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Magnetic Treatment Improves the Seedling Growth, Nitrogen Metabolism, and Mineral Nutrient Contents in *Populus* × *euramericana* 'Neva' under Cadmium Stress

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Abstract: This pot experiment was carried out to investigate the mechanism underlying nutrient metabolism and seedling growth responses to magnetic treatment following exposure to cadmium (Cd) stress. A magnetic device of 300 Gs was applied during $Cd(NO_3)_2$ solution treatment at 0 and 100 mM·L^{-1}. One-year-old seedlings of *Populus* × *euramericana* 'Neva' were treated with different $Cd(NO_3)_2$ solutions in the presence or absence of magnetic treatment for 30 days. Seedling growth and physiological–biochemical indexes were measured under Cd stress. The contents of ammonium (NH_4^+–N), nitrate (NO_3^-–N), and total nitrogen (TN) in leaves, as well as NH_4^+–N and TN in roots, were increased by magnetic treatment combined with Cd stress, although the NO_3^-–N content was decreased. The activities of nitrate reductase (NR), nitrite reductase (NiR), glutathione reductase (GR), and glutamate synthase (GOGAT) in leaves and the activities of NR, glutamine synthetase (GS), and GOGAT in roots were stimulated by magnetic treatment; conversely, the NiR activity in roots was inhibited by magnetic effects. Magnetic treatment improved the synthesis of cysteine (Cys) and glutamine (Gln) in leaves and reduced the contents of glutamic acid (Glu) and glycine (Gly), while the contents of Cys, Glu, Gln, and Gly were increased in roots. The contents of Ca, Mg, Fe, Mn, Zn, and Cu in leaves were increased by magnetic treatment under Cd stress, whereas the content of K was reduced. In roots, the contents of K, Ca, and Fe were increased by magnetic treatment under Cd stress, but the contents of Na, Mg, Mn, Zn, and Cu were decreased. Magnetization could regulate the uptake of mineral nutrients by roots and translocation from the roots to the aboveground parts by affecting root morphology. Magnetic treatment could also improve nitrogen assimilation and the synthesis of free amino acids by stimulating the activities of key enzymes.

Keywords: cadmium stress; magnetization; enzyme activities; mineral nutrient; free amino acids

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1. Introduction

Cadmium (Cd) is a widely distributed heavy metal with potential toxicity and strong mobility in the environment [1]. Although it is easily absorbed by plants, Cd is a nonessential element for plant growth, and even very low concentrations can alter a variety of physiological processes in plants [2]. More importantly, lower Cd contents present in soil can led to decreases in grain yield, otherwise, continuous consumption of crops grains will result in serious long-term health problems [3]. The accumulation of Cd in plant tissues induces oxidative stress through the production of superoxide anions (O^{2-}), hydrogen

peroxide (H_2O_2), malondialdehyde (MDA), and reactive oxygen species [4,5]. Hence, Cd exposure can lead to lipid peroxidation, impact the composition and fluidity of the cell membrane, degrade protein and nucleic acid structures [6,7], change the antioxidative defense mechanism, and impact the endogenous phytohormones contents [8]. At the same time, long-term accumulated Cd concentrations cause alterations in the structure and function of organelles, and results in diverse phytotoxicity symptoms [9]. Thereby, Cd toxicity induces the suppression of a plant's photosynthesis and respiratory processes in the chloroplasts and mitochondria, reducing the activity of key enzymes [10] such as ascorbate peroxidase, catalase, and peroxidase [11], and interfering with the direct interaction of key amino acid residues [12].

Cd stress reduces leaf relative water content [13], while it also impedes the absorption, translocation, and distribution effectivity of mineral nutrients in plants [14,15], as the amino acid contents in crops and grains of different Cd-tolerant varieties may fluctuate [16]. The presence of Cd can alter the ecological processes in the soil, affect the availability of mineral nutrients, change the utilization of nutrients, and lead to a vicious cycle in plants [17]. Nonetheless, the results reported to date are inconsistent due to differences in research materials, treatment methods, and Cd concentrations, and the mechanism by which Cd stress affects plant nutrients has not yet been clarified. Up until now, the physical, chemical, and biological measures are used for pollution repairing caused by Cd, and overall, methods for alleviating the growth damage resulting from Cd stress in plants are of great significance.

Water can be magnetized by flowing it through a magnetic device. Some of the physical and chemical properties of magnetically treated water, such as its dielectric constant, polarity, surface tension force, conductivity, electric conductivity, and salt dissolution, are different from those of pure water [18]. The response of biological systems to the magnetic treatment of water has been reported, and both the activation of ions and the polarization of dipoles in living cells are influenced by magnetic fields. Some positive results have been documented with regard to the growth and output of crops, including irrigation with low-quality water treated by a magnetic device [18]. Accordingly, the magnetic treatment of water, even the low-quality water used for agroforestry, may be feasible because it is affordable, convenient, and nonpolluting to plants, soil, and the environment [19].

Previously reported results indicated that magnetic treatment could promote seed germination, enhance stress resistance, and increase crop output and quality. First, magnetized water could enhance cellular metabolism and viability, thus promoting seed germination. For example, Morejón et al. [20] discovered that irrigating *Pinus tropicalis* seeds with magnetized water increased the germination rate and significantly enhanced seedling growth. Afzal et al. [21] revealed that treatment with magnetized water not only elevated the emergence, the seed biochemical activities, and the crop growth rate of sunflower seeds, but also promoted the crop growth rate and yield. Second, magnetized water can increase the permeability of biological membranes, the absorption of nutrients by plants, and the resistance of crops. Grewal and Maheshwari [22] showed that magnetic treatment enhanced the biomass of snow peas (*Pisum sativum* L var. *macrocarpon*) and Cabrera chickpea (*Cicer arietinum* L.) by 20%–25%, and the absorption of N, K, Ca, Mg, S, Na, Zn, Fe, and Mn was also promoted. Yao et al. [23] reported that damage to cucumber (*Cucumis sativus*) seedlings subjected to ultraviolet-B (UV-B) stress was alleviated by magnetic treatment. Finally, magnetization changes the pH, conductivity, and hardness of water, thereby promoting the dissolution of inorganic salts. Khoshravesh-Miangoleh and Kiani [24] demonstrated that soil density was effectively regulated by irrigation with magnetized water, whereas the cumulative infiltration and infiltration rates were increased. In addition, Mostafazadeh-Fard et al. [25] showed that the leaching of Cl^-, HCO_3^-, and Na^+ was promoted by long-term irrigation with magnetized water, which significantly reduced secondary soil salinization. In conclusion, all these results could provide a theoretical basis for applying the magnetic treatment of water to improve stress tolerance in plants and promote ecological restoration of the soil.

Few previous reports have focused on the responses of plants to stress conditions under magnetization and changes in the tolerance of the plants to Cd stress following exposure to a magnetic field. Liu et al. [26] found that magnetized water reduced oxidative stress by increasing the activity of superoxide dismutase (SOD) and catalase (CAT) in maize (*Zea mays*) under Cd stress. Chen et al. [27] reported that the contents of H_2O_2, O^{2-}, and malondialdehyde (MDA), as well as electrolyte leakage, were reduced by magnetic treatment in mung bean (*Vigna radiata*) seedlings under Cd exposure due to increased nitric oxide synthase (NOS) activity and improved photosynthetic characteristics. Overall, these studies have preliminarily demonstrated the possibility of reducing Cd-induced damage to plants using magnetized water, thus improving seedling growth and growing conditions. Furthermore, magnetic techniques may be applied to study the ecological restoration of Cd-contaminated soil, aiming to elevate the efficiency of Cd enrichment in plants. Therefore, the hybrid species *Populus* × *euramericana* 'Neva' (Neva hereafter) was used as the experimental material in our study. To study the feasibility of cultivating tree species in Cd-contaminated soil, we explored the possible mechanisms by which the metabolic nutrition and biological characteristics of Neva affected by Cd toxicity determine the influence of magnetic treatment of irrigation water.

2. Materials and Methods

2.1. Study Area

A pot experiment was carried out in the greenhouse at the Forestry Research Station of Shandong Agricultural University (117°08′ E, 36°11′ N) from March to May 2017. Glasshouse experiments were conducted with natural light, day and night temperatures of 20–25 °C, a relative humidity of 60–70%, and a 12-h photoperiod at 800–1000 μmol photons·m^{-2}·s^{-1} of photosynthetically active radiation per day of natural light throughout the study period.

2.2. Plant Materials

Neva is a cultivated species. It is not an endangered species. In the study, annual branches are generally used for cuttage propagation, and the experimental materials consisted of one-year-old seedlings of Neva collected from a seedling nursery garden. The sampling location belongs to the Gaoqiao state-owed forestry farm of Ningyang County, Shandong Province, China. The plant material used in this study was collected under the permission from the Gaoqiao state-owned forestry farm. In late March, the mid-stems of seedlings with a diameter of 1.52 ± 0.11 cm and a length of 12.0 cm were cut and planted in a nutrition bag (20 cm × 22 cm). The cultivation substrate was perlite, and there were two cuttings per bag. During the early stage, unified water management was performed. After germination, modified half-strength Hoagland nutrient solution was applied to irrigate the poplar seedlings. In early April, seedlings with similar development (15 cm in height with six leaves) were selected and further cultivated in half-strength modified Hoagland solution containing 0 or 100 μmol·L^{-1} $Cd(NO_3)_2$ for four weeks.

2.3. Experimental Design

A magnetic treatment device supplied by Magnetic Technologies L.C.C. (Russia, United Arab Emirates branch) with a permanent magnet was used. The device (U050 mg, 0.5 inch, output 4–6 m^3·h^{-1}) supplying a magnetic induction of approximately 300 Gs was used for treating the water.

The main irrigation strategy was initiated in early April. The potted seedlings were irrigated with half-strength Hoagland solution containing 0 and 100 μmol·L^{-1} $Cd(NO_3)_2$ solution in the presence or absence of magnetic treatment. The pots were divided into four experimental groups:

(1) (–MF, –$Cd(NO_3)_2$)—plants grown with half-strength modified Hoagland solution that were not subjected to magnetic treatment (NM_0).

(2) (+MF, –$Cd(NO_3)_2$)—plants grown with half-strength modified Hoagland solution that were subjected to magnetic treatment (M_0).
(3) (–MF, +$Cd(NO_3)_2$)—plants grown with half-strength modified Hoagland solution containing 100 μmol·L^{-1} $Cd(NO_3)_2$ that were not subjected to magnetic treatment (NM_{100}).
(4) (+MF, –$Cd(NO_3)_2$)—plants grown with half-strength modified Hoagland solution containing 100 μmol·L^{-1} $Cd(NO_3)_2$ that were subjected to magnetic treatment (M_{100}).

Half of the pots were irrigated with magnetized nutrient solution, and both groups were maintained with optimal substrate watering with irrigation every five days. Young leaves and fine roots were collected after 30 days, rinsed with deionized water, and stored at −80 °C for later testing.

2.4. Determination of Biochemical and Physiological Characteristics

2.4.1. Growth Character Analysis

For the measurement of seedling growth, each sample was gently uprooted, carefully washed under running tap water and deionized water, and then divided into leaves and roots. The individual ground diameters of the seedlings were measured using Vernier calipers, and the data were recorded. The length, surface area, diameter of roots, and number of root tips were determined using a root analysis system (WinRHIZO Pro 2007, Regent Instruments, Quebec, QC, Canada). A portable leaf area meter (CI–202; CID Bio-Science, Inc., Camas, WA, USA) was employed to analyze the leaf area.

2.4.2. Tissue Nitrogen Content Measurement

Fine roots and leaves were rinsed with deionized water, and 0.5 g of fresh sample was used to determine the ammonium nitrogen (NH_4^+–N) content using the salicylic acid–sulfuric acid (SA–H_2O_2) method, as described by Zhao and Cang [28]. Afterwards, the fresh samples were dried at 80 °C to a constant weight and 2.0 g of dry leaves and roots was used to measure the nitrate nitrogen (NO_3^-–N) content using the colorimetric method of Xu [29] based on ninhydrin. The total nitrogen (TN) content was measured using the Kjeldahl method.

2.4.3. Tissue Ion Content Measurement

Dried samples (0.1 g) were digested in a H_2SO_4–H_2O_2 solution, and the extract was used to determine elemental contents. The contents of K, Ca, Na, Mg, Fe, Mn, Zn, and Cu were measured using an atomic absorption spectrophotometer (TU–1900, Pgeneral Ltd., Peking, China) [30].

2.4.4. Enzyme Activity Determination

The activities of nitrate reductase (NR) and nitrite reductase (NiR) were assessed in accordance with the method described by Kandeler et al. [16]. Glutamine synthetase (GS) activity was determined using hydroxylamine as a substrate, and γ-glutamylhydroxamate (γ-GHM) formation was evaluated using acidified ferric chloride in accordance with the assay described by Loyala-Vergas and De Jimenez [24]. Glutamate synthase (GOGAT) was assessed through NADH oxidation and spectrophotometric measurement at 340 nm, as described by Barbosa et al. [31].

2.4.5. Amino Acid Content Estimation

Fresh roots and leaves (0.5 g) were used to determine the contents of cysteine (Cys), glutamate (Glu), glutamine (Gln), and glycine (Gly) by high-performance liquid chromatography (HPLC) [32].

2.4.6. Photosynthetic Pigment Observation

The photosynthetic pigments chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) in 5 g of fresh leaves of each sample were extracted in 5 mL of 80% acetone, and

absorbance was measured spectrophotometrically at 663, 646, and 450 nm using a TU–1900 instrument according to the method described by Singh et al. [33].

2.5. Statistical Analysis

The results for all treatments were obtained using at least three replicates. Data were statistically analyzed with SAS software (Version 8.0; SAS Institute, Cary, NC, USA). Homogeneity of variance among means was examined using one-way analysis of variance (ANOVA) with Duncan's multiple test at an alpha level of 0.05.

3. Results

3.1. Plant Growth

Compared with the M_0 and NM_0 treatments (Figure 1), the M_{100} and NM_{100} treatments showed the following changes: leaf biomass decreased by 19.72% and 33.46% (Figure 1A), respectively, root biomass decreased by 26.46% and 38.32% (Figure 1B), respectively, and leaf area significantly ($p < 0.05$) decreased by 32.83% and 29.18% (Figure 1C), respectively. Under the NM_{100} treatment, the decrease in the root biomass was the most significant, followed by the decrease in the leaf biomass. In the M_0 and NM_0 treatments, the number of new root tips was significantly enhanced by 19.79% and 20.13%, respectively ($p < 0.05$). In the M_{100} treatment, root biomass increased the most, followed by leaf biomass. In the M_0 and NM_0 treatments, the leaf area of the plants increased significantly ($p < 0.05$) at rates of 33.23% and 26.37%, respectively.

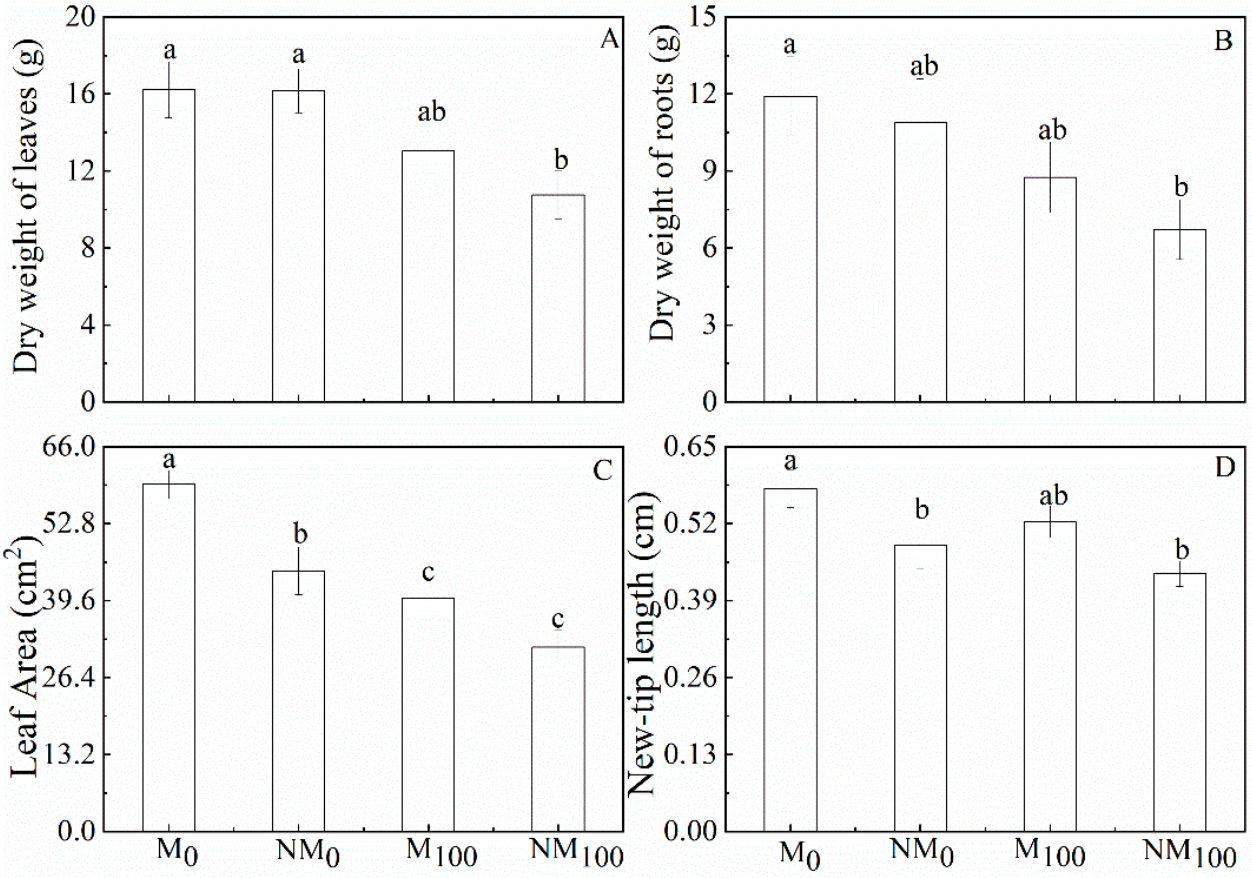

Figure 1. Changes in the biomass (dry weight) of leaves (**A**), roots (**B**), leaf area (**C**), and length of the new tip (**D**) in Neva irrigated with or without magnetized water following exposure to 0 or 100 μmol·L^{-1} cadmium stress. Values are the means ± *SE* of at least three replicates. Different lowercase letters indicate significant differences among the four treatments at the 0.05 level.

3.2. Photosynthetic Pigment Contents

The comparison of the M_0 and NM_0 treatments revealed decreases in the contents of Chl a (Figure 2A), Chl b (Figure 2B), and Car (Figure 2C) under Cd stress. Specifically, compared with those in the M_0 treatment, the contents of Chl a, Chl b, and Car in the M_{100} treatment decreased by 38.65%, 34.74%, and 31.11%, respectively ($p < 0.05$). Compared with those in NM_0, the contents of Chl a, Chl b, and Car in NM_{100} decreased by 9.27%, 18.54%, and 20.57%, respectively ($p < 0.05$). In a comparison of NM_0 and NM_{100}, we found that magnetic treatment stimulated the synthesis of photosynthetic pigments. Specifically, compared with those in NM_0, the contents of Chl a, Chl b, and Car in M_0 increased by 51.26%, 46.25%, and 35.48%, respectively ($p < 0.05$). Additionally, compared with those under NM_{100}, the photosynthetic pigment contents under M_{100} improved by 2.28%, 17.17%, and 17.50%, respectively ($p < 0.05$).

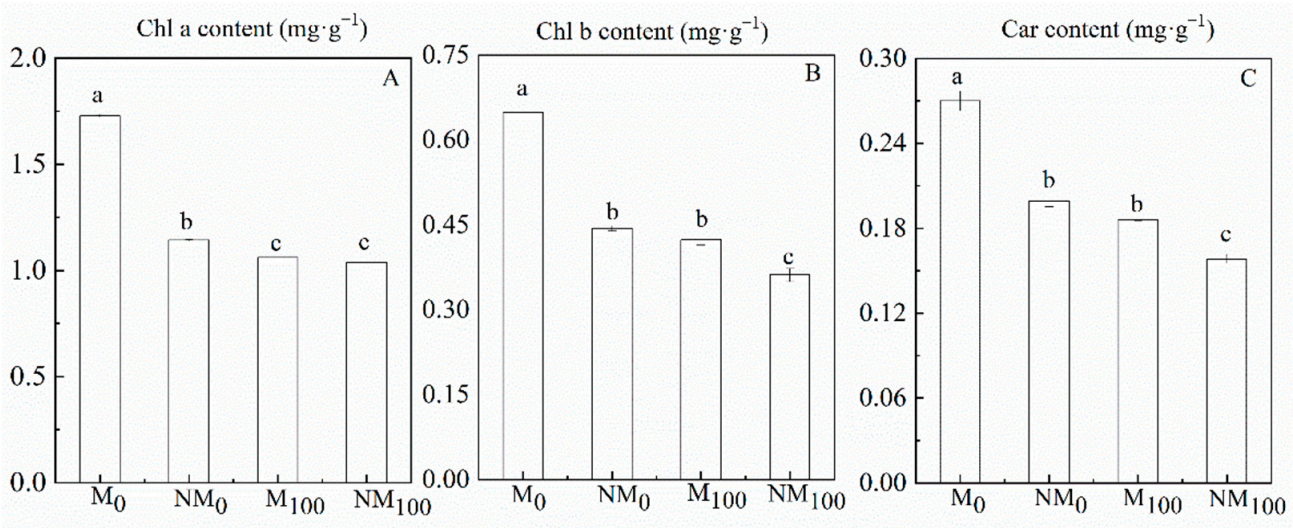

Figure 2. Changes in the contents of chlorophyll a (Chl a, (**A**)), chlorophyll b (Chl b, (**B**)), and carotenoids (Car, (**C**)) in leaves of Neva irrigated with or without magnetized water following exposure to 0 or 100 μmol·L^{-1} cadmium stress. Values are the means ± *SE* of at least three replicates. Different lowercase letters indicate significant differences among the four treatments at the 0.05 level.

3.3. NH_4^+–N, NO_3^-–N, and TN Contents

Compared with those in M_0, the NH_4^+–N, NO_3^-–N, and TN contents in M_{100} decreased by 64.04%, 31.25%, and 27.90%, respectively, in leaves (Figure 3A–C $p < 0.05$). Moreover, the NH_4^+–N, NO_3^-–N, and TN contents in the NM_{100} treatment decreased by 61.32%, 18.41%, and 25.80%, respectively, compared with those in the NM_0 treatment ($p < 0.05$). Magnetic treatment was also beneficial for nitrogen accumulation in leaves. Compared with those in NM_0, the contents of NH_4^+–N and NO_3^-–N in M_0 increased by 65.08% and 50.49%, respectively ($p < 0.05$). Furthermore, the NH_4^+–N and NO_3^-–N contents in the M_{100} treatment increased by 53.48% and 26.81%, respectively, compared with those in the NM_{100} treatment ($p < 0.05$).

However, nitrogen accumulation in roots showed a slightly different pattern (Figure 3). Compared with those in the M_0 and NM_0 treatments, the contents of NH_4^+–N and NO_3^-–N in the M_{100} treatment decreased significantly ($p < 0.05$) by 11.19% and 75.07%, respectively, and those in the NM_{100} treatment decreased by 28.69% and 10.74%, respectively. Compared with the NM_0 and NM_{100} treatments, the M_0 and M_{100} treatments caused an increase in the accumulation of NH_4^+–N and TN in the roots, with NH_4^+–N increasing significantly ($p < 0.05$). The percent increases in TN content in the M_{100} and NM_{100} treatments were relatively small, at 20.32% and 51.43%, respectively ($p < 0.05$). The NO_3^-–N content in roots decreased under magnetic treatment (in NM_{100}) (Figure 3B) by 26.62% ($p < 0.05$).

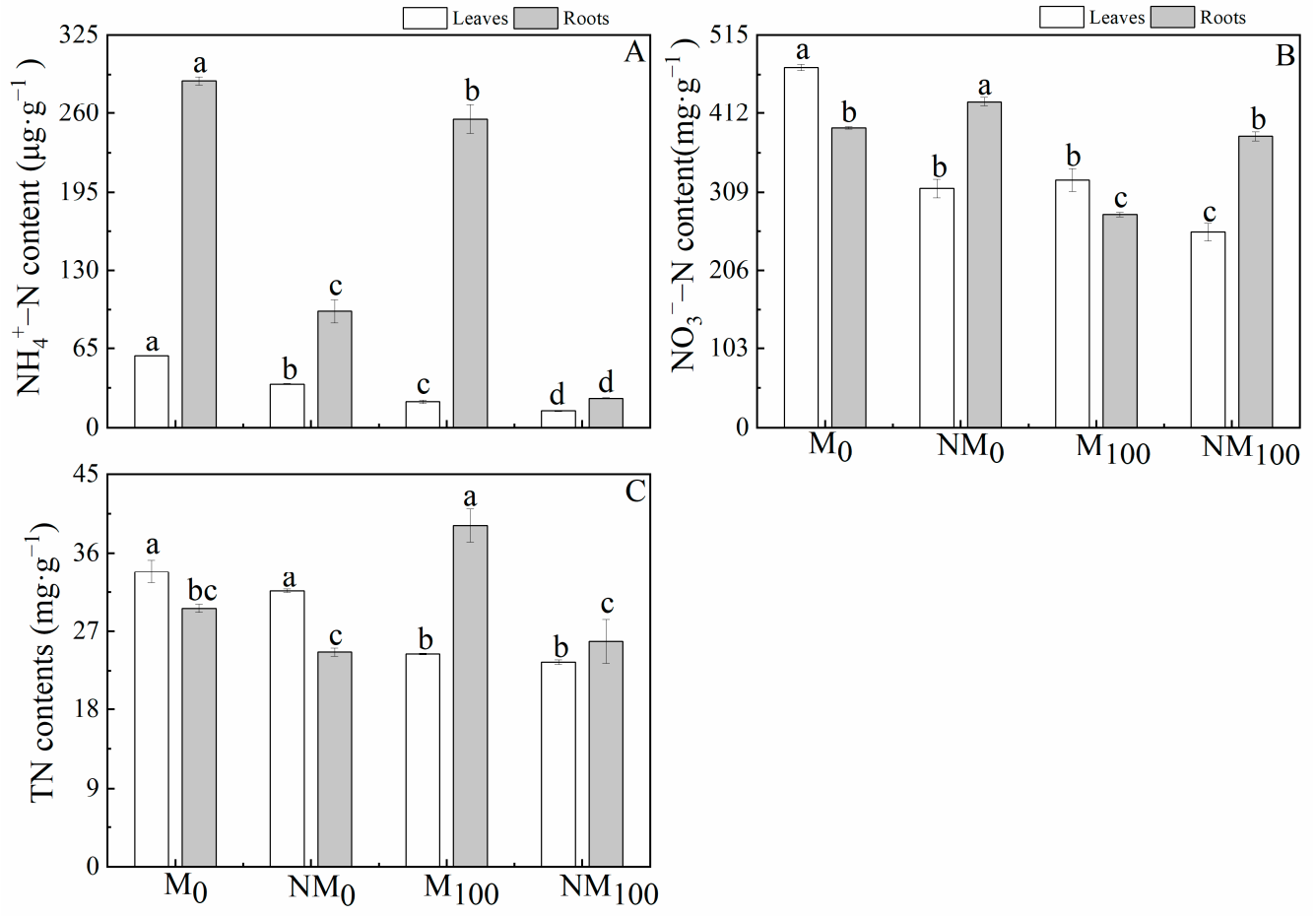

Figure 3. Changes in the contents of ammonium nitrogen (NH_4^+–N, (**A**)), nitrate nitrogen (NO_3^-–N, (**B**)), and total nitrogen (TN, (**C**)) in leaves and roots of Neva irrigated with or without magnetized water following exposure to 0 or 100 μmol·L^{-1} cadmium stress. Values are the means ± *SE* of at least three replicates. Different lowercase letters indicate significant differences among the four treatments at the 0.05 level in leaves ad roots.

3.4. Activities of Key Enzymes in Nitrogen Metabolism

Compared with the control treatments of M_0 and NM_0 (Figure 4), Cd significantly stimulated NR activity in leaves (Figure 4A), increasing it by 67.43% in the NM_{100} treatment. GS activities increased in the M_{100} treatment by 9.09% (Figure 4C), and GOGAT activities increased in the M_{100} and NM_{100} treatments by 16.69% and 9.23%, respectively (Figure 4D) ($p < 0.05$). When comparing the NM_0 and NM_{100} treatments, the NR, GS, and GOGAT activities increased by 52.47%, 16.69%, and 19.28%, respectively ($p < 0.05$). Compared with those in NM_{100}, the activities of NiR, GS, and GOGAT in M_{100} increased by 10.39%, 22.55%, and 27.43%, respectively ($p < 0.05$).

Nitrogen metabolism enzymes also responded differently to Cd stress in roots (Figure 4). Compared with the M_0 and NM_0 treatments, Cd stress had the most stimulating effect on the activity of NR, with the largest average percent increase of 29.00%. NR and GS activities were significantly different between treatments ($p < 0.05$). Compared with those in NM_0, the activities of GS and GOGAT in M0 increased by 43.06% and 4.81%, respectively ($p < 0.05$). Furthermore, the NR and GS activities in M_{100} increased by 17.98% and 61.28%, respectively, compared with those in NM_{100} ($p < 0.05$). Therefore, magnetic treatment enhanced GS activity, which showed the largest average increase of 52.17%.

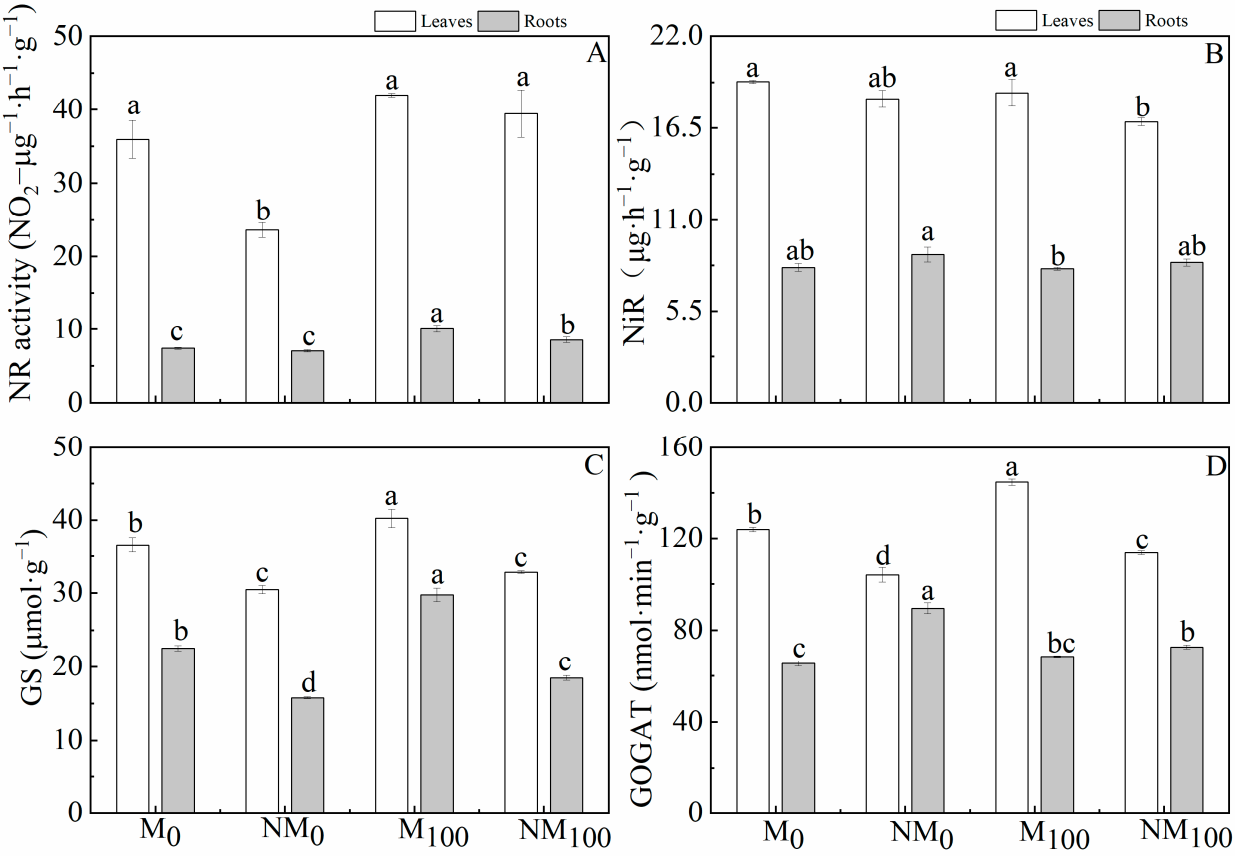

Figure 4. Changes in the activities of nitrate reductase (NR, (**A**)), nitrite reductase (NiR, (**B**)), glutamine synthetase (GS, (**C**)), and glutamate synthase (GOGAT, (**D**)) in leaves and roots of Neva irrigated with or without magnetized water following exposure to 0 or 100 μmol·L^{-1} cadmium stress. Values are the means ± *SE* of at least three replicates. Different lowercase letters indicate significant differences among the four treatments at the 0.05 level in leaves and roots.

3.5. Free Amino Acid Contents

The analysis of Cys, Glu, Gln, and Gly levels in leaves (Figure 5) revealed that the contents of Cys, Gln, and Gly in the M_{100} treatment decreased by 24.69%, 34.32%, and 33.89%, respectively, compared with those in the M_0 treatment ($p < 0.05$). Compared with those in NM_0, the contents of Cys, Gln, and Gly in NM_{100} decreased significantly ($p < 0.05$) by 20.97%, 32.27%, and 88.46%, respectively. Cd stress was beneficial for Glu synthesis (Figure 5B), which increased by 200.05% in M_{100} compared with M_0 and increased by 4.53% in NM_{100} compared with NM_0 ($p < 0.05$). Compared with those in NM_0 and NM_{100}, the contents of Cys and Gln in M_0 increased by 109.74% and 73.58%, and those in M_{100} increased by 99.88% and 11.83%, respectively ($p < 0.05$). Compared with those in NM_0, the Glu and Gly contents in M_0 decreased by 84.15% and 84.60%, and compared with those in NM_{100}, the Glu and Gly contents in M_{100} decreased by 54.50% and 11.83%, respectively ($p < 0.05$).

Different free amino acids in roots behaved differently under Cd stress. Compared with those in M_0, the contents of Cys, Glu, Gln, and Gly in M_{100} increased significantly by 67.90%, 7.64%, 0.43%, and 5.32%, respectively (Figure 5) ($p < 0.05$). Compared with those in NM_0, the Gln and Gly contents in NM_{100} decreased by 53.27% and 76.53%, respectively (Figure 5C,D) ($p < 0.05$). The Glu content in NM_{100} was 81.06% higher than that in NM_0 (Figure 5B) ($p < 0.05$). In NM_0 and NM_{100}, the contents of Gln and Gly increased dramatically, measuring 3.62 and 6.22 times higher than those in the NM_0 treatment, respectively. The contents of Glu, Gln, and Gly in the M_{100} treatment were 4.25, 8.94, and 31.42 times higher than those in the NM_{100} treatment ($p < 0.05$).

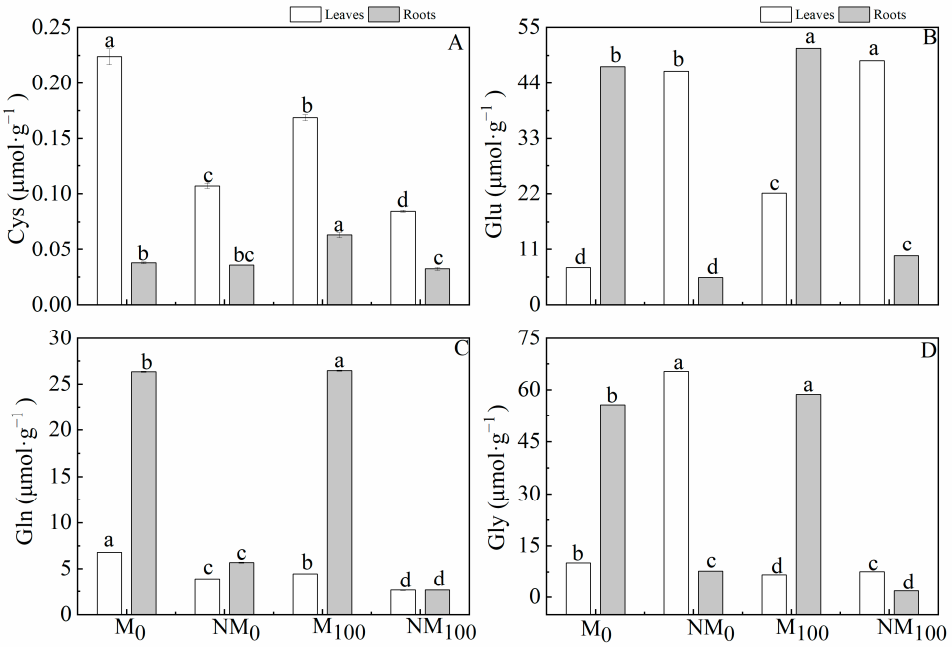

Figure 5. Contents of cysteine (Cys, (**A**)), glutamic acid (Glu, (**B**)), glutamine (Gln, (**C**)), and glycine (Gly, (**D**)) in leaves and roots of Neva irrigated with or without magnetized water following exposure to 0 or 100 μmol·L^{-1} cadmium stress. Values are the means ± *SE* of at least three replicates. Different lowercase letters indicate significant differences among the four treatments at the 0.05 level in leaves and roots.

3.6. Contents of the Elements K, Ca, Na and Mg

In the leaves of Neva (Figure 6), M_{100} induced an increase in the K content of 52.78% compared with that in M_0 (Figure 6A) ($p < 0.05$). The Ca content was significantly (17.12%) higher in NM_{100} than in NM_0 (Figure 6B) ($p < 0.05$).

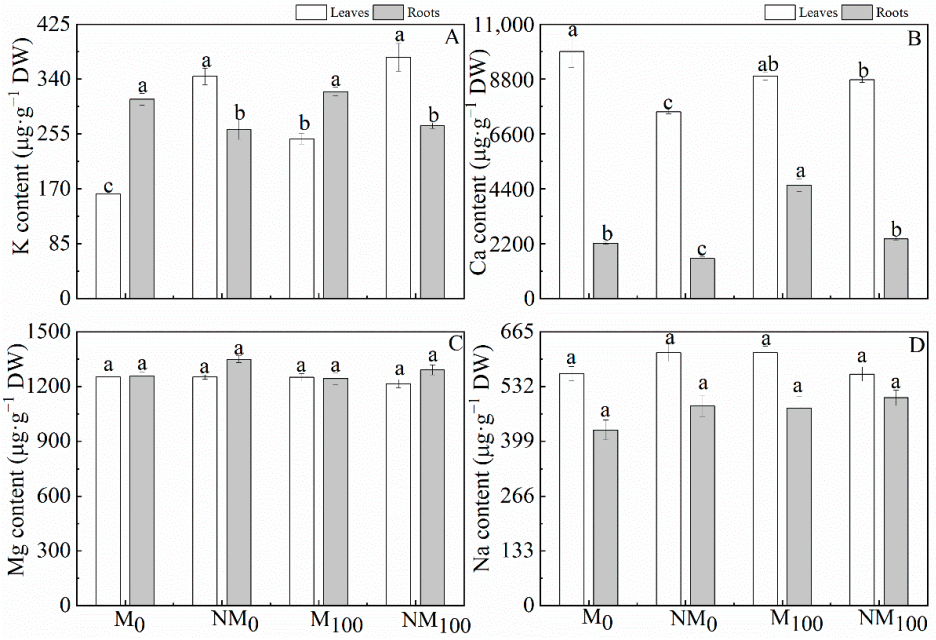

Figure 6. Changes in the contents of potassium (K, (**A**)), calcium (Ca, (**B**)), magnesium (Mg, (**C**)), and sodium (Na, (**D**)) in leaves and roots of Neva irrigated with or without magnetized water following exposure to 0 or 100 μmol·L^{-1} cadmium stress. Values are the means ± *SE* of at least three replicates. Different lowercase letters indicate significant differences among the four treatments at the 0.05 level in leaves and roots.

Compared with that in M_0, the Ca content in M_{100} increased by 104.64%, and compared with that in NM_0, the Ca content in NM_{100} increased by 49.31% in roots (Figure 6B, $p < 0.05$). The Ca content showed the greatest increase in roots, with an average increase of 64.47%.

3.7. Trace Element Contents

Compared with that in M_0, the Fe in leaves in M_{100} increased by 9.83% (Figure 7A, $p < 0.05$). Additionally, the Fe, Mn, Zn, and Cu contents in M_{100} increased by 14.44%, 36.45%, 37.05%, and 49.59%, respectively, compared with those in NM_{100} (Figure 7A–D, $p < 0.05$).

The results indicated that Cd stress inhibited the accumulation of Fe and Zn in roots (Figure 7A,C) under the NM_0 treatment. The contents of Fe and Zn in NM_{100} decreased by 34.11% and 34.09%, respectively, compared with those in NM_0 (Figure 7A,C, $p < 0.05$). Compared with those in NM_0, the contents of Mn, Zn, and Cu in M_0 decreased by 42.24%, 23.17%, and 38.40%, respectively (Figure 7B–D, $p < 0.05$). Finally, the Mn content in M_{100} decreased by 40.66% compared with that in NM_{100} (Figure 7B, $p < 0.05$).

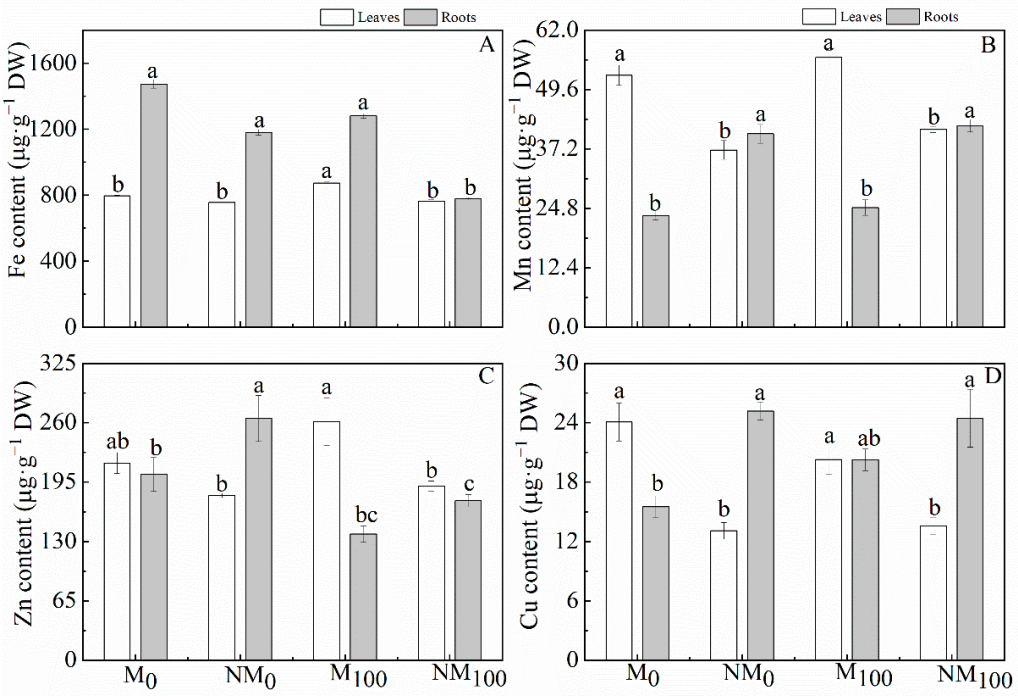

Figure 7. Changes in the contents of iron (Fe, (**A**)), manganese (Mn, (**B**)), zinc (Zn, (**C**)), and copper (Cu, (**D**)) in leaves and roots of Neva irrigated with or without magnetized water following exposure to 0 or 100 μmol·L^{-1}cadmium stress. Values are the means ± *SE* of at least three replicates. Different lowercase letters indicate significant differences among the four treatments at the 0.05 level in leaves and roots.

4. Discussion

Cd stress in plants seriously inhibits rootstock growth, dry matter accumulation, and even nutrient uptake and utilization [34]. Seedlings and/or seeds primed with magnetic treatments have been proven to elevate the photosynthetic performance of various crops species by the improved efficiency of photosystem I (PSI), photosystem II (PSII), and fluorescence dynamics (JIP test) measuring parameters, while the gas exchange indexes in nonstress and abiotic stress conditions [35,36]. In our paper, magnetic treatment combined with Cd stress significantly promoted biomass accumulation in roots and leaves, as well as stalk growth and leaf area. Maximizing leaf length and width could contribute to Neva surviving with increasing photosynthetic production. These results are consistent with those of Liu et al. [37] and Ahmad et al. [38]. Magnetic treatment stimulated the synthesis of photosynthetic pigments in response to Cd exposure. Our results are also

consistent with those of Christos et al. [39], who revealed that nitrogen application could alleviate chlorophyll reduction in the leaves of *Zoysia japonica* under Cd stress. Magnetic treatment induces the increase in the contents of photosynthetic pigments in Neva leaves by promoting chlorophyll biosynthesis, while the protective actor of nitric oxide (NO) on chlorophyll contents would be attributed to its capacity. This ability could improve the delivery and/or the utility of the basic elements in metabolism such as Fe, which are essential in the chlorophyll biosynthesis as well as in chloroplast development [40,41]. These mentioned changes affect carbon fixation, the Hill reaction, and PSII activity in Neva leaves, resulting in enhanced photochemical quenching and enhanced stomatal conductance in leaves [42].

Xu et al. [17] reported that the exogenous application of 100 $\mu mol \cdot L^{-1}$ sodium nitroprusside (SNP) effectively maintained plasma membrane integrity and reduced the absorption of Cd by alfalfa (*Medicago truncatula*) seedlings. Magnetic treatment was beneficial for root morphology (Table S1) when plants were subjected to Cd stress. The results of the present study are similar to these mentioned findings. Though Cd-stress can cause an improvement in scavenging enzymes activity, magnetization exposure could inhibit this impact [43]. Magnetization thus keeps both the capacity of intracellular antioxidation and decreases oxidative injury. Magnetization exposure helps Neva against membrane damage because of membrane transporters that eliminate excess Cd ions from the cells of roots. Simultaneously, high glutathione levels benefit cell proliferation in roots and, hence, enhances root growth [44]. Additionally, the improved root morphology properties are beneficial for the absorption of water and nutrients, representing an important adaptation for poplar to Cd-containing environments and suggesting that the growth mechanism induced by Cd is mainly responsible for the observed root activity responses to the magnetic treatment.

A portion of the nitrate is acquired by leaves, whereas the remainder is reduced to nitrite by NR in roots. Nitrite enters plastids and is reduced by NiR to ammonium, which is ultimately assimilated into amino acids and proteins [45]. The results of our study showed that 100 $\mu mol \cdot L^{-1}$ Cd promoted the activities of NR, GS, and GOGAT and restricted that of NiR. Higher leaf TN contents help maintain leaf productivity and normal metabolism through the maximum carbon fixation rate [46]. Overall, magnetic treatment induced a significant increase in nitrate nitrogen in leaves but decreased it in roots. Kataria et al. [47] found that the negative impact caused by UV-B stress could be mitigated under the effect of a static magnetic field, which is attributed to the improvement of photosynthetic parameters along with a higher NO concentration and NR activity. The much higher contents of NO and the more dynamic NR would be able to preserve the plants from the oxidative stress induced by UV-irradiation. Moreover, magnetization induced a higher NR activity in leaves and roots, and the NR activity in leaves was 4–5 times greater than that in roots. Consistent with the findings for nitrate nitrogen, the NiR activity in leaves was enhanced, and the enzyme degradation in roots was accelerated; thus, leaf activity was increased more than twofold compared with root activity. The amount of ammonium nitrogen accumulated in roots was much higher than that in leaves. Therefore, we conclude that the NH_4^+ absorbed by roots cannot be translocated directly to the leaves, which promotes the preferential transport of NO_3^- from roots to leaves and leads to a decrease in NO_3^- content in the former and an increase in the latter. NO_3^- is the main effector that induces NR and NiR, and within a certain range, the activities of NR and NiR depend on the concentration of NO_3^- in the nutrient medium. Magnetization provides a sufficient supply of NO_3^- for poplar leaves, thereby promoting the activities of both NR and NiR [48]. This result is consistent with a previous finding [30]. However, magnetization resulted in higher NR and NiR activities in leaves, which increased the efficiency of nitrate reduction to nitrite and facilitated the conversion of nitrate to nitrite. In addition, the enhanced NiR activity in leaves consumed a large amount of NH_4^+, which prevented the toxic effects of nitrite accumulation in leaf tissues. NiR in roots promoted the reduction of NO_3^- to NH_4^+ and consumed an extremely large amount of NO_2^-, which is the primary explanation for the

decrease in NiR activity in roots. Moreover, the accumulation of NH_4^+ in roots provided raw material for amino acid synthesis.

GS is the key enzyme in GS–GOGAT cycle and is involved in the regulation of various nitrogen metabolism pathways that have important effects on the metabolic efficiency of plants [2]. In our study, magnetization increased the GS and GOGAT activities in leaves and roots based on the determination of total ATPase activity in cell membranes, and significantly improved ATPase activity (Figure S1A). This finding indicates that GS induced by ATP could bind a large amount of NH_4^+ to form Gln, which is then converted to two molecules of Glu by GOGAT, and ammonium toxicity and glutamate storage supplementation can be alleviated by these transformations [49]. Moreover, magnetization induced a significant increase in the contents of Glu and Gln in root tissues. Glu and Gln are the main donors for nitrogen-containing organic compounds such as amino acids, nucleic acids, chlorophyll, and polyamines, and increased Glu and Gln contents promote the synthesis of macromolecules, such as amino acids and proteins. We also found that the contents of Cys and particularly Gly synthesized in roots under magnetic treatment were approximately 18 times higher than those under nonmagnetic treatment in Neva, and these changes promoted the transport of NH_4^+ from roots to leaves in the form of organic nitrogen, e.g., amino acids [50].

Cd exposure promoted the absorption of K and Mn by roots and the transport of K and Mn from roots to leaves, increasing the K and Mn contents in leaves. Hernández et al. [51] noted that the concentration of Cd in the medium was related to the absorption and transport of K by plants. Under Cd stress, the K concentration in roots increased with the accumulation of Cd and was higher than that in leaves, possibly due to interactions between Cd and K in Neva roots [52]. Additionally, magnetization enhanced ATPase activity in cell membranes (Figure S1A), thus providing an explanation for the increase in K uptake. Under magnetization, the K content in roots increased with the increasing Cd content (Figure S2A), and the translocation of K to the aboveground parts decreased with an increasing biological transfer coefficient of Cd (Figure S2B). These results suggest that magnetic treatment stimulated the binding of excess Cd with ATP in roots, resulting in less available energy for the transmembrane transport system and thus reducing K uptake by the aboveground parts of Neva [52]. Cd stress also promoted the accumulation of Mn in leaves. González et al. [53] found that excess Mn mainly damages the aboveground parts of plants, inducing leaf brown spot formation, chlorosis, and leaf shrinkage. We postulate that this phenomenon is due to the Cd-induced rapid transport of Mn in plants [54], which also explains the observation of leaf chlorosis and shrinkage during Neva growth.

Under Cd stress, magnetic treatment alleviated the substitution effect of Cd^{2+} for Fe^{2+}, Zn^{2+}, and Mg^{2+} by regulating the absorption and transport of Fe, Zn, and Mg in roots and leaves. Cd and Zn have similar extranuclear electron configurations and can be substituted for each other. The improved Zn content in poplar leaves reduced the damage caused by the inactivation of chlorophyll due to compositional changes in the central ion of chlorophylls. Chlorophylls are the vital pigments that assimilate suitable quantities of light energy and establish photosynthetic reactions in plants [55]. The ions changes in Zn, demonstrating the considerable adaptation of poplar to Cd conditions. Moreover, this study showed that magnetization promotes the development of root morphology in Neva, which is important for improving the absorption and accumulation of Fe in roots. All biochemical reactions involving Fe are completed in the chloroplast, which contains the largest iron pool in plant cells. After magnetic treatment, both the leaf Fe and Mg and K contents increased, which promoted the maintenance of chloroplast structure and the number of thylakoids, photosynthetic electron transfer in chloroplasts, and the alleviation of photochemical damage caused by Cd stress [56]. Magnetization also stimulated the uptake of Mn, Zn, and Cu by poplar roots, promoted Mn, Zn, and Cu transport to the aboveground parts, and enhanced the contents of these elements in leaves. These effects could reduce the substitution of Cd for the metal elements in the reaction center and lead to the production of the heavy metal-binding protein Cu/Zn–SOD in poplar cells. Excessive

Cd can increase the activity of Cu/Zn–SOD, which in turn eliminates superoxide anions in plants and reduces reactive species (ROS) accumulation [57], thus maintaining the normal permeability and stability of 'Neva' cells. K and Na contents are important indicators of the ion balance and symptoms of ion damage in plant cells [58]. With magnetic treatment after the addition of exogenous Cd, the Na content generally decreased, and the K/Na ratio was reduced in leaves and increased in roots (Figure S1B). These findings indicate that Cd stress affects the ability of leaves to regulate the ion balance influenced by magnetization, thereby further affecting leaf–mediated ion regulation in the whole plant.

5. Conclusions

The results indicate that magnetization can improve seedling growth and accelerate the synthesis of photosynthetic pigments in Neva under Cd stress at a concentration of 100 μmol·L^{-1}. Additionally, it could contribute to root development, which was in favour of the absorption and transportation of the mineral nutrient elements such as Ca, Fe, and K. Tolerance against Cd stimulated the activity of NR and NiR in leaves, which seemed to reduce the content of nitrite; otherwise, the accumulation of NH_4^+ in roots could supply abundant raw materials for the synthesis of amino acids induced by irrigation with the magnetic treatment of water. The enhancement of the GS–GOGAT cycle by magnetization could promote nitrogen metabolism efficiency by stimulating the activities of GS and GOGAT. Thus, the magnetic treatment of water applying helps to alleviate the negative effect of plants induced by Cd toxicity; for another, it would be a promising strategy to promote the growth, development, and economical production in crops by improving the growing environment in soil. Therefore, molecular mechanisms stimulated by magnetic treatment in plants and soil microecosystems under Cd exposure should be the future investigations, aiming to explore the precise involvement of magnetic treatment in modifying tolerance in Cd-poisoning plants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13060947/s1, Figure S1 Activities of cytomembrane adenosine triphosphatase (ATPase, A) and ratios of K and Na (K/Na ratio) in tender leaves and fine roots irrigated with or without magnetized water and exposed to cadmium stress, Figure S2 Changes in cadmium contents (Cd, A) in tender leaves and fine roots and the biological transfer coefficient of Cd from leaves to roots (S/R, B) of 'Neva' irrigated with or without magnetized water and exposed to cadmium stress, Table S1 Changes in root morphological characteristics of 'Neva' irrigated with or without magnetized water and exposed to cadmium stress.

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