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NH_4^+-N and Low Ratios of NH_4^+-N/NO_3^--N Promote the Remediation Efficiency of *Salix linearistipularis* in Cd- and Pb-Contaminated Soil

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Abstract: (1) Background: the utilization of fast-growing trees for phytoremediation in heavy-metal-contaminated soil is increasingly recognized as an effective remediation method. Nitrogen (N) fertilizer enhances plant tolerance to heavy metals, yet the impact of various N levels and ammonium (NH_4^+-N)/nitrate (NO_3^--N) ratios on the remediation of cadmium (Cd) and lead (Pb) by trees remains unclear. (2) Methods: the efficiency of *Salix linearistipularis* in remediating Cd- and Pb-contaminated soil was investigated using a pot experiment with three N levels (60, 120, 200 kg hm^{-1} $year^{-1}$) and five NH_4^+-N/NO_3^--N ratios (6/0, 4/2, 3/3, 2/4, 0/6) employed, resulting in 16 treatments including a control. (3) Results: the levels and ratios of NH_4^+-N/NO_3^--N significantly affected the Cd and Pb uptake by *S. linearistipularis*. The highest increases in Cd and Pb in *S. linearistipularis* were observed for the N_{120}-6/0 treatment, which increased by 104.36% and 95.23%, respectively. In addition, in the N_{120}-6/0 treatment, the stem and leaf bioconcentration factors of Cd were significantly enhanced by 28.66% and 40.11%, respectively. Structural equation modeling revealed that the uptake of Cd and Pb was predominantly influenced by plant traits (biomass and root traits) rather than soil properties. (4) Conclusions: Our findings highlight the potential of the NH_4^+-N/NO_3^--N ratio to regulate plant traits, thereby improving the phytoremediation efficiency of heavy-metal-contaminated soil.

Keywords: willow; nitrogen; heavy metals; phytoremediation; plant trait; soil contamination

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1. Introduction

Heavy metal (HM) contamination in soil has occurred globally due to intensive anthropogenic activities and emissions, such as cadmium (Cd) and lead (Pb) [1,2]. Cd and Pb are non-degradable toxicants that can accumulate in plants, cause chlorosis, affect plant photosynthesis [3], reduce the absorption and translocation of essential elements [4], and pose a threat to human health via the food chain [5]. Therefore, the remediation of Cd- and Pb-contaminated soil is an essential and urgent issue concerning environmental safety, food security, and human health [6,7].

Phytoremediation has garnered recognition for its environmentally friendly nature, aesthetic appeal, efficiency, cost-effectiveness, and high level of acceptance [8,9]. Numerous hyperaccumulators that accumulate high levels of HMs have been screened, such as *Sedum alfredii*, *Thlaspi caerulescens*, and *Amaranthus Hypochondriacus* [10,11]. However, the herbaceous traits of hyperaccumulators, which result in relatively low biomass and high costs for

annual plants and harvests, restrict their widespread application [12]. Conversely, there has been considerable interest in the use of woody species for phytoremediation in recent years. Among them, willow (genus: *Salix*) was most extensively studied [13]. *Salix* has excellent traits such as rapid growth, ease of cultivation, hypoxia tolerance, a deep root system, large biomass, and high metal accumulation [14–16]. However, the growth of woody plants is mainly limited by their low nitrogen (N) content in urban contaminated soil, which restricts plant growth, especially root development and remediation efficiency [17]. As a component of amino acids, enzymes, and hormones, N plays an important role in stress resistance [3]. In addition, sufficient N increases transpiration and water flow within plants, resulting in a high uptake of mineral elements, including HMs [18–21]. Ammonium (NH_4^+-N) and nitrate (NO_3^--N) are the main forms of N taken up by plants [19]. Therefore, regulating N levels represents an effective strategy to improve the efficiency of HM remediation.

Multiple studies have emphasized species-specific responses to different N sources in plants [22–24]. Research has predominantly focused on herbaceous species, with notable findings such as Cheng's study demonstrating increased Cd accumulation in the herbaceous plant *Solanum nigrum* when exposed to NH_4^+-N [25]. Conversely, increased Cd uptake was observed in the cereal crop *Oryza sativa* and the metal hyperaccumulator *Thlaspi caerulescens* under NO_3^--N conditions [26,27]. The combined application of NH_4^+-N and NO_3^--N has been shown to enhance plant growth by influencing various nitrogen-related physiological processes [28]. Recent decades have seen a growing consensus that the NH_4^+-N/NO_3^--N ratio significantly impacts the biomass production and phytoremediation capabilities of various species, including agricultural crops like wheat [18,29], *Brassica napus*, *Pakchoi*, *Ricinus communis*, and *Panicum maximum* [24,30–32]. Wu's research indicated that higher NH_4^+-N ratios reduce Cd levels in rice shoots and roots, enhance biomass, and diminish total rice Cd accumulation at NH_4^+-N/NO_3^--N ratios of 2/1 and 1/0 [33]. Additionally, *Tanzania guinea* grass grown at NH_4^+-N/NO_3^--N ratios of 1/1 demonstrated the increased uptake, transport, and accumulation of Cd [24]. However, the specific effects of this ratio on the growth and phytoremediation efficiency of willow, a fast-growing woody plant, have not been adequately explored, suggesting a potential difference in response compared to the commonly studied herbaceous and agricultural species.

Willow demonstrates considerable proficiency in accumulating Cd and Pb [34,35]. This characteristic can be enhanced through suitable amendments, including fertilizer applications. The effects of N fertilizer on HMs in soil and plant accumulation have been extensively studied [21]. It was found that N fertilizer application increased metal accumulation in plants by altering soil properties and promoting plant growth [36]. Moreover, it was established that N regulates soil pH and HMs' bioavailability, thereby altering remediation efficiency [37]. Previous studies have also shown that Cd and Pb are more mobile in acidic soil than in neutral or alkaline soil [38]. Arnaud showed that the application of the N fertilizer enhanced the accumulation of HMs by increasing the biomass of *N. caulescens* [39]. Additionally, Sterckeman demonstrated that roots are the main driver of HM accumulation, with root mass being positively correlated with plant metal uptake [40]. However, the underlying mechanisms by which N levels and ratios regulate Cd and Pb accumulation in *S. linearistipularis* remain unclear.

In this study, we investigated the effects of the levels and ratios of NH_4^+-N/NO_3^--N on the remediation efficiency of *S. linearistipularis* in Cd- and Pb-contaminated soil. The objective of this study was twofold: (1) to investigate the effects of different NH_4^+-N/NO_3^--N levels and ratios on the growth of *S. linearistipularis* and its accumulation of HMs in Cd- and Pb-contaminated soil, and (2) to explore the underlying mechanism by which the N fertilizer application influences the phytoremediation efficiency of willow. The outcomes of this study are anticipated to provide valuable insights into enhancing fertilization methods in order to optimize the phytoremediation efficiency of woody plants in soil contaminated with HMs.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

One-year-old *S. linearistipularis* branches with uniform sizes were collected from the experimental station of Hebei Agricultural University (38°45′21″ N, 115°24′31″ E). In June 2020, branches measuring 15 cm in length were planted in pots filled with 20 kg of soil. The dimensions of the pots were 34 cm in diameter and 25 cm in height. The soil was classified as meadow cinnamon soil (surface soil, depth 0–20 cm) and spiked with $CdCl_2$ and $Pb(NO_3)_2$ for more than 5 years prior to our experiment [41]. The soil Cd and Pb concentrations were 7 mg kg^{-1} and 210 mg kg^{-1}, respectively. The other soil properties are listed in Table A1. Soil water content was maintained at 60% of the maximum field capacity throughout the experiment.

2.2. Experimental Design

Four N levels (0, 60, 120, and 200 kg hm^{-2} $year^{-1}$) and five NH_4^+-N/NO_3^--N ratios (6/0, 4/2, 3/3, 2/4, and 0/6) were used in this study (Table 1). Nitrogen application levels were established based on the standard nitrogen application rate of 120 kg·hm^{-2}·$year^{-1}$ for one-year seedlings [42]. To minimize the influence of other experimental conditions on plant growth, NH_4 Cl was chosen as the source of NH_4^+-N, and $NaNO_3$ was chosen as the source of NO_3^--N, considering the relatively minor effects of Cl^- and Na^+ on plant growth. All reagents were of analytical grade.

Table 1. Fertilization treatments.

Treatments	Nitrogen Levels (kg·hm^{-2}·$year^{-1}$)			
	0	60	120	200
NH_4^+-N/NO_3^--N = 6/0	Control	N_{60}-6/0	N_{120}-6/0	N_{200}-6/0
NH_4^+-N/NO_3^--N = 4/2		N_{60}-4/2	N_{120}-4/2	N_{200}-4/2
NH_4^+-N/NO_3^--N = 3/3		N_{60}-3/3	N_{120}-3/3	N_{200}-3/3
NH_4^+-N/NO_3^--N = 2/4		N_{60}-2/4	N_{120}-2/4	N_{200}-2/4
NH_4^+-N/NO_3^--N = 0/6		N_{60}-0/6	N_{120}-0/6	N_{200}-0/6

The experiment was designed with 16 distinct treatments to comprehensively assess the impact of various fertilization treatments on the growth of *Salix*, and each treatment consisted of 4 replicates. One-year-old branches approximately 0.6 cm in diameter were cut into 15 cm in length and were then transplanted into the pot. The nitrogen fertilizer was applied in four equal portions each year, at intervals of 10 days. The fertilizer, once dissolved in water, was evenly sprayed onto the surface of the pots using a small sprayer, followed immediately by watering. To ensure uniform conditions throughout the growing season, all pots were randomly arranged and received identical care, including watering, soil loosening, weeding, and were harvested 150 days after planting.

2.3. Experimental Analysis

2.3.1. Gas Exchange Measurements

Prior to harvest, six leaves of each plant were selected for the measurement of gas exchange parameters such as the photosynthetic rate (Pn, μmol CO_2·m^{-2} s^{-1}), transpiration rate (E, mmol H_2O·m^{-2} s^{-1}), stomatal conductance (Gs, mol H_2O·m^{-2} s^{-1}), and intercellular CO_2 concentration (CO_2 int, μmol CO_2·mol^{-1}). They were determined using a photosynthesis analysis system (Li-6400, Lincoln, NE, USA) with 1200 μmol m^{-2} s^{-1} of photons and 400 μmol mol^{-1} of CO_2 concentration [43]. All measurements were performed outdoors between 9:00 and 11:00 a.m. on a sunny day with relatively stable environmental conditions.

2.3.2. Root Morphological Parameters and Biomass Determination

Upon harvest, each plant was carefully removed from the growth substrate, rinsed with tap and deionized water, and separated into roots, stems, and leaves. Roots were collected for the measurement of morphological parameters, including the average root diameter, surface area, total volume, and total root length, using an automatic root scanner (LA-S Plant image analyzer system). Finally, each sample (roots, stems and leaves) was oven-dried at 65 °C to constant weight for dry biomass determination.

2.3.3. Rhizosphere Soil Analyses

Rhizosphere soil was collected via shaking during the harvesting of plants [43]. The soil was subsequently air-dried, pulverized, and sieved using 20- and 100-mesh sieves. Soil pH, soil organic matter (SOM), cation exchange capacity (CEC), soil urease activity (Ure), and soil catalase activity (S-CAT) were determined using soil sieved through a 20-mesh sieve. Soil pH was measured at a volume ratio of 1/2.5 (soil/water) using a pH meter (PHSJ-3F). SOM was determined by oxidation with potassium dichromate and titration with a ferrous sulfate reagent [44]. The CEC was determined using the barium chloride-sulfuric acid exchange method. Urea content was determined via phenol hypochlorite colorimetry using a spectrophotometer (AA-680, Shimadzu, Kyoto, Japan) at 578 nm [45]. The potassium permanganate titration method was used for S-CAT [46]. Ammonium and nitrate concentrations were determined using a COMIN kit [47]. The total concentrations of Cd and Pb were determined by acid digestion (HCL, HNO_3, and $HClO_4$) [48], and chemical fractions were extracted with the sequential extraction method (BCR) described by Rauret using soil sieved through a 100-mesh sieve [49]. Atomic absorption spectrophotometry (AA-680; SHIMADZU, Kyoto, Japan) was used to determine Cd and Pb concentrations.

2.3.4. Plant Tissue Analysis

Dried plant samples (roots, stems, and leaves) were powdered and sieved through a 60-mesh sieve before digestion with concentrated $HNO_3/HCLO_4$ (v/v, 4:1) at 170 °C for approximately 3.5 h until the solution became clear [50]. The solution was filtered through a 0.45 µm membrane, diluted to 50 mL, and analyzed for Cd and Pb concentrations using flame atomic absorption spectrometry (AA-680, Shimadzu, Kyoto, Japan).

2.4. Statistical Analysis

Data preprocessing included tests for normality, homogeneity of variances, and independence to meet the assumptions required for two-way ANOVA. Following the successful validation of these assumptions, two-way ANOVA was performed at a significance level of 0.05 using IBM SPSS version 20.0. and the analysis primarily focused on examining the effects of varying levels and ratios on N fertilizer as independent variables. Subsequently, post hoc comparisons were carried out employing Duncan's multiple range tests to ascertain the differences in means. A principal component analysis (PCA) was performed applying the "FactoMineR" and "factoextra" packages in R (4.2.1). Additionally, SEMs were developed to investigate the direct and indirect influences of nitrogen fertilizer levels and ratios on the total bioaccumulation of Cd or Pb using the "lavaan" and "semPlot" packages in R (4.2.1).

The bioconcentration factor (BCF) was computed to evaluate the plant's capability to accumulate HMs from its surroundings [51]. Furthermore, the translocation factor (TF) was calculated to assess the efficiency of metal translocation from the roots to the aerial parts of the plant [52]. This provided a comprehensive understanding of the plant's response to different nitrogen treatments in terms of heavy metal accumulation and translocation.

$$\text{BCF} = \frac{\text{Cd or Pb concentration in the harvested tissues}}{\text{Cd or Pb concentration in the soil}}$$

$$\text{TF} = \frac{\text{Cd or Pb concentration in the aboveground tissues}}{\text{Cd or Pb concentration in the roots}}$$

3. Results

3.1. Chemical Properties of Rhizosphere Soil

The application of N fertilizer significantly decreased soil pH, and this effect was significantly associated with both N levels and ratios ($p < 0.01$; Table 2). The impact of a high N level or balanced NH_4^+-N and NO_3^--N ratios on pH was pronounced, with pH reaching a minimum of 7.66 for the N_{200}-3/3 treatment. However, N fertilizer increased the concentrations of NH_4^+-N and NO_3^--N, as well as the SOM content and urea activity (Table A2). N levels, N ratios, and their interactions had a significant impact on the concentrations of Cd and Pb in rhizosphere soil ($p < 0.01$; Table 2). The N_{200}-0/6 treatment revealed the highest Cd and Pb concentrations of 6.09 mg kg^{-1} and 201.92 mg kg^{-1}, respectively. In contrast, the N_{60}-6/0 treatment had the lowest Cd concentration (2.65 mg kg^{-1}). The N_{120}-6/0 and N_{120}-3/3 treatments showed the lowest Pb concentrations, measuring 103.24 mg kg^{-1} and 102.26 mg kg^{-1}, respectively. Additionally, the NH_4^+-N-dominated treatment and the N_{200} group increased the acid-soluble concentrations of Cd and Pb. The N_{120}-6/0 treatment recorded the highest Cd concentration, measuring 0.83 mg kg^{-1}, whereas the N_{200}-6/0 treatment exhibited the highest Pb concentration at 27.62 mg kg^{-1}.

Table 2. pH, Cd and Pb concentrations of rhizosphere soil.

Nitrogen Levels (kg hm^{-2} $year^{-1}$)	Ratios	pH	Total Cd (mg kg^{-1})	Total Pb (mg kg^{-1})	Acid-Soluble Cd (mg kg^{-1})	Acid-Soluble Pb (mg kg^{-1})
0	Control	7.96 ± 0.05 ^{a}	4.09 ± 0.56 ^{ef}	184.17 ± 11.04 ^{abc}	0.77 ± 0.04 ^{a}	20.78 ± 2.22 ^{de}
60	6/0	7.88 ± 0.06 ^{bc}	2.65 ± 0.17 ^{g}	132.24 ± 19.28 ^{fgh}	0.81 ± 0.13 ^{a}	22.82 ± 2.53 ^{bcd}
	4/2	7.92 ± 0.03 ^{ab}	2.77 ± 0.15 ^{g}	121.61 ± 23.95 ^{ghi}	0.81 ± 0.05 ^{a}	18.91 ± 1.61 ^{e}
	3/3	7.78 ± 0.06 ^{de}	2.71 ± 0.24 ^{g}	159.21 ± 10.82 ^{de}	0.82 ± 0.1 ^{a}	22.43 ± 3.51 ^{bcde}
	2/4	7.77 ± 0.05 ^{e}	3.06 ± 0.42 ^{g}	136.74 ± 9.19 ^{efg}	0.37 ± 0.03 ^{c}	22.24 ± 1.87 ^{cde}
	0/6	7.84 ± 0.01 ^{cd}	4.29 ± 0.46 ^{de}	110.34 ± 18.48 ^{hi}	0.41 ± 0.09 ^{c}	19.42 ± 2.48 ^{de}
120	6/0	7.88 ± 0.02 ^{bc}	4.74 ± 0.44 ^{cd}	103.24 ± 12.79 ^{i}	0.83 ± 0.05 ^{a}	19.53 ± 2.23 ^{de}
	4/2	7.78 ± 0.08 ^{de}	5.14 ± 0.16 ^{bc}	125.9 ± 10.48 ^{ghi}	0.81 ± 0.08 ^{a}	19.22 ± 1.19 ^{de}
	3/3	7.76 ± 0.03 ^{e}	4.54 ± 0.45 ^{cde}	102.26 ± 18.05 ^{i}	0.41 ± 0.03 ^{c}	19.85 ± 1.15 ^{de}
	2/4	7.80 ± 0.03 ^{de}	4.57 ± 0.48 ^{cde}	135.74 ± 12.25 ^{efg}	0.48 ± 0.04 ^{bc}	19.11 ± 1.64 ^{e}
	0/6	7.80 ± 0.03 ^{de}	3.64 ± 0.35 ^{f}	155.35 ± 15 ^{def}	0.55 ± 0.08 ^{b}	14.32 ± 2.78 ^{f}
200	6/0	7.78 ± 0.04 ^{de}	4.33 ± 0.46 ^{de}	132.97 ± 14.31 ^{fgh}	0.5 ± 0.07 ^{bc}	27.62 ± 0.69 ^{a}
	4/2	7.74 ± 0.04 ^{ef}	5.55 ± 0.48 ^{ab}	194.67 ± 12.03 ^{ab}	0.78 ± 0.07 ^{a}	25.9 ± 0.59 ^{ab}
	3/3	7.66 ± 0.03 ^{g}	5.09 ± 0.33 ^{bc}	173.13 ± 15.84 ^{bcd}	0.77 ± 0.05 ^{a}	25.81 ± 3.66 ^{ab}
	2/4	7.69 ± 0.04 ^{fg}	5.62 ± 0.17 ^{ab}	168.91 ± 19.3 ^{cd}	0.8 ± 0.07 ^{a}	25.37 ± 2.87 ^{abc}
	0/6	7.73 ± 0.05 ^{ef}	6.09 ± 0.62 ^{a}	201.92 ± 16.63 ^{a}	0.73 ± 0.17 ^{a}	25.18 ± 0.77 ^{abc}
L		**	**	**	**	**
R		**	**	**	**	**
L × R		ns	**	**	**	ns

Note: All data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's test for comparison with the controls. N levels are denoted as "L", the N ratio as "R", and the interactive effect of N levels and ratios as "L × R". Different superscript lowercase letters indicate significant differences among the treatments at $p < 0.05$ in specific tissues. Data represent means ± SD ($n = 4$), "ns" denotes non-significance and double asterisks (**) represent significant differences at $p < 0.01$.

3.2. Salix linearistipularis Traits under Different N Treatments

The N level, N ratio, and their interactions significantly influenced the plant traits of *S. linearistipularis*, including photosynthesis, root morphology, and biomass ($p < 0.01$, Figures 1 and 2). Specifically, when NH_4^+-N was added alone, it significantly enhanced the photosynthetic and transpiration rates of *S. linearistipularis*. Furthermore, the NH_4^+-N treatment markedly increased the root surface area, volume, and length, resulting in a substantial enhancement of root growth (Figures 1 and A2). Significant increases in total biomass were noted, with particular prominence in the N_{60}-6/0 and N_{120}-6/0 treatments, where the values reached 33.45 and 36.42 g $plant^{-1}$, respectively (Figure 2D). Compared

to the control, the biomass increased by 38.91% and 51.25%, respectively. Conversely, the introduction of NO_3^--N led to divergent outcomes, resulting in diminished growth (Figure A3), a significant reduction in photosynthetic activity (Figure A1), and a decrease in *S. linearistipularis* biomass (Figure 2). Additionally, higher N levels resulted in an evident decrease in the photosynthetic rate of plants, thereby limiting root expansion and compromising overall plant vitality. Notably, leaves in the N_{200} treatment, characterized by a high N content, exhibited diminished size and yellowing and even displayed signs of toxicity (Figure A3).

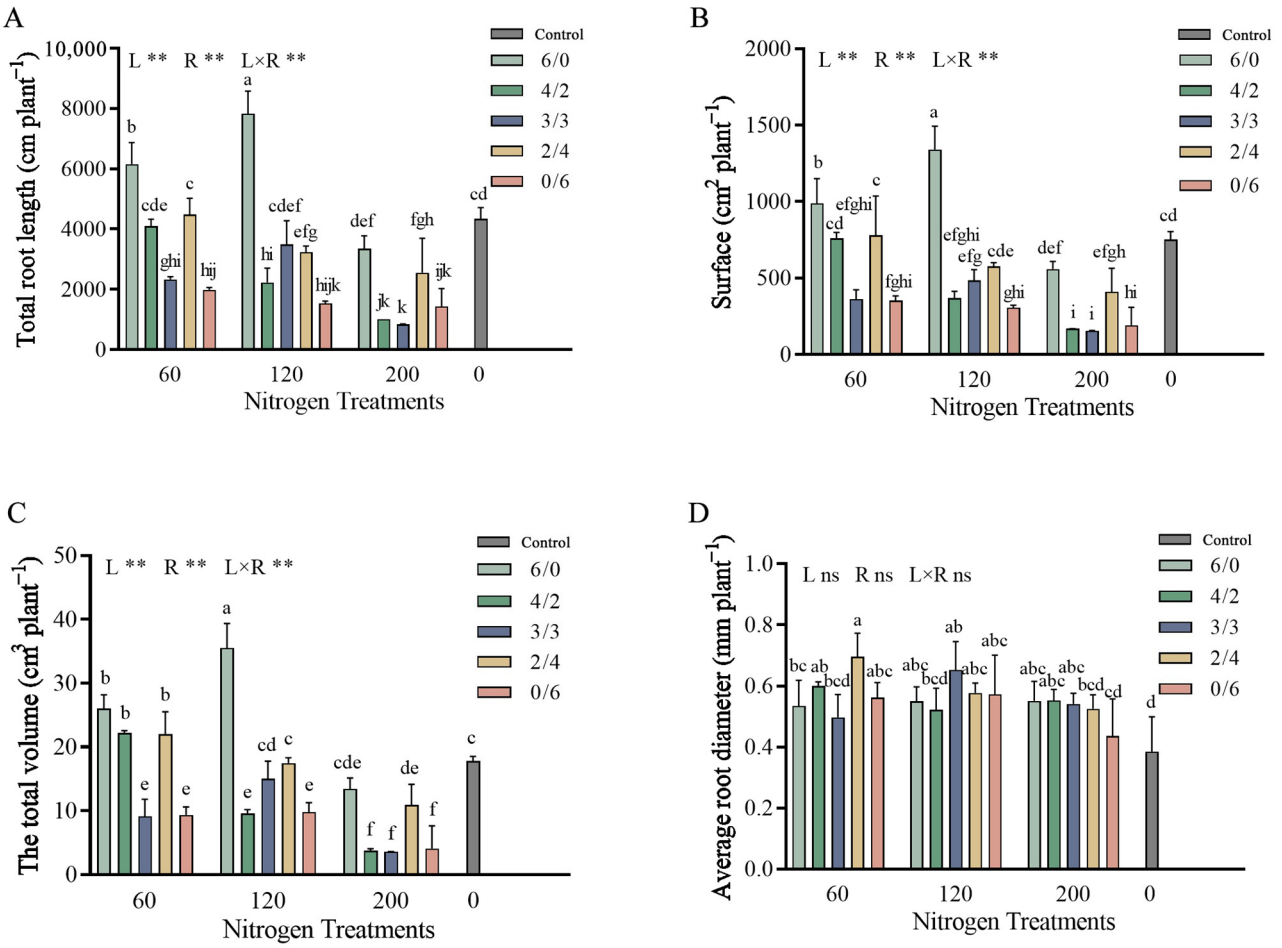

Figure 1. Root traits of *S. linearistipularis* under different N treatments. Note: All data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's test for comparison with the controls. N levels are denoted as "L", the N ratio as "R", and the interactive effect of N levels and ratios as "L × R". Different superscript lowercase letters indicate significant differences among the treatments at $p < 0.05$ in specific tissues. Data represent means ± SD ($n = 4$), "ns" denotes non-significance and double asterisks (**) represent significant differences at $p < 0.01$.

3.3. Distribution of Cd and Pb in Salix linearistipularis

The concentrations of Cd and Pb within *S. linearistipularis* were significantly influenced by the interactions between N levels and ratios ($p < 0.01$, Figure 3). In the N_{120} and N_{200} groups, the predominance of NH_4^+-N (NH_4^+-N/NO_3^--N = 6/0 and 4/2) resulted in a substantial increase in the Cd concentration in the roots, with a range of 60.31%–83.81% (Figure 3A). The highest Cd concentrations in the stems and leaves were observed in the N_{120}-6/0 treatment, reaching 26.43 and 64.35 mg kg^{-1} (Figure 3B,C), representing increases of 28.60% and 40.03%, respectively, compared to the control. Nevertheless, a decrease in the Cd concentration was observed in the stems and leaves, which was primarily induced by NO_3^--N treatments (NH_4^+-N/NO_3^--N = 3/3, 2/4, and 0/6). The N_{200}-3/3 treatment

displayed the lowest Cd concentration in stems (10.47 mg kg^{-1}), whereas the N_{120}-0/6 treatment demonstrated the lowest Cd concentration in leaves (20.23 mg kg^{-1}). The Pb concentration in the roots significantly increased under the NO_3^--N and high N treatments (Figure 3D). The N_{120}-0/6 treatment recorded the highest concentration at 14.85 mg kg^{-1}, followed by the N_{60}-2/4 treatment at 14.24 mg kg^{-1}, representing increases of 103.59% and 95.15% compared to the control, respectively. Moreover, N fertilization increased Pb concentrations in both the stem and leaf tissues (Figure 3E,F). The N_{60}-2/4 treatment exhibited the highest stem Pb concentration of 10.45 mg kg^{-1}, whereas the N_{200}-3/3 treatment displayed the highest leaf Pb concentration of 12.10 mg kg^{-1}.

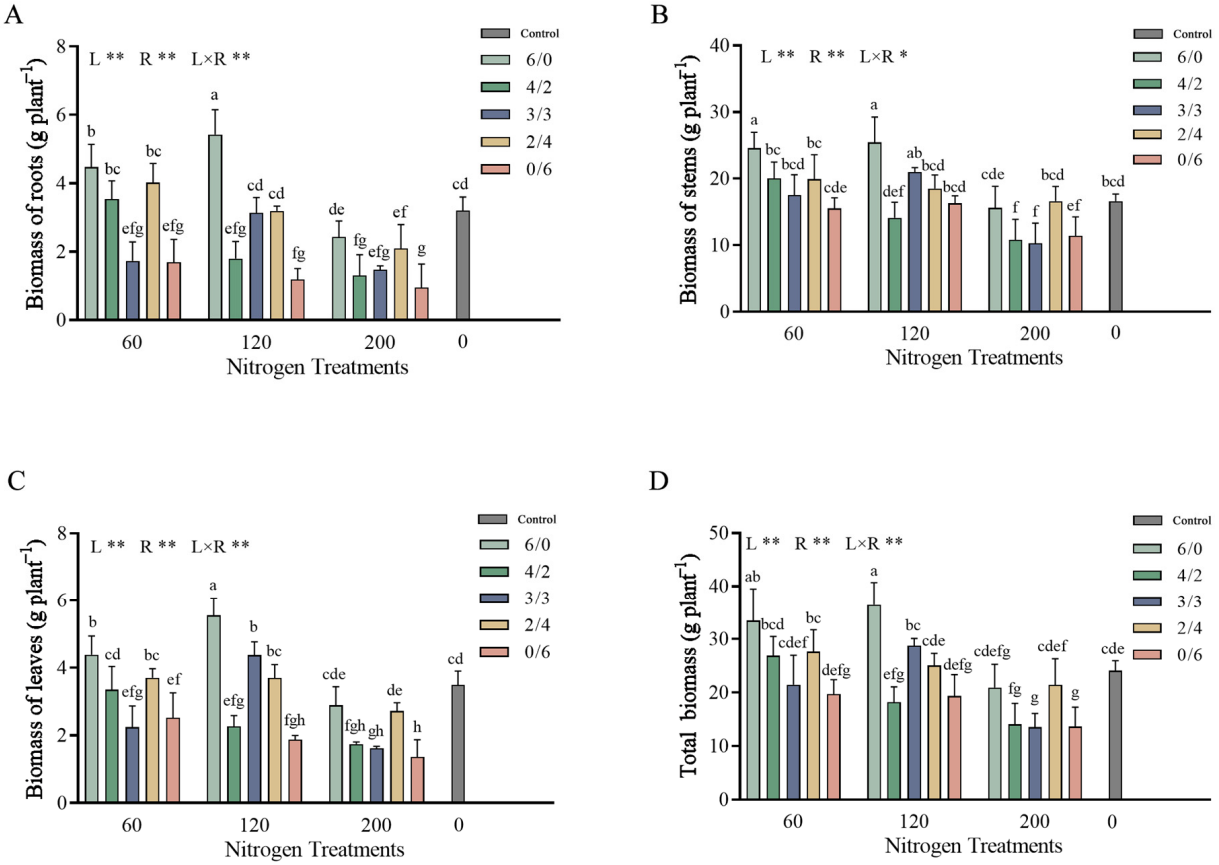

Figure 2. Biomass of *S. linearistipularis*. Note: All data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's test for comparison with the controls. N levels are denoted as "L", the N ratio as "R", and the interactive effect of N levels and ratios as "L × R". Different superscript lowercase letters indicate significant differences among the treatments at $p < 0.05$ in specific tissues. Data represent means ± SD (n = 4), a single asterisk (*) represents significant differences at $p < 0.05$, and double asterisks (**) represent significant differences at $p < 0.01$.

The N level, N ratio, and their interactions significantly influenced the BCF of Cd in *S. linearistipularis* ($p < 0.001$; Table 3). The use of a N fertilizer significantly increased the BCF of Cd in root tissues, with the N_{200}-4/2 treatment attaining the highest value of 3.69, followed by the N_{120}-6/0 treatment at 3.33. Furthermore, the N_{120}-6/0 treatment significantly increased stem BCF (4.13) and leaf BCF (10.06), representing increases of 28.66% and 40.11%, respectively. Conversely, stem BCF was significantly reduced under treatments such as N_{120}-3/3, 0/6, and N_{200}-4/2, 3/3, 2/4, 0/6. Similarly, leaf BCF exhibited significant reductions under various treatments, including N_{60}-0/6, N_{120}-3/3, 2/4, 0/6, and N_{200}-6/0, 4/2, 3/3, 0/6, with reductions ranging from 23.36% to 48.91% for stem BCF and from 7.02% to 55.99% for leaf BCF compared to the control.

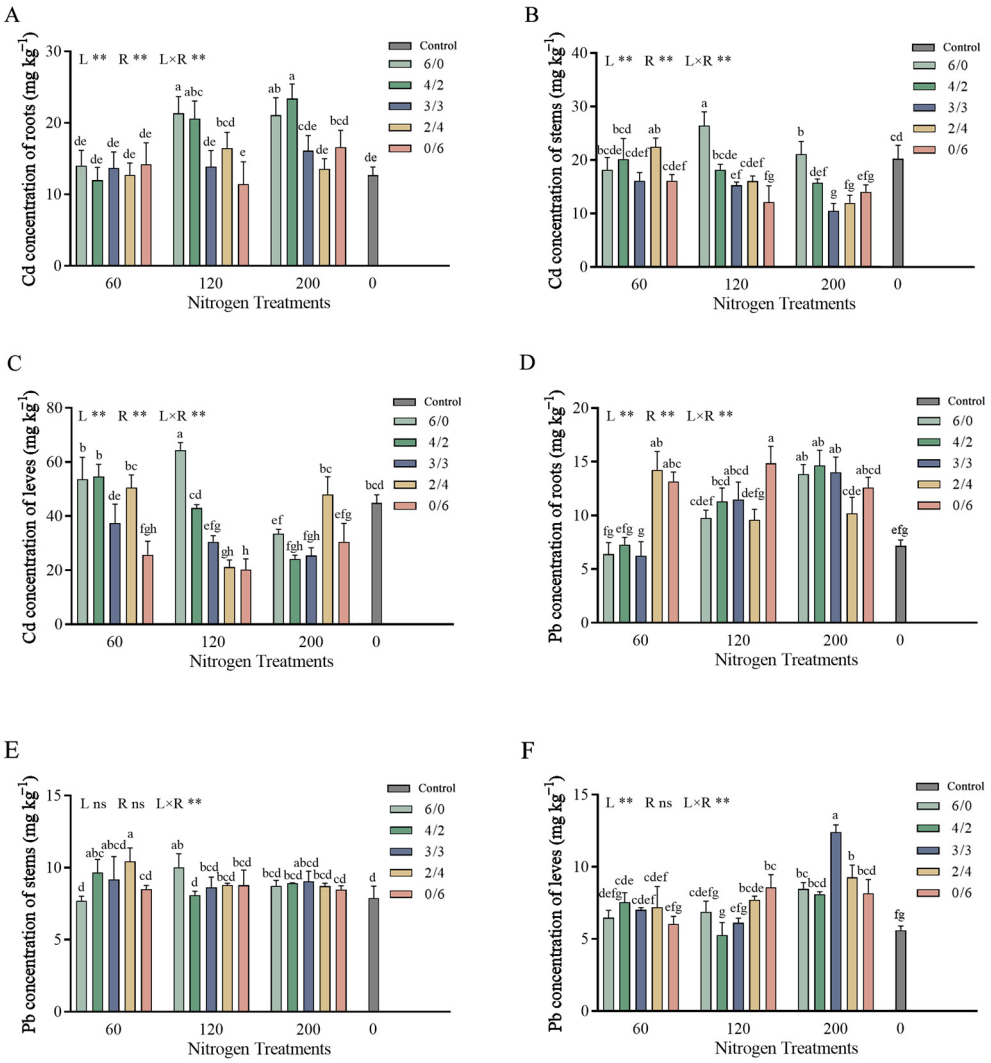

Figure 3. Pb and Cd concentration in *S. linearistipularis* under different treatments. Note: All data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's test for comparison with the controls. N levels are denoted as "L", the N ratio as "R", and the interactive effect of N levels and ratios as "L × R". Different superscript lowercase letters indicate significant differences among the treatments at $p < 0.05$ in specific tissues. Data represent means ± SD ($n = 4$), "ns" denotes non-significance, and double asterisks (**) represent significant differences at $p < 0.01$.

Table 3. BCF of Cd and Pb in *S. linearistipularis*.

Nitrogen Levels (kg hm^{-2} year^{-1})	Ratios	Cd			Pb		
		BCF of Roots	BCF of Stems	BCF of Leaves	BCF of Roots	BCF of Stems	BCF of Leaves
0	Control	2.01 ± 0.19 ^{de}	3.21 ± 0.4 ^{bc}	7.18 ± 0.49 ^{bcd}	0.04 ± 0.01 ^{e}	0.04 ± 0.01 ^{b}	0.03 ± 0.01 ^{fg}
60	6/0	2.19 ± 0.47 ^{de}	2.84 ± 0.37 ^{bcde}	8.37 ± 1.28 ^{b}	0.03 ± 0.01 ^{e}	0.04 ± 0.01 ^{b}	0.03 ± 0.01 ^{defg}
	4/2	1.87 ± 0.28 ^{de}	3.15 ± 0.6 ^{bcd}	8.55 ± 0.69 ^{b}	0.04 ± 0.01 ^{e}	0.05 ± 0.01 ^{ab}	0.04 ± 0.01 ^{cde}
	3/3	2.14 ± 0.35 ^{de}	2.52 ± 0.24 ^{cdef}	5.85 ± 1.1 ^{de}	0.03 ± 0.01 ^{e}	0.05 ± 0.01 ^{ab}	0.04 ± 0.01 ^{cdef}
	2/4	1.99 ± 0.26 ^{de}	3.52 ± 0.25 ^{ab}	7.9 ± 1.51 ^{bc}	0.07 ± 0.01 ^{ab}	0.05 ± 0.01 ^{a}	0.04 ± 0.01 ^{cdef}
	0/6	2.22 ± 0.47 ^{de}	2.52 ± 0.18 ^{cdef}	4.01 ± 0.79 ^{fgh}	0.07 ± 0.01 ^{abc}	0.04 ± 0.01 ^{b}	0.03 ± 0.01 ^{efg}
120	6/0	3.33 ± 0.52 ^{a}	4.13 ± 0.85 ^{a}	10.06 ± 0.44 ^{a}	0.05 ± 0 ^{cde}	0.05 ± 0.01 ^{a}	0.03 ± 0.01 ^{cdefg}
	4/2	3.22 ± 0.39 ^{abc}	2.84 ± 0.16 ^{bcde}	6.72 ± 0.2 ^{cd}	0.06 ± 0.01 ^{bcd}	0.04 ± 0.01 ^{b}	0.03 ± 0.01 ^{g}
	3/3	2.17 ± 0.35 ^{de}	2.39 ± 0.09 ^{ef}	4.77 ± 0.35 ^{efg}	0.06 ± 0.01 ^{bcd}	0.04 ± 0.01 ^{b}	0.03 ± 0.01 ^{efg}
	2/4	2.57 ± 0.34 ^{cd}	2.51 ± 0.15 ^{cdef}	3.31 ± 0.41 ^{gh}	0.05 ± 0.01 ^{de}	0.04 ± 0.01 ^{b}	0.04 ± 0.01 ^{bcde}
	0/6	1.79 ± 0.49 ^{e}	1.9 ± 0.47 ^{fg}	3.16 ± 0.61 ^{h}	0.07 ± 0.02 ^{ab}	0.04 ± 0.01 ^{b}	0.04 ± 0.01 ^{bc}

Table 3. *Cont.*

Nitrogen Levels (kg hm^{-2} year^{-1})	Ratios	Cd			Pb		
		BCF of Roots	BCF of Stems	BCF of Leaves	BCF of Roots	BCF of Stems	BCF of Leaves
200	6/0	3.3 ± 0.38 ^{ab}	3.3 ± 0.38 ^{b}	5.24 ± 0.24 ^{ef}	0.07 ± 0 ^{ab}	0.04 ± 0.01 ^{b}	0.04 ± 0.01 ^{bc}
	4/2	3.69 ± 0.66 ^{a}	2.46 ± 0.11 ^{def}	3.78 ± 0.22 ^{fgh}	0.08 ± 0.01 ^{a}	0.04 ± 0.01 ^{b}	0.04 ± 0.01 ^{bcd}
	3/3	2.52 ± 0.33 ^{cde}	1.64 ± 0.22 ^{g}	3.98 ± 0.45 ^{fgh}	0.08 ± 0.01 ^{a}	0.05 ± 0.01 ^{b}	0.06 ± 0.01 ^{a}
	2/4	2.12 ± 0.22 ^{de}	1.87 ± 0.22 ^{fg}	7.51 ± 1.02 ^{bc}	0.05 ± 0.01 ^{cde}	0.04 ± 0.01 ^{b}	0.05 ± 0.01 ^{b}
	0/6	2.6 ± 0.36 ^{bcd}	2.19 ± 0.21 ^{efg}	4.77 ± 1.06 ^{efg}	0.06 ± 0.01 ^{abcd}	0.04 ± 0.01 ^{b}	0.04 ± 0.01 ^{bcd}
L		**	**	**	**	ns	**
R		**	**	**	**	ns	ns
L × R		**	**	**	**	**	**

Note: All data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's test for comparison with the controls. N levels are denoted as "L", the N ratio as "R", and the interactive effect of N levels and ratios as "L × R". Different superscript lowercase letters indicate significant differences among the treatments at $p < 0.05$ in specific tissues. Data represent means ± SD ($n = 4$), "ns" denotes non-significantce, and double asterisks (**) represent significant differences at $p < 0.01$.

The application of N fertilizer increased the BCF of Pb within root tissues, resulting in increases ranging from 25% to 100%. Notably, the N_{200}-4/2 and N_{200}-3/3 treatments exhibited the highest BCF values. The increase in stem BCF of Pb was relatively modest, with only the N_{60}-2/4 and N_{120}-6/0 treatments showing a 25% increase compared to the control. In terms of the leaf BCF of Pb, significant increases were observed under the N_{60}-4/2, N_{120}-2/4, N_{120}-0/6, and N_{200} treatments. Among these, the N_{200}-3/3 treatment demonstrated the most substantial increase, with a 100% increase compared with the control.

3.4. Cd and Pb Accumulation in Salix linearistipularis

The N level, N ratio, and their interactions significantly increased the accumulation of Cd and Pb in *S. linearistipularis* tissues ($p < 0.01$, Figure 4). Most Cd and Pb concentrations accumulated in the stems, which were substantially higher than those in the roots and leaves. The total Cd accumulation in *S. linearistipularis* significantly increased in the N_{120}-6/0 treatment, reaching 0.11, 0.66, 0.36, and 1.13 mg plant^{-1} in roots, stems, leaves, and the entire plant, respectively. These values represented increases of 174.67%, 93.05%, 110.06%, and 104.36%, respectively, compared to the control. This was followed by N_{60}-6/0 treatment, with Cd accumulation rates of 0.06, 0.44, 0.23, and 0.74 mg plant^{-1} in the roots, stems, leaves, and entire plant, respectively, corresponding to increases of 49.44%, 29.03%, 37.83%, and 33.25%. Meanwhile, Cd accumulation was lower in the N_{60}-0/6, N_{120}-4/2, 0/6, and N_{200}-4/2, 3/3, 2/4, and 0/6 treatments, ranging from 0.16 to 0.39 mg plant^{-1}.

The total Pb accumulation significantly increased in the N_{60}-6/0, 4/2, 2/4, and N_{120}-6/0, 3/3 treatments compared to the control (Figure 4H). In the N_{120}-6/0 treatment, maximum Pb accumulation was observed, with accumulation rates of 0.05, 0.26, 0.04, and 0.35 mg plant^{-1} in the roots, stems, leaves, and entire plant, respectively, exhibiting increases of 124.79%, 92.45%, 80.76%, and 95.23%, respectively, compared to the control. The N_{60}-2/4 treatment ranked second in Pb accumulation, with accumulation rates of 0.06, 0.21, 0.03, and 0.29 mg plant^{-1} in the roots, stems, leaves, and whole plant, respectively, signifying increases of 42.31%, 55.01%, 27.14%, and 63.92% compared to the control. However, a decrease in Pb accumulation was observed in the N_{120}-4/2 and N_{200}-4/2, 3/3, and 0/6 treatments, with measurements of 0.11, 0.12, and 0.13 mg of the plant^{-1}, respectively.

3.5. The Direct and Indirect Mechanisms Regulating the Accumulation of Cd and Pb

An SEM was constructed that consisted of the level and ratio of N, soil properties, plant traits, and the total accumulation of Cd and Pb in *S. linearistipularis* (Figure 5). Plant traits were characterized using the first principal component of the PCA. The Cd interpretation rate was 0.92, whereas that of Pb was 0.87. The total accumulation of Cd and Pb was mainly regulated by plant traits. The level and ratio of N indirectly affected plant traits by affecting NH_4^+-N. In addition, the effect of the N fertilizer ratio on Pb accumulation was more

significant in *S. linearistipularis*. The total absorption of Cd and Pb by *S. linearistipularis* increased with increasing NH_4^+-N/NO_3^--N ratios.

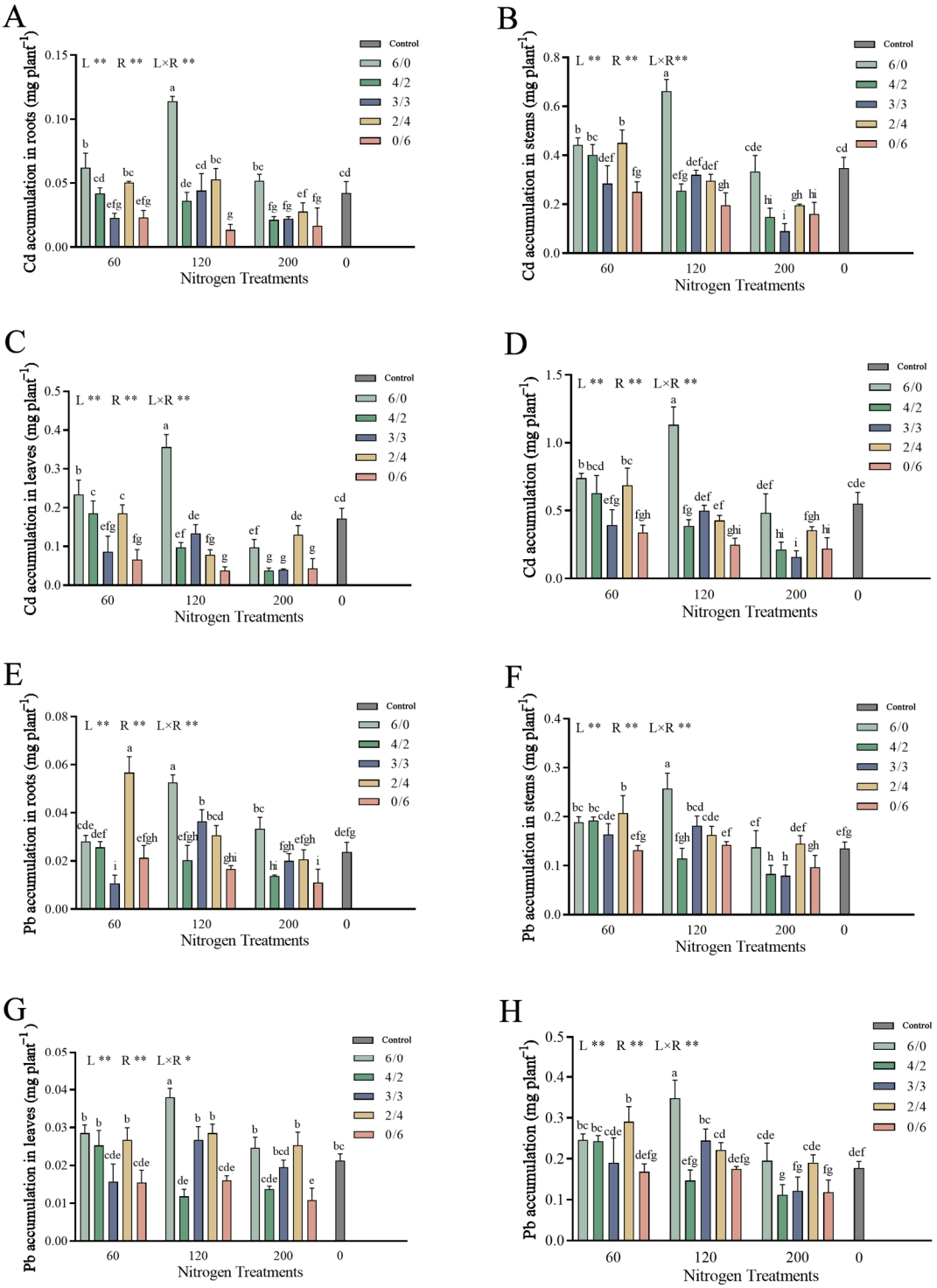

Figure 4. Pb and Cd accumulation in *S. linearistipularis* under different treatments. Note: All data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's test for comparison with the controls. N levels are denoted as "L", the N ratio as "R", and the interactive effect of N levels and ratios as "L × R". Different superscript lowercase letters indicate significant differences among the treatments at $p < 0.05$ in specific tissues. Data represent means ± SD ($n = 4$), a single asterisk (*) represents significant differences at $p < 0.05$, and double asterisks (**) represent significant differences at $p < 0.01$.

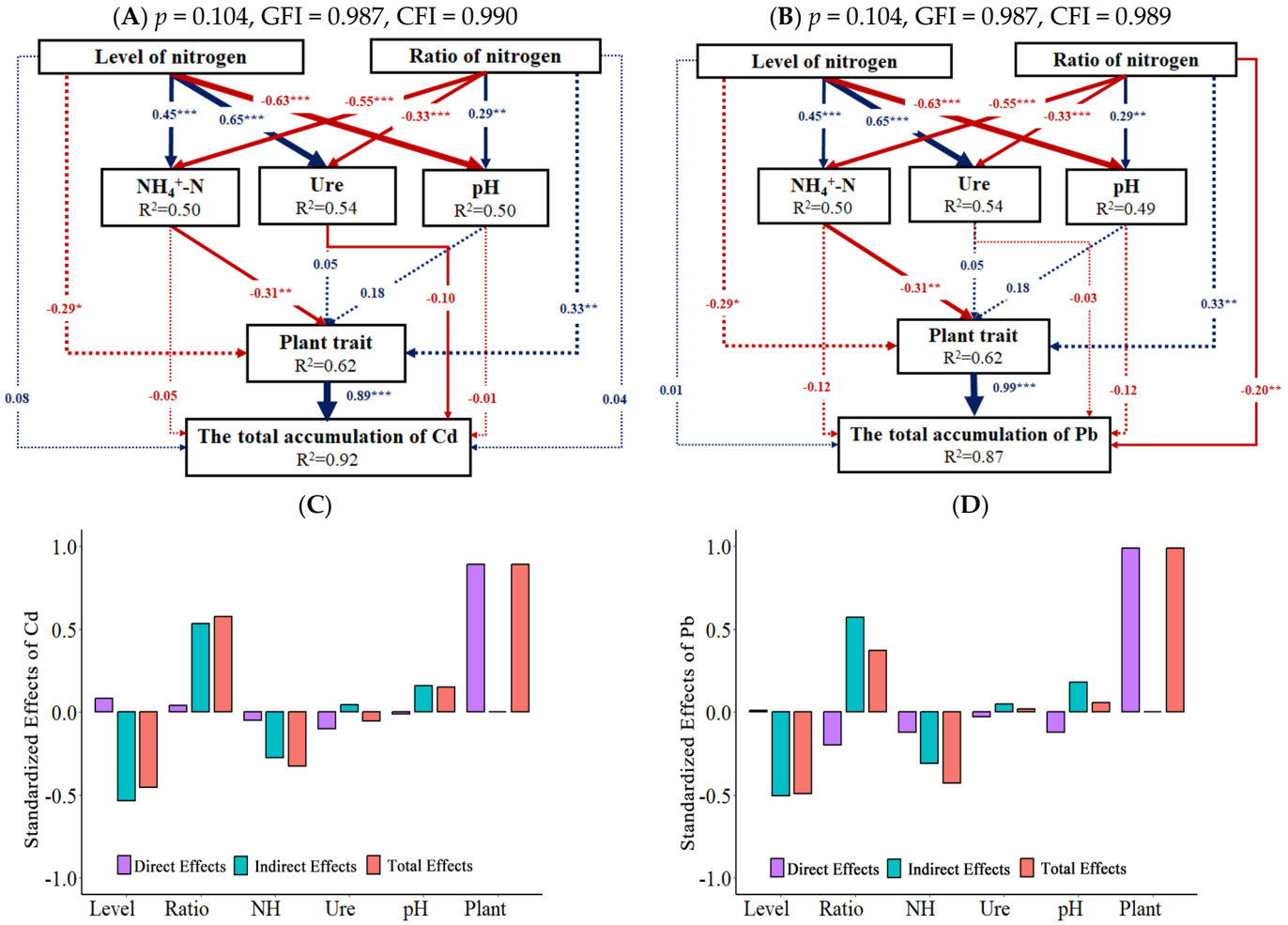

Figure 5. A structural equation model was employed to illustrate the direct and indirect effects of N application, soil properties (NH_4^+-N, Ure, and pH), and plant traits on the total accumulation of Cd (**A**) and Pb (**B**) in *S. linearistipularis*, along with the effect value of the total Cd accumulation (**C**) and Pb accumulation (**D**). Solid black and red arrows indicate significantly positive and negative correlations, respectively, while dotted arrows indicate insignificant correlations. The numbers adjacent to the arrows represent standardized path coefficients (λ). Significance levels are denoted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

4.1. Nitrogen Reduced Soil pH and Improved Soil Quality

Appropriate N application can increase soil fertility and improve soil quality. Our study demonstrated that the application of N a fertilizer to meadow cinnamon soil enhanced urea activity while concurrently increasing the contents of NH_4^+-N, NO_3^--N, and SOM. This improvement in soil quality can be attributed to the promotion of nutrient cycling due to the addition of N [53]. This observation is consistent with most findings in the field [18]. However, excessive N affects plant health and poses environmental risks, necessitating the control of N fertilizer application [54]. In this study, N application resulted in soil acidification within the rhizosphere (Table 2). However, some studies have shown that NO_3^--N addition can increase the soil's pH [55]. This variation may be related to soil characteristics because the application of inorganic N in alkaline soil can result in the accumulation of phenolic acids [56]. Furthermore, willows release organic acids during their growth cycle [57], which contribute to soil pH reduction as N fertilization accelerates protein synthesis in soil contaminated with HMs, resulting in the secretion of various organic acids by plants [58]. At a constant N application ratio (NH_4^+-N/NO_3^--N = 3/3),

the pH decreased significantly (Table 1). This was attributed to the nitrification of NH_4^+-N and the absorption of NH_4^+-N by plants, which resulted in the release of H^+. Moreover, NH_4^+-N/NO_3^--N = 3/3 further acidifies the soil through the leaching of acid-soluble alkali ions [59,60].

The bioavailability of HMs is linked to their chemical form [61]. Acid-soluble HMs were identified as the primary forms absorbed and utilized by plants [62]. The NH_4^+-N treatment increased the acid-soluble Cd concentrations while decreasing the total concentrations of Cd and Pb in the rhizosphere soil (Table 2). In alkaline soil, a decreased pH facilitates the dissolution of acid-soluble Cd and Pb [63]. Additionally, complexes readily form between Cl^- and Cd^{2+}, thereby promoting an increase in acid-soluble Cd [64]. This ultimately leads to an increase in soluble metal ions, with more metals being taken up by plants, resulting in a decrease in soil Cd and Pb.

4.2. NH_4^+-N Promotes Plant Growth

Photosynthesis and plant traits are important indicators of the tolerance or resistance of plants to various stressors [32]. N fertilization significantly affected the photosynthesis, root morphology, and growth of *S. linearistipularis*. High N levels and separate NO_3^--N strongly inhibited *S. linearistipularis* growth, whereas this inhibitory effect was mitigated by the addition of NH_4^+-N at low and medium N levels (Figures 1 and 2). Comparable outcomes have been documented in other studies of *Solanum nigrum*, *Carpobrotus rossii*, *Oryza sativa*, and *Carpobrotus rossii* [25,33,60]. The acceleration of NH_4^+-N can be attributed to increased cell division and subsequent root branching, which consequently enhances root vitality [33]. Additionally, NH_4^+-N is directly absorbed by plants as a protein, reducing energy and photosynthetic product consumption and thereby providing more energy for plant growth [28]. Additionally, high N fertilizer application can result in metabolic imbalance, oxidative stress, lipid peroxidation, the disruption of root respiration, cell osmotic balance, and plant hormone homeostasis [65]. High N levels led to a decrease in pH and an increase in the soluble HM content (Table 2), thereby increasing toxicity to *S. linearistipularis* [66]. Additionally, the low biomass of NO_3^--N might have been due to the high BCF of HMs in the roots (Table 2), which generated large amounts of HMs, inhibited nutrient absorption, and impeded root growth, thereby causing adverse damage to plant traits [32].

A low NH_4^+-N/NO_3^--N ratio also promoted *S. linearistipularis* growth, such as NH_4^+-N/NO_3^--N = 2/4. Carbon and N fluxes were stimulated by applying NH_4^+-N and NO_3^--N concurrently and reducing the carbon loss caused by respiration, which can directly contribute to biomass accumulation [23,28]. It has also been observed that stimulated root development promotes enzyme activity, productivity, and fruit quality in peppers through a low NH_4^+-N/NO_3^--N ratio (1/3) [67]. We believe that *S. linearistipularis* prefers NH_4^+-N in isolation and low NH_4^+-N/NO_3^--N ratios. In summary, at N levels of 60 and 120 kg hm^{-2} $year^{-1}$, applying NH_4^+-N or low NH_4^+-N/NO_3^--N can alleviate HM stress and promote root growth, thereby affecting the biomass accumulation and uptake of HMs [3,68].

4.3. NH_4^+-N Increases Cd and Pb Accumulation

N application significantly affected Cd and Pb concentrations in *S. linearistipularis*. NH_4^+-N increased Cd concentrations and their accumulation in plant tissues, especially in the N_{60}-6/0 and N_{120}-6/0 treatments, where *S. linearistipularis* showed the highest Cd accumulation. The total absorption of Cd by *S. linearistipularis* was 0.6 mg pot^{-1}, which is three times and twenty times higher than that of the herbaceous plants *Amaranthus Hypochondriacus* and *Pentas lanceolata*, respectively [11,69]. Furthermore, the TF of HMs ranged from 1.6 to 3.56, approaching that of hyperaccumulator plants. Appropriate NH_4^+-N application increased the transpiration and water flow of plants (Figure A1B), facilitating the absorption of various elements, including HMs, from the soil. Chai found that NH_4^+-N competes with Cd^{2+}, expediting Cd transport from the root cell walls to the aboveground

parts, thereby promoting Cd translocation from the root to the stem (TF) [70]. Several studies have concluded that higher protein levels in NH_4^+-N-fed plant roots, resulting from N assimilation, can upregulate the expression of genes related to Cd loading and transport them in the xylem [25]. Additionally, NH_4^+-N is believed to stimulate cell division, root branching, and root elongation (Figure 1) and promote nutrient uptake and biomass production, ultimately leading to increased Cd accumulation. *S. linearistipularis*, treated with the N_{120}-6/0 and N_{60}-2/4 treatments, accumulated the highest levels of Pb. Moreover, for *S. linearistipularis*, the N_{60}-2/4 treatment may help *S. linearistipularis* relieve Pb stress and increase metal ion concentrations and plant growth, thereby improving remediation efficiency. Similar results were obtained by *Zerche*, who suggested that the N form shifts hormone levels and carbon flux, which, in turn, affects the plant form [71].

N application may increase plant Cd uptake by promoting root growth and increasing Cd and Pb bioavailability in the soil–plant system. In the present study, plant traits were the main factors promoting HM accumulation, but not the available Cd and Pb in the soil, which directly affected the nutrient absorption and health of the plants (Figure 5). NH_4^+-N is the main factor affecting plant traits and can be directly absorbed by plants, accelerating cell division, reducing energy and photosynthetic product usage, and ultimately promoting plant growth [28]. In conclusion, *S. linearistipularis* prefers NH_4^+-N, which can change root traits, increase *S. linearistipularis* biomass, and alleviate HM stress, thereby improving plant resistance and increasing total absorption. For *S. linearistipularis*, we proposed a potential fertilization strategy: N_{120}-6/0 is the most effective at improving the remediation efficiency in Cd- and Pb-contaminated soil. Further validation can be conducted through field-based, in situ experiments. However, our study specifically targeted *S. linearistipularis*, acknowledging that phytoremediation capabilities vary across willow species. The applicability of our results to the phytoremediation effectiveness of other willow types remains uncertain. Additionally, our study was limited to examining Cd and Pb as pollutants despite the fact that contaminated soil frequently comprises a mix of pollutants [1,16,36]. Furthermore, the short-term nature of our experimental design leaves the long-term impacts of N fertilizer's use on soil quality and phytoremediation effectiveness unexplored. Future investigations should delve into its combined remediation efficiency against various pollutants and prioritize longitudinal studies to thoroughly understand the persistent effects of N in phytoremediation and its environmental implications.

5. Conclusions

The levels and ratios of NH_4^+-N and NO_3^--N significantly affected the uptake of Cd and Pb by *S. linearistipularis*. The highest accumulation rates of Cd and Pb in plants were observed under the N_{120}-6/0 treatment, which increased by 104.36% and 95.23%, respectively, compared to that of the control. In addition, under the N_{120}-6/0 treatment, the stem and leaf BCF of Cd significantly increased by 28.66% and 40.11%, respectively. Furthermore, the N_{60}-2/4 treatment significantly enhanced the plant growth and remediation efficiency of HMs, resulting in an increase of 63.92% in the total Pb uptake in the whole plant compared to the control. Plant traits, rather than soil properties, were the main factors affecting Cd and Pb uptake. The levels and ratios of NH_4^+-N and NO_3^--N affect Cd and Pb accumulation by regulating plant traits. Our findings highlight the potential of the ratio of NH_4^+-N and NO_3^--N to regulate plant traits, thereby improving the phytoremediation efficiency of HMs in contaminated soil.

Author Contributions: Methodology, visualization, investigation, formal analysis, writing original draft, D.D.; software, formal analysis, methodology, S.W.; review and editing, Supervision, G.C.; data curation, J.Z. and Q.W.; resources, writing—review and editing, supervision, X.N. and D.H. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Soil properties and metal concentrations before planting.

Soil Properties	Value
pH	8.21
Soil organic matter	15.67 g kg^{-1}
Total phosphorus	185.25 mg kg^{-1}
Total potassium	6.12 g kg^{-1}
Pb concentration	210 mg kg^{-1}
Cd concentration	6.5 mg kg^{-1}

Table A2. Chemical properties of rhizosphere soil.

Nitrogen Concentratioin ($kg\ hm^{-2}\ year^{-1}$)	Ratios	SOM ($g{\cdot}kg^{-1}$)	CEC ($cmol{\cdot}Kg^{-1}$)	Ure ($mg\ g^{-1}\ d^{-1}$)	S-CAT ($ml{\cdot}g^{-1}$)	NO_3^--N ($mg\ kg^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	Cd ($mg\ kg^{-1}$)	Pb ($mg\ kg^{-1}$)
0	0	17.71 ± 1.81^{cd}	12.52 ± 0.06^{a}	1 ± 0.09^{cd}	0.94 ± 0.14^{efg}	0.64 ± 0.09^{fg}	0.24 ± 0.13^{h}	4.09 ± 0.56^{ef}	184.17 ± 11.04^{abc}
60	6/0	19.6 ± 0.4^{ab}	11.86 ± 0.83^{a}	0.91 ± 0.05^{de}	1.03 ± 0.13^{cdef}	1.47 ± 0.1^{efg}	0.37 ± 0.07^{gh}	2.65 ± 0.17^{g}	132.24 ± 19.28^{fgh}
	4/2	19.42 ± 0.66^{ab}	10.46 ± 0.28^{a}	0.73 ± 0.07^{f}	0.77 ± 0.12^{g}	5.08 ± 0.45^{e}	0.31 ± 0.07^{gh}	2.77 ± 0.15^{g}	121.61 ± 23.95^{ghi}
	3/3	18.56 ± 0.8^{bc}	11.52 ± 1.25^{a}	0.89 ± 0.08^{e}	1.09 ± 0.08^{bcde}	2.75 ± 0.19^{efg}	0.56 ± 0.09^{de}	2.71 ± 0.24^{g}	159.21 ± 10.82^{de}
	2/4	17.35 ± 0.35^{de}	11.52 ± 0.58^{a}	0.99 ± 0.04^{cd}	0.96 ± 0.1^{defg}	10.59 ± 1.14^{d}	0.4 ± 0.05^{fg}	3.06 ± 0.42^{g}	136.74 ± 9.19^{efg}
	0/6	19.07 ± 0.66^{ab}	12.19 ± 0.69^{a}	0.97 ± 0.05^{de}	1.03 ± 0.16^{cdef}	11.76 ± 0.92^{d}	0.65 ± 0.06^{cd}	4.29 ± 0.46^{de}	110.34 ± 18.48^{hi}
120	6/0	16.32 ± 0.35^{e}	11.03 ± 1.45^{a}	0.91 ± 0.08^{de}	0.87 ± 0.04^{fg}	0.35 ± 0.03^{g}	0.33 ± 0.05^{gh}	4.74 ± 0.44^{cd}	103.24 ± 12.79^{i}
	4/2	18.56 ± 0.01^{bc}	11.87 ± 0.79^{a}	1.19 ± 0.05^{a}	0.96 ± 0.11^{defg}	19.91 ± 1.88^{c}	0.52 ± 0.05^{ef}	5.14 ± 0.16^{bc}	125.9 ± 10.48^{ghi}
	3/3	18.55 ± 0.68^{bc}	12.55 ± 1.1^{a}	1.16 ± 0.08^{a}	0.95 ± 0.07^{defg}	22.71 ± 0.86^{c}	0.31 ± 0.06^{gh}	4.54 ± 0.45^{cde}	102.26 ± 18.05^{i}
	2/4	18.73 ± 0.66^{bc}	12.18 ± 0.99^{a}	1.16 ± 0.06^{a}	1.18 ± 0.09^{abc}	36.41 ± 4.92^{b}	0.77 ± 0.14^{b}	4.57 ± 0.48^{cde}	135.74 ± 12.25^{efg}
	0/6	19.41 ± 0.35^{ab}	12.43 ± 0.86^{a}	1.15 ± 0.04^{a}	1.34 ± 0.08^{a}	23.04 ± 3.49^{c}	0.98 ± 0.08^{a}	3.64 ± 0.35^{f}	155.35 ± 15^{def}
200	6/0	19.25 ± 0.57^{ab}	11.55 ± 0.72^{a}	1.06 ± 0.06^{bc}	0.91 ± 0.07^{efg}	4.47 ± 0.52^{ef}	0.33 ± 0.06^{gh}	4.33 ± 0.46^{de}	132.97 ± 14.31^{fgh}
	4/2	20.16 ± 0.4^{a}	11.98 ± 1.75^{a}	1.13 ± 0.03^{ab}	1.11 ± 0.14^{bcde}	39.46 ± 2.76^{b}	0.72 ± 0.08^{bc}	5.55 ± 0.48^{ab}	194.67 ± 12.03^{ab}
	3/3	18.79 ± 0.79^{bc}	10.78 ± 0.4^{a}	1.18 ± 0.02^{a}	1.28 ± 0.14^{ab}	14.09 ± 1.68^{d}	1.35 ± 0.12^{a}	5.09 ± 0.33^{bc}	173.13 ± 15.84^{bcd}
	2/4	18.73 ± 0.86^{bc}	12.55 ± 0.83^{a}	1.16 ± 0.04^{a}	1.11 ± 0.1^{bcde}	58.17 ± 5.38^{a}	0.52 ± 0.04^{def}	5.62 ± 0.17^{ab}	168.91 ± 19.3^{cd}
	0/6	20.15 ± 0.39^{a}	12.74 ± 0.79^{a}	1.16 ± 0.04^{a}	1.15 ± 0.16^{abcd}	38.59 ± 2.26^{b}	1.05 ± 0.04^{a}	6.09 ± 0.62^{a}	201.92 ± 16.63^{a}
L		**	ns	**	**	**	**	**	**
R		**	ns	**	**	**	**	**	**
L × R		**	ns	**	**	**	**	**	**

Note: All data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's test for comparison with the controls. N levels are denoted as "L", the N ratio as "R", and the interactive effect of N levels and ratios as "L × R". Different superscript lowercase letters indicate significant differences among the treatments at $p < 0.05$ in specific tissues. Data represent means ± SD ($n = 4$), "ns" denotes non-significance, and double asterisks (**) represent significant differences at $p < 0.01$.

Table A3. TF of Cd and Pb.

Nitrogen Level ($kg\ hm^{-2}\ year^{-1}$)	Ratios	Cd		Pb	
		TF of Stems	TF of Leaves	TF of Stems	TF of Leaves
0	0	1.6 ± 0.21^{abc}	3.59 ± 0.4^{abc}	1.11 ± 0.14^{bcd}	0.78 ± 0.04^{bcdef}
60	6/0	1.35 ± 0.41^{abcd}	3.86 ± 0.24^{abc}	1.23 ± 0.24^{abc}	1.04 ± 0.24^{ab}
	4/2	1.7 ± 0.34^{ab}	4.62 ± 0.75^{a}	1.33 ± 0.01^{ab}	1.05 ± 0.18^{ab}
	3/3	1.21 ± 0.32^{cde}	2.86 ± 1.09^{cde}	1.54 ± 0.5^{a}	1.15 ± 0.24^{a}
	2/4	1.79 ± 0.34^{a}	4.08 ± 1.26^{ab}	0.74 ± 0.06^{ef}	0.51 ± 0.16^{fg}
	0/6	1.18 ± 0.32^{cde}	1.86 ± 0.56^{ef}	0.66 ± 0.12^{f}	0.47 ± 0.11^{g}
120	6/0	1.25 ± 0.27^{bcde}	3.08 ± 0.58^{bcd}	1.03 ± 0.11^{bcde}	0.71 ± 0.1^{cdefg}
	4/2	0.89 ± 0.07^{def}	2.11 ± 0.3^{def}	0.72 ± 0.09^{ef}	0.47 ± 0.08^{g}
	3/3	1.12 ± 0.14^{def}	2.23 ± 0.33^{def}	0.76 ± 0.09^{def}	0.54 ± 0.05^{efg}
	2/4	0.99 ± 0.16^{def}	1.29 ± 0.1^{f}	0.92 ± 0.08^{cdef}	0.81 ± 0.08^{bcde}
	0/6	1.08 ± 0.26^{def}	1.84 ± 0.56^{ef}	0.65 ± 0.3^{f}	0.63 ± 0.27^{defg}

Table A3. *Cont.*

Nitrogen Level (kg hm^{-2} year^{-1})	Ratios	Cd		Pb	
		TF of Stems	TF of Leaves	TF of Stems	TF of Leaves
200	6/0	$1 \pm 0.02^{\text{def}}$	$1.61 \pm 0.19^{\text{f}}$	$0.63 \pm 0.05^{\text{f}}$	$0.61 \pm 0.04^{\text{defg}}$
	4/2	$0.68 \pm 0.09^{\text{f}}$	$1.03 \pm 0.13^{\text{f}}$	$0.61 \pm 0.06^{\text{f}}$	$0.56 \pm 0.06^{\text{efg}}$
	3/3	$0.65 \pm 0.01^{\text{f}}$	$1.58 \pm 0.03^{\text{f}}$	$0.65 \pm 0.01^{\text{f}}$	$0.88 \pm 0.06^{\text{bcd}}$
	2/4	$0.89 \pm 0.18^{\text{def}}$	$3.58 \pm 0.78^{\text{abc}}$	$0.87 \pm 0.16^{\text{def}}$	$0.91 \pm 0.05^{\text{abc}}$
	0/6	$0.85 \pm 0.12^{\text{ef}}$	$1.84 \pm 0.32^{\text{ef}}$	$0.69 \pm 0.16^{\text{ef}}$	$0.65 \pm 0.05^{\text{cdefg}}$
L	**	**	**	**	**
R	ns	ns	**	ns	ns
L × R	**	ns	**	ns	**

Note: All data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's test for comparison with the controls. N levels are denoted as "L", the N ratio as "R", and the interactive effect of N levels and ratios as "L × R". Different superscript lowercase letters indicate significant differences among the treatments at $p < 0.05$ in specific tissues. Data represent means ± SD ($n = 4$), "ns" denotes non-significance, and double asterisks (**) represent significant differences at $p < 0.01$.

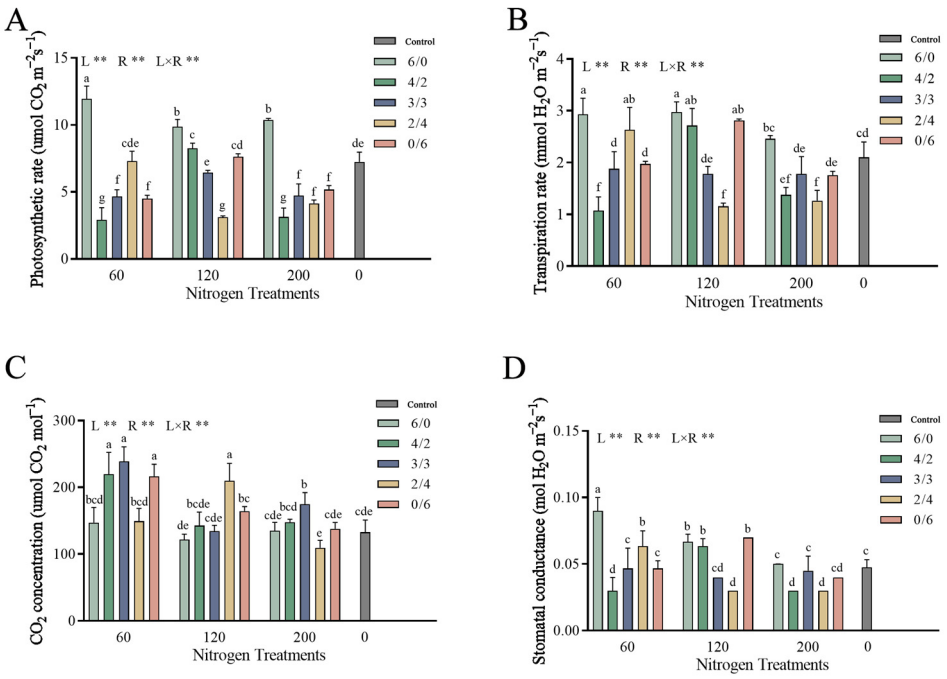


Figure A1. Leaf gas exchange characteristics. Note: All data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's test for comparison with the controls. N levels are denoted as "L", the N ratio as "R", and the interactive effect of N levels and ratios as "L × R". Different superscript lowercase letters indicate significant differences among the treatments at $p < 0.05$ in specific tissues. Data represent means ± SD ($n = 4$), double asterisks (**) represent significant differences at $p < 0.01$.

Figure A2. Root phenotype.

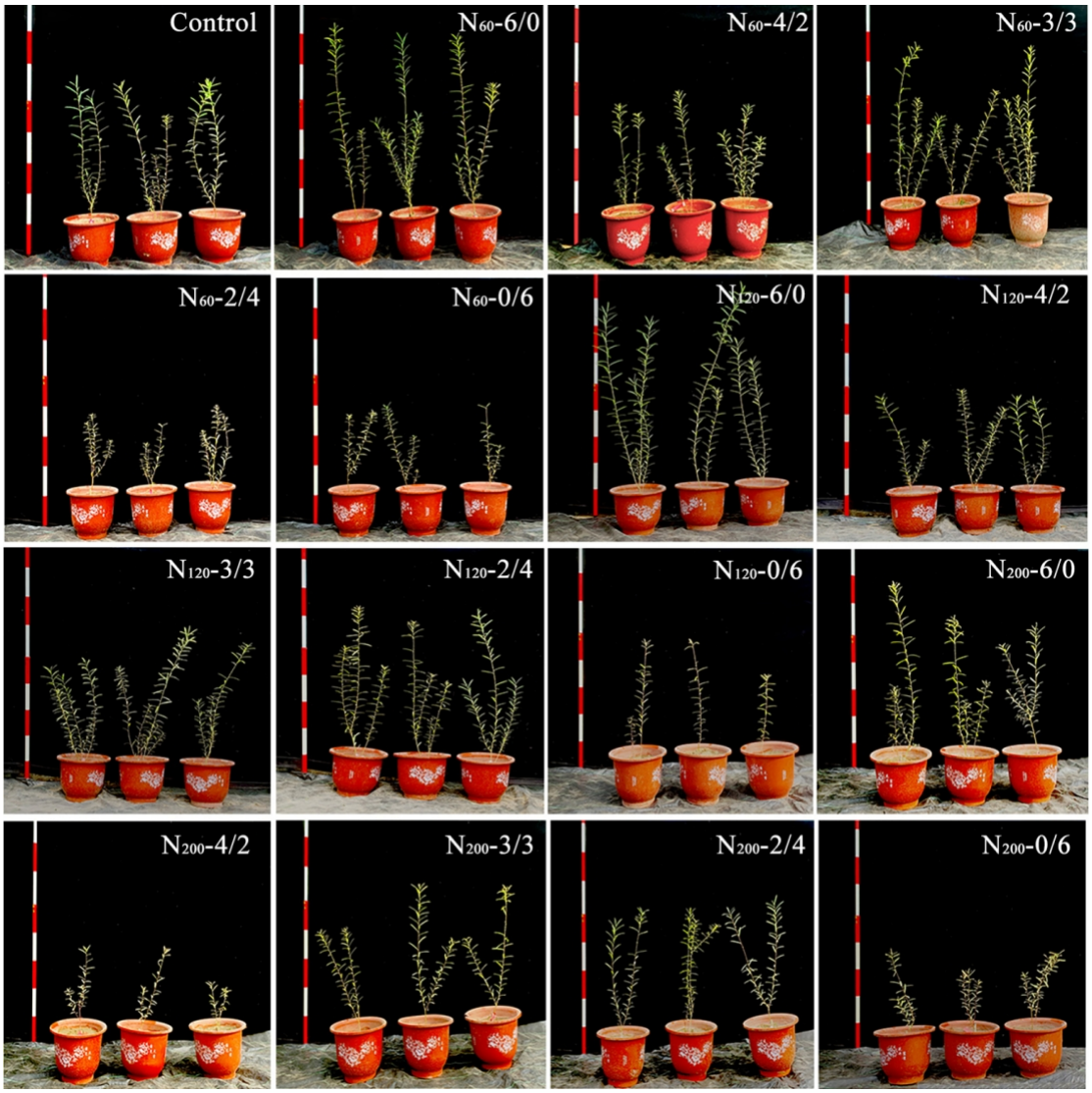
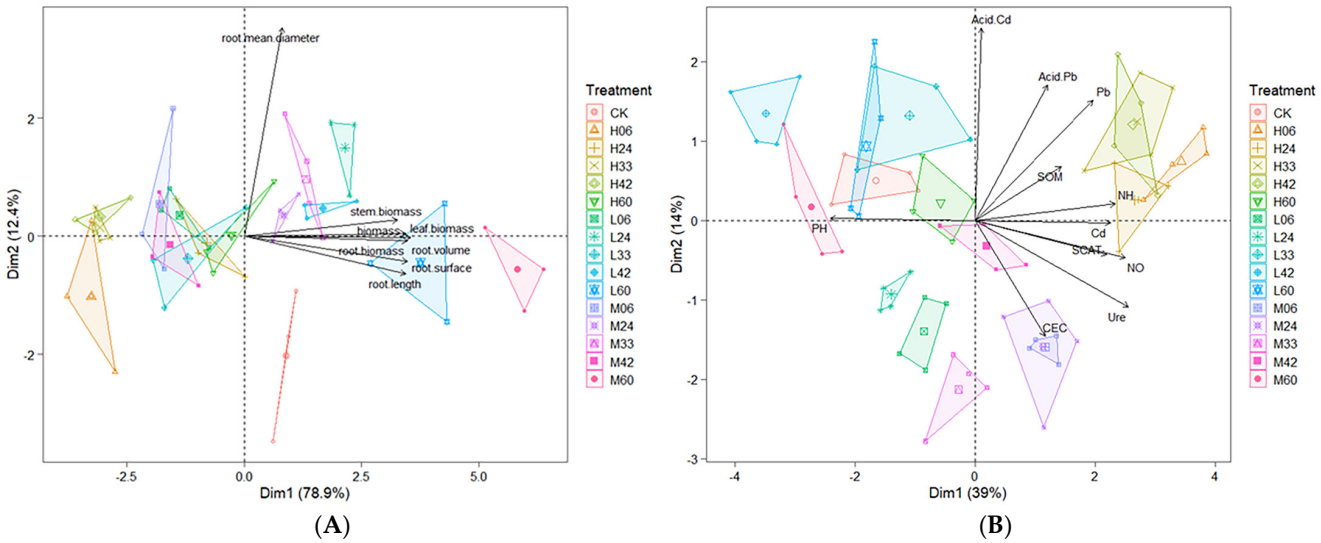


Figure A3. Plant growth status.



(**A**) (**B**)

Figure A4. PCA of plant traits (**A**) and soil properties (**B**).

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