Nitrogen Addition Alleviates Cadmium Toxicity in Eleocarpus glabripetalus Seedlings

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Abstract: Cadmium (Cd) accumulation in soil is a serious form of heavy metal pollution affecting environmental safety and human health. In order to clarify the tolerance mechanisms to Cd-contaminated soils under N deposition, changes in plant growth, root architecture and physiological characteristics of Eleocarpus glabripetalus seedlings under combined nitrogen (N) and cadmium (Cd) treatments were determined in this study. The results indicated that Cd-induced negative effects inhibited the growth of E. glabripetalus seedlings through increased underground biomass allocation, and affected transpiration and respiratory processes, resulting in a decreased soluble sugars concentration in leaves and non-structural carbohydrates (NSC) in the roots. Root systems might play a major role in Cd absorption. Cd stress restricted the growth of fine roots (<0.5 mm), and affected the uptake of N and P. N addition alleviated the Cd-induced negative effect on plant growth through improving the root system, increasing starch and NSC contents in the roots and increasing total biomass. These findings have important implications for understanding the underlying tolerance mechanisms of Cd pollution under N deposition in arbor species.

Keywords: cadmium; nitrogen deposition; plant growth; physiological characteristics; Eleocarpus glabripetalus

1. Introduction

The rapid development of industrialization and urbanization has led to increasingly serious soil heavy metal contamination. The accumulation of heavy metals could affect plant growth and even cause ecological imbalance and environmental deterioration [1]. Cadmium (Cd) is a highly toxic heavy metal pollutant in agricultural soil [2] that could be accumulated in plant organs after being absorbed by plant roots, causing serious damage to plant growth [3,4]. Cd ions could affect the uptake, transportation and subsequent distribution of nutrient elements in plants [5]. When Cd²⁺ is excessively accumulated by plants, it can affect the physiology and biochemical activities of plants, such as photosynthesis, respiration, transpiration, and even the expression of related genes [6–8].

Global atmospheric nitrogen (N) deposition is steadily increasing with the increased burning of fossil fuels and the use of man-made fertilizers, and it has considerable effects on terrestrial ecosystems [9]. Moderate N deposition can contribute to an increase in plant biomass [10,11]. However, excessive N input can inhibit plant photosynthesis [12], and cause changes in soil physicochemical properties, resulting in decreased root growth and affecting plant root morphology [13]. As is widely known, nitrogen is essential for plant growth and development [14,15]. Nitrogen also plays a crucial role in plant stress resistance [15,16], and its supply may influence the plant’s ability to cope with abiotic stress, e.g., heavy metal pollution [17,18]. Adequate N application could enhance plant
adaptability and resilience under various environmental conditions through promoting the synthesis of amino acids, enzymes and hormones [16,19,20].

Some research has revealed that N can effectively decrease the Cd toxicity for plants [21–23]. This alleviation effect of N addition on Cd toxicity differs among plant species and according to concentrations of Cd and N. It has been reported that appropriate N addition could promote root development and enhance Cd accumulation in plants [24]. High-concentration N supplementation could enhance plants’ growth, as well as the antioxidant enzymes’ activities, which may partially alleviate AOS accumulation induced by Cd stress [25,26]. However, the exact mechanism of how additional N alleviates cadmium-induced stress responses remains unknown. Additionally, many of the previous studies on Cd-stress in plants focused on vegetables [1,27–29] and crops [30,31], and only a few on woody plants, which are more susceptible to soil contamination because of their slow growth and the long generation time.

Eleocarpus glabripetalus is a dominant evergreen tree species in the subtropical forest of China. According to our previous studies, E. glabripetalus seedlings showed a strong plasticity to Cd stress and acid rain [32]. However, this could not fully explain the adaption of E. glabripetalus to soil Cd pollution. Therefore, in this study we aimed to understand the effects of Cd pollution on the morphology and physiology of E. glabripetalus under N deposition. We hypothesized that (1) N addition could influence eco-physiological traits under Cd stress, and (2) N addition could alleviate cadmium-induced stress responses.

2. Materials and Methods

2.1. Experiment Design

The experiment was carried out in a greenhouse of the Botanical Garden of Zhejiang A&F University (119°44’ E, 30°16’ N), East China. One-year-old healthy E. glabripetalus seedlings with a height of 60 cm and a ground diameter of about 0.9–1.0 cm were planted in plastic pots with 30 cm inner diameter and 40 cm depth. There was one seedling in each pot, with a PVC plate under the pot. E. glabripetalus seedlings were treated with Cd treatments (no Cd and 100 mg·kg\(^{-1}\) Cd), N additions (no N addition and 90 kg N·ha\(^{-1}\)·yr\(^{-1}\) N addition), and a combined treatment of Cd and N. In each treatment, 12 seedlings were used as replicates to minimize sampling errors.

According to the reported average soil Cd concentration [33,34], Cd concentrations of 100 mg·kg\(^{-1}\) were selected. The Cd\(^{2+}\) solutions were prepared using CdCl\(_2\)·5H\(_2\)O (Chemical Co. Ltd., Shanghai, China) [32] and were sprayed evenly on the air-dried, reddish-brown forest soil and mixed well until the soil Cd concentration reached 100 mg kg\(^{-1}\). Then, the soil was potted, with 10 kg per pot. The same volume of soil with no Cd was also potted as the control. Then, E. glabripetalus seedlings were planted in pots in January 2019. After two months of growth, simulated nitrogen deposition treatment was carried out. According to the atmospheric N deposition of 59.1–70.5 kg N·ha\(^{-1}\)·yr\(^{-1}\) in eastern China [35], the average total N deposition of 80 kg N·ha\(^{-1}\)·yr\(^{-1}\) [36], and the projected local N deposition value by 2050 [37], we applied 90 kg N·ha\(^{-1}\)·yr\(^{-1}\) as N addition treatments in this study. Average weekly N deposition rate and N input amounts for each pot were calculated. A dissolved ammonium nitrate (NH\(_4\)NO\(_3\)) solution was sprayed evenly onto leaves and soil twice a week during the experimental period. The N treatments lasted 180 days, from April to October.

2.2. Measurements of Gas Exchange Parameters

Three individuals from each treatment were chosen randomly to measure leaf gas exchange from 8:00 a.m. to 11:30 a.m. after 180 days of treatments. The 3rd to 5th leaves with the same orientation were selected for gas exchange parameter measurements using LI-6400 system (Li-Cor Inc., Lincoln, NE, USA). The conditions were 25 °C leaf temperature, 50% relative air humidity, 400 ± 5 µmol·mol\(^{-1}\) CO\(_2\) concentration and 1000 µmol m\(^{-2}\)·s\(^{-1}\) light intensity. The net photosynthetic rate (\(P_n\)), transpiration rate (\(T_r\)), stomatal conductance
(gₚ) and intercellular CO₂ concentration (Cᵢ) of plants were recorded. Then, the intrinsic water use efficiency (WUE) was calculated with the following formula:

\[ \text{WUE} = \frac{P_{n}}{T_{r}} \]

2.3. Measurements of Root Morphology and Biomass

At the end of treatments, four individuals of each treatment were harvested and separated into roots, leaves and stems after washing with deionized water. The root system was imaged using a scanner (LA2400 Scanner, Seiko Epson Corp., Nagano, Japan). A WinRHIZO System (Version 2012b, Regent Instruments Inc., Québec, QC, Canada) was used to analyze the total root length, average diameter, root diameter classes, root surface area, and root volume. Then, the samples were oven-dried to reach a constant dry weight. Total biomass (TB) was the sum of root biomass (RB), stem biomass (SB) and leaf biomass (LB). Biomass distributions were calculated with the following formula:

\[ \text{Root to shoot ratio} (\text{RSR}) = \frac{\text{root biomass}}{\text{stem biomass} + \text{leaf biomass}} \]

Then, dried leaves, stems and roots were ground separately and sieved through a 100-mesh sieve for determining the contents of N, phosphorus (P), Cd, starch and soluble sugar contents.

2.4. Measurements of N, P and Cd Contents, and Non-Structural Carbohydrate (NSC) Contents

The semi-micro-Kjeldahl acid-digestion method was used to determine the N content in leaves, stems and roots using an Alpkem Auto Analyzer (Kjektec System 2300 Distilling Unit, Tecator AB, Hoganas, Sweden). H₂SO₄-H₂O₂ decoction and molybdenum-antimony colorimetry was used to determined P content. ICP-MS was used to determine the Cd contents.

The total NSCs contents were considered as the sum of soluble sugars and starch. Soluble sugars were extracted from dried leaves, stems, and roots in 80% (v/v) ethanol. Then, the extraction was centrifuged at 5000×g for 20 min. Then, 80% (v/v) ethanol was added to the pellet, and centrifugated for 5 min at 5000×g. The soluble sugars content in the supernatant was measured colorimetrically at 620 nm using the phenol-sulfuric method. Then, starch content was determined from the remaining pellet after the soluble sugars were extracted. After incubation in sodium acetate and amyloglucosidase solution, starch content was measured colorimetrically at 650 nm using the phenol-sulfuric method, as described by Newell et al. [38].

2.5. Data Analysis

Statistical analyses were performed using SPSS v22.0 software (SPSS, Inc., Chicago, IL, USA). The normality of the data were tested using Levene’s test before analysis, and, if necessary, natural log transformations were performed. One-way analysis of variance (ANOVA) was used to analyze the significant difference between the N treatments or Cd treatments. Two-way ANOVA was used to analyze the interactions of Cd and N treatments. Statistically significant difference was set at a 95% confidence level. All data were expressed as means ± standard error (SE, \( n \geq 3 \)). Correlation analyses between two variables were tested with Spearman’s rho correlation coefficients using Origin 2020 (Origin 2020, Origin Lab, Northampton, MA, USA). Graphs were prepared in Origin 2020.

3. Results

3.1. Gas Exchange

The \( P_{n} \), \( g_{s} \) and \( T_{r} \) of *E. glabripetalus* seedlings decreased significantly under Cd treatment and N treatment compared with the control group (\( p < 0.05 \)) (Figure 1). However, the combined N + Cd treatment increased the \( P_{n} \), \( g_{s} \) and WUE of *E. glabripetalus* seedlings significantly compared with the Cd-treated group (\( p < 0.05 \)). A significant interactive effect between Cd treatment and N treatment on \( P_{n} \), \( g_{s} \), and \( T_{r} \) was detected (\( p < 0.05 \)).
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**Figure 1.** Effects of Cd and N addition on leaf net photosynthesis rate (\( P_n \)) (A), stomatal conductance (\( g_s \)) (B), intercellular CO\(_2\) concentration (\( C_i \)) (C), transpiration (\( T_r \)) (D) and water use efficiency (WUE) (E) in \( E. glabripetalus \) seedlings. Different letters indicate significant differences between groups (mean ± SE, \( n = 3 \)) (\( p < 0.05 \)).

\( \text{PCd} \), Cd effect; \( \text{PN} \), N effect; \( \text{PN} \times \text{Cd} \), the interactive effect of N and Cd.

**3.2. Biomass**

The total biomass and leaf and stem biomass in the Cd-treated group were significantly decreased compared with control plants (\( p < 0.05 \)) (Figure 2). N addition significantly increased the leaf biomass, while the combined N and Cd treatment significantly increased the leaf biomass, root biomass and total biomass, compared to the plants under single cadmium treatment (\( p < 0.05 \)). Compared with control plants, RSR was significantly increased under the Cd treatment and combined N + Cd treatment. The Cd and N treatment had a significant interactive effect on root biomass (\( p < 0.05 \)).
Figure 2. Effects of Cd and N addition on leaf biomass (A), stem biomass (B), root biomass (C), total biomass (D) and root to shoot ratio (E) in *E. glabripetalus*. Different letters indicate significant differences between groups (mean ± SE, n = 4) (*p* < 0.05). *P*<sub>Cd</sub>, Cd effect; *P*<sub>N</sub>, N effect; *P*<sub>N × Cd</sub>, the interactive effect of N and Cd.

3.3. Root Architecture

Cd treatment, N addition and their combination significantly altered the root phenotypic traits (Table 1). Cd treatment significantly increased the root average diameter, and the proportion of the length of fine roots with 0.5–1 mm- and 1–2 mm-diameter classes to total root length, but decreased the proportion of the length of <0.5 mm-diameter fine roots to the total root length. N treatment increased the total root length and total root surface area significantly compared with control groups (*p* < 0.05). Compared to the Cd- treated seedlings, the combined N + Cd treatment increased the total root length, total root surface area, total root volume and the proportion of fine roots (<0.5 mm in diameter) significantly (*p* < 0.05), but decreased the root mean diameter and length of roots with 1–2 mm diameter.
Table 1. Effects of Cd and N addition on root morphology of *E. glabripetalus*.

<table>
<thead>
<tr>
<th>N (kg N ha$^{-1}$ yr$^{-1}$)</th>
<th>Cd (mg kg$^{-1}$)</th>
<th>Total Root Length (cm)</th>
<th>Total Root Surface Area (cm$^2$)</th>
<th>Average Root Diameter (mm)</th>
<th>Total Root Volume (cm$^3$)</th>
<th>The Proportion of the Length of Fine Roots with Different Diameter Classes to Total Root Length (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0–0.5 mm</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>6236.20 ± 626.70 b</td>
<td>1080.60 ± 34.40 b</td>
<td>0.52 ± 0.02 b</td>
<td>66.88 ± 1.00 ab</td>
<td>24.97 ± 0.04 bc</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>6445.24 ± 1815.82 b</td>
<td>1342.86 ± 389.49 b</td>
<td>0.64 ± 0.01 a</td>
<td>56.53 ± 0.16 c</td>
<td>31.40 ± 0.26 a</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
<td>15991.44 ± 1747.08 a</td>
<td>2318.75 ± 250.20 a</td>
<td>0.50 ± 0.02 b</td>
<td>72.09 ± 3.01 a</td>
<td>21.88 ± 2.16 c</td>
</tr>
<tr>
<td>90</td>
<td>100</td>
<td>14579.63 ± 3224.05 a</td>
<td>2602.361 ± 549.36 a</td>
<td>0.55 ± 0.01 b</td>
<td>64.37 ± 1.32 b</td>
<td>27.86 ± 0.59 ab</td>
</tr>
</tbody>
</table>

$P_{Cd}$, Cd effect; $P_N$, N effect; $P_N \times Cd$, the interactive effect of N and Cd. NS, no significance; * $p < 0.05$ and ** $p \leq 0.01$.

Mean values ± SE (n = 4) are shown. Different letters indicate significant differences between groups ($p < 0.05$).

3.4. N, P and Cd Contents

N treatment and the combined N + Cd treatment significantly increased the leaf N content and N/P ratio (Figure 3). The phosphorus content in stems and leaves of *E. glabripetalus* seedlings grown under Cd treatment decreased compared with the control group, while the nitrogen–phosphorus ratio of stems and leaves was significantly increased. The highest Cd content was found in the roots of the Cd treatment group (Table 2). Compared to Cd treatment, Cd content in the above-ground parts was significantly increased under the combined N + Cd treatment, indicating combined N + Cd treatment had a significant impact on Cd accumulation in above-ground parts ($p < 0.05$).

Table 2. Effects of Cd and N addition on Cd contents in the root, stem and leaves of *E. glabripetalus* seedlings.

<table>
<thead>
<tr>
<th>N (kg N ha$^{-1}$ yr$^{-1}$)</th>
<th>Cd (mg kg$^{-1}$ Dry Soil)</th>
<th>Root Cd (mg kg$^{-1}$)</th>
<th>Stem Cd (mg kg$^{-1}$)</th>
<th>Leaf Cd (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.95 ± 0.35 c</td>
<td>1.78 ± 0.19 c</td>
<td>0.18 ± 0.05 c</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>1701.01 ± 53.06 b</td>
<td>12.2 ± 0.64 b</td>
<td>2.08 ± 1.13 b</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
<td>2.85 ± 0.02 c</td>
<td>2.78 ± 0.71 c</td>
<td>0.48 ± 0.11 bc</td>
</tr>
<tr>
<td>90</td>
<td>100</td>
<td>2451.07 ± 392.77 a</td>
<td>15.62 ± 0.93 a</td>
<td>5.43 ± 0.14 a</td>
</tr>
</tbody>
</table>

$P_{Cd}$, Cd effect; $P_N$, N effect; $P_N \times Cd$, the interactive effect of N and Cd. NS, no significance; * $p < 0.05$ and ** $p \leq 0.01$.

Mean values ± SE (n = 3) are shown. Different letters indicate significant differences between groups ($p < 0.05$).
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Table 1. Effects of Cd and N addition on root morphology of E. glabripetalus. Different letters indicate significant differences between groups (mean ± SE, n = 3) (p < 0.05). PCd, Cd effect; PN, N effect; PN × Cd, the interactive effect of N and Cd.

3.5. Non-Structural Carbohydrate Content

Cd and N treatment decreased the soluble sugar contents in the roots and leaves of E. glabripetalus (p < 0.05) (Figure 4). However, soluble sugars in the stems remained relatively stable compared with control plants. Cd treatment decreased the stem starch content but increased the leaf starch content. Combined N and Cd treatments increased the root starch content but decreased the leaf starch content compared to Cd treatment. The NSCs contents in root and stem decreased significantly under Cd treatment (p < 0.05). N treatment increased NSCs contents in the root under Cd treatment. The soluble sugar/starch ratio in leaves was significantly decreased, by 30%, under Cd treatment compared with the control group. Cd and N treatment had a significant interactive effect on soluble sugars and the non-structural carbon of leaves, as well as the non-structural carbon of roots (p < 0.05).

3.6. Correlation between the Indicators in E. glabripetalus Seedlings

The leaf biomass and stem biomass were negatively correlated with leaf non-structural carbohydrate, leaf starch and root mean diameter, and positively correlated with N and P contents in plants. Root biomass was negatively correlated with root soluble sugar/starch ratio. Root length and root surface area were negatively correlated with leaf non-structural carbohydrate and root soluble sugar/starch ratio, and positively correlated with N contents in plants. PN was positively correlated with non-structural carbohydrate in plants. Cd

Figure 3. Effects of Cd and N addition on N contents (A), P contents (B), and N/P ratio (C) in roots, stem and leaves of E. glabripetalus. Different letters indicate significant differences between groups (mean ± SE, n = 3) (p < 0.05). PCd, Cd effect; PN, N effect; PN × Cd, the interactive effect of N and Cd.
content in roots, stems and leaves was positively correlated with WUE, root biomass and root surface area, and negatively correlated with root soluble sugars and non-structural carbohydrates (Figure 5).

Figure 4. Effects of Cd and N addition on soluble sugars (A), starch (B), non-structural carbohydrate (C) and sugar/starch ratio (D) in roots, stems and leaves of *E. glabripetalus*. Different letters indicate significant differences between groups (mean ± SE, n = 3) (p < 0.05). P<sub>Cd</sub>, Cd effect; P<sub>N</sub>, N effect; P<sub>N × Cd</sub>, the interactive effect of N and Cd.

Figure 5. Correlation between the indicators in *E. glabripetalus* seedlings. Data are Spearman correlation coefficients. Positive correlations are shown in red and negative correlations are shown in blue, and the darker the color, the stronger the correlation. * indicates a significant correlation at the 0.05 level.
4. Discussion

4.1. The Impact of N and Cd Treatments on the Gas Exchange of E. glabripetalus Seedlings

It has been reported that Cd has adverse effects on the gas exchange system in plants [39–41]. Our results also found that E. glabripetalus seedlings responded to Cd with an inhibited gas exchange system, which makes them vulnerable to environmental stress. Previous studies have revealed that Cd could decrease gas exchange attributes [42,43]. Cd stress decreased the $P_n$, $g_s$, and $T_r$ of E. glabripetalus in this study, indicating that Cd has deleterious effects on transpiration and respiratory processes and stomatal conductance, ultimately leading to a decline in photosynthesis [44,45]. Inhibited photosynthesis induced by Cd can be attributed mainly to stomatal and non-stomatal restriction [46]. The former refers to Cd causing stomatal closure, while the latter refers to the regulation of the plant’s intrinsic mechanisms. In addition, there was a significant linear correlation ($p < 0.05$) between $P_n$, $g_s$, and $T_r$ in seedlings, suggesting that Cd treatments may limit $P_n$ by affecting the stomatal factors of the plant. Since $g_s$ was not significantly correlated with $C_i$, the significant decrease in $P_n$ may be due to a combination of stomatal and non-stomatal factors [46]. $C_i$ was not significantly changed, indicating that leaf stomatal conductance was decreased in order to reduce leaf transpiration and prevent excessive tissue water deficit, keeping the rate of carbon dioxide, which indirectly led to the decrease in $P_n$. These results indicated that declined $P_n$, $g_s$, and $T_r$ are closely related to plant growth [47,48].

N addition altered the negative effects induced by Cd stress, which was reflected by a significant increase in $P_n$, $g_s$, and WUE. This indicated that N addition could mitigate the damage of the gas exchange system induced by Cd. N fertilization could have a positive effect on $P_n$, $g_s$, and $T_r$, which are positively related to plant yield [48]. Nitrogen supplementation helps stomatal opening, slightly restores the level of photosynthesis, improves the water use efficiency, and reduces stomatal limitations induced by Cd stress. These results indicate that N addition could help plants to increase their sensitivity to other stresses [49–51].

4.2. The Impact of Cd and N Treatments on N, P and Cd Contents of E. glabripetalus Seedlings

In the present study, Cd accumulation in E. glabripetalus seedlings was mainly concentrated in the root system. Most studies have shown that plant root systems have a greater capacity to accumulate cadmium than the aboveground parts [52–54]. Normally, Cd absorbed by plants accumulates first in the root system and is subsequently distributed to individual tissues [53]. To avoid adverse effects of Cd on the normal physiological metabolism of the plant, Cd is usually stored in the underground parts, far from sensitive photosynthetic organs [52,55]. Most of the Cd accumulated in the roots can reduce the toxic effect of Cd on aboveground tissues and improve the Cd tolerance of seedlings to a certain extent, which is a self-protection mechanism [53,56]. These results showed that root systems have a major impact on heavy metal uptake. Root characteristics, such as root size, root surface area and the fineness of roots, may play the key role in Cd absorption.

In order to avoid unfavorable environmental stresses, plants can adopt some adaptation strategies to adapt and protect themselves from stress [57]. For example, plants could regulate nutrient allocation strategies to meet the nutrient requirements of different organs under Cd stress [58]. The essential elements absorbed by plant root systems could be transported to stems and leaves, in which equilibrium can be disturbed by the presence of heavy metals, thus affecting the elements’ uptake and distribution in plant. This can be explained by the competition for the same transporters, a disturbance in water uptake as a result of Cd stress or disturbed key enzymes processes [59]. All these processes have effects on plant growth, such as on photosynthesis, non-structural carbon distribution and biomass allocation. According to the correlation analysis, the Cd content in plants was negatively correlated with N and P content in plants. Thus, it is hypothesized that the allocation of N and P in various plant parts may be affected by the accumulation of Cd through influencing the changes and equilibrium regulation in biomass and nonstructural carbon.
4.3. The Impact of N and Cd Treatments on the Non-Structural Carbohydrates of E. glabripetalus Seedlings

As is well known, NSCs represent the photosynthesis products and have important functions in plant development, carbon–nitrogen metabolism and resistance [60]. As the energy supply substances in plant growth metabolism, NSCs comprise mainly soluble sugars involved in nonstructural carbohydrate transport in plants or temporary storage, and starch stored for a long time in different plant tissues for future use, maintaining function when carbon demand is higher than supply under abiotic stress [61–63]. In this study, no matter what kind of treatment, starch was the main fraction of NSCs (>69%) in the roots and was the main storage system of carbohydrates in the roots. However, soluble sugars are the main fraction of leaf NSCs (>63%) of E. glabripetalus seedlings. Most of the NSCs were stored in the leaves and the roots, suggesting their importance in carbohydrate production and NSCs' storage in E. glabripetalus. Carbon storage in the root plays a critical role in supporting plant growth under abiotic stress. Cd treatment decreased root NSCs contents, which may be because the adaption strategies of E. glabripetalus respond to Cd stress by decreasing carbohydrate temporary storage in the roots to restrict the new fine root growth, which inhibits Cd uptake. Soluble sugars contribute to the osmotic adjustment and can indicate an ability to adapt to environmental changes [64]. The decreased soluble sugars concentration induced by Cd in roots and leaves reflected a decrease in carbohydrates produced by photosynthesis, shown by the decreased $P_n$, and also as a result of carbohydrate conversion. These results indicated that the NSCs’ allocation pattern changes in roots and leaves reflected the dynamic balance between assimilated savings and growth investment allocation, and plant adaptation strategies to environmental changes [65,66].

N treatment increased the root starch and NSC contents under Cd stress, with a decrease in the soluble sugars to starch ratio, indicating the increased starch content to maintain carbon demand under abiotic stress [67]. N alleviated the adverse effects on root induced by Cd, since a large portion of starch and NSC were supplied to support the production of new tissues (such as new fine roots). Furthermore, some of starch and NSC were also used for plant respiration. The decrease in leaf starch and NSC under the combined N and Cd treatment indicated that increased stomatal conductance and photosynthetic rate resulted in less carbon synthesis than consumption [68], showing that N addition alleviated leaf growth restriction by Cd. These results indicated that carbohydrates invested in growth or storage may be affected by environmental changes.

4.4. The Impact of Cd and N Treatments on the Growth of E. glabripetalus Seedlings

It has been reported that Cd could have negative effects on plant growth [27,69], often as the indicator for the toxicity assessment of heavy metals in plants [70,71]. Similar to the findings of previous studies [32], this study also found that E. glabripetalus seedlings responded to Cd with decreased above-ground biomass and total biomass. Interestingly, Cd significantly increased the root biomass allocation, showing that more resources were allocated to underground parts to resist Cd stress, which reflected the optimal allocation partitioning in plant biomass [72]. More biomass allocation to the root system may be associated with a reduction in resource uptake and photosynthesis. In general, heavy metals may affect the ability of resources (e.g., carbon, nutrients, or water) to be transported upward from the roots, thereby affecting plant growth [64,73]. The decreased leaf and stem biomass might result from the large root Cd accumulation, which restricted the nutrient transportation to stems and leaves. Single N addition has no effect on total biomass accumulations. However, N addition improved the plant growth under Cd stress by increasing the total biomass. Meanwhile, nitrogen has a positive influence on root growth and development, as N treatment significantly increased root biomass and carbohydrate storage in the root, with increased starch and NSC contents. These results revealed that N addition effectively alleviated the inhibition of Cd on plant growth [74–76].
Root morphology is an important indicator for environmental stress tolerance assessment [77,78]. Roots have phenotypic plasticity under stress conditions, which allows plants to obtain more resources from the surrounding soil environment [79]. In this study, the roots of *E. glabripetalus* seedlings became significantly thicker, and the fine roots (<0.5 mm) were significantly inhibited under Cd treatment, as reported in other studies, where high Cd could inhibit root hair development and fine root production [80,81]. Lateral and fine roots could increase the volume of soil reached by the root. However, Cd reduced the proportion of the length of <0.5 mm fine roots to the total root length, making the root system shorter and thicker and reducing root vigor, which in turn affected the nutrient uptake [80].

As is well known, root systems are extremely important in responding to heavy metals because root systems can regulate plants’ responses to stress through coordinated nutrients absorption and distribution in the whole plant [82]. There was a significant correlation between the root morphology and biomass of *E. glabripetalus* seedlings, and the root system of *E. glabripetalus* seedlings was inhibited by Cd treatment, which affected the nutrient uptake and thus affected plant growth.

N treatment increased the root growth of *E. glabripetalus* under Cd treatment, which was beneficial for increasing water and nutrient absorption. In fact, many studies have found that moderate N addition could positively alleviate Cd’s adverse effects on plants [50,76,83]. The reason might be that adequate N supply could alter the bioavailable Cd concentration in the soil [84], which is favorable for plants to absorb more Cd from the soil. More importantly, N could regulate cell wall isolation, chelation capacity and oxidative resistance to regulate Cd accumulation in plants [50]. Whether for N addition or Cd stress, roots are pivotal for elemental iron absorption. Improved root systems indicate that N addition alleviated Cd-induced toxicity.

Plants under adverse environmental conditions may show effective ecological strategies [85,86]. The changes after N addition indicated that N improved plant stress resistance by increasing photosynthesis and improving root growth, and affected carbon conversion. These indicated plants needed response time to adverse environments for plant growth, and often adopted the physiology-to-phenotype strategy. However, other influencing factors, e.g., interactions of plant–microbes in the soil, may also participate in Cd stress responses in *E. glabripetalus*, and should be considered in the future studies.

5. Conclusions

Our results suggested that Cd stress inhibited the growth of *E. glabripetalus*. Cd treatment affected transpiration and respiratory processes and stomatal conductance, resulting in a decline in photosynthesis, which led to decreased soluble sugar concentrations in leaves and NSC in the roots. Cd affected biomass allocation by decreasing the above-ground biomass and increasing underground biomass allocation. The growth of fine roots (<0.5 mm) was restricted by Cd. Most Cd was retained in roots, which affected the distribution of N and P in the plant. N addition improved the root system by increasing root growth, and increasing starch and NSC contents in the roots under Cd stress. With increased total biomass, these results indicated that N addition alleviated Cd-induced growth inhibition. These findings have important implications for understanding the underlying tolerance mechanisms of Cd pollution under N deposition in arbor species.

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