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RESEARCH ARTICLE

# Biochar application for enhancing water and nitrogen use efficiency of understory acacia species in a suburban native forest subjected to nitrogen deposition in Southeast Queensland

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**Abstract**
*Purpose* The fuel reduction prescribed burning and biochar application can have significant impacts on water and nitrogen (N) use efficiency of understory acacia species as well as soil carbon (C) and N pools in a suburban native forest subject to N deposition in Southeast Queensland, Australia.
*Methods* We evaluated the impact of biochar application rates (0, 5.0 and 10.0 t biochar per hectare) and prescribed burning on soil-plant interactions in carbon (C) and N cycling in a suburban native forest in the first two years of biochar application or three and half years of the recently prescribed burning.
*Results* Anthropogenic N deposition not only enhanced N losses caused by N leaching and denitrification, but also inhibited biological N fixation (BNF) by increasing N availability in forest systems. The *Acacia leiocalyx* with higher water use efficiency was more inclined to utilize easily available N resources (from N deposition), compared with *A. disparismma*. In this study, biochar application could indeed reduce N loss in forest soil and improve soil fertility by improving plant water and N use efficiency. Meanwhile, soil moisture content affected by biochar application also influenced soil N transformations by affecting soil microbial activity.
*Conclusion* For urban forest soils, the high N availability caused by N deposition could inhibit the BNF in a suburban native forest ecosystem. The high-porosity physical structure of biochar applied increased the soil water content and soil N retention capacity.

**Keywords** N deposition · Water use efficiency · N use efficiency · Forest system · Soil moisture content · $\delta^{15}N$

Weiling Sun and Yinan Li contributed equally to this work.

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## Introduction

Since the industrial revolution and rapid development in the 150 years, we have dramatically altered the global nitrogen (N) cycle, with widespread and often divergent effects on ecological systems and environmental quality (Galloway et al. 2008; Xu et al. 2009; Succarie et al. 2022). The N cycling in terrestrial ecosystems is mainly driven by three primary sources: biological N fixation (BNF), N mineralization, and atmospheric N deposition. Among them,

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the potential threat of atmospheric N deposition to forest health (Binkley and Högberg 1997) may cause N saturation, which increases the risk of N leaching, soil acidification and N denitrification (Conley et al. 2009; Huang et al. 2015; Zhang et al. 2018a, b, c), which is causing concern about significant impacts on global forest health and plant biodiversity (Vitousek et al. 1997; Sala et al. 2000; Phoenix et al. 2006; Cecchini et al. 2021). At the same time, forest N dynamics interact closely with other biogeochemical cycles and greater N emissions contribute to a litany of well-documented and undesirable environmental changes, including loss of biodiversity (Bobbink et al. 2010; Aryal et al. 2022), soil and surface water acidification (Moldan and Wright 2011; Zhou et al. 2023) and reductions in air quality (Wolfe and Patz 2002; Wang et al. 2023).

Biochar is formed by heating organic material under low oxygen concentrations in a process known as pyrolysis (Lehmann et al. 2011; Rady et al. 2016; Haldar and Purkait 2021). Biochar is enriched in C, considered relatively stable in the soil, and has better nutrient retention than other forms of organic matter (Woolf et al. 2010; Nguyen et al. 2017; Farrar et al. 2022). Hence, adding biochar to the soil becomes an option for carbon (C) sequestration, potentially improving soil quality and reducing environmental pollutant release (Lehmann 2007; Gurwick et al. 2013; Das et al. 2023). Moreover, biochar can act as a soil amendment to affect nutrient cycling and plant growth by affecting microbial community composition and activity, water holding capacity and pH, as well as improving root growth (Kuzyakov et al. 2009; Liang et al. 2010; Robertson et al. 2012; Biederman and Harpole 2013; Thomas and Gale 2015; Liu et al. 2021). In addition, labile C fractions of biochar may also accelerate the decomposition of old soil organic matter through the priming effect (Cross and Sohi 2011; Zimmerman et al. 2011; Wang et al. 2016; Rasul et al. 2022).

Recently, although people have been interested in biochar as a soil amendment to improve soil quality, improve and maintain soil fertility, and increase soil C sequestration (Glaser et al. 2002; Das et al. 2021; Luo et al. 2023), there is still a lack of clear understanding of the characteristics of biochar produced from different raw materials and under different pyrolysis conditions and its interaction with soil. In addition, some researchers believe that biochar can be used as a fertilizer to provide N for plant growth (Wu et al. 2016, 2017). By improving the pH, cation exchange capacity (CEC), organic carbon and ash content of the soil (Rahim et al. 2020; Tomczyk et al. 2020), biochar may further reduce N loss from N leaching and ammonium volatilization (Deluca et al. 2015). In addition to helping the soil retain water and nutrients, the oxygen-containing functional groups on the biochar surface also improve the soil's water holding capacity (WHC) and nitrogen use efficiency (NUE) (Yu et al. 2018), promoting plant productivity and improving soil fertility (Palansooriya et al. 2019; Bai et al. 2022). The ability of biochar to directly supply nutrients is limited, but its application to soil can improve soil fertility by changing the availability of soil N (Zhu et al. 2017; Asadyar et al. 2021). The high-temperature biochar (600 °C) used in this study usually has a higher surface area, porosity and alkalinity, thereby enhancing the soil water holding capacity and nutrient use efficiency of the soil and improving soil nutrient uptake by plants (Zhang et al. 2015; Burrell et al. 2016; Ding et al. 2016; Zornoza et al. 2016). However, the optimal application rate of high-temperature biochar and its relationship with plant-soil interactions have not been studied well (Zhang et al. 2015; Lan et al. 2018).

Prescribed burning has been widely used as a forest management tool to reduce the risk of wildfire spread (May and Attiwill 2003; Reverchon et al. 2011; Bai et al. 2012; Francos and Úbeda 2021). Prescribed burning may cause N losses through volatilization and release terrestrial carbon (C) into the atmosphere (Thonicke et al. 2010; Muqaddas et al. 2016). *Acacia* species can help soil nutrient recovery by biological N fixation (BNF) and increasing carbon (C) sequestration, especially in forests after prescribed burning and fire (Guinto et al. 2000; Bai et al. 2014; Witt et al. 2017; Reverchon et al. 2020). Most forest soil N is in organic form, but N mineralization rates are low, and tree growth is N-limited (Sponseller et al. 2016). Biochar application has been shown to stimulate soil organic matter decomposition by increasing net N mineralization and nitrification rates (Ameloot et al. 2015; Case et al. 2015; Gundale et al. 2016).

Based on previous studies, plant-soil $\delta^{13}C$ and $\delta^{15}N$ values are related to soil N and water availability and are closely related to C and N cycling of soil-to-plant (Werth and Kuzyakov 2010; Nogués et al. 2023). There is a significant correlation between plant $\delta^{13}C$ and soil moisture content, which makes the $\delta^{13}C$ a powerful tool to evaluate plant water use efficiency (Klaus et al. 2016). By analysing $\delta^{15}N$ in soil and plants, we can better understand the retention and use efficiency of N in forest ecosystems (Schlesinger 2009; Hietz et al. 2011; Ibell et al. 2013; Mao et al. 2022). According to the reports, in the process of microbial transformation, the soil substrate is usually rich in $^{15}N$ due to the rapid movement of the substrate in the process of N loss (Kuzyakov and Xu 2013; Nogués et al. 2023). However, the altered global N cycle will have important consequences. The N deposition will increase the N losses via denitrification and leaching of nitrate and the production of reactive N, adversely affecting the environment, climate, biodiversity and human health (Robertson and Vitousek 2009; Fowler et al. 2013; Cheng et al. 2020; Song et al. 2020). This study aimed to quantify the impact of biochar application rates (5.0 and 10.0 t biochar per hectare) on soil-plant interactions in C and N cycling in a suburban native forest in the first two years of biochar application or three and half years of the recent prescribed burning. Generally, biochar always causes a short-term limited positive priming effect, but long-term field experiments about the impacts of biochar in urban forest ecosystems subjected to prescribed burning are rare (Bruckman et al. 2015; Mitchell et al. 2015; Page-Dumroese et al. 2016).

## Materials and methods

### Study area and experiment design

The study site was located in Toohey Forest, Brisbane, South Queensland, Australia (27⁰32′53"S; 153⁰03′21″E). This area is one of Australia's native forests dominated by *Eucalyptus* species. Toohey forest is a subtropical climate area with an average annual temperature of 27 °C and an average annual rainfall of 1350 mm (Bai et al. 2012). Toohey forest has been subject to prescribed burning since 1993, and the risk of wildfire spread has been reduced by dividing areas for prescribed burning (Catterall et al. 2001; Wang et al. 2020a). The experimental site in this study was last burned in August 2017, and a field biochar application trial was established in this area in May 2019 after 20 months of prescribed burning. The test area was randomly placed in a site, which included a mixture of understorey legumes *Acacia leiocalyx, Acacia disparismma* and overstorey such as *Eucalyptus psammitica*, which is typical of the area.

In brief, the experimental design is a random complete block design, with 4 plots as 4 replicates. These four circular plots with a radius of 12.62 m, giving an internal area of 500 $m^2$, were randomly located within the site, offering a total sampling area of one-fifth of a hectare. Each plot has 13 sub-samples, including two understorey legume species of Acacia with *Eucalyptus planchoniana* as a reference plant. Each plant area is defined by four steel pegs providing an area of 4 $m^2$ (2 m × 2 m). Biochar was applied to each acacia plant at three rates of 0, 5 and 10 t $ha^{-1}$. In each plot, there are 3 different treatment methods: 1) control (no biochar), 2) biochar 5 t $ha^{-1}$ (2 kg/plant), and 3) biochar 10 t $ha^{-1}$ (4 kg/plant), respectively. Biochar was manually scattered on the soil surface in May 2019. First, the grass in each plant area was manually removed, and the biochar was homogeneously distributed and mixed manually on the soil surface to minimise biochar loss. Soil samples were collected from the central area of each plant area after the removal of the litter layer to avoid edge effects.

### Characterization and application of biochar

Biochar material used in this experiment was produced from pine wood (*Pinus radiata*) through slow pyrolysis at 600 °C introduced in Western Australia. The N isotope composition ($\delta^{15}N$) and C isotope composition ($\delta^{13}C$) of the chosen high-temperature biochar were 2.3‰ and −27.5‰ respectively in this study. It is pertinent to note that we chose high-temperature biochar that was made above 500 °C because this study region is in a natural forest with high N deposition pollution (Bai et al. 2012). In terms of sustainability, the two biochar rates were selected in this study, as it has been confirmed and reported that 5 t $ha^{-1}$ and 10 t $ha^{-1}$ are ideal and economically feasible application rates (Williams and Arnott 2010; Bruckman et al. 2016). The biochar properties are summarized in Table S1.

### Soil and foliage sample collection

After 24 months of the field establishment, soil samples were collected from three different depths of 0–5, 5–10 and 10–20 cm using a soil auger with a diameter of 7.5 cm at various points across each plant area in May 2021. After collecting soil and plant samples, the soil samples were sieved by using a 2 mm sieve and mixed thoroughly, then subsampled were collected for the following analyses: 1) air drying a part of the soil samples to analyse the soil pH value; 2) oven drying (60 °C) a part of soil samples for mass spectrometry analysis and determination of soil moisture content.

Similarly, plant samples (foliage) were oven-dried to a constant weight for 72 hours at 60 °C to a constant weight. After that, the dried foliage samples were ground into a fine powder using the Rocklabs™ ring grinder and weighted for mass spectrometry analyses. For foliage samples and oven-dried soil samples, we have evaluated the total C, total N, and their isotope composition (e.g., $\delta^{13}C$ and $\delta^{15}N$) (Xu et al. 2000).

## Data collection and measurements

### *Measurements of soil physiochemical properties*

After collecting and processing soil samples, some initial soil characteristics were determined, such as soil moisture content and pH value. Soil moisture content (SMC) was determined by drying the field moist soil at 105 °C for 24 hours. The value of SMC was determined using the following equation (Voroney 2019):

$$\textit{Soil moisture content}\ (\%) = \frac{\left[W_{wet\ soil} - W_{dry\ soil}\right]}{W_{dry\ soil}} \times 100$$

The ratio of soil and water mass was 1:5 to determine soil pH. The 5 g of air-dried soil was taken into a 50 ml falcon and 25 ml of deionized water was added. The samples were then shaken in an end-to-end shaker for an hour, and then they were allowed to stand to settle down the solution for 20 minutes. After calibrating the machine with two buffer solutions, the soil pH was measured with a pH electrode.

Oven-dried soil samples were ground into fine powder by using the Rocklabs™ ring grinder. Then approximately 40–50 mg of soil (0–5 and 5–10 cm), about 50–60 mg (10–20 cm) and about 6–7 mg foliage samples were transferred into tin capsules for total C total N, $\delta^{13}C$ and $\delta^{15}N$ analyses by using the isotope ratio mass spectrometer (IRMS, Elementar, Langenselbold, Hesse, Germany).

The value of $\delta^{13}C$ and $\delta^{15}N$ were determined using the following two equations:

$$\delta^{13}C_{sample} = \frac{\left[R_{sample} - R_{VPDB}\right]}{R_{VPDB}} \times 1000$$

$$\delta^{15}N_{sample} = \frac{\left[R_{sample} - R_{air}\right]}{R_{air}} \times 1000$$

where,

R the isotope ratio

$R_{sample}$ the ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of sample respectively

$R_{VPDB}$ the ratio of $^{13}C/^{12}C$ of the international standard (Vienna Pee Dee. Belemnite (VPDB))

$R_{air}$ the ratio of $^{15}N/^{14}N$ of the international standard (atmospheric $N_2$)

The results for $\delta^{13}C$ and $\delta^{15}N$ were expressed as parts per thousand (‰).

### *Measurements of plant growth and physiochemical properties*

Plant height and diameter at ground level (DGL) were measured in May 2021 at four plots by using diameter tape and height measurements hypsometer (Vertex IV), respectively. All understory plants, such as *Acacia* and *Eucalyptus* species for all plots, were included for growth measurements. Four plots contain an average of 52 plants, 48 plants for each *Acacia* spp. (*A. leiocalyx* = 24; *A. disparimma* = 24) and 4 reference plants of *E. psammitica*.

The percentage of N derived from atmospheric $N_2$ (%Ndfa) was calculated using the following equation (Shearer and Kohl 1986).

$$\%Ndfa = \left[\frac{\left(\delta^{15}N_{ref} - \delta^{15}N_{acacia}\right)}{\delta^{15}N_{ref} - Bvalue}\right] \times 100$$

where:

| | |
|---|---|
| $\delta^{15}N_{ref}$ | The $\delta^{15}N$ value of the reference plants |
| $\delta^{15}N_{acacia}$ | The $\delta^{15}N$ value of the Acacia spp. |
| B value | The relative isotopic abundance of Acacia spp. growing under an N-free nutrient condition |

The B values reported from a large array of sources vary from −2.9‰ to 1.0‰ for woody plants (Shearer and Kohl 1986; Boddey et al. 2000). In the present study, using a B value of −1.3‰ resulted in an estimation of %Ndfa that was greater than 100%. Assuming B = −1.3‰ resulted in 75.0% of all cases having the %Ndfa greater than 100% ($n = 30$ out of 40), while this percentage dropped to 5.0% when B was assumed to be −0.8‰ ($n = 2$ out of 40). None of the cases were greater than 100% when B values were either −0.3‰ or 1.0‰. Therefore, different B values varying from −1.3‰ to 1.0‰ were examined to determine an applicable B value for *Acacia* spp. under the experimental condition. In our study, we chose a B value of −0.3‰ to report the results of BNF determination based on the $^{15}N$ natural abundance method.

### Statistical analyses

A general linear model was used to analyse treatment effects (SPSS 19.0, IBM Crop., Armonk, NY, US) (Bruckman et al. 2016). All data were tested for homogeneity of variance and normality of distribution. A three-way ANOVA was performed to test for these three factors (i.e., soil depths, biochar rates and plant species) and their interactions on each measured parameter. Pearson's correlation analysis was used to assess relationships between soil properties across all data of three biochar rates at different soil depths. Statistical significance was set at $\alpha = 0.05$.

## Results

### Chemical and physical properties of plant samples

The results from Table 1 show that after biochar application at the Toohey forest for 2 years, the foliar N isotope compositions ($\delta^{15}N$) and C isotope composition ($\delta^{13}C$) were significantly different between the two understorey acacia species ($P < 0.05$). The foliar $\delta^{15}N$ of *A. disparimma* (−1.46‰) was significantly higher than that of *A. leiocalyx* (−2.01‰), and foliar $\delta^{13}C$ of *A. disparimma* (−32.1‰) was also significantly higher than that of *A. leiocalyx* (−32.8‰) (Table 1). The foliar total N was also significantly increased by these two biochar application rates. The %Ndfa of *A. disparimma* was 58.0%, which was significantly higher than the %Ndfa of *A. leiocalyx* (37.4%) ($P < 0.05$) (Table 1).

There were no significant differences in plant height, diameter at ground level (DGL), basal area (BA) and volume between the three different biochar application rates and the two species ($P > 0.05$) (Table 2). Relative to the control, biochar addition increased plant growth in natural forests and

**Table 1** Effects of biochar application rates and understorey acacia species on foliar total carbon (total C), total nitrogen (total N), C and N isotope composition ($\delta^{13}C$ and $\delta^{15}N$), and biological N fixation (BNF) of *Acacia leiocalyx* and *Acacia disparimma* in Toohey forest after 2 years of biochar application

| Treatments | Total C (%) | Total N (%) | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | BNF (%) |
|---|---|---|---|---|---|
| Biochar rates (t ha$^{-1}$) | | | | | |
| 0 | 48.2 a | **1.99 b** | −32.0 a | −1.93 a | 51.7 a |
| 5 | 48.2 a | **2.45 a** | −32.4 a | −1.80 a | 46.3 a |
| 10 | 48.0 a | **2.42 a** | −32.6 a | −1.76 a | 45.1 a |
| Species | | | | | |
| *Acacia leiocalyx* | **47.4 b** | 2.57 a | **−32.8 b** | **−2.01 b** | **37.4 b** |
| *Acacia disparrima* | **49.0 a** | 2.31 a | **−32.1 a** | **−1.46 a** | **58.0 a** |

Lower case letters indicate significant differences among biochar rates and/or acacia species. All differences were considered significant at $P < 0.05$

biochar applied at a rate of 10 t ha$^{-1}$ led to the greatest improvement in plant growth ($0.05 < P < 0.10$). Further details are documented in the Supplementary Material. Table S3 provides the interactive effects of the biochar application rates and species on plant height, diameter at ground level (DGL), basal area (BA) and volume.

## Soil chemical properties

As the soil depth increased, soil total C was 6.81%, 4.77%, and 3.27% for 0–5, 5–10 and 10–20 cm respectively, while the corresponding soil total N was 0.250%, 0.168% and 0.106% respectively, significantly decreased as the soil depth increased ($P < 0.05$) (Table 3). Soil $\delta^{13}$C values were −27.10‰, −25.86‰ and −25.63‰ respectively for the 0–5, 5–10 and 10–20 cm soil depths (Table 3). For soil $\delta^{15}$N from 5 to 10 cm depth (1.044‰) was significantly higher than those of the other two soil depths ($P < 0.05$) (Table 3). The results of Table 3 also show that soil total C and total N were significantly higher only when the biochar application rate was 10 t/ha ($P < 0.05$) (Table 3).

After two years of biochar application, the soil moisture content of the biochar application rate of 10 t/ha was 14.3% and 13.8% higher than that under the control treatment at 0–5 cm and 5–10 cm soil depth ($0.05 < P < 0.10$) (Table 4). At the 0–5 cm soil, the results of Table 4 also showed that soil total C for the biochar application rate at 10 t/ha was significantly higher than that of the biochar application rate at 0 t/ha ($P < 0.05$). The soil total N at 5 and 10 t/ha biochar application rates (0.262% and 0.265%) were higher than that of without biochar application (0.230%) (Table 4).

**Table 2** Effects of biochar application rates and understorey acacia species on plant height (cm), diameter at ground level (DGL, cm), basal area (BA, cm$^2$) and volume (cm$^3$) of *Acacia leiocalyx* and *Acacia disparimma* after 2 years of biochar application in Toohey Forest, Australia

| Treatments | Height (cm) | DGL (cm) | BA (cm$^2$) | Volume (cm$^3$) |
|---|---|---|---|---|
| Biochar rates (t ha$^{-1}$) | | | | |
| 0 | 131.6 a | **1.31 B** | **1.43 B** | **68.02 B** |
| 5 | 130.4 a | **1.41 AB** | **1.70 AB** | **75.49 B** |
| 10 | 153.0 a | **1.67 A** | **2.29 A** | **124.42 A** |
| Species | | | | |
| *Acacia leiocalyx* | 151.4 a | 1.50 a | 1.85 a | 99.74 a |
| *Acacia disparrima* | 125.3 a | 1.43 a | 1.76 a | 78.88 a |

Lower case letters indicate significant differences among biochar rates and/or acacia species. All differences were considered significant at $P < 0.05$

**Table 3** Effects of soil depths, biochar application rates and understorey acacia species on soil total carbon (total C), total nitrogen (total N), C and N isotope composition ($\delta^{13}$C and $\delta^{15}$N) after two years of biochar application in Toohey forest, Australia

| Treatments | Total C (%) | Total N (%) | $\delta^{13}$C (‰) | $\delta^{15}$N (‰) |
|---|---|---|---|---|
| Depth (cm) | | | | |
| 0–5 | **6.81 a** | **0.250 a** | **−27.10 c** | **−0.236 b** |
| 5–10 | **4.77 b** | **0.168 b** | **−25.86 b** | **1.044 a** |
| 10–20 | **3.27 c** | **0.106 c** | **−25.63 a** | **−1.066 c** |
| Biochar rates (t ha$^{-1}$) | | | | |
| 0 | **4.62 b** | **0.165 b** | −26.19 a | −0.092 a |
| 5 | **5.01 ab** | **0.177 ab** | −26.24 a | −0.220 a |
| 10 | **5.39 a** | **0.186 a** | −26.16 a | 0.057 a |
| Species | | | | |
| *Acacia leiocalyx* | 4.98 a | 0.176 a | −26.24 a | 0.058 a |
| *Acacia disparrima* | 5.13 a | 0.177 a | −26.16 a | −0.212 a |

Lower case letters indicate significant differences among biochar rates and/or acacia species. All differences were considered significant at $P < 0.05$

**Table 4** Effects of biochar application rates and understorey acacia species on soil total carbon (total C), total nitrogen (total N), C and N isotope composition ($\delta^{13}C$ and $\delta^{15}N$), soil moisture content and pH value at different soil depths (0–5, 5–10, 10–20 cm) after two years of biochar application in Toohey forest, Australia

| Treatments | Total C (%) | Total N (%) | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | Soil Moisture Content (%) | pH |
|---|---|---|---|---|---|---|
| 0–5 cm | | | | | | |
| Biochar rates (t ha$^{-1}$) | | | | | | |
| 0 | **6.10 b** | **0.230 B** | −27.08 a | −0.205 a | **12.74 B** | 5.29 a |
| 5 | **7.20 ab** | **0.262 A** | −27.21 a | −0.424 a | **13.53 AB** | 5.27 a |
| 10 | **7.49 a** | **0.265 A** | −27.01 a | −0.093 a | **14.56 A** | 5.28 a |
| Species | | | | | | |
| *Acacia leiocalyx* | 6.75 a | 0.249 a | −27.11 a | −0.114 a | 13.80 a | 5.30 a |
| *Acacia disparrima* | 7.20 a | 0.255 a | −27.04 a | −0.306 a | 13.44 a | 5.25 a |
| 5–10 cm | | | | | | |
| Biochar rates (t ha$^{-1}$) | | | | | | |
| 0 | 4.50 a | 0.161 a | −25.85 a | 0.958 a | **12.47 B** | 5.28 a |
| 5 | 4.78 a | 0.171 a | −25.88 a | 1.068 a | **13.06 AB** | 5.25 a |
| 10 | 5.15 a | 0.177 a | −25.85 a | 1.149 a | **14.19 A** | 5.25 a |
| Species | | | | | | |
| *Acacia leiocalyx* | 4.84 a | 0.170 a | −25.95 a | 1.098 a | 13.54 a | 5.26 a |
| *Acacia disparrima* | 4.89 a | 0.171 a | −25.82 a | 1.048 a | 13.36 a | 5.25 a |
| 10–20 cm | | | | | | |
| Biochar rates (t ha$^{-1}$) | | | | | | |
| 0 | 3.25 a | 0.105 a | −25.64 a | −1.029 a | 13.65 a | 5.21 a |
| 5 | 3.04 a | 0.098 a | −25.61 a | −1.304 a | 13.26 a | 5.17 a |
| 10 | 3.54 a | 0.115 a | −25.63 a | −0.885 a | 14.30 a | 5.15 a |
| Species | | | | | | |
| *Acacia leiocalyx* | 3.33 a | 0.109 a | −25.65 a | −0.810 a | 13.82 a | 5.16 a |
| *Acacia disparrima* | 3.29 a | 0.105 a | −25.62 a | −1.378 a | 13.62 a | 5.17 a |

Lower case letters indicate significant differences among biochar rates and/or acacia species. All differences were considered significant at $P < 0.05$

## Relationship plant physiological variables

Regression analysis showed that there was a significant positive relationship between foliar $\delta^{15}N$ (‰) and plant BNF capacity, which depended on different acacia species. It has been shown from Fig. 1 that *Acacia disparrima* foliar $\delta^{15}N$ was more strongly related to plant BNF capacity ($R^2 = 0.863$, $P < 0.001$) than that of *Acacia leiocalyx* ($R^2 = 0.661$, $P < 0.001$).

## Relationships between soil physiological variables

In the Toohey Forest, it has been shown from Fig. 2, soil $\delta^{13}C$ was positively related to soil $\delta^{15}N$ at soil depths of 5–10 cm, and soil $\delta^{13}C$ was strongly related to soil $\delta^{15}N$ on biochar rate of 10 t/ha ($R^2 = 0.641$, $P < 0.001$) than those of biochar rates of 5 t/ha ($R^2 = 0.388$, $P = 0.01$) (Fig. 2). There were strong correlations between soil total N and soil $\delta^{15}N$ at soil depth of 0–5 and 5–10 cm, but the relationship

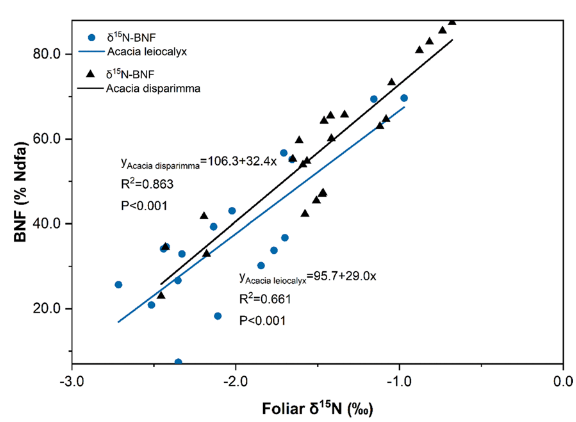


**Fig. 1** Linear relationship between foliar $\delta^{15}N$ (‰) and biological nitrogen fixation rate (% Ndfa) of *Acacia leiocalyx* and *Acacia disparrima*. This relationship represents the variation of two acacia species and shows the contribution of foliar $\delta^{15}N$ in changing the BNF rate of *Acacia leiocalyx* and *Acacia disparrima* respectively

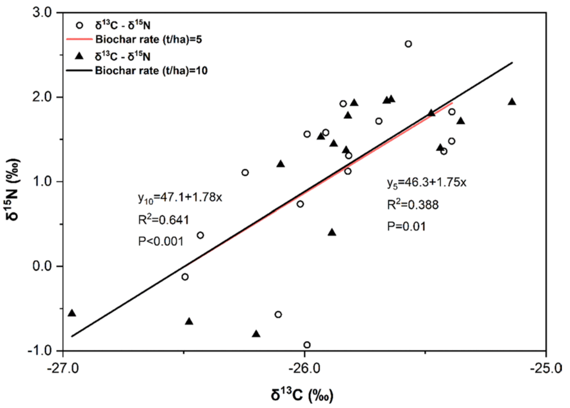


**Fig. 2** Linear relationship between soil $\delta^{13}C$ (‰) and soil $\delta^{15}N$ (‰) of two biochar rates (5 and 10 t/ha) at soil depths of 5–10 cm

depended on the biochar application rates (Fig. 3). At soil depth of 0–5 cm, there was a tight non-linear relationship between soil total N and soil $\delta^{15}N$ ($R^2=0.424$, $P<0.05$) when the biochar application rate is 5 t/ha (Fig. 3a). In the 5–10 cm soil layer, the correlations between soil total N and soil $\delta^{15}N$ became stronger and corresponding peaks also move backward as the biochar application rates increased (Fig. 3b).

In addition, a single regression analysis explained the non-linear relationship between soil moisture content and soil $\delta^{15}N$ at soil depth of 5–10 cm ($R^2=0.407$, $P<0.001$) (Fig. 4). Biochar application rate also affects the correlation between soil moisture content and soil $\delta^{15}N$. It could be seen from Fig. 5 that when the biochar application rate was 10 t/ha, the non-linear relationship between soil moisture content and soil $\delta^{15}N$ ($R^2=0.750$, $P<0.001$) was stronger than those of the biochar application rates of 0 and 5 t/ha ($R^2=0.197$, $P=0.08$; $R^2=0.377$, $P=0.046$).

## Discussions

### The effect of N deposition and biochar application on plants

The negative $\delta^{15}N$ values in the surface soil and foliage observed in this study are unusual for native forests (Tables 1, 3 and 4), as the isotope discrimination usually demonstrates a level of $^{15}N$ enrichment in soil and plant (Robinson 2001; Blumfield et al. 2006; Burton et al. 2007; Murray 2013; Reis et al. 2017). In this study, the negative soil $\delta^{15}N$ may result from background soil in the Toohey forest near a busy highway, being affected by atmospheric N deposition. Because the $\delta^{15}N$ in $NO_x$ emitted from vehicles ranges from −13‰ to −2‰ (Heaton 1990; Zong et al. 2020), this resulted in the negative soil $\delta^{15}N$ we observed in this experiment. Meanwhile, high N availability would inhibit the BNF of forest systems (Marcarelli and Wurtsbaugh 2007; Barron et al. 2009;

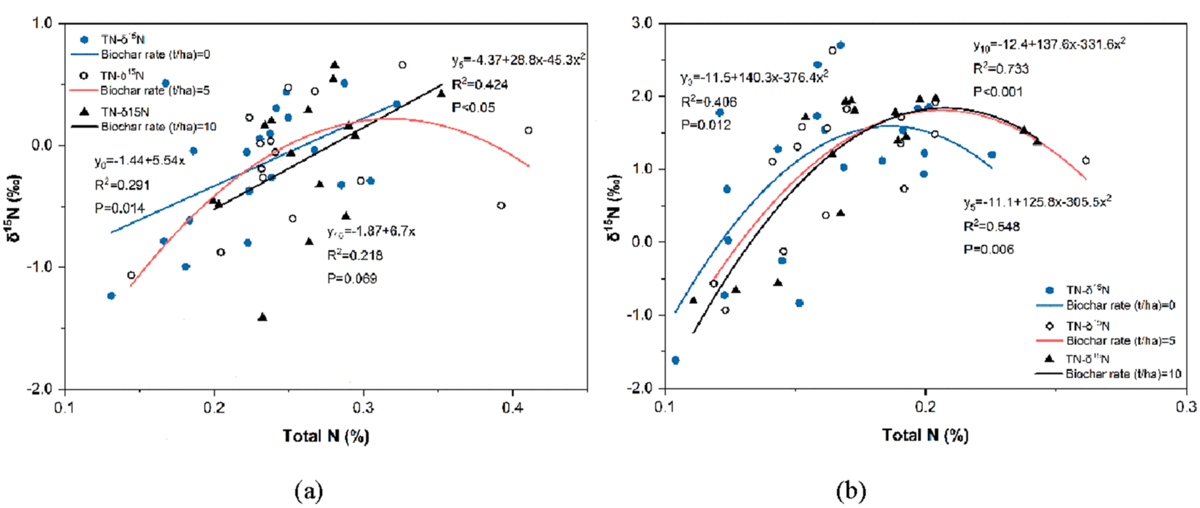


**Fig. 3** Non-linear relationship between soil total nitrogen (N) and soil $\delta^{15}N$ (‰) of three biochar rates (0, 5 and 10 t/ha) at soil depth of 0–5 cm (**a**) and 5–10 cm (**b**) respectively

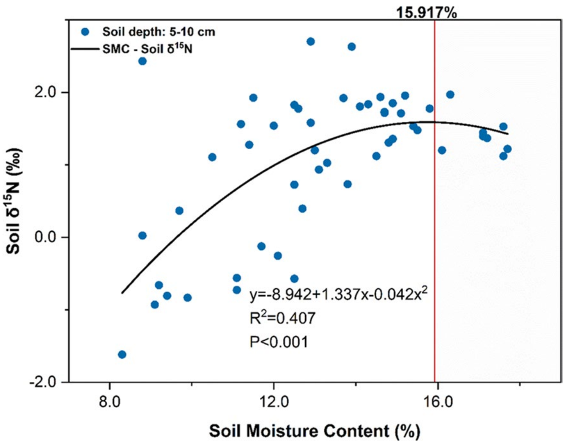


**Fig. 4** Linear relationships between soil moisture content (%) and soil δ$^{15}$N (‰) at soil depths of 5–10 cm

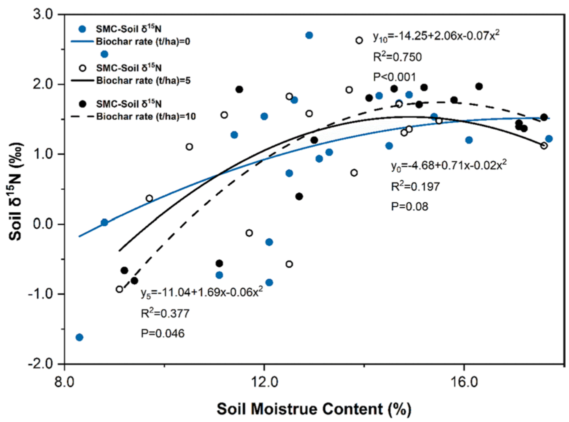


**Fig. 5** Non-linear relationship between soil moisture content (%) and soil δ$^{15}$N (‰) of three biochar rates (0, 5 and 10 t/ha) at soil depth of 5–10 cm

Avila Clasen et al. 2023). It could be seen from the results of Table 1 that for the two selected legumes, *Acacia leiocalyx* and *Acacia disparrima*, BNF of *Acacia disparrima* was significantly higher than that of *Acacia leiocalyx*. It can also be seen in Fig. 1 that the linear relationship between foliar δ$^{15}$N and BNF was also closer in *Acacia disparrima*. In the previous studies, the higher BNF in *Acacia leiocalyx* may be due to its location in less fertile soil, which provided more N for photosynthesis (Bai et al. 2012; Taresh et al. 2021). In this study, in the N deposition soil, as can be seen from Table 1, the total C value of *Acacia leiocalyx* was significantly lower than that of *Acacia disparrima*, indicating that *Acacia leiocalyx* grew faster than *Acacia disparrima*, and the rhizome system was also more developed than that of *Acacia disparrima*. At the same time, plants are more inclined to preferentially absorb N resources that are more readily available (from N deposition) and do not need to consume much energy (Gurmesa et al. 2016; Xie et al. 2021). From what has been observed in other ecosystems, increased N availability (such as N deposition) may inhibit BNF in forest systems (Compton et al. 2004; Saiz et al. 2021). Therefore, the BNF of *Acacia leiocalyx* was relatively lower in this study, which is consistent with the results obtained in other studies. Meanwhile, under high N deposition, biochar applications in this study significantly increased the total N of *Acacia* foliage (Table 1). Several studies have shown that biochar improves N dynamics in soil, thereby improving plant growth and yield and having a positive impact on plant nutrient content, which may increase plant N uptake and utilization (Rondon et al. 2007; Uzoma et al. 2011; Qian et al. 2019; Ali et al. 2020).

Meanwhile, in the previous studies, *Acacia leiocalyx* generally showed a higher δ$^{13}$C value than that of *Acacia disparrima*, suggesting higher water use efficiency. Additionally, within plant species, there is a genetic component to the distinction in the δ$^{13}$C value, which may be as great as 3‰ (Tieszen 1991; Audiard et al. 2018; Hussain et al. 2022), and the higher δ$^{13}$C value of *Acacia leiocalyx* indicated that it has a better water use strategy under drought stress (Raddad and Luukkanen 2006; Taresh et al. 2021). In contrast, in this study, *Acacia leiocalyx* showed a significantly lower δ$^{13}$C value, so it can be inferred that the forest soil is not restricted by moisture, and the water can be used freely by plants. In contrast, foliar δ$^{15}$N of *Acacia leiocalyx* with much lower BNF in this study suggests that the $^{15}$N signature of deposition N can alter plant δ$^{15}$N by direct uptake in the canopy and by altering the signature of available N in the soil. In contrast, the total inorganic N in deposition was $^{15}$N-depleted (−10‰) (Craine et al. 2015). The addition of N due to N depositional contamination resulted in the δ$^{15}$N of plants towards the $^{15}$N signature of the added N, indicating the incorporation of added N into plants. Thus, foliar δ$^{15}$N values were lower for *Acacia leiocalyx*, which is more likely to retain and take N from N deposition, than for *Acacia disparrima*. This again highlights the importance of the $^{15}$N signature of input N in controlling ecosystem $\delta^{15}$N.

## The effect of N deposition on soil C and N isotope compositions

The $\delta^{13}$C and $\delta^{15}$N in the soil can be used as an overall indicator of soil environmental processes in forest ecosystems, thereby providing comprehensive insights into the long-term changes of soil C and N cycles (Amelung et al. 2008; Brunn et al. 2014; Guillaume et al. 2015; Wang et al. 2018). In the entire ecosystem, soil $\delta^{15}$N has been used as a potential indicator of soil N status (Schulze et al. 1998; Craine et al. 2009; Ladd et al. 2010; Zhang et al. 2022). They usually accumulate as soil N availability increases, and human disturbance and climatic factors will change the N cycle process in forest soil (Pardo et al. 2002; Wang et al. 2014; Mgelwa et al. 2019).

In the previous studies, soil $\delta^{15}$N and $\delta^{13}$C values usually increase with increasing depth (Eshetu 2004; Schneider et al. 2021). In this study, soil $\delta^{13}$C was significantly enriched with the increase of soil depth, which may be because deeper soil layers have greater decomposition and humification of organic matter or mainly increase the residence time of organic C in the soil (Bird et al. 2002; Lorenz et al. 2020), which is consistent with the previous research results (Brunn et al. 2014). The value of surface soil $\delta^{13}$C was close to the $\delta^{13}$C of the applied biochar in this study, which may be because of surface-applied biochar on soil C pool (Table 3). However, in this study, with the increase of soil depth, the changing trend of soil $\delta^{15}$N value is different from the results of the previous studies. The negative $\delta^{15}$N value of the surface soil may be the result of atmospheric N deposition because this forest is close to a busy highway. The $\delta^{15}$N of NOx emitted by vehicles could lead to the negative soil $^{15}$N values observed in this study at soil depths of 0–5 and 10–20 cm (Savard et al. 2009; Su et al. 2020). However, soil $\delta^{15}$N value showed an abnormally positive value (1.044‰) at 5–10 cm soil depth, which was significantly higher than those of the other two depths. In these N-rich ecosystems, the fate of the deposited N may be different, and most of the deposited N may be directly lost in humid climates (Amundson et al. 2003; Templer et al. 2012; Rivero-Villar et al. 2021). The forest system in this study has a large amount of N losses via denitrification and N leaching, so there is a large amount of N loss at a soil depth of 5-10 cm, which is consistent with the results of previous studies (Fang et al. 2008; Liu et al. 2023). In addition, we found that soil C and N content decreased with increasing soil depth as expected.

## Responses of the relationship between soil moisture content and soil $\delta^{15}$N to biochar application

The critical role of soil moisture content in N leaching and denitrification is demonstrated from the non-linear relationship between soil $\delta^{15}$N and SMC in this study (Fig. 4), where higher SMC drives more soil N loss initially due to soil nitrification since N transformations such as nitrification, denitrification and N mineralization produce mobile, $^{15}$N-deleted N, it is easy to enrich soil $\delta$ $^{15}$N by leaching or gaseous emission of soil N (Bai et al. 2013). However, the soil N loss rate slows as the soil moisture content continues to increase. The nitrification rates decline under these conditions since oxygen is essential for nitrification (Zhu et al. 2011; Ouyang et al. 2017). Meanwhile, the heavy rainfall event before sampling significantly increased leachate volume in the soil in this study (Yang et al. 2015). With increasing biochar application rate (10 t/ha), a stronger non-linear relationship between soil $\delta^{15}$N enrichment and soil moisture content could be found in our study (Fig. 5). The reason for this is that applying biochar with a porous structure and higher surface area to the soil in this study, the ability of the soil to absorb and retain more water can be improved, thus providing more moisture to the soil-plant system (Zhang et al. 2008; Li et al. 2018). Moreover, it can also be seen in Table 2 that with the biochar application, the soil total N content was higher. The regression relationship between soil total N and $\delta^{15}$N was also closer, and the enrichment of soil $\delta^{15}$N slows down (Fig. 3). This may be because biochar addition can reduce nitrate leaching by retain more soil water and total N loss from forest soils by modifying the soil characteristics (Kanthle et al. 2016; Xu et al. 2016; Sun et al. 2018).

In Table 4, soil moisture content from the 0–5 and 5–10 cm soil layers increased with the increasing biochar application rates. This can be attributed to the fact that the soil was added with biochar, which has a highly porous structure to retain water physically (Atkinson et al. 2010; Kang et al. 2022). Meanwhile, water movement down into the deep soil is called infiltration. The application of biochar leads to the creation of more pores in the soil matrix and the formation of tortuous interstitial spaces between the soil and the biochar particles, thereby increasing the moisture penetration rate, which is consistent with previous research results (Ajayi et al. 2016; Liu et al. 2016).

### Responses of soil $\delta^{13}C$ and $\delta^{15}N$ to biochar application

Previous studies showed that biochar application increased soil cation exchange capacity and absorbed N compounds, thereby reducing $NH_4^+$ leaching (Glaser et al. 2002; Liang et al. 2006; Sun et al. 2017). The influence of biochar applications on soil N cycling is especially complex and may have a significant impact on soil N transformation processes by altering soil microbial activities and community structures (Streubel et al. 2011; Song et al. 2014). It can be seen from Fig. 3 that at soil depths of 0–5 cm and 5–10 cm, the addition of biochar was an important adjustment factor for soil N loss on the corresponding variability of biochar. Significant differences were observed in this study. With the increase in the level of biochar addition, the influence of biochar on soil $\delta^{15}N$ increased significantly (Fig. 3). The reason may be that the biochar addition can promote soil N transformation by increasing the abundance of soil ammonia-oxidizing bacteria and the activity of nitrifying bacteria, while retaining $NH_4^+$ through its acidic functional groups, thus offsetting the rapid soil N loss from biochar amended with soil N transformations (Zheng et al. 2013; Zhang et al. 2020). At the same time, as can be seen from Fig. 3b with the gradual increase in the biochar application rate, the trend of soil N loss gradually flattened, and the apex of the parabola appears later with the biochar addition of 5 t/ha. The results of the study are consistent (Cao et al. 2014). When the biochar application rate reached 10 t/ha, the observed positive correlation between the soil $\delta^{13}C$ and $\delta^{15}N$ was tighter (Fig. 2), which also illustrated that biochar application could improve the activity and structure of soil microorganisms, thereby providing new information on soil C and N turnover controlled by biochar application rates (Dijkstra et al. 2006; Werth and Kuzyakov 2010; Gerschlauer et al. 2019). These results highlight the importance of appropriate biochar application rates in soil N improvement.

Compared with the treatment without biochar application, a significant increase in the soil total C and total N content was observed under biochar application, especially for the surface soil (Tables 3 and 4), which is consistent with the results of previous studies (Qiao-Hong et al. 2014). It was shown that the biochar application improved soil characteristics. In addition to soil samples, the total N content of foliage also increased significantly under biochar application (Table 1). These results are consistent with previous studies (Ibrahim et al. 2020). Among them, the biochar application has been proven to increase N uptake in plant and soil N content (Fiorentino et al. 2019; Wang et al. 2020b). Previous studies have proposed that biochar application generates a large amount of ion exchange capacity and improves the adsorption and immobilization of N forms required for plant uptake. When these N forms are adsorbed by biochar, they can be released again and become available for plant absorption and utilization (Xue et al. 2017), thus improving N use efficiency. Applying biochar to soil is a potential way to improve the bioavailability of nutrients and reduce soil nutrient loss (Lehmann et al. 2003; Shen et al. 2016).

## Conclusions

The N deposition existing in urban forest soils had an impact on the internal N cycle of the soil-plant system, and the resulting high N availability significantly inhibited the BNF capacity of the forest system. The biochar application significantly reduced N losses due to leaching and denitrification, and significantly increased the potential of soil to retain N. This also improved N uptake by plants and N utilization efficiency. In this study, when the biochar application rate was applied at 10 t/ha, it had the best effect on improving soil N transformations while reducing soil N loss as well as improved plant growth.

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**Declarations**

**Competing interests** All authors certify that they have no relevant financial or non-financial interests to disclose.



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