Environ Sci Pollut Res (2018) 25:1460–1469
https://doi.org/10.1007/s11356-017-0574-1

RESEARCH ARTICLE

Biochar addition induced the same plant responses as elevated CO_2 in mine spoil

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Received: 31 May 2017 / Accepted: 24 October 2017 / Published online: 31 October 2017

Abstract Nitrogen (N) limitation is one of the major constrain factors for biochar in improving plant growth, the same for elevated atmospheric carbon dioxide (CO_2). Hence, we hypothesized that (1) biochar would induce the same plant responses as elevated CO_2 under N-poor conditions; (2) elevated CO_2 would decrease the potential of biochar application in improving plant growth. To test these hypotheses, we assessed the effects of pinewood biochar, produced at three pyrolytic temperatures (650, 750 and 850 °C), on C and N allocation at the whole-plant level of three plant species (*Austrostipa ramosissima*, *Dichelachne micrantha* and *Isolepis nodosa*) grown in the N poor mine spoil under both ambient (400 μL L^{-1}) and elevated (700 μL L^{-1}) CO_2 concentrations. Our data showed that biochar addition (1) significantly decreased leaf total N and $\delta^{15}N$ ($P < 0.05$); (2) decreased leaf total N and $\delta^{15}N$ more pronouncedly than those of root; and (3) showed more pronounced effects on improving plant biomass under ambient CO_2 than under elevated CO_2 concentration. Hence, it remained a strong possibility that biochar addition induced the same plant physiological responses as elevated CO_2 in the N-deficient mine spoil. As expected, elevated CO_2 decreased the ability of biochar addition in improving plant growth.

Keywords Biochar application · Elevated atmospheric CO_2 · Leaf C and N · Leaf $\delta^{13}C$ and $\delta^{15}N$ · Nitrogen use efficiency · Plant biomass

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11356-017-0574-1) contains supplementary material, which is available to authorized users.

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Introduction

Biochar is a carbon-rich product that is produced via pyrolysis of a wide range of biomass sources (Lehmann et al. 2006). It has been well documented that biochar application to soils brings a number of benefits, including improving soil water storage (Reverchon et al. 2015; Wang et al. 2016), liming effect (Van Zwieten et al. 2010b) and reducing nutrient leaching (Bai et al. 2015; Biederman and Harpole 2013). The improved soil quality by biochar addtion is expected to increase plant growth (Jones et al. 2012; Rajkovich et al. 2012).

However, biochar has a limited capacity in improving nitrogen (N) availability for plant uptake (Biederman and Harpole 2013; Bruun et al. 2012; Nelson et al. 2011). Hence, there is a broad spectrum of plant growth responses to biochar additon, including positive, neutral and negative results (Biederman and Harpole 2013; Kammann et al. 2015; Rajkovich et al. 2012). Previous studies have shown that positive effects of biochar application on plant growth usually appeared in the following cases: (1) biochar produced from specific feedstocks (e.g. manure) has relatively high contents of N (Hass et al. 2012; José

and Knicker 2011; Nguyen et al. 2017); (2) the combination of biochar amendment with N fertilizers (Kammann et al. 2015; Nguyen et al. 2017), or (3) N-sufficient soils, mostly are agricultural soils (Olmo et al. 2014). However, it has also been reported that different plant species show different responses to the same biochar addition (Biederman and Harpole 2013; Van Zwieten et al. 2010a). Moreover, some plants can even maintain growth stimulation under N-deficient conditions following biochar application (Jeffery et al. 2011; Liu et al. 2013). N availability following biochar addition is commonly invoked to explain these diverse responses (Biederman and Harpole 2013; Bruun et al. 2012; Nelson et al. 2011; Rajkovich et al. 2012).

Similarly, N availability was found to affect the responses of plant biomass to elevated CO_2 in massive body of research, and has been ascribed as the primary factor for the diverse plant growth responses to elevated CO_2 (McMurtrie et al. 2008; Oren et al. 2001). Therefore, we hypothesized that biochar, with negligible amounts of N, would induce the same plant responses as elevated CO_2 in the N-poor soil. If so, this probably would provide insights to elucidate the diverse responses of plant growth to biochar amendment from the perspective of plant physiology.

Moreover, in the context of steadily rising atmospheric CO_2 concentrations (Stocker et al. 2013), it is necessary to evaluate if elevated CO_2 concentration would influence the potential of biochar application in improving plant growth. Since elevated CO_2 would also increase plant demand for N (Finzi et al. 2006; Langley and Megonigal 2010), it is highly likely that under N-limited conditions, the disadvantages of biochar application in improving N availability would be exacerbated by elevated atmospheric CO_2. The CO_2-enrichment studies have shown that limited N availability would constrain plant production (Elser et al. 2007; LeBauer and Treseder 2008). Furthermore, if N availability is lower than the N demand, plants would increase their loss of carbon (C) through root turnover, respiration or exudation (Drigo et al. 2007, 2008; Hungate et al. 1997). Therefore, if our hypothesis that biochar addition would induce the same responses as elevated CO_2 under N-limited conditions is tenable, elevated CO_2 would probably deprive the potential of biochar application in improving plant biomass.

Hence, the objectives of the current study were to (1) investigate if biochar with low level of N would induce the same plant responses as elevated CO_2 under N-deficient conditions; and (2) evaluate the influence of elevated CO_2 on the potential of biochar addition in improving plant growth. To achieve these goals, we grew three Australian indigenous plant species adapted to harsh environmental conditions, including *Austrostipa ramosissima* (*A. ramosissima*), *Dichelachne micrantha* (*D. micrantha*) and *Isolepis nodosa* (*I. nodosa*) (Table 1), with application of three types of biochar in the N-depleted mine spoil under both ambient (400 μL L^{-1}) and elevated (700 μL L^{-1}) CO_2 atmospheric concentrations. We assessed the effects of biochar on C and N allocation at the whole-plant level to elucidate the physiological implications. To avoid the N supply from biochar itself, we used N-depleted biochar in the current study with N content of ≤ 0.1% (Table S1).

Material and methods

Mine spoil sampling and biochar production

The sampling site of mine spoil used in the current study was Mount Owen Coal Mine Complex (Thiess Pty Ltd), located in the central Hunter valley, 20 km northwest of Singleton, New South Wales (NSW), Australia. The mine spoil was composed of coarse rocks that were not suitable for plant growth because of deficiency of water and essential nutrients, extreme pH, up to 9.7, coarse texture and compacted structure (Fisher 2010).

We divided the sampling site into 40 m × 40 m subplots in September 2013 and collected 1 tonne of spoil from each subplot. Then we transported the mine spoil samples in a cooling truck to the laboratory at the Hawkesbury Institute for the Environment (Western Sydney University, Penrith, NSW, Australia). The material (10 tons) was sieved (104 mm mesh), homogenized and stored at 4 °C until use (within 1 week after sampling).

Biochar was produced from pinewood (*Pinus radiata*) through slow pyrolysis at three maximum temperatures 650 °C (B650), 750 °C (B750) and 850 °C (B850). Specifically, the pinewood sawdust with a particle size of 25 mm was fed into the reactor continuously at 180 kg h^{-1}, with a rotation of 2 rpm, for 8 h per temperature. This allowed for a biomass residence time of 25 min. The biochar produced was released via a jacketed cooling screw.

Establishment of plants

We firstly sterilized the seeds of *A. ramosissima*, *D. micrantha* and *I. nodosa* (Greening Australia, Richmond, NSW, Australia) with 70% alcohol and 6% sodium hypochlorite and germinated them on sterilized glass beads (3 mm diameter) in a growth chamber set at 25 °C light (16 h) and 15 °C dark (8 h). Uniform 24-week-old seedlings (plumule length 10 cm) were selected and transferred to plastic containers (three seedlings per container) upon detection. The three types of biochar were thoroughly mixed with the mine spoil in the plastic containers (750 cm^3) at the rate of 8% by dry weight, totally ~ 6 kg in each plastic container and the moisture kept at ~ 10% (based on dry weight) using demineralized water. The control pots received the same weight of mine spoil (~ 6 kg) with no biochar addition. Within each chamber, all pots were shuffled after each

watering period to reduce potential position effects within the growth chambers.

CO_2 controlled cabinets

The experiment consisted of four environmentally controlled CO_2 flow cabinets (4 m × 5 m × 3 m; Climatic Chambers, Vancouver, Canada), with two cabinets CO_2 exposed to elevated CO_2 at 700 μL L^{-1}, and the remaining two at 400 μL L^{-1}. An airtight system (13,000 L airtight units) integrated with mass flow controllers (Brooks Smart, DMFC, Emmerson process Management, Pittsburg, PA, USA) in each cabinet enabled the maintenance of a constant atmospheric CO_2 level of 400 (ambient) or 700 (elevated) μL L^{-1}. A board infrared gas analyser (IRGA, CARBOCAP, GMT222, Dual Wavelength NDIRsensor, Vaisala, Oyj, Finland) was fitted in each of the flow cabinets to control the injection of scrubbed, pure CO_2 gas. The elevated CO_2 concentration (700 μL L^{-1}) was obtained by injection of CO_2 from a pressurized cylinder and the ambient (400 μL L^{-1}) by removal of CO_2 from the air by a solid carbon soda filter (Sofnoline, Sigma, St Louis, MO, USA). Inside each CO_2 flow cabinet, the maximum and minimum daily temperatures were 26 and 16 °C, respectively, a relative humidity was 70%. Light intensity was averagely kept at 250 μE. Climate data for the cabinets were stored digitally during the entire incubation period (3 years).

Experimental design

The experiment was set-up according to a split-plot design. In this design, we considered the CO_2 flow cabinets as whole plot, while the plant species (Table 1) and four types of biochar treatments (without biochar addition (B0), B650, B750 and B850) were considered as sub-treatments.

Sample collection and analysis

A. ramossissima, *D. micrantha* and *I. nodosa* were harvested at 3 years after germination, when plants were dominated by shoot growth. At harvesting, plant biomass was divided into different plant components, including leaves and roots, and roots were shaken gently to remove loosely adhering soil. Plant samples were dried at 65 °C to a constant weight, and the dry weights of leaves and roots were measured. We definded the N-use-efficiency (NUE) as the weight (g) of plant biomass produced per N (g). That is, the inverse of the N concentration in the biomass (Berendse and Aerts 1987). The oven-dried samples were ground to fine powder by a RocklabsTM ring grinder. Approximately 8 mg of the ground root or leaf samples was then transferred to 5 mm × 8 mm tin capsules. The powders were then assessed for total C (TC), total N (TN), and C and N isotope compositions ($\delta^{13}C$ and $\delta^{15}N$, respectively) using a Eurovector Elemental Analyser (Isoprime-EuroEA 3000, Milan, Italy). Stable isotope ratios were expressed in conventional δ notation as:

$$\delta^{13}C_{sample} \text{ or } \delta^{15}N_{sample} = \left(\left(R_{sample} - R_{std}\right)/R_{std}\right)$$

where $\delta^{13}C_{sample}$ or $\delta^{15}N_{sample}$ was the sample of interest, R_{sample} was its $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio, R_{std} was the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of standard, specifically, PDB (Pee Dee Belemnite) standard for $\delta^{13}C$ and the atmospheric air standard for $\delta^{15}N$. The results for $\delta^{13}C$ and $\delta^{15}N$ were reported as parts per thousand (‰) deviations from the PDB standard and atmospheric air, respectively. The analysis of TC, TN, $\delta^{13}C$ and $\delta^{15}N$ for biochar was conducted in the same way as biomass samples (Table S1). Biochar physical properties were determined by the laboratory of School of Engineering, University of Western Australia (Wang et al. 2016).

Statistical analysis

We assessed biochar effects under ambient and elevated CO_2 concentrations separately. A one-way ANOVA was conducted to determine if plant properties measured in the current study were significantly different under the four biochar treatments (B0, B650, B750 and B850) ($n = 4$). We used Shapiro-Wilk test to assess whether the data were normally distributed ($P > 0.05$). The homogeneity of variances was assessed by Levene's test for equality of variances ($P > 0.05$). If homogeneity of variances was met, we kept the results of the standard one-way ANOVA. When homogeneity of variances was

Table 1 List of the three plant species used in the current study

Latin name	Common name	Provenance	Properties	Photosynthetic pathway
Austrostipa ramossissima	Stout bamboo grass	The Ponds	AMF/not-N-fixing	C_3
Dichelachne micrantha	Shorthair plume grass	Erskine Park	AMF/not-N-fixing	C_3
Isolepis nodosa	Knobby club rush	Rouse Hill	Not AMF/ not-N-fixing	C_3

All the places in Provenance column are located in New South Wales, Australia and AMF, arbuscular mycorrhizal fungi

violated, we carried out the analysis with a one-way Welch ANOVA. The mean values were given along with the standard error for replicate measurements. Means and standard errors were based on the four replicates for each biochar treatment. Tukey test ($P < 0.05$) was used to determine the significance of differences among the treatments. All analyses were performed using SPSS 22.

Results

The effects of biochar addition on plant C and N

Leaf TN concentrations significantly decreased following the addition of all the three types of biochar for species *D. micrantha* under ambient CO_2 and *I. nodosa* under both ambient and elevated CO_2, where significant increases in leaf total C content (TC_{weight}) were also detected (Tables 2 and 3; Fig. 1b, c). In contrast, no significant alteration in leaf TN concentrations was observed for *A. ramossissima* under both CO_2 concentrations or *D. micrantha* under elevated CO_2, accompanied by the absence of increase in leaf TC_{weight} (Tables 2 and 3; Fig. 1a, b). *A. ramossissima* under elevated CO_2 even exhibited reduction in leaf TC_{weight}, especially significant with the application of biochar produced at 650 and 850 °C (Fig. 1a). On the other hand, biochar addition exerted negligible changes in root TN concentrations across the three plant species and the CO_2 treatments, except for *D. micrantha* that the application of biochar produced at 750 °C significantly increased root TN concentration (Tables 2 and 3).

Biochar addition increased root total N content (TN_{weight}) for species *D. micrantha* and *I. nodosa* under both CO_2 treatments, although the significances varied with biochar pyrolysis temperature. In contrast, the presence of biochar exhibited no significant influence on leaf TN_{weight} (Tables 2 and 3). In contrast, *A. ramossissima* showed no significant changes in either leaf or root TN_{weight} following biochar amendment under both CO_2 concentrations (Tables 2 and 3). Significant improvements in NUE following biochar addition were detected across the plant species and CO_2 treatments, except for *A. ramossissima* under elevated CO_2 concentration and *D. micrantha* with application of biochar produced at 750 °C under both CO_2 treatments not statistically significant (Fig. 2). Biochar addition decreased leaf $\delta^{15}N$ values across the plant species and CO_2 treatments, except for *I. nodosa* and *A. ramossissima* under ambient CO_2, although the significances varied with biochar pyrolysis temperature (Tables 2 and 3). However, there were no significant effects of biochar application on root $\delta^{15}N$ values across plant species and CO_2 treatments, except for the application of biochar produced at 650 °C significantly decreasing root $\delta^{15}N$ value for *I. nodosa* under elevated CO_2 (Tables 2 and 3). While biochar addition significantly decreased leaf $\delta^{13}C$ values for *I. nodosa* under both CO_2 concentrations, no significant changes appeared in leaf $\delta^{13}C$ for *A. ramossissima* and *D. micrantha* under both CO_2 treatments (Fig. 3).

Table 2 Leaf and root total nitrogen concentration (TN), stable nitrogen isotope ratio ($\delta^{15}N$) and total nitrogen content (TN_{Weight}) of *A. ramossissima*, *D. micrantha* and *I. nodosa* under different biochar treatments exposed to ambient CO_2 concentration (400 μL L^{-1})

Species		TN (%)		TN_{Weight} (mg)		$\delta^{15}N$ (‰)	
		Leaf	Root	Leaf	Root	Leaf	Root
A. ramossissima	*P* value	0.523	0.151	0.203	0.979	0.081	0.053
	B0	0.903 (0.381) a	0.765 (0.188) a	11.70 (3.81) a	12.18 (6.95) a	0.593 (0.266) a	0.338 (0.632) a
	B650	0.460 (0.070) a	0.255 (0.021) a	9.40 (2.17) a	12.40 (2.06) a	− 0.835 (0.518) a	− 1.628 (0.417) a
	B750	0.608 (0.087) a	0.245 (0.012) a	16.75 (3.03) a	14.38 (2.31) a	− 1.248 (0.524) a	− 0.473 (0.468) a
	B850	0.485 (0.054) a	0.325 (0.055) a	8.75 (1.03) a	12.58 (2.91) a	− 1.173 (0.645) a	− 1.485 (0.454) a
D. micrantha	*P* value	< 0.001	0.053	0.496	0.008	0.015	0.187
	B0	0.910 (0.044) a	0.408 (0.035) a	8.18 (1.51) a	2.18 (0.42) b	2.158 (0.498) a	0.803 (0.395) a
	B650	0.518 (0.067) b	0.298 (0.014) a	9.50 (2.33) a	11.33 (1.89) a	− 0.693 (0.491) ab	− 0.658 (0.479) a
	B750	0.485 (0.035) b	0.515 (0.099) a	6.50 (0.20) a	7.90 (1.83) ab	− 0.733 (0.666) ab	− 0.403 (0.367) a
	B850	0.293 (0.014) c	0.355 (0.046) a	4.53 (2.53) a	5.78 (1.51) ab	− 1.615 (1.064) b	3.798 (4.630) a
I. nodosa	*P* value	0.033	0.179	0.197	< 0.001	0.152	0.159
	B0	0.920 (0.170) a	0.330 (0.058) a	17.01 (1.57) a	2.53 (1.59) c	1.345 (0.182) a	0.883 (0.519) a
	B650	0.398 (0.016) b	0.225 (0.010) a	20.33 (3.11) a	12.63 (1.55) b	− 0.248 (0.767) a	0.440 (0.590) a
	B750	0.348 (0.030) b	0.248 (0.013) a	14.40 (2.42) a	18.83 (1.49) a	− 0.920 (0.738) a	− 0.858 (0.598) a
	B850	0.318 (0.020) b	0.308 (0.038) a	13.85 (1.15) a	19.65 (0.54) a	− 0.100 (0.745) a	0.615 (0.429) a

Values shown are mean (SE) (n = 4). B0: without biochar addition; and B650, B750 and B850: biochar produced via slow pyrolysis at 650, 750 and 850 °C, respectively. Different lower case letters indicated significant differences

Table 3 Leaf and root total nitrogen concentration (TN), stable nitrogen isotope ratio ($\delta^{15}N$) and total nitrogen content (TN_{Weight}) of *A. ramossissima*, *D. micrantha* and *I. nodosa* under different biochar treatments exposed to elevated CO_2 concentration (700 μL L^{-1})

Species		TN (%)		TN_{Weight} (mg)		$\delta^{15}N$ (‰)	
		Leaf	Root	Leaf	Root	Leaf	Root
A. ramossissima	*P* value	0.586	0.449	0.065	0.185	0.009	0.115
	B0	0.793 (0.097) a	0.298 (0.021) a	30.78 (10.72) a	10.15 (4.88) a	0.670 (0.388) a	− 0.473 (0.412) a
	B650	0.715 (0.123) a	0.353 (0.067) a	5.35 (1.07) a	4.95 (2.06) a	− 0.740 (0.405) ab	− 2.058 (0.344) a
	B750	0.518 (0.048) a	0.310 (0.038) a	12.13 (2.03) a	14.33 (3.68) a	− 1.283 (0.342) b	− 0.710 (0.474) a
	B850	0.758 (0.251) a	0.433 (0.097) a	9.95 (2.15) a	4.40 (2.22) a	− 1.400 (0.273) b	− 1.275 (0.550) a
D. micrantha	*P* value	0.137	0.007	0.209	0.015	< 0.001	0.224
	B0	0.678 (0.075) a	0.330 (0.012) b	13.15 (2.93) a	3.70 (1.92) b	1.538 (0.099) a	0.783 (0.700) a
	B650	0.465 (0.046) a	0.345 (0.027) b	5.40 (0.99) a	8.98 (0.72) ab	− 0.485 (0.523) b	− 0.783 (0.830) a
	B750	0.578 (0.086) a	0.533 (0.064) a	7.35 (0.89) a	13.60 (1.36) a	− 0.868 (0.075) b	− 0.820 (0.598) a
	B850	0.490 (0.039) a	0.365 (0.019) b	7.23 (0.83) a	12.00 (2.87) a	− 1.495 (0.693) b	− 1.088 (0.433) a
I. nodosa	*P* value	< 0.001	0.107	0.498	0.017	0.004	0.034
	B0	0.738 (0.067) a	0.258 (0.003) a	16.33 (0.68) a	16.90 (0.33) b	1.548 (0.104) a	1.240 (0.208) a
	B650	0.303 (0.031) b	0.213 (0.017) a	20.10 (3.38) a	34.35 (3.17) a	− 1.175 (0.381) b	− 0.675 (0.365) b
	B750	0.335 (0.019) b	0.213 (0.018) a	16.95 (1.70) a	21.53 (3.96) ab	− 0.108 (0.832) ab	0.065 (1.087) a
	B850	0.358 (0.011) b	0.210 (0.015) a	20.93 (3.15) a	24.35 (4.13) ab	− 0.213 (0.422) ab	0.293 (1.023) a

Values shown are mean (SE) (n = 4). B0: without biochar addition; and B650, B750 and B850: biochar produced via slow pyrolysis at 650, 750 and 850 °C, respectively. Different lower case letters indicated significant differences

The effects of biochar addition on plant growth

Biochar amendment significantly increased leaf biomass for *I. nodosa* under both ambient and elevated CO_2, whereas biochar addition exhibited no significant effects on leaf biomass for *D. micrantha* under both CO_2 treatments (Table 4). Moreover, biochar application even significantly decreased leaf biomass for *A. ramossissima* under elevated CO_2 (Table 4). For root biomass, significant improvements were observed for *I. nodosa* under elevated CO_2 but only statistically significant for *I. nodosa* and *D. micrantha* with application of biochar produced at 650 °C under ambient CO_2 (Table 4). Total biomass showed significant improvement across the plant species and CO_2 treatments, except for *A. ramossissima* and *D. micrantha* under elevated CO_2 concentration, although the significances varied with biochar pyrolysis temperature (Fig. 4).

Discussion

Biochar addition induced the same plant responses as elevated CO_2

Our results showed that biochar addition significantly decreased leaf TN concentrations for species *D. micrantha* under ambient CO_2 and *I. nodosa* under both ambient and elevated CO_2 (Tables 2 and 3). There are few studies that reported decreases in leaf TN concentrations following biochar addition (Kammann et al. 2011; Lehmann et al. 2003; Noguera et al. 2010; Rondon et al. 2007), which have been ascribed to adsorption or immobilization of N induced by biochar incorporation (O'Toole et al. 2013; Rajkovich et al. 2012). Decreases in leaf TN concentration are commonly seen under elevated CO_2 and the dilution due to accumulation of non-structural carbohydrates (NSC) has been proposed as the primary reason (Gifford et al. 2000; Taub and Wang 2008). Interestingly, the corresponding significant increases in leaf TC_{weight} in these cases suggested that dilution of NSC provides a likely explanation for the significant decrease in leaf TN following biochar addition (Fig. 1). This dilution mechanism is further confirmed by the concurrence of the absences of significant decrease in leaf TN and increase in leaf TC_{weight} of *A. ramossissima* under both CO_2 concentrations and *D. micrantha* when exposed to elevated CO_2 (Tables 2 and 3; Fig. 1).

However, it has been widely proved that dilution is not solely responsible for decreasing leaf TN cencentration under elevated CO_2 (Poorter et al. 1997; Taub and Wang 2008). Alternatively, many studies suggested that plants decreased N investment in photosynthetic apparatus, particularly Ribulose 1,5 bisphosphate carboxylase (rubisco) and thus, potentially distributes more N in root, which was involved in obtaining the resources that would be needed most (Hermans et al. 2006). In this way, plants could achieve optimal N distribution and thus obtain greater NUE to meet the higher N demand under elevated CO_2 (Cotrufo et al. 1998; Taub and Wang 2008; Wolfe et al. 1998).

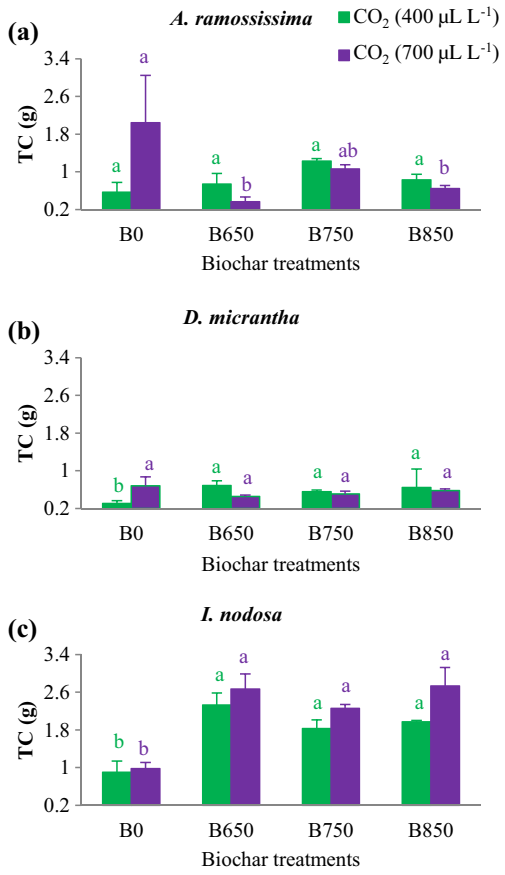

Fig. 1 Effects of biochar addition on leaf total carbon content (TC (g)) of **a** *A. ramossissima*, **b** *D. micrantha* and **c** *I. nodosa* under ambient (400 μL L^{-1}) and elevated (700 μL L^{-1}) CO_2 concentrations. Values are mean + SE (n = 4). B0, without biochar addition; B650, B750 and B850: biochar produced via slow pyrolysis at 650, 750 and 850 °C, respectively. Different lower case letters above the bars indicated significant difference at $P < 0.05$

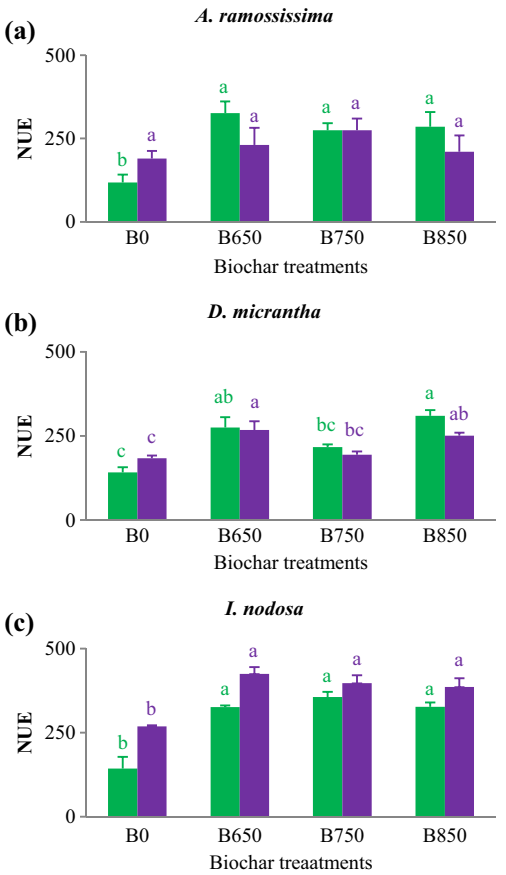

Fig. 2 Effects of biochar addition on nitrogen-use-efficiency (NUE) of **a** *A. ramossissima*, **b** *D. micrantha* and **c** *I. nodosa* under ambient (400 μL L^{-1}) and elevated (700 μL L^{-1}) CO_2 concentrations. Values are mean + SE (n = 4). B0, without biochar addition; B650, B750 and B850: biochar produced via slow pyrolysis at 650, 750 and 850 °C, respectively. Different lower case letters above the bars indicated significant difference at $P < 0.05$

The significant increase in root TN_{weight}, along with the absence of significant alteration in leaf TN_{weight} of *D. micrantha* and *I. nodosa* (Tables 2 and 3), suggested that the shift in N partitioning from leaves to roots in the presence of biochar probably also played a role in reducing leaf N concentration. The exception of *A. ramossissima* (Tables 2 and 3) probably suggested the relatively lower potential for improving NUE. The near-ubiquitous NUE improvement in this study (Fig. 2) lent further qualified support to this conclusion, which also offered a possibility for the plant total biomass enhancement (Fig. 4) in spite of the N deficiencies of biochar and mine spoil used in this study (Fisher 2010). Therefore, our results at least partially explained why some plants could even maintain growth stimulation under N-deficient conditions following biochar application (Jeffery et al. 2011; Liu et al. 2013).

Further evidence in support of the hypothesis that biochar addition induced closely resembled plant responses to elevated CO_2 comes from leaf $\delta^{15}N$ values. Significant depletion in plant $\delta^{15}N$ values under elevated CO_2 has been observed in many previous studies (BassiriRad et al. 2003; Billings et al. 2002). This was primarily attributed to increased plant reliance on mycorrhizal fungi for N uptake with the rising of CO_2 since substantial N isotopic fractionation occurred during transferring from mycorrihizal fungi to plant (BassiriRad et al. 2003; Garten et al. 2011; Polley et al. 2015). Our data showed significant decreases in leaf $\delta^{15}N$ of *A. ramossissima* and *D. micrantha* following biochar addition, although the decrease in case of *A. ramossissima* under ambient CO_2 was not statistically significant (Tables 2 and 3). Moreover, the absence of significant decrease in leaf $\delta^{15}N$ of *I. nodosa* under ambient CO_2 concentration (Table 2) supported our hypothesis in a different perspective in view of the fact that *I. nodosa* relied on root for N uptake while *A. ramossissima* and *D. micrantha* were associated with arbuscular mycorrhizal (AM) fungi (Table 1). However, the significant decrease in leaf $\delta^{15}N$ of *I. nodosa* when exposed to elevated CO_2 (Table 3) released a signal of some level of N limitation (Garten et al. 2011). This is not out of expectation since both CO_2 enrichment and biochar addition would induce higher N demand.

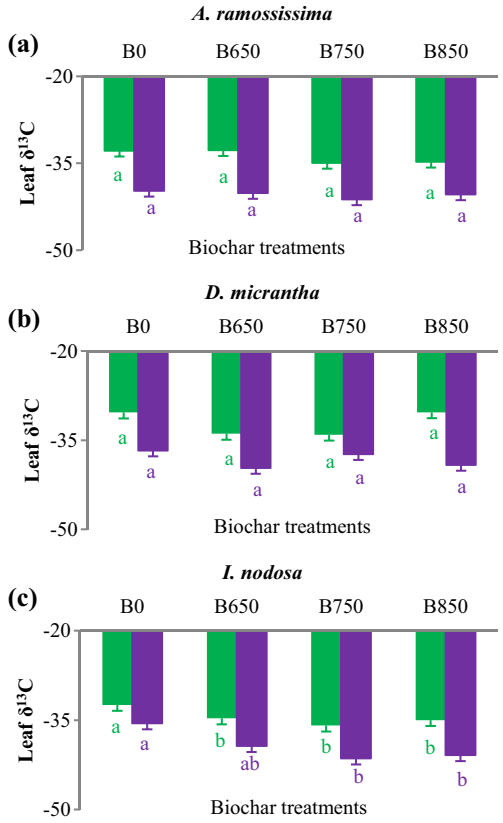

Fig. 3 Effects of biochar addition on leaf stable carbon isotope ratio ($\delta^{13}C$) of **a** *A. ramosissima*, **b** *D. micrantha* and **c** *I. nodosa* under ambient (400 μL L^{-1}) and elevated (700 μL L^{-1}) CO_2 concentrations. Values are mean + SE ($n = 4$). B0, without biochar addition; B650, B750 and B850: biochar produced via slow pyrolysis at 650, 750 and 850 °C, respectively. Different lower case letters above the bars indicated significant difference at $P < 0.05$

Another line of evidence derived from our observation that biochar application tended to reduce TN and $\delta^{15}N$ of leaves more pronouncedly than those of roots (Tables 2 and 3), which were commonly seen under elevated CO_2 (BassiriRad et al. 2003; Cotrufo et al. 1998). Hence, it remains a strong possibility that biochar addition induced the same plant responses as elevated CO_2 in the N-deficient environment in this study, which deserves further investigation.

What is the influence of elevated CO_2 on the potential of biochar application in improving plant growth?

As expected, when exposed to elevated CO_2, *A. ramosissima* exhibited no significant improvements in plant total biomass in the presence of biochar (Fig. 4a). Additionally, although not statistically significant, root biomass of *A. ramosissima* even showed some level of decrease in response to biochar addition (Table 4). This suggested that N demand was probably not fully met and thus, *A. ramosissima* might increase its root turnover, which is consistent with our hypothesis that disadvantages of biochar application in improving N availability would be

Table 4 Leaf and root biomass of *A. ramosissima*, *D. micrantha* and *I. nodosa* under different biochar treatments in combination with ambient (400 μL L^{-1}) and elevated (700 μL L^{-1}) CO_2 concentrations

Species		Ambient CO_2 (400 μL L^{-1})		Elevated CO_2 (700 μL L^{-1})	
		Leaf (g)	Root (g)	Leaf (g)	Root (g)
A. ramosissima	*P* value	0.116	0.057	0.020	0.178
	B0	1.49 (0.37) a	1.42 (0.62) a	4.61 (2.20) a	3.52 (1.63) a
	B650	2.09 (0.47) a	4.98 (0.86) a	0.82 (0.23) b	1.77 (0.89) a
	B750	2.70 (0.21) a	5.73 (0.64) a	2.33 (0.21) b	4.92 (1.39) a
	B850	1.85 (0.50) a	4.60 (1.68) a	1.45 (0.14) b	1.25 (0.67) a
D. micrantha	*P* value	0.099	0.029	0.169	0.100
	B0	0.89 (0.14) a	0.57 (0.16) b	2.05 (0.50) a	1.09 (0.56) a
	B650	1.80 (0.28) a	3.89 (0.75) a	1.13 (0.10) a	2.69 (0.42) a
	B750	1.36 (0.10) a	1.78 (0.56) ab	1.34 (0.20) a	2.69 (0.42) a
	B850	1.55 (0.86) a	1.85 (0.63) ab	1.47 (0.09) a	3.37 (0.88) a
I. nodosa	*P* value	0.031	< 0.001	0.002	0.011
	B0	2.08 (0.46) b	0.80 (0.48) b	2.31 (0.30) b	6.64 (0.21) b
	B650	5.06 (0.59) a	5.65 (0.80) a	6.61 (0.68) a	16.40 (1.79) a
	B750	4.10 (0.52) a	7.76 (0.89) a	5.00 (0.24) a	10.23 (2.06) ab
	B850	4.34 (0.09) a	6.64 (0.69) a	5.86 (0.84) a	11.67 (1.91) ab

Values shown are mean (SE) ($n = 4$). B0: without biochar addition; and B650, B750 and B850: biochar produced via slow pyrolysis at 650, 750 and 850 °C, respectively. Different lower case letters indicated significant differences

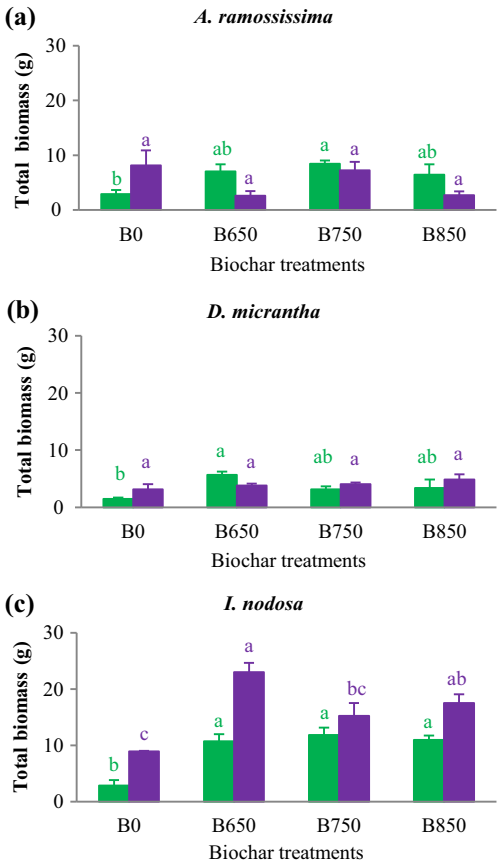

Fig. 4 Effects of biochar addition on plant total biomass of **a** *A. ramossissima*, **b** *D. micrantha* and **c** *I. nodosa* under ambient (400 μL L^{-1}) and elevated (700 μL L^{-1}) CO_2 concentrations. Values are mean + SE (n = 4). B0, without biochar addition; B650, B750 and B850: biochar produced via slow pyrolysis at 650, 750 and 850 °C, respectively. Different lower case letters above the bars indicated significant difference at $P < 0.05$

exacerbated under elevated atmospheric CO_2 and thus, elevated CO_2 would deprive the potential of biochar application in improving plant biomass. Similarly, while biochar amendment significantly improved total biomass of *D. micrantha* under ambient CO_2 concentration, no significant increase existed under elevated CO_2 (Fig. 4b), which further confirmed our hypothesis.

Conversely, biochar addition significantly increased total biomass of *I. nodosa* under both CO_2 concentrations (Fig. 4c). It was interesting to notice that biochar amendment triggered significant decrease in leaf $\delta^{13}C$ of *I. nodosa* (Fig. 3c), which is commonly seen under elevated CO_2 and could be partially ascribed to improved photosynthetic capacity (Leakey et al. 2009; Meyers 2014; Schubert and Jahren 2015). This might suggest that *I. nodosa* would have larger potential for increasing NUE compared to *A. ramossissima* or *D. micrantha* and thus, allowed greater improvement in plant photosynthesis. Further support for this view was derived from our observation that *I. nodosa* exhibited the only significant increase in leaf biomass in response to biochar addition (Table 4). Therefore, the higher potential for improving NUE of *I. nodosa* made it is possible that biochar addition continued to improve biomass of *I. nodosa* under elevated CO_2. This is probably why different plant species show different responses to the same biochar addition (Biederman and Harpole 2013; Van Zwieten et al. 2010a).

Conclusion

Our results suggested that in the N-depleted mine spoil, the addition biochar with extremely low level of N induced species-specific decreases in leaf total N concentrations, which was commonly seen under elevated CO_2. Under N-deficient conditions, plants could decrease N investment in leaf and thus, potentially distribute more N in root, via which plants could achieve optimal N distribution and thus obtain greater N-use-efficiency to sustain plant growth improvement even under N-limited conditions. Hence, the species-specific decreases in leaf total N with biochar addition among the three plant species used in this study probably reflect different potential in improving N-use-efficiency in an increasing order as follows: *A. ramossissima* < *D. micrantha* < *I. nodosa*. This offers an optional explanation for the broad spectrum of plant growth responses to biochar additon from the perspecitve of plant physiology. Biochar addition also decreased leaf $\delta^{15}N$ as elevated CO_2, which probably due to increasing plant reliance on mycorrihizal fungi for N uptake to adapt to N deficiency. Hence, it remains a strong possibility that biochar addition induced similar plant physiology responses to elevated CO_2 in the N-deficient mine spoil. Moreover, since elevated CO_2 would also increase plant N demand, the lower potential for improving N-use-efficience of *A. ramossissima* and *D. micrantha* probably only allowed significant increase in total biomass in presence of biochar under ambient CO_2, whereas the higher N-use-efficience of *I. nodosa* could sustain the biomass improvement under both CO_2 concentrations. Hence, elevated CO_2 decreased the ability of biochar addition in improving plant growth.

Acknowledgements The spoil mine soil was provided by Newcastle University (Newcastle, NSW, Australia) and Thiess Pty Ltd. (Singleton, NSW, Australia) and the biochar was provided by the University of Western Australia (Perth, WA, Australia).

Funding information This work was funded by the Australian Research Council at the Hawkesbury Institute for the Environment (Western Sydney University, Penrith, NSW, Australia) and by the Griffith University PhD scholarships and operating funding.

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