

EFFECTS OF SOIL EXOGENOUS NITROGEN ON BAMBOO (*DENDROCALAMUS LATIFLORUS* MUNRO) SHOOTS, PHOTOSYNTHETIC CHARACTERISTICS, AND NITROGEN METABOLISM

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Abstract. Nitrogen (N) is an essential nutrient element that is involved in almost every aspect of a plant's physiological mechanism. Therefore, the current research aims to determine the optimal amount of N fertilizer for bamboo seedlings for better nutrient management practices to minimize N pollution in bamboo forests. We evaluated the physiological response of *Dendrocalamus latiflorus* Munro grown under five varying levels of N fertilizer; such as N0, N1, N2, N3, N4, and N5 (0, 1.50, 3.00, 4.50, 6.00, and 7.50 g·pot⁻¹, respectively). N4 treatment had a significant effect on the number of shoots, which was greatly correlated with net photosynthetic rate (P_n) and photosynthetic pigment including carotenoid (Car), total chlorophyll (Chls), and Chl a per b ratio (Chl a/b) as well as N-related indices [leaf N, leaf ammonium N (NH₄⁺-N), and nitrate reductase (NR)]. N supply significantly increased soil carbon and N contents, which could be conducive to the accumulation of leaf chlorophyll content, improving leaf photosynthesis mechanism, and accelerating N metabolism and conversion through an enzymatic reaction. Overall, the N application of 6.00 g·pot⁻¹ was advantageous to improving physiological characteristics and shoot production of seedlings, which provided a theoretical basis for improving bamboo nitrogen use efficiency and high-yield cultivation. **Keywords:** nitrogen application, bamboo seedlings, photosynthetic physiological characteristics, nitrogen-related enzyme activity

Introduction

Nitrogen (N) is an essential macronutrient element that is required by plants in higher amounts relative to other essential nutrients (Kirova et al., 2005). N is involved in almost every aspect of a plant's physiological metabolism being a most essential nutritional component (Kishorekumar et al., 2020a). At the physiological level, N may trigger both the nitrate (NO₃⁻) assimilatory mechanism and the regulation of carbon (C) metabolism that can provide C skeletons and reductants for this process (Stitt, 1999). At the developmental stage, N regulates activities such as leaf expansion (Walch-Liu et al., 2000), root branching (Forde and Lorenzo, 2002), and resource allocation between axillary bud and root growth (Scheible et al., 1997). N and other elements in the plant are responsible for the synthesis of amino acids, proteins, nucleic acids, chlorophyll, and other compounds (Talukder et al., 2016; Wen et al., 2020). Furthermore, N can also regulate the adaptation mechanism of plants to the environment and play an important role in the adaptation of plants to adversity (Krouk et al., 2010). Simultaneously, exogenous N can significantly affect the N metabolism in plants as well as a certain

degree of impact on its related enzymatic activities, thereby affecting plant growth (Xing et al., 2018).

N, being a necessary component, has the potential to change plant proteins as well as plant photosynthesis (Kishorekumar et al., 2020b; Liu et al., 2020). In general, when the N concentration increases, the leaf's photosynthetic rate (P_n) and transpiration rate (T_r) increase while the intercellular CO₂ concentration (C_i) decreases (Li et al., 2017; Mu and Chen, 2021). Furthermore, increasing the amount of N fertilizer can also increase the leaf's chlorophyll contents and alleviate the reduction in photosynthetic efficiency (Zhou et al., 2017a). Previous studies have shown that N fertilizer application can effectively increase the chlorophyll contents and net photosynthetic rate of agricultural or horticultural crops, such as rice (Kurai et al., 2011; Zhou et al., 2017b), wheat (Kataria and Guruprasad, 2015), peanut (Liu et al., 2019), etc. As a result, in the current research, we attempt to gain insight into the photosynthetic capacity of *Dendrocalamus latiflorus* Munro grown under varying N levels.

Soil is a part of the ecosystem, where the contents of essential nutrients can not only reveal the utilization of soil nutrients but also explore the regulatory and metabolic balance mechanisms of elements such as C and N in plants and soil (Kleinhenz et al., 2003). Since, both C and N are essential nutrient elements in the soil and are required for plant growth and development (Krapp and Castaings, 2012). Plants uptake the nutrients from the soil via roots, which are greatly influenced by the external environment, especially by fertilizers (Bargaz et al., 2018). Therefore, the nutritional changes (C and N) in plants can explore the nutrient distribution ratio and status of plants, as well as the internal connection between soil and plants.

N metabolism is one of the important physiological metabolic processes in plants (O'Brien et al., 2016). Nitrate reductase (NR) is the main N metabolizing enzyme of higher plants. The metabolic enzyme activity of plant leaves and roots is related to the accumulation of N in plants (Fu et al., 2020a). Glutamine synthetase (GS) is the first enzyme to be isolated, purified, and identified from plants, and it is also the first enzyme to be found to be related to the storage form of plant N (Seabra and Carvalho, 2015). GS converts the inorganic ammonia absorbed by plants into an organic form in glutamine (Gln) and glutamic acid (Glu) as N donors in the biosynthesis of N-containing organics in higher plants (Yang et al., 2016). NR and GS are all inducible enzymes that affect the metabolism and transformation of N in plants, which play an important role in the absorption and transformation of nutrient elements to affect plant growth.

Fertilization is an important measure to improve bamboo cultivation. As one of the main factors affecting bamboo growth, N has a great influence on the growth and development of bamboo. Previous research has shown that optimum N fertilization can effectively improve the photosynthetic capacity, growth, and productivity of shoots and timber in bamboo forests (Xu et al., 2014; Gao et al., 2016). At present, N fertilization in bamboo forests is relatively systematic in China, but the phenomenon of soil deterioration and environmental degradation caused by fertilization still exists (Zhu and Chen, 2002). Increased use of N fertilizers will also exacerbate the increase in nitrous oxide (N₂O) emissions from the soil (Xu et al., 2014). In order to increase the yield of bamboo forests and protect the ecological environment, it is necessary to further improve the utilization efficiency of N fertilizer in bamboo forests.

D. latiflorus Munro has become the main cultivated bamboo species for shoots in China by the features of its strong adaptability, long shoot period, and high yield of bamboo shoots. *D. latiflorus* Munro has a large leaf area and continuous rainfall in the

early stage of bamboo shoots in southern China, which would exacerbate the lack of sunlight in the forest understory. Nutrient management can also alleviate the stress caused by low light, improve the photosynthetic capacity of bamboo, and increase its productivity. Therefore, in the current study, *D. latiflorus* Munro seedlings were established under various levels of N-based fertilizer, to assess its efficacy on shoot growth. The study will provide valuable insights into optimum N requirements for the growth of *D. latiflorus* Munro seedlings. The main goals of our experiment were (i) to distinguish the effect of different levels of N on physiological and biochemical attributes of the seedlings; (ii) to identify the effect of N application on soil properties and the growth pattern in the seedlings; (iii) to determine the reasonable amount of N applied to bamboo shoots.

Material and Methods

Study sites

The greenhouse experiment was carried out in the greenhouse of the College of Forestry, Fujian Agriculture and Forestry (*Fig. 1*). The study site was located (119°13'51.18" E, 26°05'4.35" N) in Fuzhou, Fujian province, China, where the average solar radiation per year was 1246 kW·hm⁻². The maximum temperature in the greenhouse was controlled below 35°C, and the relative air humidity was kept above 85%.

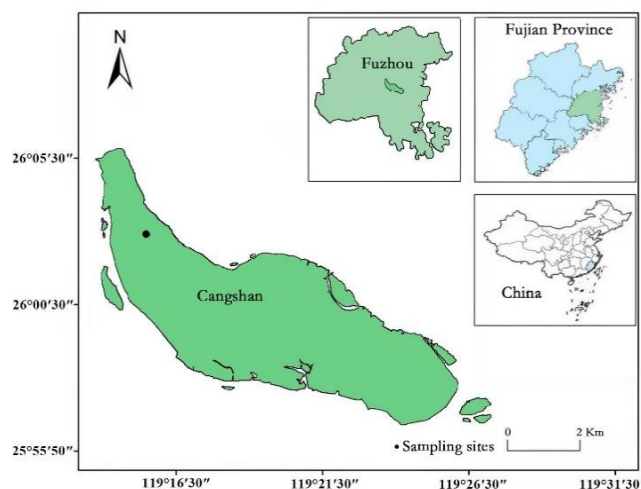


Figure 1. The geographical location of Fujian Agriculture and Forestry University

Plant material and soil

Three-year-old seedlings of *D. latiflorus* were grown in PVC pots with a diameter of 30 cm and a height of 33 cm. The potting substrate material was yellow soil and peat (volume ratio 3:1) with a weight of 15 kg per pot. The basic properties of soil were as follows: pH value, 5.77; organic C, 13.67 g·kg⁻¹; total N, 0.35 g·kg⁻¹; total phosphorus (P), 0.50 g·kg⁻¹; total potassium (K), 50.01 g·kg⁻¹. The basic growth attributes of *D. latiflorus* Munro seedlings cultured from October 2018 were as follows: mean height, 105.51 cm; mean DBH, 4.67 mm; north-south crown, 71.11 cm; east-west crown, 68.95 cm.

Experiment design

In the current research, we used five N treatment combinations; N0, N1, N2, N3, N4, and N5, like 0, 1.50, 3.00, 4.50, 6.00, and 7.50 g of N in each pot, respectively. N was applied three times such as April (30%), May (40%), and June (30%), respectively in 2019. The amount of exogenous N applied to the seedlings of *D. latiflorus* Munro has been presented in *Table 1*. All treatments were replicated 15 times. Three months later when nitrogen was completely added, leaf gas exchange parameters were measured, after that, the leaf tissues were collected for the estimation of chlorophyll content, N-related indices, and C content. Soil samples were also collected to measure N-related indices and C content. The experiment started in April and ended in October.

Table 1. The amount of exogenous N ($\text{g}\cdot\text{pot}^{-1}$) applied to *D. latiflorus* Munro seedlings

Fertilization times	Treatments ($\text{g}\cdot\text{pot}^{-1}$)					
	CK	N1	N2	N3	N4	N5
I (April)	0	0.45	0.90	1.35	1.80	2.25
II (May)	0	0.60	1.20	1.80	2.40	3.00
III (June)	0	0.45	0.90	1.35	1.80	2.25
Total amount	0	1.50	3.00	4.50	6.00	7.50

Urea (mass fraction 46%) was applied as N fertilizer, calcium superphosphate (P_2O_5 , mass fraction 12%), and potassium chloride (K_2O , mass fraction 60%) were used as phosphate and potassium fertilizer, respectively. Among them, phosphate and potassium fertilizer were applied only once, and the dosages were 6.00 and 1.50 $\text{g}\cdot\text{pot}^{-1}$, respectively. Weeding and other cultural practices were carried out regularly during the entire experiment.

Investigation of the number of bamboo shoots

After every two days, the number of shoots from each replicate was recorded from beginning to end. The bamboo shoots (semi-lignified state) were harvested after they attained a height of approximately 50 cm (*Fig. 2*). The bamboo shoots were not retained in shooting initial-phase and metaphase, and new bamboo shoots were retained at the end of shooting. The purpose of cutting bamboo shoots was to remove the apical dominance of the bamboo shoots and stimulate the germination of dormant shoots at the base of the bamboo stump.



Figure 2. Schematic diagram of bamboo shoots growth of *D. latiflorus* Munro seedlings

Determination of leaf gas exchange parameters and chlorophyll content

Li-6400 photosynthesis system (Li-Cor Inc., Lincoln, USA) was used to determine the gas exchange parameters, including P_n , C_i , T_r , stomatal conductance ($Cond$), and water use efficiency (WUE) from 2-3 mature functional leaves during consecutive sunny days. The LED (red and blue) light source was used with $1600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation (PAR). The seedlings were measured from all replicates, however, before the measurement, the sampling leaves were induced under a light intensity of $1600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR for 20-30 min.

Following the assessment of gas exchange parameters, fresh leaves were cut to determine the concentrations of the photosynthetic pigments. After the separation of the midrib, the fresh leaves were cut into pieces and weighed 0.20 g. A mixed solution (pure acetone: absolute ethanol: distilled water = 4.5: 4.5:1) of 25 ml (Gao, 2006) was used to extract the photosynthetic pigments directly in the dark for 48 h. Chlorophyll a (Chl a), b (Chl b), total chlorophylls (Chls), and carotenoid (Car) contents were calculated by optical density (OD) values according to the equations of Lichtenthaler (1987), and the ratios of chlorophyll a per b ratio (Chl a/b) and carotenoid per total chlorophyll ratio (Car/Chls) were further calculated.

Determination of leaf N indices and C content

After the extraction of leaf chlorophyll, part of fresh leaves was chopped, mixed, weighed, stored in liquid N, and then stored at -80°C refrigerator for measuring. Fresh leaves were used to estimate the activities of leaf Gs and NR, leaf ammonium N ($\text{NH}_4^+\text{-N}$), and nitrate N ($\text{NO}_3^-\text{-N}$) using the kit manufactured by Suzhou Keming Biotechnology Co., Ltd. After chopping and mixing, another part of fresh leaves was oven-dried at 105°C for 15 minutes and later at 85°C to dry to constant weight. Furthermore, the dried samples were grinded with an ultra-high-speed pulverizer (HUANGCHENG HC-300Y, China) and sieved through a 0.15 mm sieve. The total C and N contents were determined using an element analyzer (VARIO MAX, ELEMENTAR, Germany), and their ratio was further calculated.

Determination of soil C and N indices

Similarly, the fresh soil samples were taken from a distance of 10 cm and a depth of 10 cm around the bamboo stump. After removing stones and roots, each soil sample was separated into two portions: One portion was used to determine soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ using the chemical kits. The other was air-dried, grinded, and sieved to 0.15 mm to determine total C and N contents using an element analyzer (VARIO MAX, ELEMENTAR, Germany), and their ratios were also calculated.

Data analysis

All the data were expressed as means and standard errors. Analysis of variance (one-way ANOVA) was performed using SPSS 20.0 to determine the effect of N treatments, and Tukey HSD test was used to identify significant differences ($\alpha=0.05$) between mean values. The statistical data was adopted for principal component analysis (PCA) to analyze relationships among the number of shoots, leaf biochemical attributes, and soil C and N properties under different N treatments. Origin 9.5 and Prism 8.0 were used for graphical illustrations and Microsoft Excel-2016 was used for the table.

Results

Impact of N application on the number of bamboo shoots

We noticed that N application significantly influenced the number of bamboo shoots (Fig. 3). *D. latiflorus* Munro established under N4 treatment increased their shoots up to 68.75% ($P < 0.05$) compared to N0.

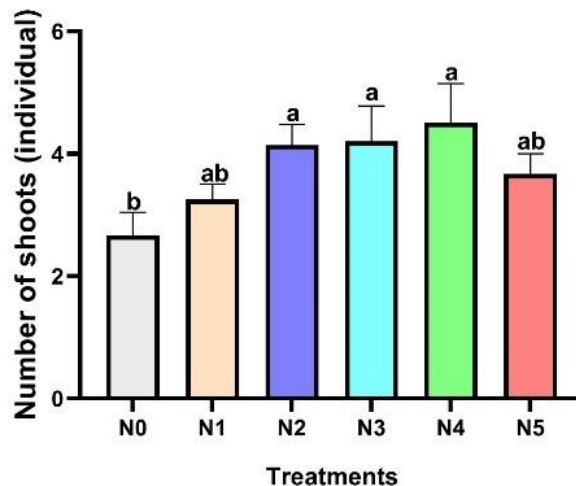


Figure 3. Impact of various N application rates on the number of bamboo seedlings. N0: 0 g·pot⁻¹, N1: 1.50 g·pot⁻¹, N2: 3.00 g·pot⁻¹, N3: 4.50 g·pot⁻¹, N4: 6.00 g·pot⁻¹, and N5: 7.50 g·pot⁻¹, respectively. Different letters indicate significant differences ($P < 0.05$) in the mean between different nitrogen treatments and \pm denotes the standard errors of the means (SE) ($n = 4$)

Leaf photosynthetic pigments concentrations under various N application rates

Under various N application rates, the leaves photosynthetic pigments (Car, Chls, and Chl a/b) concentrations increased as depicted in Figure 4. Compared to N0, under N4 and N5 treatments, significant increases of 100.00% and 109.60%, 99.08% and 110.52% ($P < 0.05$) respectively were observed for Chls and Car (Fig. 4A–B). N3 treatment exhibited the maximum Chl a/b compared to other treatments (Fig. 4C). Besides, for Car/Chls, seedlings established under the N application did not show any difference (Fig. 4D).

Leaf gas exchange parameters under various N application rates

The N application had a significant ($P < 0.05$) effect on leaf P_n (Fig. 5A). Specifically, N4 and N5 treatments resulted in a significant rise in leaf P_n relative to N0, whereas N4 was found the most effective over all other treatments combinations. In addition, the rise in $Cond$ was parallel to the N application rate (Fig. 5B) and seedlings established under N4 treatment greatly ($P < 0.05$) enhanced their leaf $Cond$ (106.57%) as compared to N0. In contrast, compared to N0, none of N based treatments influenced the C_i and T_r significantly (Fig. 5C and D). Besides, seedlings under N4 treatment exhibited a relatively greater ($P < 0.05$) WUE compared to N1 and N2 treatments (Fig. 5E).

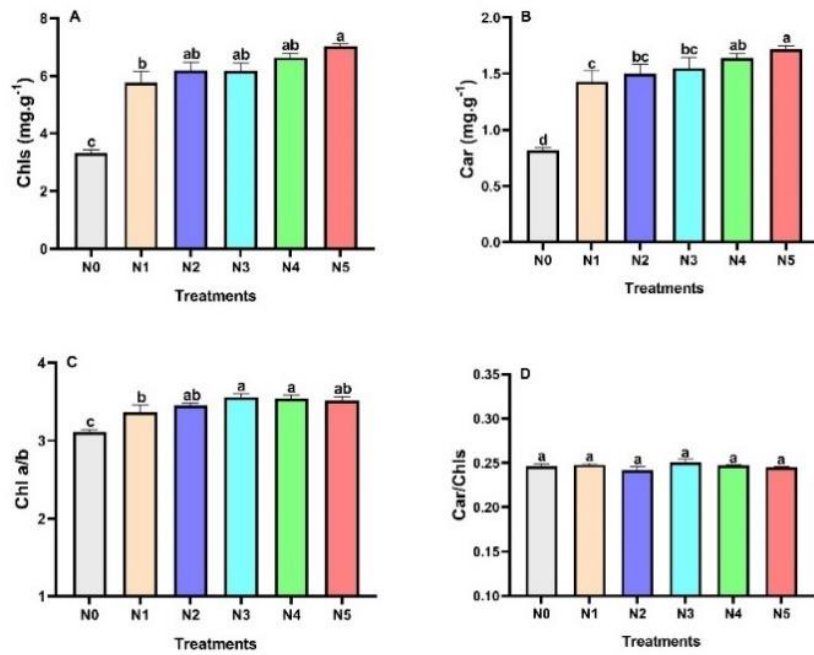


Figure 4. Leaf photosynthetic pigments under different N application rates. (A) Chls – total chlorophylls, (B) Car – carotenoids, (C) Chl a/b – chlorophyll a per b ratio, and (D) Car/Chls – carotenoid per total chlorophylls ratio, respectively. N0: 0 g·pot⁻¹, N1: 1.50 g·pot⁻¹, N2: 3.00 g·pot⁻¹, N3: 4.50 g·pot⁻¹, N4: 6.00 g·pot⁻¹, and N5: 7.50 g·pot⁻¹, respectively. Different letters indicate significant differences ($P < 0.05$) in the mean between different N treatments and \pm denotes the standard errors of the means (SE) ($n = 4$)

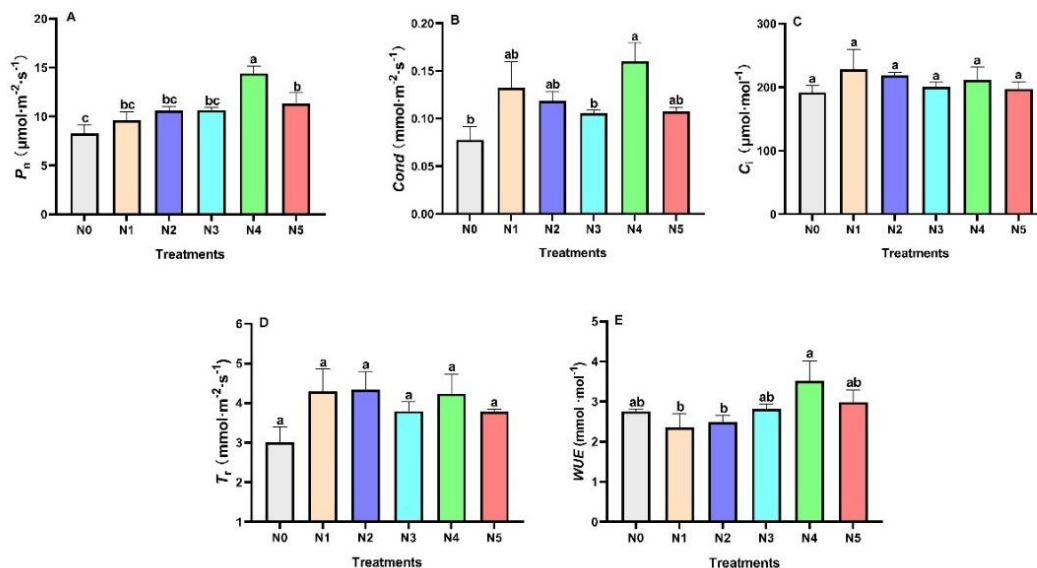


Figure 5. Leaf gas exchange parameters under different N application rates. (A) P_n – net photosynthetic rate, (B) Cond – stomatal conductance, (C) C_i – intercellular CO_2 concentration, (D) T_r – transpiration rate, and (E) WUE – Water use efficiency, respectively. N0: 0 g·pot⁻¹, N1: 1.50 g·pot⁻¹, N2: 3.00 g·pot⁻¹, N3: 4.50 g·pot⁻¹, N4: 6.00 g·pot⁻¹, and N5: 7.50 g·pot⁻¹, respectively. Different letters indicate significant differences ($P < 0.05$) in the mean between different N treatments and \pm denotes the standard errors of the means (SE) ($n = 4$)

The N and C indices of leaf and soil under various N application rates

Compared to N0, N-treated seedlings accumulated significantly ($P < 0.05$) greater soil N and C contents (Fig. 6). Overall, the seedlings treated in N4 treatment responded with maximum concentrations for leaf N and C contents (31.97 and 433.23 $\text{g}\cdot\text{kg}^{-1}$, respectively) (Fig. 6A and B). In contrast, N5 treatment for the contents of soil N and C were significantly ($P < 0.05$) higher relative to other treatments (Fig. 6D and E). Except for N5, the leaf C/N decreased under N application as the level of concentration increased (Fig. 6C). Additionally, seedlings amended with N4 treatment responded with maximum soil C/N (Fig. 6F).

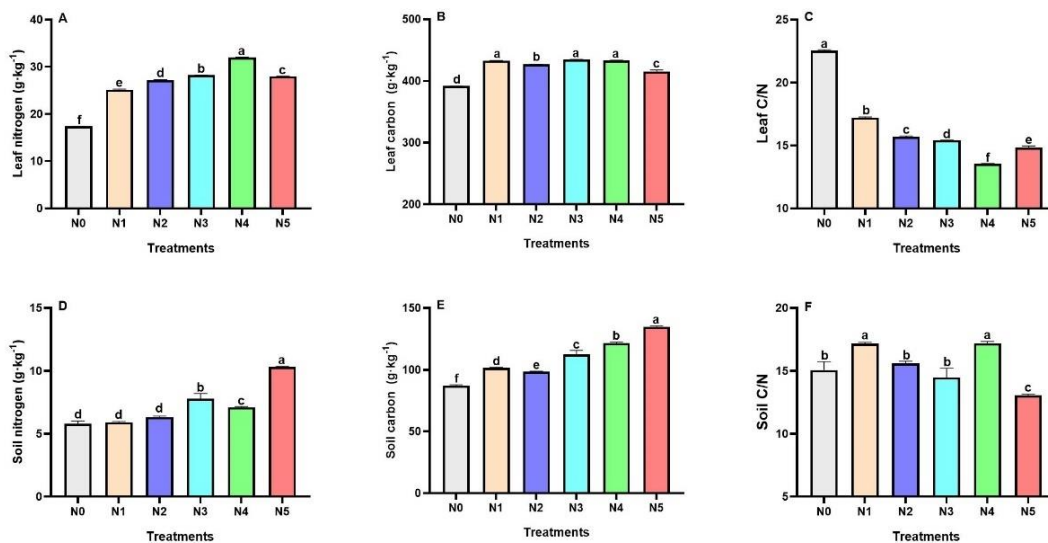


Figure 6. Total nitrogen and carbon indices of leaf and soil under different N application rates. (A) leaf nitrogen content, (B) leaf carbon content, (C) leaf C/N - leaf carbon per nitrogen ratio, (D) soil total nitrogen content, (E) soil total carbon content, and (F) soil C/N – soil carbon per nitrogen ratio, respectively. N0: 0 $\text{g}\cdot\text{pot}^{-1}$, N1: 1.50 $\text{g}\cdot\text{pot}^{-1}$, N2: 3.00 $\text{g}\cdot\text{pot}^{-1}$, N3: 4.50 $\text{g}\cdot\text{pot}^{-1}$, N4: 6.00 $\text{g}\cdot\text{pot}^{-1}$, and N5: 7.50 $\text{g}\cdot\text{pot}^{-1}$, respectively. Different letters indicate significant differences ($P < 0.05$) in the mean between different N treatments and \pm denotes the standard errors of the means (SE) ($n = 4$)

Leaf GS and NR activities under various N application rates

The activities of leaf GS and NR were influenced by N application (Fig. 7). Compared to N0, GS activities greatly ($P < 0.05$) increased by 140.55% and 121.13% under N3 and N4 treatments (Fig. 7A). However, seedlings established under the N application did not show any significant difference for NR (Fig. 7B).

Leaf and soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ under various N application rates

N application determined higher leaf $\text{NH}_4^+\text{-N}$ in seedlings compared to N0 (Fig. 8A). N1, N2, and N3 treatments showed a significant ($P < 0.05$) impact on soil $\text{NH}_4^+\text{-N}$, while N4 decreased soil $\text{NH}_4^+\text{-N}$ by 18.88% compared to N0 (Fig. 8C). In contrast, there was a decline under N application amended seedlings for leaf and soil $\text{NO}_3^-\text{-N}$ (Fig. 8B and D).

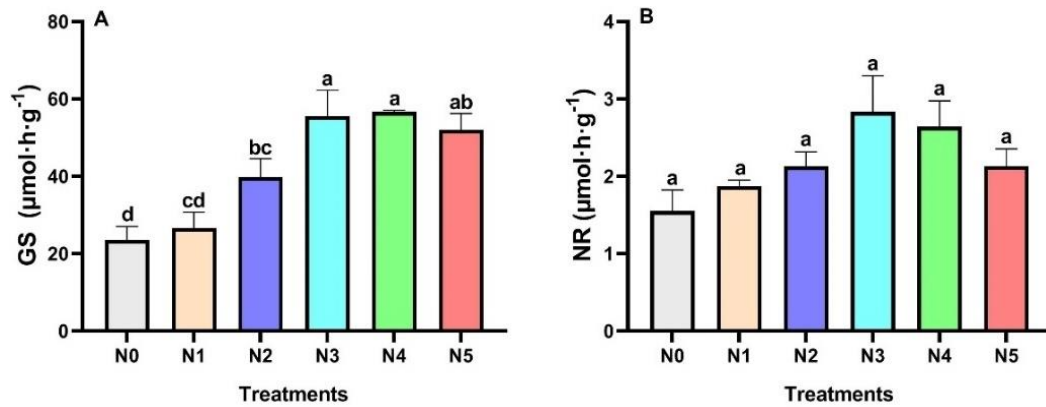


Figure 7. The activities of leaf GS and NR under different N application rates. (A) GS – glutamine synthetase and (B) NR – nitrate reductase. N0: 0 g·pot⁻¹, N1: 1.50 g·pot⁻¹, N2: 3.00 g·pot⁻¹, N3: 4.50 g·pot⁻¹, N4: 6.00 g·pot⁻¹, and N5: 7.50 g·pot⁻¹, respectively. Different letters indicate significant differences ($P < 0.05$) of mean between different N treatments and \pm denotes the standard errors of the means (SE) ($n = 4$)

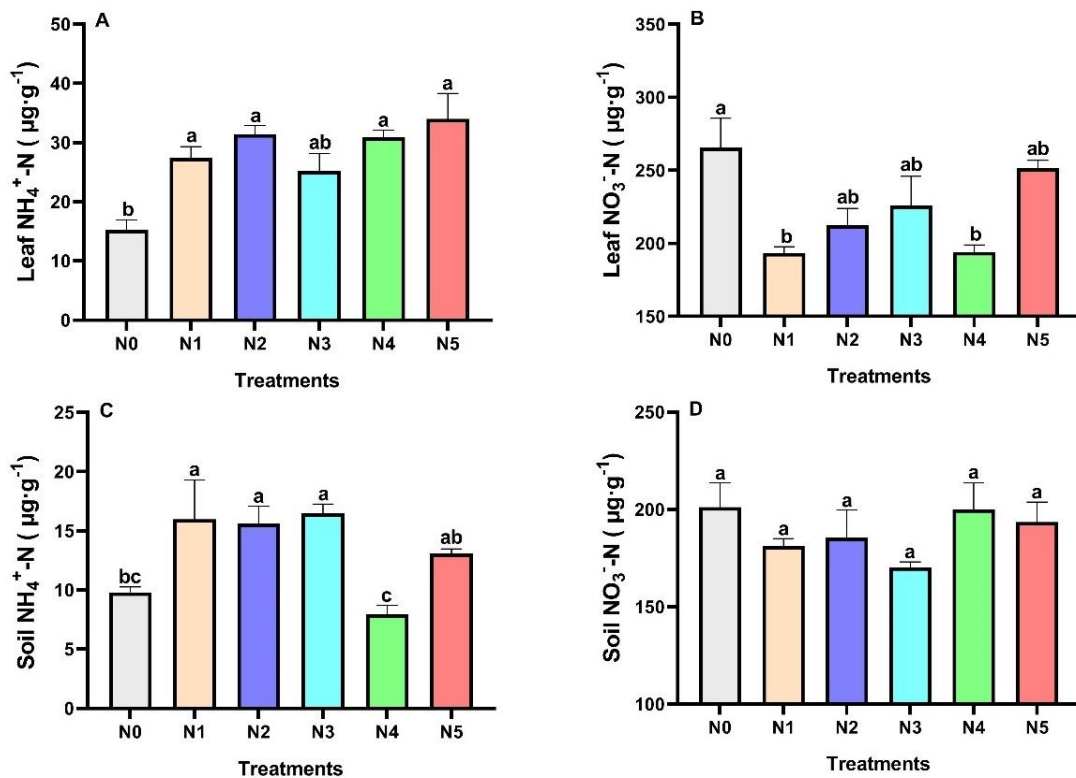


Figure 8. The contents of NH₄⁺-N and NO₃⁻-N of leaf and soil under different N application rates. (A) leaf NH₄⁺-N – leaf ammonium N, (B) leaf NO₃⁻-N – leaf nitrate N, (C) soil NH₄⁺-N – soil ammonium N, and (D) soil NO₃⁻-N – soil nitrate N, respectively. N0: 0 g·pot⁻¹, N1: 1.50 g·pot⁻¹, N2: 3.00 g·pot⁻¹, N3: 4.50 g·pot⁻¹, N4: 6.00 g·pot⁻¹, and N5: 7.50 g·pot⁻¹, respectively. Different letters indicate significant differences ($P < 0.05$) in the mean between different N treatments and \pm denotes the standard errors of the means (SE) ($n = 4$)

Evaluation of all indices under different N treatments by principal component analysis (PCA)

PCA revealed that the cumulative variance contribution rate of the first two principal components reached 74.47%, suggesting an overall variation of the data (Fig. 9). PC1 showed that the number of shoots had a highly positive correlation with photosynthetic pigments (Car, Chls, and Chl a/b), gas exchange parameters (P_n , Cond, T_r , and WUE), and C and N metabolism related indices (GS, NR, LC, LN, $L-NH_4^+$ and $S-NH_4^+$). Except for LC/N, $L-NO_3^-$, and $S-NO_3^-$, all indices exhibited with PC1 were favorable following N4 treatment, whereas N0 was correlated with LC/N, $S-NO_3^-$, and $S-NO_3^-$.

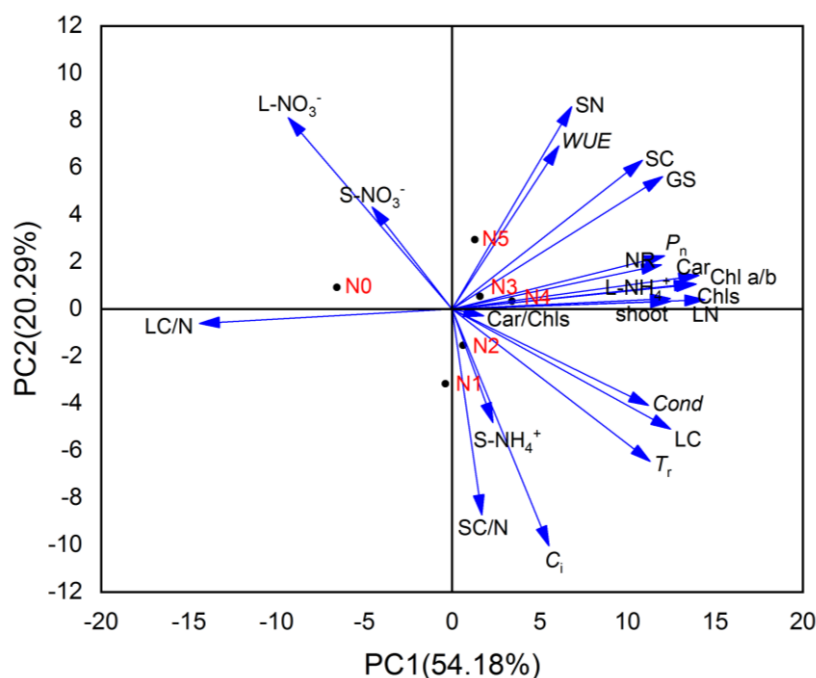


Figure 9. Biplot of principal component analysis of the first two principal components of all tested parameters and N levels. Shoot – the number of shoots, Chls – total chlorophylls, Car – carotenoid, Chl a/b – chlorophyll a per b ratio, Car/Chls – carotenoid per total chlorophyll ratio, P_n – net photosynthetic rate, Cond – stomatal conductance, C_i – intercellular CO_2 concentration, T_r – transpiration rate, WUE – water use efficiency, GS – glutamine synthetase, NR – nitrate reductase, LN – leaf nitrogen, LC – leaf carbon, LC/N – leaf C per N ratio, SN – soil nitrogen, SC – soil carbon, SC/N – soil C per N ratio, $L-NH_4^+$ – leaf ammonium N, $L-NO_3^-$ – leaf nitrate N, $S-NH_4^+$ – soil ammonium N, and $S-NO_3^-$ – soil nitrate N, respectively. N0: 0 $g \cdot pot^{-1}$, N1: 1.50 $g \cdot pot^{-1}$, N2: 3.00 $g \cdot pot^{-1}$, N3: 4.50 $g \cdot pot^{-1}$, N4: 6.00 $g \cdot pot^{-1}$, and N5: 7.50 $g \cdot pot^{-1}$, respectively

Discussion

In the current study, we noticed that different N levels impacted the number of shoots, leaf photosynthetic characteristics, and soil and leaf N-related indices in *D. latiflorus* Munro seedlings. The optimal N application can substantially increase leaf N and photosynthetic pigments, as well as improve plant photosynthetic efficiency and leaf physiological activities (Peng et al., 2021). The increase in soil C and N contents is conducive to the accumulation of leaf chlorophyll content, improving leaf photosynthetic

capacity, and accelerating C and N metabolism and conversion (Manna et al., 2005; Zhang et al., 2019) ultimately increasing the number of shoots, which was also confirmed by our research.

The formation of bamboo shoots is inseparable from the intensity of photosynthesis, N utilization, and transportation (Dordas and Sioulas, 2008). Increasing the application of N fertilizer to some extent may increase the chlorophyll contents, prolong the function period of the leaves, and improve the photosynthetic efficiency, thereby increasing the production capacity of the plant (Liu et al., 2019; Fu et al., 2020b). The current study concluded that N application enhanced the chlorophyll accumulation and improved the photosynthetic efficiency in leaves. For instance, N4 treatment had a positive effect on the accumulation of leaf chlorophylls and P_n in leaves, indicating that optimal N supply can effectively increase leaf chlorophyll contents, which was conducive to improving the photosynthesis mechanism of *D. latiflorus* Munro seedlings.

In plants, nutrient elements play a vital role in maintaining leaf C balance and sustaining photosynthetic efficiency (Matthews et al., 2017). In the current research, N application significantly increased P_n to fix relatively large amounts of C for photosynthesis, which is closely related to N assimilation and metabolic processes (Huang et al., 2013; Zhang et al., 2017). We reported that N4 was advantageous compared to other N treatments in terms of increasing P_n to enhance the C sequestration ability of *D. latiflorus* Munro. Higher *Cond* leads to enhancing leaf photosynthetic biochemical pathways and the accumulation of photosynthetic products (Lichtenthaler et al., 2007a,b). Compared with N0, the values of *Cond*, C_i , T_r , and *WUE* increased to a certain degree of N treatments, which can improve photosynthetic performance in the growth of the seedlings.

N application can effectively increase the contents and reserves of soil organic C and total N content (Huang et al., 2013). The C/N in the soil can reflect the utilization efficiency of the soil, and the application of fertilizer can affect the nutrient element in the soil (Vitousek, 1982). In our research, N treatments significantly improved N availability and increased soil N contents. Similarly, N provided a carbon source and improved the environment in the soil, which promoted the conversion of organic C and increased the organic C content (Cheng et al., 2020), where all N treatments significantly increased soil C content compared to N0. Additionally, previous studies have shown that there is a positive correlation between soil C and N content and crop yield (Wang et al., 2019; Zhang et al., 2019). Our research found that the number of shoots, SC, and SN all had positive correlations. In addition, some studies have shown that the application of N fertilizer significantly increases the N accumulation in leaves, thereby reducing the C/N of the leaves (Liu et al., 2014; Wu et al., 2019), which is consistent with our research. These changes would be conducive to increasing productivity, as well as changing the degradation and mineralization of leaves, which in turn would be conducive to the return of C and N to the soil (Stubbs et al., 2009; Pierik et al., 2011), to ensure the supply essential nutrient to meet the needs of bamboo shoot production.

NR and GS are the key enzymes for N assimilation and ammonia assimilation in plant physiological processes (Xie et al., 2014; Lin et al., 2017). Zhu et al. (2016) found that fertilization can effectively promote the N metabolism of *Phyllostachys edulis*, and enhance the activities of both NR and GS, which is related to the direct promotion of fertilizers and the absorption of N by plants. Our research came to the same conclusion that the activities of NR and GS were enhanced with the application of N fertilization, which can improve the transformation of N metabolism directly. Furthermore, N

application would encourage plants to absorb more N to synthesize more NR and GS indirectly.

Compared to N0, the soil and leaf $\text{NH}_4^+\text{-N}$ was at a significant level under N supply. Soil $\text{NH}_4^+\text{-N}$ was greatly reduced under N4 treatment in comparison to soil $\text{NO}_3^-\text{-N}$, indicating that roots of seedlings had higher absorption of $\text{NH}_4^+\text{-N}$. The enhanced NR activity under N application could promote the reduction of NO_3^- to more NH_4^+ (Maeda et al., 2014), which possibly promoted to accumulation of $\text{NH}_4^+\text{-N}$ of leaves in our research. Additionally, compared with other N treatments, the enhanced activity of GS under N4 treatment could be conducive to catalyzing inorganic $\text{NH}_4^+\text{-N}$ to produce more organic N, which could provide sufficient N not only for bamboo shoot production but also to synthesize more chlorophyll to ensure the photosynthesis of seedlings (Cruz et al., 1993). However, leaf and soil $\text{NO}_3^-\text{-N}$ had an insignificant negative correlation with bamboo shoots, which had an adverse effect on shoots. Our research was consistent with the previous findings that $\text{NH}_4^+\text{-N}$ can promote leaf chlorophyll synthesis and increase plant production (Sanchez-Zabala et al., 2015; Heuermann et al., 2021).

The PCA analyzed with strong correlations may be screened out and can be used to assess *D. latiflorus* Munro adaptation to varying N levels. According to the PCA results, N4 treatment seems to be advantageous for shoots growth, owing to increased leaf photosynthetic characteristics and soil nutrient availability. In addition, the increased number of bamboo shoots under N4 treatment exhibited strong correlations with chlorophylls pigments and leaf N-related indices, which may regulate the photosynthesis mechanism and promote N accumulation in *D. latiflorus* Munro to fulfill the requirements of a greater number of bamboo shoots.

Conclusions

Exogenous N has varying degrees of influence on the photosynthetic characteristics, nutritional element contents, and N metabolism-related indices of *D. latiflorus* Munro seedlings. We concluded that applying N fertilizer ($6 \text{ g} \cdot \text{pot}^{-1}$) to maximize the growth and shoot production of *D. latiflorus* Munro seedlings was advantageous. We elucidated the N absorption mechanism of the bamboo seedlings as well as the related physiological change mechanism after the application of N fertilizer. These findings not only provide a theoretical basis for improving the N utilization efficiency of *D. latiflorus* Munro but also the production of high-yield and high-quality bamboo shoots in the future.

Competing interests. The authors declare that they have no competing interests.

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