig. 2. Dynamic variation in β-glucosidase activity observed in the different treatments over 108 days of incubation. The vertical bars in the figures represent the standard errors of the means (n = 3). N0 and N1 refer to the no-urea condition and the condition with urea addition at 0.2 g kg^{-1}, respectively. B0, B1, B2 and B3 refer to no biochar input and the incorporation of biochar into the soil at 1%, 2% and 4% by mass, respectively.

($P < 0.05$). However, the β-GAs of N0B1 and N0B2 were 20.85% and 10.52% higher than that of N0B0 at day 108 ($P < 0.05$). In general, the β-GA dynamics in the treatments under the N1 condition exhibited greater fluctuations than under the N0 condition throughout the incubation period (Fig. 2). As shown in Fig. 2, the β-GAs of B0 and B1 showed a rise-decline-rise tendency, whereas a consistent gradually increasing trend was observed under the B3 treatment. The variations in N1B0 and N1B3 peaked at day 108, and those in N1B1 and N1B2 peaked at day 28. Notably, the β-GAs of N1B1 and N1B2 during the 7-to-28-day incubation stage were significantly higher than that of N1B0 (by an average of 60.36% and 54.99%, respectively; $P < 0.05$), whereas the β-GA of N1B3 was not significantly different from that of N1B0. However, after 28 days, the β-GA of N1B3 steadily increased, finally yielding the highest value among all treatments at day 108, which was 24.41% higher than that of N1B0 ($P < 0.05$).

Similar to the β-GA results, in N0 soil, the mean β-NAs of B1, B2 and B3 were significantly lower than that of B0 by 20.95%, 33.50% and 18.94% ($P < 0.05$), respectively (Table 1). In N1 soil, the mean β-NAs of B1, B2 and B3 were significantly higher than that of B0 by 7.91%, 27.68% and 4.90%, respectively (Table 1). Additionally, although the variation trends in β-NA found for N0 and N1 were different, the trends obtained for β-NA under the biochar amendment treatments were consistent with those observed under the biochar-free treatments (with the exception of B3; Fig. 3). In N0 soil, the β-NAs of all treatments suddenly decreased at 14 days but recovered at 56 days (with the exception of N0B3). The β-NAs of B1, B2 and B3 were significantly lower than that of N0B0 at day 56 ($P < 0.05$), and N0B3 showed a positive effect on β-NA at days 14 and 108. In N1 soil, the β-NAs of all of the treatments gradually increased during the 14-to-28-day incubation stage, then declined at the 28-to-56-day incubation stage and increased again at the 56-to-108-day incubation stage (with the exception of N0B3). At day 108, the B1 and B2 treatments increased β-NA by 58.31% and 127.11%, respectively, compared with N1B0 ($P < 0.05$). The β-NA of N1B3 gradually increased from day 7 to day 56, reaching levels that were significantly higher than those of B0, B1 and B2 at day 56, and then declined suddenly at day 108 to a level that was significantly lower than those of B0, B1 and B2.

The applied biochar and the interaction of biochar and N levels had no significant effects on UA ($P > 0.05$), but UA was significantly affected by N levels, incubation time and the interactions of incubation time, N levels and biochar (Supplementary Table S3). The mean UAs of N0B1 and N0B3 were significantly lower than that of

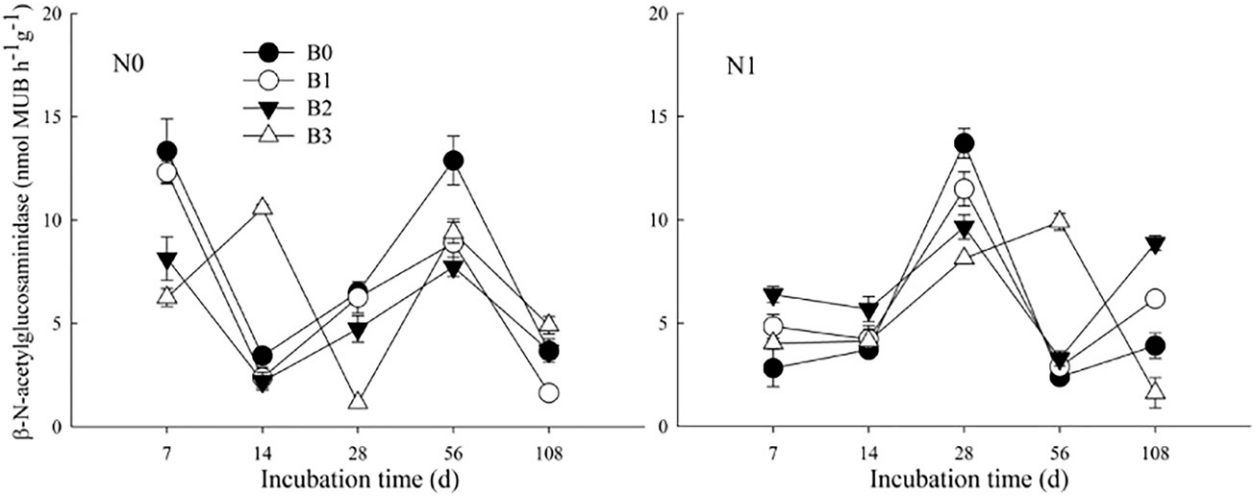

Fig. 3. Dynamic variation in β-*N*-acetylglucosaminidase activity observed in the different treatments over 108 days of incubation. The vertical bars in the figures represent the standard errors of the means (n = 3). N0 and N1 refer to the no-urea condition and condition with urea addition at 0.2 g kg^{-1}, respectively. B0, B1, B2 and B3 refer to no biochar input and the incorporation of biochar into the soil at 1%, 2% and 4% by mass, respectively.

N0B0 by 8.25% and 7.73%, respectively ($P < 0.05$). However, in N1 soil, the mean UAs of B1, B2 and B3 were not significantly different from that of N0B0 ($P > 0.05$) (Table 1). Furthermore, UA in N1 soil exhibited a gradually declining trend early during incubation (7–56 days) and an increasing trend at both moderate and high biochar rates (B2 and B3) in the later stage (56–108 days) (Fig. 4). UA was significantly stimulated (48% and 32%) by N1B3 and N1B2 compared with N1B0 ($P < 0.05$) at day 108. However, at day 108, the UAs of N0B2 and N0B3 were not significantly different from that of N0B0 ($P > 0.05$), and N0B1 had a significantly negative effect on UA ($P < 0.05$).

Compared with the biochar-free treatments (B0), the B2 and B3 biochar treatments significantly increased AlkPA in both N0 and N1 soils, whereas B1 only significantly increased AlkPA in N1 soil ($P < 0.05$; Table 1). The applied biochar, N levels and incubation time as well as their interactions had significant effects on AlkPA ($P < 0.05$; Supplementary Table S3). A positive effect of biochar on AlkPA was observed after 14 days in both N0 and N1 soils, but the dynamics of AlkPA were different. Under N0 conditions, AlkPA increased with the rate of biochar addition at the 28-to-108-day stage. The AlkPAs of N0B2 and N0B3 were significantly higher than that of N0B0 after 28 days ($P < 0.05$), whereas the AlkPA of N0B1 was significantly higher than that of N0B0 after 56 days ($P < 0.05$; Fig. 5). Under N1 conditions, AlkPA showed a sudden decrease in all of the treatments at day 14 and then slowly increased until the end of the incubation period. After day 14, the AlkPAs of the biochar addition treatments (N1B1, N1B2 and N1B3) were significantly higher than that of N1B0 ($P < 0.05$), and the N1B2 treatment exerted a more prominent effect than the other rates of biochar addition (Fig. 5).

3.4. Microbial biomass C, N and P

Compared with the B0 treatments, the biochar treatments (B1, B2 and B3) significantly increased the SMBC content, regardless of the N level ($P < 0.05$). There was no significant difference between the SMBCs of N0B2 and N0B3 ($P > 0.05$). However, the SMBC of N1B3 was 13.41% and 15.18% higher than those of N1B2 and N0B3, respectively ($P < 0.05$; Fig. 6A). The SMBN of all treatments under the N0 condition was significantly higher than that of the treatments under the N1 condition (with the exception of N0B3; ($P < 0.05$); it was noteworthy that the SMBN contents of N0B1 and N0B2 were significantly higher than those of the other treatments ($P < 0.05$; Fig. 6B). Under the N0 condition, SMBP increased with increasing rates of biochar addition. The SMBP values of N0B2 and N0B3 were 73.86% and 141.71% higher than that of N0B0, respectively ($P < 0.05$). Nevertheless, under the N1 condition, the SMBP contents of all biochar amendment treatments were consistently higher than that of N1B0, and this positive effect varied little with the rate of biochar addition (Fig. 6C).

3.5. Changes in soil nutrients

SOC was affected by the rate of biochar addition as well as the interaction of the rate of biochar addition and N levels ($P < 0.05$), and SOC increased with increasing rates of biochar addition (Fig. 7A). Under the N0 condition, the SOC contents of B1, B2 and B3 were 48.22%, 204.61% and 350.73% higher than that of B0 ($P < 0.05$), respectively, while under the N1 condition, the SOC contents of B1, B2 and B3 were 154.36%, 324.71% and 610.17% higher than that of B0, respectively ($P < 0.05$). In addition, the SOC content of N1B3 was 13.63% higher than that of N0B3 ($P < 0.05$; Fig. 7A).

Similar to the changes in SOC content, TN increased with increasing rates of biochar addition (Fig. 7B). TN was affected by the rate of biochar addition and the N level ($P < 0.05$). Under the N0 condition, the TN contents of B1, B2 and B3 were 10.42%, 35.42% and 54.17% higher than that of B0 ($P < 0.05$), respectively, while under the N1 condition, the TN contents of B1, B2 and B3 were 18.19%, 36.37% and 54.54% higher than that of B0, respectively ($P < 0.05$). The soil nitrate (NO_3^-) and soil ammonium (NH_4^+) contents were determined by the N level and the rate of biochar addition as well as their interaction ($P < 0.05$). The NO_3^- content of N0B3 was 28.74% lower than that of N0B0 ($P < 0.05$), and the NO_3^- contents of N1B3 and N1B2 were 36.74% and 13.91% lower than that of N1B0, respectively ($P < 0.05$). In contrast, the N1B1 treatment significantly increased the NO_3^- content by 7.45% compared with N1B0 ($P < 0.05$) (Fig. 7C). The NH_4^+ contents of N0B2 and N1B2 were significantly higher than those of the other treatments ($P < 0.05$; Fig. 7D). The NH_4^+ content of N0B3 was 28.79% lower than that of N0B0 ($P < 0.05$), but those of N1B1 and N1B3 were 22.34% and 16.58% higher than that of N1B0, respectively ($P < 0.05$).

The soil TP content depended on the soil N level and the rate of biochar addition as well as their interaction ($P < 0.05$). Notably, N0B3 significantly increased the TP content compared with N0B0 ($P < 0.05$), whereas the TP content recorded in N1B3 was the lowest among the treatments under the N1 condition (Fig. 7E). The available P content (AP) was closely dependent only on the rate of biochar addition ($P < 0.05$). The AP contents of N0B1, N1B1 and N1B2 were lower than that of N0B0 ($P < 0.05$), while N0B2, N0B3 and N1B3 had no effect on the AP content ($P > 0.05$). In general, biochar amendment negatively affected the AP content, but the magnitude of the reduction in AP content decreased with increasing rates of biochar addition (Fig. 7F).

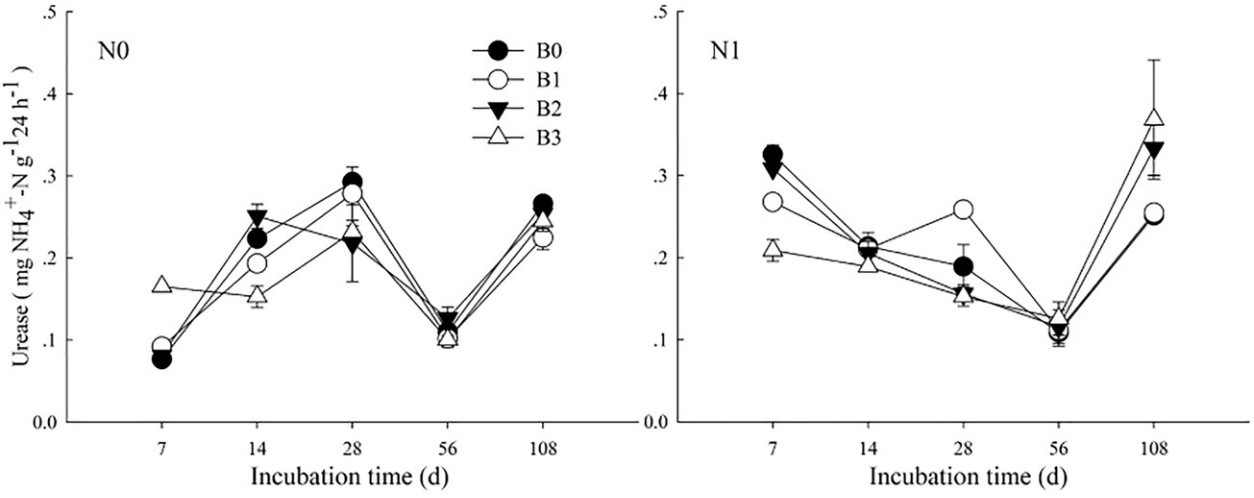

Fig. 4. Dynamic variation in urease activity observed in the different treatments over 108 days of incubation. The vertical bars in the figures represent the standard errors of the means (n = 3). N0 and N1 refer to the no-urea condition and the condition with urea addition at 0.2 g kg^{-1}, respectively. B0, B1, B2 and B3 refer to no biochar input and the incorporation of biochar into the soil at 1%, 2% and 4% by mass, respectively.

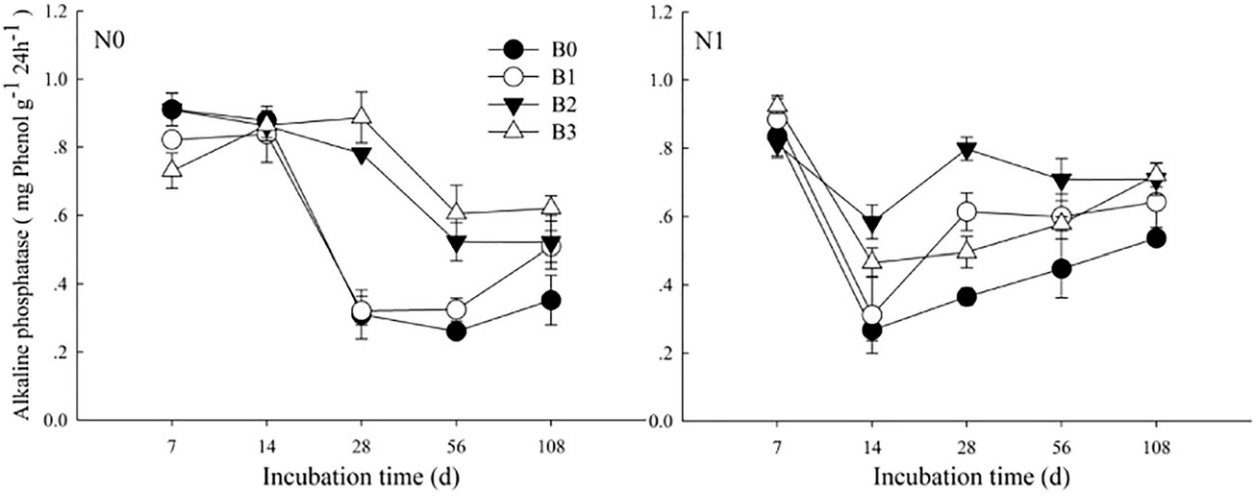

Fig. 5. Dynamic variation in alkaline phosphatase activity observed in the different treatments over 108 days of incubation. The vertical bars in the figures represent the standard errors of the means (n = 3). N0 and N1 refer to the no-urea condition and the condition with urea addition at 0.2 g kg^{-1}, respectively. B0, B1, B2 and B3 refer to no biochar input and the incorporation of biochar into the soil at 1%, 2% and 4% by mass, respectively.

4. Discussion

We conducted a 108-day incubation experiment to investigate the soil respiration rate, the primary enzymes involved in C, N and P cycling, and the soil microbial biomass and nutrient contents in response to the addition of apple branch biochar in soils with two levels of N. This comprehensive study preliminarily explored the effects of apple branch biochar on soil C, N and P cycling.

4.1. Soil C mineralization

The production of CO_2 through soil organic matter decomposition is regulated by both the activity of the microbial communities and the availability of C substrates (Six et al., 2006), and previous studies have shown that biochar addition can either increase or decrease soil C mineralization (Purakayastha et al., 2016; Wang et al., 2016). In this study, we did not use isotopic labeling to separate the different SOC pools involved; thus, the CO_2 emissions measured in the present study were actually the integrated result of the mineralization of biochar and SOC. Under both N0 and N1 conditions, B1 significantly decreased R_s compared with B0, whereas B2 and B3 significantly increased R_s. Similar results were obtained in previous studies. For example, Sui et al. (2015) and Song et al. (2016) found that the addition of biochar enhanced CO_2 emissions, with an increasing trend being observed under increasing rates of biochar addition. Some studies have suggested that short-term positive priming effects due to increased SOC mineralization are largely due to the stimulation of microbial activity by the labile C contained within biochar or the abiotic release of CO_2 from carbonates in ash (Luo et al., 2017). The higher levels of SMBC and SOC found in the biochar amendment treatments (particularly in N1 soil) indicated that the application of biochar may contribute to the growth and activation of soil microorganisms through an increased supply of nutrients (by biochar or added N), all of which are responsible for the higher CO_2 emissions observed (Luo et al., 2011; Song et al., 2016). The positive influence of slow pyrolysis biochar (production temperatures of 400–600 °C) in promoting soil aggregation has been reported in soils ranging in texture from sandy loam to clay loam (Gul et al., 2015), and previous studies have attributed the negative priming effects to the physical protection of soil organic matter by biochar (Maestrini et al., 2015). Therefore, physical protection through soil aggregation, a lower content of biochar liable C and stabilization of soil organic matter via the sorption of dissolved organic C onto biochar (Chen et al., 2017) might have led to the decrease in R_S under B1. However, other studies have indicated that physical protection through soil aggregation might be weakly linked to biochar-induced priming effects (Kerré et al., 2016). Thus, the mechanism whereby the low rate of apple branch biochar amendment decreased the emission of soil CO_2 requires further study.

4.2. Soil enzyme activity

The effect of apple branch biochar on soil enzyme activity was found to be determined by the soil N content, the rate of biochar addition, and the incubation time and varied with the type of enzyme, as observed in similar studies (Bhaduri et al., 2016; Ouyang et al., 2014). In the present

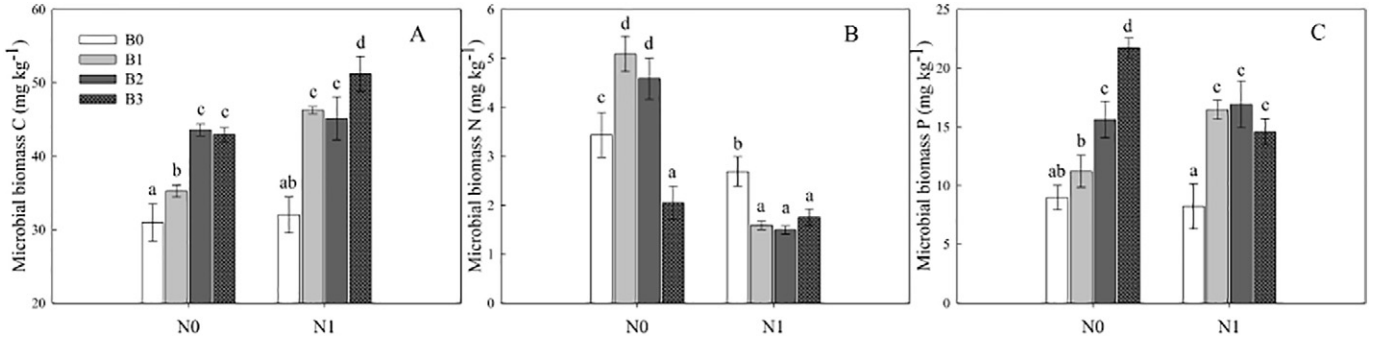

Fig. 6. Soil microbial biomass C, N and P observed in the different treatments at the last incubation stage. A: Soil microbial biomass C, B: soil microbial biomass N, C: soil microbial biomass P. The vertical bars in the figures represent the standard errors of the means (n = 3). N0 and N1 refer to the no-urea condition and the condition with urea addition at 0.2 g kg^{-1}, respectively. B0, B1, B2 and B3 refer to no biochar input and the incorporation of biochar into the soil at 1%, 2% and 4% by mass, respectively.

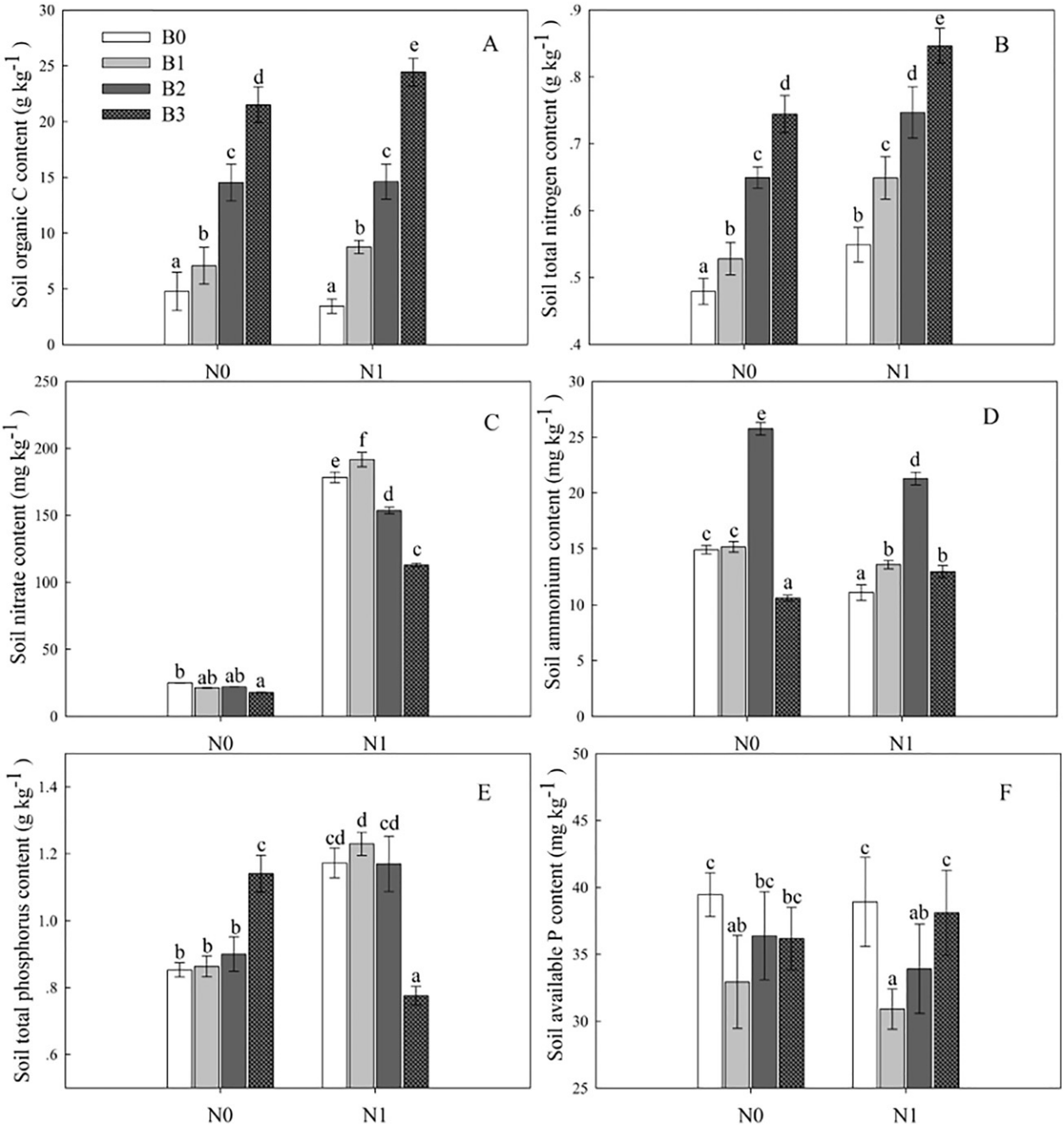

Fig. 7. The soil organic C contents (A), soil total N contents (B), soil nitrate contents (C), soil ammonium contents (D), soil total P contents (E) and soil available P contents (F) observed under the different treatments at the last incubation stage. The vertical bars in the figures represent the standard errors of the means (n = 3). N0 and N1 refer to the no-urea condition and the condition with urea addition at 0.2 g kg^{-1}, respectively. B0, B1, B2 and B3 refer to no biochar input and the incorporation of biochar into the soil at 1%, 2% and 4% by mass, respectively.

study, the applied apple branch biochar significantly decreased β-GA, β-NA and UA in N0 soil and increased β-GA and β-NA in N1 soil. However, apple branch biochar significantly increased AlkPA, regardless of the soil N level.

It is noteworthy that the methods for analyzing enzyme activities in soil containing biochar remain far from specifically adapted, optimized and standardized. In this study, traditional soil analytical methods were used to investigate the activities of enzymes associated with C, N and P cycling in soil containing biochar. Such analyses following extraction face the problem of the potential capture and adsorption of the substrates or products of a specific enzyme by biochar during enzymes assays (Elzobair et al., 2015). However, if only the adsorption behavior of biochar is taken into consideration in enzyme assays, the results may more accurately reveal the effects of apple branch biochar on the activities of these enzymes.

In general, soil enzyme activity depends on the interaction of the substrate and enzyme with biochar (Gul et al., 2015). The functional groups present in biochar tend to bind substrates and extracellular enzymes, thus interfering with the rate of substrate diffusion to the active site of enzyme catalysis; thus, biochar with a high porosity and surface area is expected to reduce extracellular enzyme activity (Bailey et al., 2011; Lammirato et al., 2011). Biochar addition has generally been found to reduce soil enzyme activities associated with soil C mineralization (Lehmann et al., 2011); however, in the current study, the effects of apple branch biochar on the β-GA and β-NA were found to depend on soil N levels. A reduction of β-GA and β-NA in N0 soil and an increase

in their activities in N1 soil were observed, which contradicts the results reported by Chen et al. (2017), XB Wang et al. (2015) and Tian et al. (2016), who showed that the activities of β-glucosidase, α-glucosidase, β-cellobiosidase and *N*-acetyl-β-glucosaminidase are markedly decreased by biochar amendment during either short-term laboratory incubation or long-term field studies. However, Elzobair et al. (2015) used fluorescence-based assays (the same assays used in the present study) and found that hardwood biochar did not affect the activities of β-glucosidases and *N*-acetyl-β-glucosaminidase, suggesting that biochar did not absorb these enzymes or their substrates/products during the enzyme assay. Hence, the observed reductions were likely due to the sorption of enzymes by biochar and the subsequent isolation of active sites (Bailey et al., 2011; Lammirato et al., 2011), both of which are related to the porosity and surface area of the biochar (Gul et al., 2015), rather than the sorption of substrates or byproducts during the assay. The applied biochar promoted β-GAs and β-NAs in N1 soil, which suggests that the interaction of biochar and N had positive effect on β-GAs and β-NAs. Could biochar protect these enzymes from degradation or facilitate substrate-enzyme reactions by acting as a platform in N-rich soil, as suggested by Elzobair et al. (2015)? Further research is needed to understand the exact underlying mechanisms.

Previous studies have indicated that the addition of biochar to soil can potentially increase the activities of a series of enzymes related to N utilization (Bailey et al., 2011; XB Wang et al., 2015). However, in the present study, apple branch biochar significantly decreased the mean UA in N0 soil (Table 1). The limited amount of the urease substrate present in the treatments without urea addition and the sorption of the substrate and urease onto the biochar (Gul and Whalen, 2016) led to the negative effects of the apple branch biochar on UA in N0 soil. However, in N1 soil, the addition of 0.2 g kg^{-1} urea to the soil guaranteed an adequate amount of the substrate for urease and promoted its activity; thus, the protection and stabilization of urease resulting from enzyme-biochar interactions might have led to the positive effect of apple branch biochar on UA (Nannipieri et al., 2012). Although our results indicated that the biochar treatments had no significant effect on the mean UA in N1 soil (Table 1), UA was significantly stimulated (48% and 32%) by N1B3 and N1B2 compared with N1B0 at day 108 (Fig. 4). In addition, the sorption of NH_4^- (a product of urease) by biochar in the urease assay might have led to underestimation of the positive effects (in N1 soil) and overestimation of the negative effects (in N0 soil) of biochar on UA (Hagemann et al., 2017; Haider et al., 2016). Therefore, apple branch biochar has the potential to increase the UA in N-rich soil.

Organic P hydrolysis is carried out by extracellular enzymes (i.e., phosphatases), and this enzymatic activity and microbial biomass are the most important determinants of P mineralization (Bohme et al., 2005). In the current study, apple branch biochar was found to significantly increase AlkPA in N0 and N1 soils, which is consistent with the previous findings reported by Chen et al. (2013) and Du et al. (2014), who suggested that the addition of biochar to soil is beneficial for AlkPA; this finding was most likely due to increased microbial abundance and, thus, increased enzyme levels in response to biochar addition. Furthermore, the modification of soil pH by biochar might affect P hydrolysis because higher soil pH values have been reported to enhance AlkPA (Du et al., 2014; Jin et al., 2015). However, the soil pH values did not differ significantly between any of the treatments in the current study (data not shown), indicating that a change in pH might not have been the main factor affecting P cycling in this study. Additionally, SMBP and SMBC increased with the rate of biochar addition, suggesting that biochar might introduce bioavailable P to soils through labile fractions (Takaya et al., 2016), which could also promote AlkPA. Because a greater microbial biomass requires more ortho-P to sustain its metabolic functions according to the principles of stoichiometric homeostasis, biochar-amended soil with a high SMBC concentration will also exhibit a high rate of P mineralization (Gul and Whalen, 2016; Masto et al., 2013).

4.3. Soil nutrients

Apple branch biochar significantly increased SOC and TN contents. SOC and TN were found to increase with the rate of biochar addition, which is consistent with previously published results (Liang et al., 2014; XB Wang et al., 2015). This increase might be due to the following reasons: 1) biochar contains labile C and N and can release organic C and N into the soil (Liang et al., 2014; XB Wang et al., 2015); and 2) biochar produced from lignocellulosic feedstocks with a low nutrient content is expected to cause net N immobilization in the short term (Gul and Whalen, 2016). Moreover, the numerous carbonaceous bonds with highly crosslinked networks present in the biochar (confirmed by FTIR spectra) also contribute to the enhancement of the soil C content (Bhaduri et al., 2016). The addition of apple branch biochar at a lower (B1 and B2) amendment rate had no significant effect on TP, but the observation that the highest rate (B3) increased the TP content in N0 soil and decreased the TP content in N1 was confusing (Fig. 7E). We used the molybdenum blue method (after digestion with H_2SO_4-$HClO_4$ at 300 °C for 2 h) to investigate the TP content of the soil containing biochar, but biochar P might not always be completely extractable with concentrated acid (Mukherjee and Zimmerman, 2013). The biochar might have been incompletely digested, and P adsorption by the residual biochar might have resulted in the uncertain TP results (Takaya et al., 2016). Therefore, the true TP content of N1B3 might be higher than indicated by the results.

Based on the adsorption of biochar during the assays, it can be hypothesized that NO_3^- decreased greatly with an increase in the amount of biochar applied (Fig. 7C). Previous studies have found that 30 min of KCl extraction (as performed in this study) might extract only 13% of the nitrate captured by the biochar (Haider et al., 2016). Nevertheless, the results of this study showed that N1B1 could significantly increase the NO_3^- content and that N0B2, N1B1 and N1B2 could significantly increase the NH_4^- content, which indicates that apple branch biochar has the potential to improve the capacity of the soil to supply mineral N to plants. The enhancement of UA and the interaction of biochar and N likely resulted in the significant increase in the NH_4^+ content (Mandal et al., 2015). However, SMBN in the N1 soil was not affected by the rate of biochar addition, indicating that the reduction of SMBN resulted from the interaction of biochar and N, rather than N adsorption by the biochar during the assay, in addition, SMBN was significantly higher in N0B1 and N0B2 than in N0B0 (Fig. 6B), which indicated that biochar may promote the utilization of N by microorganisms in N-poor soil, while restricting the use of N by microorganisms in N-rich soil. Regarding AP, its contents in the biochar addition treatments (B1, B2 and B3) were lower than those found in biochar-free soil (B0), regardless of the N level. In addition, AP increased in the biochar amendment treatments with an increase in the rate of biochar addition (Fig. 7F), similar to the results reported by Zhai et al. (2015), who found that the application of biochar had a greater effect on AP, resulting from increased application rates. These authors suggested that the increase in AP was mainly due to high concentrations of P in the ash fraction. The availability of P in soil is highly dependent on the P-sorptive capacity of the soil; metal-phosphate precipitation reactions play a more important role in the phosphate-sorptive capacity than the surface area (Zheng et al., 2011). The influence of the biochar surface area on AP adsorption is unclear, but some studies have suggested that its influence might be minor compared with the adsorbent elemental composition (Takaya et al., 2016). Takaya et al. (2016) observed some positive correlations between AP adsorption and the Ca or Mg contents of biochar, and the presence of surface MgO and other cations, including Ca^{2+} and Al^{3+}, is also known to improve phosphate adsorption (Z. Wang et al., 2015; Yao et al., 2011).

5. Conclusions

This study examined soil respiration, soil nutrient contents, and the activities of C-, N- and P-cycling enzymes in response to the application

of apple branch biochar at different rates in soils with two N levels. The results yield three salient conclusions:

1) Apple branch biochar amendment at rates of 2% and 4% primed the C-mineralization rate, whereas 1% biochar amendment decreased the C-mineralization rate, regardless of the N level. SOC and microbial biomass C increased with an increase in the rate of biochar addition. Therefore, apple branch biochar amendment might contribute to the long-term sequestration of C. The biochar had negative effects on the activities of β-GA and β-NA in N-poor soil, but a positive effect on the activities of these enzymes in N-rich soil.

2) Apple branch biochar increased UA in N-rich soil but might have decreased UA in N-poor soil. The total N content increased with an increase in the rate of biochar addition. The observed reductions in NO_3^- might have been due to adsorption by biochar during the assays.

3) Apple branch biochar increased AlkPA and the microbial biomass P content, regardless of the soil N level. The AP contents under all of the biochar addition treatments were lower than those found under the biochar-free treatments.

Positive changes in enzymatic activities in biochar-amended soil might result in better soil biological health and improved nutrient cycling. Apple branch biochar has the potential to capture and sequester C and improve soil fertility, but the responses of soil biological processes and soil nutrients to apple branch biochar are affected by soil N levels.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.06.275.

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