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Nickel Oxide Nanoparticles Improve Soybean Yield and Enhance Nitrogen Assimilation

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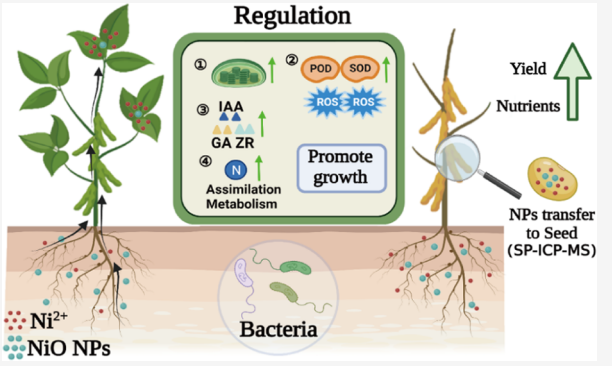
ABSTRACT: Nickel (Ni) is a trace element beneficial for plant growth and development and could improve crop yield by stimulating urea decomposition and nitrogen-fixing enzyme activity. A full life cycle study was conducted to compare the long-term effects of soil-applied NiO nanoparticles (n-NiO), NiO bulk (b-NiO), and $NiSO_4$ at 10−200 mg kg^{-1} on plant growth and nutritional content of soybean. n-NiO at 50 mg kg^{-1} significantly promoted the seed yield by 39%. Only 50 mg kg^{-1} n-NiO promoted total fatty acid content and starch content by 28 and 19%, respectively. The increased yield and nutrition could be attributed to the regulatory effects of n-NiO, including photosynthesis, mineral homeostasis, phytohormone, and nitrogen metabolism. Furthermore, n-NiO maintained a Ni^{2+} supply for more extended periods than $NiSO_4$, reducing potential phytotoxicity concerns. Single-particle inductively coupled plasma mass spectrometry (sp-ICP-MS) for the first time confirmed that the majority of the Ni in seeds is in ionic form, with only 28−34% as n-NiO. These findings deepen our understanding of the potential of nanoscale and non-nanoscale Ni to accumulate and translocate in soybean, as well as the long-term fate of these materials in agricultural soils as a strategy for nanoenabled agriculture.



KEYWORDS: *NiO, nanoparticles, glycine max, life cycle, micronutrient delivery, nutrition quality*

1. INTRODUCTION

Nanoscale NiO is widely applied as a catalyst sensor and is used in battery electrodes due to the materials' unique optical and electrochemical properties.[1−3] Gomes et al. reported that the estimated production of NiO nanoparticles (n-NiO) is approximately 20 tons per year in the United States alone.[4] Nanomaterials produced at this scale will inevitably be released into the environment during production, packaging, transportation, and use, posing unknown potential adverse impacts on the environment and human health.[5] Ni contamination has been one of increasing concern as its concentration in polluted soils has been reported to be up to 26,000 mg kg^{-1}, which is orders of magnitude higher than in unpolluted soils (10−50 mg kg^{-1}).[6] In China, 4.8% of soils in China are contaminated at levels above the tolerance, making Ni second among inorganic pollutants of concern in farmlands after cadmium.[7] The addition of n-NiO to this global pool of contamination may cause additional concerns due to their possible distinct behavior and biological effects compared with ionic Ni.[8,9]

On the other hand, Ni is known to be an essential micronutrient for plant growth.[10] Though the exact role of Ni in plant metabolism is not fully understood, it is clear that Ni is a critical structural component of some key plant enzymes and an essential catalytic cofactor,[11] particularly in urease and [NiFe] hydrogenase. The former enzyme hydrolyzes urea into ammonia, which is the form of nitrogen that plants can effectively accumulate and metabolize.[12] Without sufficient Ni, excessive urea can accumulate in plants, causing tissue necrosis in plant leaves.[13] Conversely, hydrogenase is commonly present in symbiotic bacteria and is involved in hydrogen oxidation and production in a side reaction of catalytic nitrogenase that accelerates biological nitrogen fixation.[14] Therefore, leguminous plants that rely on N_2 fixation through nitrogen-fixing bacteria may be particularly vulnerable to deficiency. Although overt symptoms of modest Ni deficiency are typically not noticeable, a significant reduction of yield can occur.[15] Addition of small amounts of Ni fertilizers (Ni sulfate or organic Ni) in the soil can effectively enhance biological

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nitrogen fixation and urease activity, thereby increasing yield. For example, Freitas et al. reported a 2.6-fold increase in soybean yield upon amendment.[16] Previous studies have explored how Ni fertilization affects a responsive soybean genotype,[16] and the physiological effects of nano-Ni[17] have been explored. However, the physiological effects of n-NiO on soybean, including the effects on nitrogen-fixation enzymes and carbon and nitrogen cycling in plants, are not studied. Therefore, we chose n-NiO as the experimental object in this pilot study.

In the current study, we investigated if n-NiO can be used as a more effective and targeted Ni fertilizer than conventional Ni^{2+} sources ($NiSO_4$) to improve the growth and yield of soybean. We hypothesized that n-NiO would release Ni^{2+} in a slow and potentially controllable fashion, thus improving the use efficiency of this Ni fertilizer compared to $NiSO_4$. In order to explore the agricultural application of n-NiO, a wide range of concentrations should be used to cover those that are both beneficial and detrimental to plants. Therefore, concentrations from 10 mg kg^{-1} (background value in soil) to 200 mg kg^{-1} (which might cause phytotoxicity) were used. The pod yield and nutritional quality of the seeds were assessed. Additional endpoints, including key enzymes that are involved in urea hydrolysis and nitrogen fixation and metabolism, levels of phytohormones, and antioxidative responses, were tested. The uptake and distribution of Ni in the plants were investigated by sp-ICP-MS. This life cycle study demonstrates the potential of n-NiO as an effective micronutrient fertilizer to sustainably enhance crop production.

2. MATERIALS AND METHODS

2.1. Materials and Characterization of n-NiO. Nanoscale NiO (n-NiO) (99.99%, 40 nm) was purchased from Hefei Zhonghang Nanometer Technology Development Co., Ltd (China). Powdered bulk NiO (b-NiO) (99.99%, 1 μm) was obtained from Shanghai ST-Nano Science and Technology Co., Ltd. $NiSO_4{\cdot}7H_2O$ was purchased from Sinopharm Chemical Reagent Co., Ltd. All other compounds used were of analytical grades. The morphology and primary sizes of n-NiO and b-NiO were characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) (Figure S1, SI). ζ Potential and hydrodynamic sizes of n-NiO and b-NiO in deionized water (100 mg L^{-1}) were analyzed with a Zetasizer (Nano ZS90, Malvern, U.K.) (Table S1).

2.2. Pot Experiment Design. The plant exposure experiments were conducted in a greenhouse with controlled environmental conditions at the China Agricultural University (Beijing, China). The soybean seeds (Hedou 13) were a common late maturing variety in the north area of China and were purchased from Shouguang Seeds & Seedling Co., Ltd. Soil sampling and pretreatment details are shown in Text S1 (SI), and the detailed properties of the surface soil are presented in Table S2. The prepared soil was separately mixed with n-NiO and b-NiO or $NiSO_4$ $7H_2O$ to achieve initial concentrations of 10, 50, 100, and 200 mg kg^{-1}. Each pot was filled with 2.0 kg of soil. Soil with no addition of Ni (0 mg kg^{-1}) was used as a control (CK). Seeds were sterilized with 5% NaClO for 5 min and rinsed with deionized water thoroughly before sowing. The seeds were placed in a Petri dish containing 10 mL of deionized water and germinated in a climate incubator (25 °C, dark environment) for 7 days. Soybean seedlings of uniform size were selected and planted into pots containing soils. Portions of the soybean plants were harvested at 80 days after sowing (DAS) and chlorophyll content was measured (Text S2). Samples were divided into shoots (including leaves and stems), roots, and seeds. The fresh samples were stored at −80 °C for the determination of physiological and biochemical indicators as noted below. Another set of soybean plants was harvested at 140 DAS to evaluate full life cycle endpoints. Four replicates were used for each treatment. At harvest, deionized water and 0.1% HNO_3 were used to rinse the plants thoroughly so as to remove the adhered soil particles or Ni-based materials (NBMs) on the plant surfaces. Root length and height were measured, and the dry weight of shoot and root tissues were determined after oven drying (105 °C for 1 h and 80 °C for 24 h).

2.3. Antioxidant System and Phytohormones. The fresh roots or shoots collected at 80 DAS were processed for antioxidant and phytohormone determination according to Wang et al.[18] The activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) antioxidant enzymes and the contents of malondialdehyde (MDA) and proline (Pro) contents were determined according to the manufacturer's instructions (Nanjing Jiancheng Biotechnology Co., Ltd). Enzyme-linked immunosorbent assay (ELISA) was used to quantify the level of nine phytohormones in the shoot tissues, according to the previously described methods.[18] The detailed experimental protocols and quality control endpoints are described in Texts S3 and S4.

2.4. Nitrogen Metabolism-Related Enzymes. In the fresh shoots and roots (collected at 80 DAS), key enzymes involved in N assimilation were measured using an assay kit (Beijing Boxbio Co., Ltd.), including nitrate reductase (NR), glutamine synthetase (GS), glutamate synthetase (GOGAT), and urease (UE). The total carbon and nitrogen accumulation in plants were determined according to Hu et al.[19] Specifically, total C and total N in oven-drying powders of roots and shoots (collected at 140 DAS) were determined using an organic elemental analyzer (Vario EL, Elementar, Germany). Nodule nitrogenase activity was measured using the acetylene reduction assay (see details in SI, Text S5).[20]

2.5. Content of Nickel and Mineral Elements in Plants. Approximately, 0.2 g of oven-drying powders of roots, shoots, or seeds (removed and collected from the pods at 140 DAS) was digested in HNO_3 (70%) using a microwave digestion system (MARS6, CEM). The digesta was then diluted with ultrapure water to 50 mL. The total content of Ni and other mineral nutrients was then determined by ICP-MS (DRCII, PerkinElmer, and Norwalk). Methodological details are described in Text S6. QA/QC for the ICP-MS measurement is described in Table S3.

2.6. Organic Nutrient Composition in Seeds. At 140 DAS, soybean seeds were collected from the pods, and the content of soluble sugars, starch, amino acids, and fatty acids was measured. Sugar and starch content were measured according to Ma et al.[21] The amino and fatty acids were determined according to Zhou et al.[22] Details of sample preparation and subsequent measurements are provided in Text S7−S9.

2.7. sp-ICP-MS Measurement of In-Planta n-NiO. Plant roots, shoots, and seeds collected at 140 DAS were digested with Macerozyme R10 at pH 6 (adjusted with HCl and NaOH) for 24 h to release the accumulated n-NiO from the plant tissue.[23] After enzymatic digestion, samples were diluted 100-fold and then analyzed by sp-ICP-MS using an Agilent

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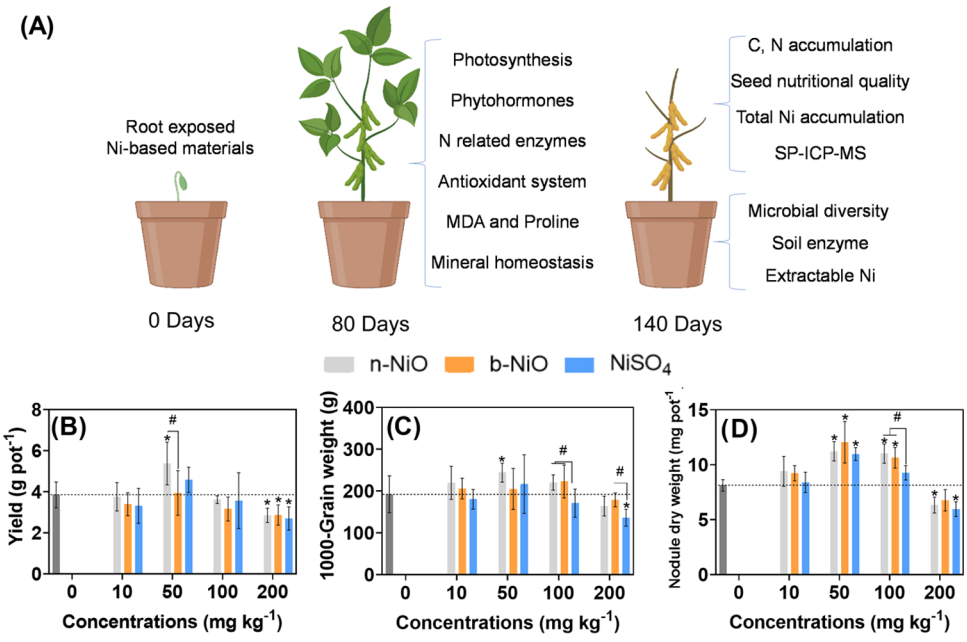

Figure 1. (A) Illustration of the experimental design. Various parameters were evaluated at R5 (80 DAS) and R8 (140 DAS) phases. The impact of Ni-based materials on yield per pot (B), 1000-grain weight (C), and nodule dry weights (D) after full life cultivation (R8). * and # indicate significant differences at $p < 0.05$ ($n = 6$) compared with control and between groups, respectively.

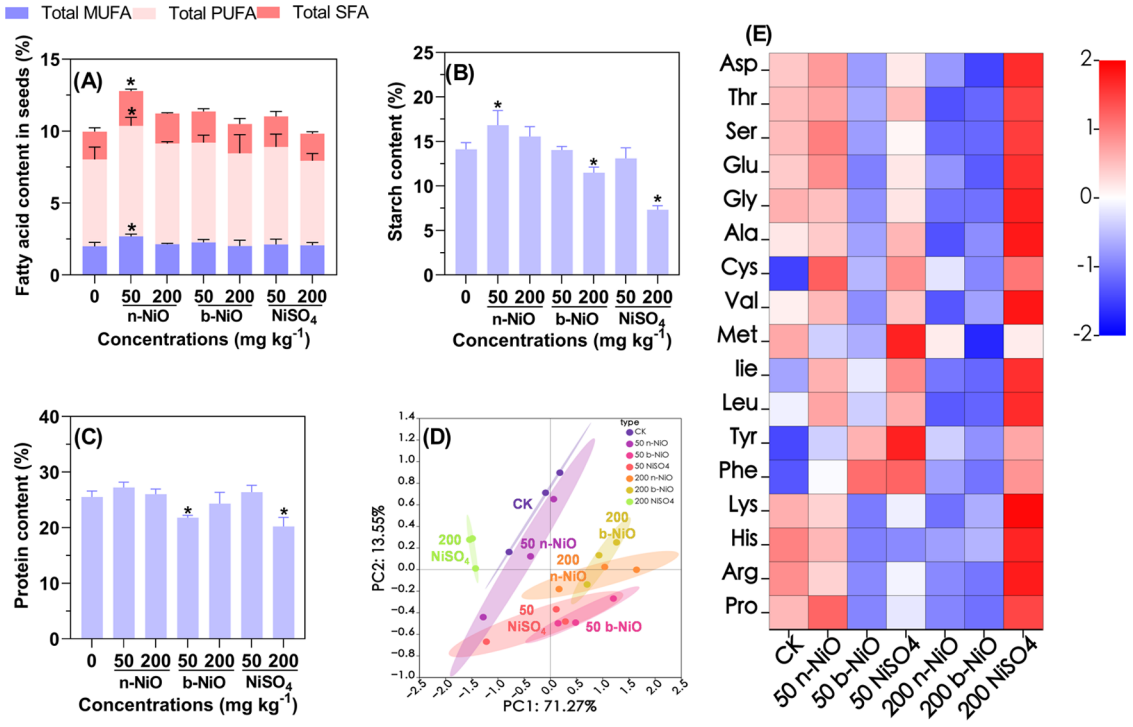

Figure 2. Impact of Ni-based materials on (A) fatty acid content, (B) starch content, and (C) protein content in seeds after full life cycle cultivation (140 DAS). Principal component analysis (D). Originating lines from the center show the positive or negative correlations of different variables, and the closeness of lines shows correlation strength with specific treatment. * indicates a significant difference at $p < 0.05$ ($n = 3$) compared with the control (CK). The heatmap (E) of amino acids under different Ni-based materials at various concentrations (50 and 200 mg kg^{-1}) is presented. The color in (E) represents the normalized amino acid content.

8900 ICP-MS in a single-particle mode. The instrument configuration was that of Tan et al. with appropriate modifications.[24] The particle size distribution, particle concentration, and ionic Ni concentration in the samples were measured simultaneously in each analysis. The samples were measured using an integration residence time of 100 μs and a sampling time of 60 s. The calibration standards (0−5 μg L^{-1} dissolved Ni) were prepared in a 100-fold diluted control plant digestate to match the sample matrix for each plant tissue. The sp-ICP-MS transfer efficiency (6.7%) in enzymatically digested plant matrices was determined using 56 nm monodispersed Au NPs (NIST, Gaithersburg, MD) as a

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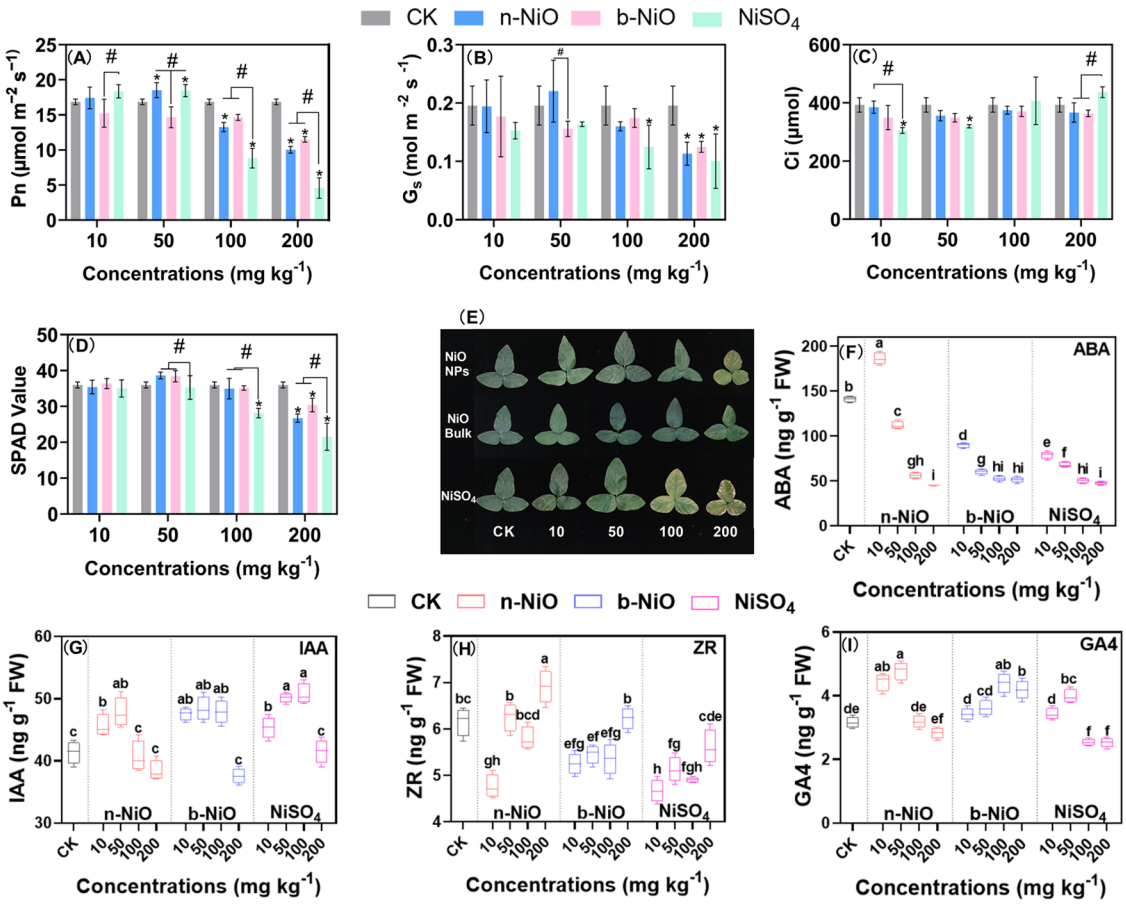

Figure 3. Impact of Ni-based materials on leaves as determined by (A) net photosynthesis (Pn) and (B) stomatal conductance (G_s), (C) intercellular carbon dioxide concentration (C_i), (D) relative chlorophyll content, (E) leaf phenotypes of different Ni-based material treatments, (F) ABA, (G) IAA, (H) ZR, and (I) GA4 at 80 DAS. Different letter indicates a significant difference at $p < 0.05$ ($n = 6$) between groups, respectively.

reference. Additional instrument settings and conditions are shown in Table S5.

2.8. Statistical Analysis. Statistical analyses were performed using one-way analysis of variance (ANOVA) in SPSS 20.0. The mean values for each treatment were compared using the least significant difference (LSD) multiple comparison test at $p < 0.05$. Data are expressed as means ± standard deviation (SD). The symbol "*" indicates a significant difference at $p < 0.05$ ($n = 6$) compared with controls.

3. RESULTS AND DISCUSSION

3.1. Low-Dose n-NiO Improves Pod Yield and Nutrients. *3.1.1. Yield and Phenotype.* The effect of NBMs on plant growth was evaluated at two critical stages during the soybean life cycle: R5 and R8 (Figure 1A). The R5 stage (80 DAS) is also known as the seed-filling phase, where soybean seeds start development and any alteration of physiological processes can affect the seed yield and nutritional quality. At the R8 stage (140 DAS in this study), the seeds are mature and the seed weight remains constant. At this latter stage, the yield and quality of the seeds were evaluated. The results show that all NBMs influenced pod yield in a concentration-dependent manner. At 50 mg kg^{-1}, n-NiO significantly improved soybean seed yield in each pot by 39% (Figure 1B), while $NiSO_4$ and b-NiO showed no significant effect. All of the treatments suppressed crop yield as the concentration increased to 200 mg kg^{-1}. The yields were further assessed by 1000-grain weight following the international seed testing rule. The 1000-grain weight is an indicator of seed size and plumpness, reflecting overall seed quality. Notably, only 50 mg kg^{-1} n-NiO significantly increased the 1000-grain weight (by 26%), with the remaining treatments showing no effect, the exception being that $NiSO_4$ at 200 mg kg^{-1} reduced by 29% (Figure 1C). Since seed formation and reproduction of soybean largely relies on nitrogen fixation through Rhizobium in the nodule,[25] we further examined the impacts of NBMs on the nodules. The weight of nodules per pot increased after treatment with all of the NBMs at doses lower than 200 mg kg^{-1}, with the particles showing stronger positive effects than the ionic form (Figure 1D). At 50 mg kg^{-1}, n-NiO, b-NiO, and $NiSO_4$ increased the weight of nodules by 38, 48, and 35%, respectively. However, at 200 mg kg^{-1}, the nodule weights were significantly reduced by n-NiO and $NiSO_4$. Since the strongest effects were observed at 50 and 200 mg kg^{-1}, these concentrations were used to evaluate treatment effects on seed nutritional quality.

3.1.2. Seed Nutritional Quality. The nutritional quality of the seeds was evaluated by measuring the content of fatty acids (FAs), amino acids, starch, and soluble sugar. FAs are an essential energy source and a component of cell membrane lipids and are involved in the protection of plants against abiotic stresses.[26] Moreover, the FAs and in particular, unsaturated FAs (UFAs), are known to be sensitive to ambient stresses such as heavy metals and toxic nanomaterials (e.g., CuO[27]). At 50 mg kg^{-1}, n-NiO elevated the FA content; specifically, the content of monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and saturated

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fatty acids (SFAs) was increased by 34, 26, and 24%, respectively (Figure 2A). The elevation of SFAs was attributed to the increase in palmitic and stearic acids (Figure S4A), while the elevation of UFAs was attributed to oleic and linoleic acid increases (35 and 28%, respectively) (Figure S4B). Linoleic acid is an essential UFA for human beings and can only be acquired from the diet.[28] These findings suggest that n-NiO improved FA nutrition. In contrast, both $NiSO_4$ and b-NiO treatments at 50 and 200 mg kg^{-1} concentrations had no significant effects on the total content and composition of the UFAs.

n-NiO at 50 mg kg^{-1} also increased the levels of starch content by 19%(Figure 2B). At a concentration of 200 mg kg^{-1}, b-NiO and $NiSO_4$ significantly reduced the content of starch by 19 and 48%, respectively. The content of total seed protein was significantly reduced by 50 mg kg^{-1} b-NiO and 200 mg kg^{-1} $NiSO_4$ treatments (Figure 2C). Soluble sugar contents were reduced by all treatments except for 50 mg kg^{-1} n-NiO (Figure S5). We also analyzed the amino acids using a principal component analysis (PCA) and generated a heatmap. There was a clear separation between the 200 mg kg^{-1} $NiSO_4$ treatment and the other treatments, demonstrating that 200 mg kg^{-1} $NiSO_4$ could significantly increase the amino acid content to a greater extent than n-NiO and b-NiO (Figure 2D). Venn diagrams were used to summarize the NBM-induced changes in amino acid and fatty acid composition (Figure S6). Exposure to 200 mg kg^{-1} b-NiO and 200 mg kg^{-1} n-NiO significantly decreased the content of 10 separate amino acids (Figure 2E).

We also examined the mineral nutrients in the harvested seeds (Figure S7). Exposure to the low concentration of NBMs showed little effect, the exception being that Zn and Fe levels were increased by 42 and 36% after treatment with 10 mg kg^{-1} n-NiO and 50 mg kg^{-1} b-NiO, respectively. At high concentrations, n-NiO reduced the levels of Mn and Cu, while $NiSO_4$ caused more extensive decreases in mineral content. For example, 200 mg kg^{-1} $NiSO_4$ significantly decreased Mg, K, Ca, and Mn elements by 22, 22, 39, and 37%, respectively (Figure S7). Overall, the above results suggest that low-dose exposure to n-NiO can improve soybean pod yield and nutritional quality and that these benefits exceed those of the conventional ionic Ni fertilizer.

3.2. Molecular Mechanism of the Phytoeffects Induced by n-NiO. *3.2.1. Modulation of Photosynthesis, Antioxidant Systems, and Phytohormones.* The nutritional quality and yield of soybean seeds depend largely on growth at the seed-filling stage (R5). We, therefore, examined physiological parameters at this critical stage, given that changes in physiological processes here may significantly impact seed maturation. The increased productivity and quality of soybean seeds are often a direct function of higher photosynthetic rates. The photosynthetic parameters of soybean were slightly improved by the low concentration of NBMs. Specifically, n-NiO and $NiSO_4$ at 50 mg kg^{-1} enhanced the net photosynthetic rate (Pn) by 10 and 9%, respectively (Figure 3A). Similar to the phenotypic results, high concentrations of NBMs inhibited various photosynthetic parameters (Figure 3A,B), with $NiSO_4$ showing greater toxicity than n-NiO and b-NiO. Stomatal conductance (G_s) and intercellular CO_2 concentration (C_i) were decreased by NBM exposure, likely through an altered K^+ flux across cell membranes, which subsequently inhibited gas exchange across the leaf surface.[29] Interestingly, high concentrations of NBMs did not reduce C_i (Figure 3C), likely because more CO_2 is produced due to more energy for respiration so as to relieve stress.[21] The overall chlorophyll contents in the n-NiO and b-NiO treatments showed the same trend. Compared with CK, the total chlorophyll contents slightly increased by 8 and 7%, respectively, after treatment with 50 mg kg^{-1} n-NiO and b-NiO, while decreased by 25 and 16%, respectively, at 200 mg kg^{-1} (Figure 3D). Again, $NiSO_4$ exhibited the greatest reduction at 200 mg kg^{-1}, with a decrease of 40%. Moreover, the leaf area was significantly enhanced after treatment with 50 mg kg^{-1} n-NiO and $NiSO_4$ (Figure 3E). These findings correlate well with the phenotypic data, clearly suggesting that enhanced photosynthesis by n-NiO contributes to improved seed yield.

The oxidative response of soybean seedlings at the R5 stage was examined. Both b-NiO and $NiSO_4$ increased the root MDA content at 50 mg kg^{-1} and leaf content even at 10 mg kg^{-1} (Figure S8A,E). This impact triggered the activation of the antioxidative enzyme systems, including SOD and POD, suggesting the occurrence of oxidative damage and plant defense response. Conversely, n-NiO increased the MDA content only at high concentrations (200 mg kg^{-1}). The content of proline, another important indicator of plant stress (Figure S8D,H), was also determined. The effects of the NBMs on proline content showed similar trends to MDA, with the greatest stress observed for $NiSO_4$. The proline level is enhanced as a response to ambient stress such as exposure to toxic substances. The addition of n-NiO did not lead to an increase in the proline level, but significantly decreased proline content at 200 mg kg^{-1}, indicating that n-NiO reduced the stress.[15] However, $NiSO_4$ increased the proline level at all concentrations except for 50 mg kg^{-1}, suggesting that the plants were under high stress.

Phytohormones are signaling molecules that play crucial roles in plant growth and modulate plant adaption to various environmental conditions, including abiotic and biotic stresses.[30,31] We examined nine major phytohormones in plant leaves, including ABA, IAA, ZR, GA4, GA3, DH-ZR, JA-ME, BR, and IPA. In general, n-NiO at 50 mg kg^{-1} significantly increased the content of six phytohormones in leaves (IAA, GA4, GA3, DH-ZR, JA-ME and BR), whereas b-NiO (IAA and DH-ZR) and $NiSO_4$ (IAA and GA4) each only increased the amount of two analytes (Table S4). $NiSO_4$ treatment at a concentration of 200 mg kg^{-1} significantly reduced all phytohormones except for IAA and ZR.

ABA is known for its role in leaf abscission but it also plays an important role in plant response to environmental stress.[32] NBM exposure reduced ABA levels in a concentration-dependent manner. The overall ABA content in n-NiO-treated plants was greater than the other treatments (Figure 3F), suggesting that the n-NiO-treated group had greater resistance to abiotic stresses than was conveyed to plants exposed to b-NiO and $NiSO_4$. IAA is known to promote growth, specifically by stimulating elongation at the cellular level.[33] Low concentrations (50 mg kg^{-1}) of all NBMs increased IAA by 16−21%, but there were no differences between the treatments (Figure 3G). ZR belongs to the cytokinin family and is typically an antagonist of ABA.[30] Typically, in the current study, the trend in ZR content was the opposite of ABA (Figure 3H).

GA regulates plant shoot elongation and flowering and is involved in seed production.[34] Interestingly, GA may be critical to the hormonal differences among NBM treatments. For example, n-NiO and $NiSO_4$ at 50 mg kg^{-1} caused GA4 to

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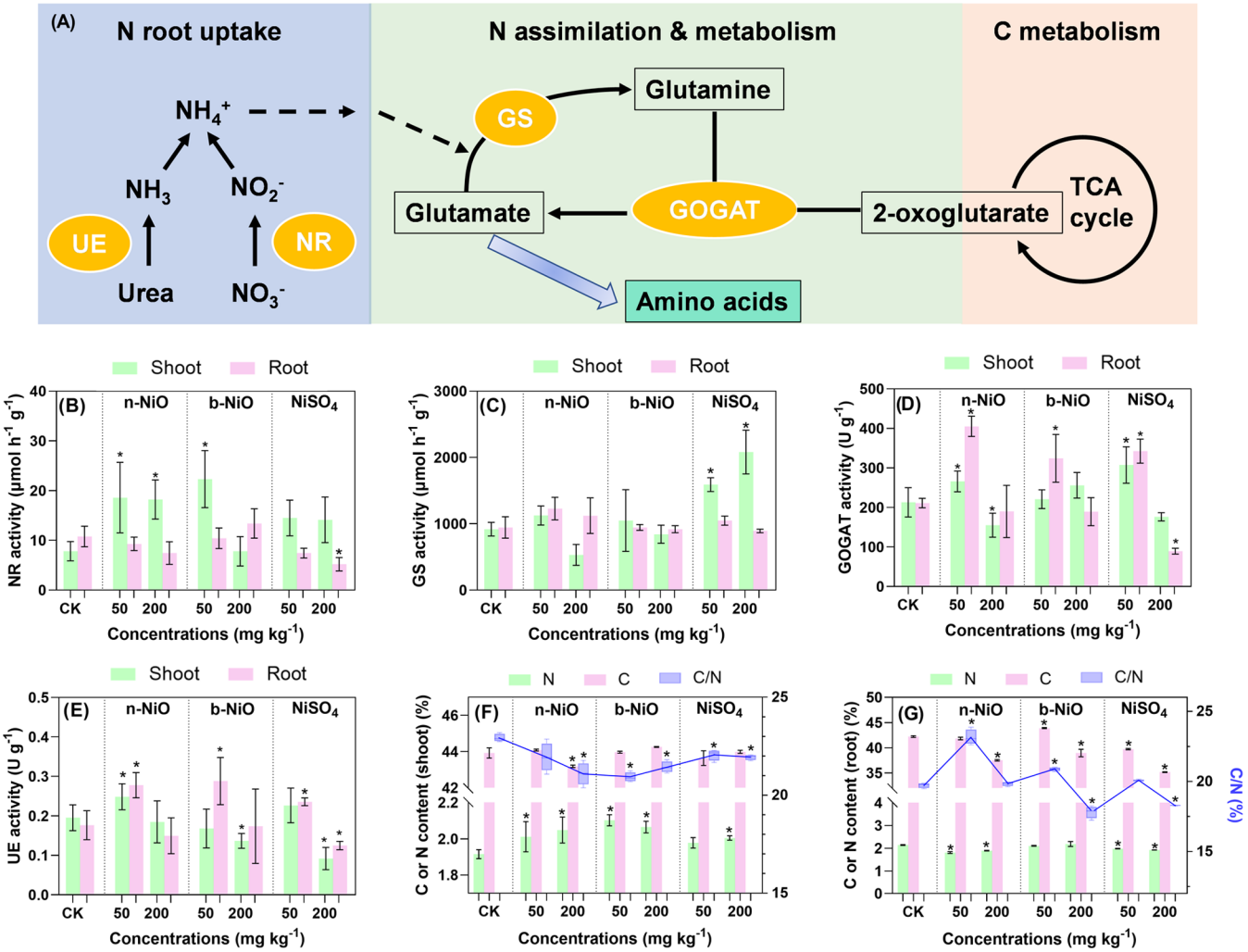

Figure 4. (A) Schematic diagram of the action of enzymes related to nitrogen metabolism and assimilation. The impact of Ni-based materials on the activity of (B) NR, (C) GS, (D) GOGAT, and (E) UE at 80 DAS. (F, G) C and N contents in the shoot and the root after full life cultivation. * indicates a significant difference at $p < 0.05$ ($n = 6$) compared with the control.

increase by 51 and 26% (Figure 3I), respectively, and a similar trend was observed for GA3 (Figure S9B). In addition, n-NiO at 50 mg kg^{-1} significantly increased the levels of DH-ZR, JA-ME, and BR by 32, 29, and 28%, respectively, while no significant differences were evident in the b-NiO and $NiSO_4$ treatments. Importantly, JA and BR play a key role in regulating abiotic stress tolerance and promoting growth,[35] highlighting that 50 mg kg^{-1} n-NiO induced hormonal changes that promoted soybean development. Overall, our findings demonstrate that low-dose n-NiO can not only improve photosynthesis but also regulate antioxidative systems and phytohormone activity to increase plant tolerance to ambient stress, which then contributed to increased seed yield. The regulation of plant hormones by NPs is a dynamic and rather complex process, the mechanisms of which remain unclear. One of the mechanisms that have been reported is that the expression of hormone synthesis-related genes and hormone pathways may be regulated by NPs.[36,37] Therefore, further studies are required in the future.

3.2.2. Nitrogen Metabolism and Elemental Homeostasis. We examined if added Ni would impact nitrogen assimilation and metabolism at the R5 phase, when this process is particularly intense, thereby improving seed maturation and nutrition. Nitrogen assimilation and metabolism involve several crucial enzymes, including urease (UE), nitrate reductase (NR), glutamine synthetase (GS), and glutamate synthetase (GOGAT).[38] In soil, urea can be transformed to NH^{4+} by NR and UE (Figure 4A), with subsequent NH^{4+} use by the plant and then assimilation to amide N by GOGAT and GS for the synthesis of amino acids.[16,39] n-NiO at 50 mg kg^{-1} significantly increased NR, GOGAT, and UE activities in both roots and shoots, except for NR in roots. Specifically, NR, GOGAT, and UE shoot activities were increased by 138, 25, and 27%, respectively (Figure 4B–E). GS activity in the shoot was increased by 73 and 126% after 50 and 200 mg kg^{-1} $NiSO_4$ treatments (Figure 4C). We also measured the activity of nitrogenase, which catalyzes nitrogen fixation. (Figure S10). Results showed that at the R5 stage, 50 mg kg^{-1} n-NiO enhanced the nitrogenase activity by 138%, while no significant effects were observed for other treatments. Alterations in nitrogen metabolism and assimilatory enzymes may lead to changes in nitrogen content in plants and potentially affect closely related carbon metabolism.[40] Total nitrogen content in the shoots was significantly increased by 6 and 9% after exposure to 50 mg kg^{-1} n-NiO and b-NiO, respectively, indicating increased nitrogen assimilation in plant shoots

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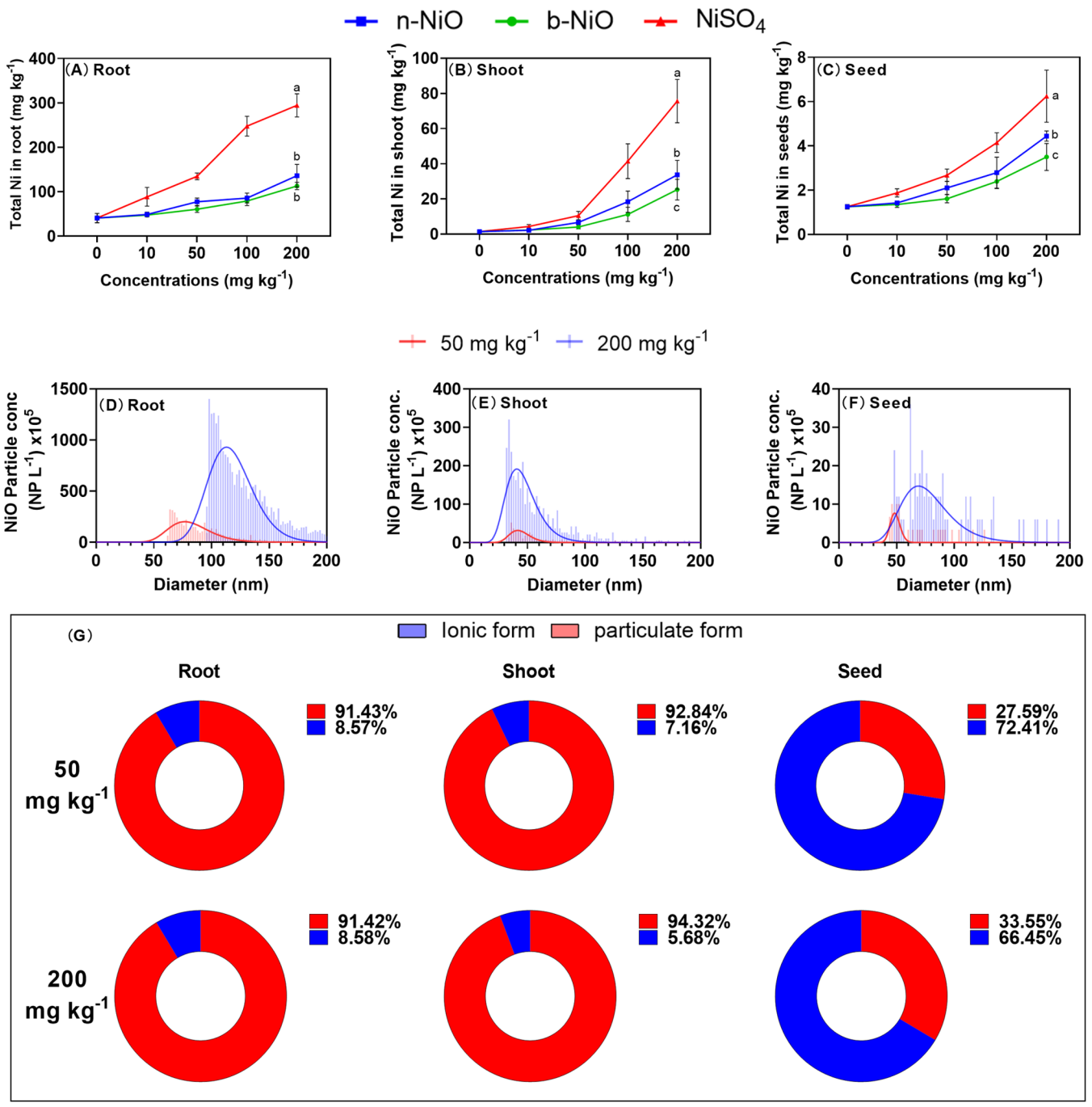

Figure 5. Ni concentrations in the (A) roots, (B) shoots, and (C) seeds of soybean plants after full life cultivation (140 DAS) exposed to Ni-based materials at 0, 10, 50, 100, and 200 mg kg^{-1}. Particle size distribution of n-NiO as measured by sp-ICP-MS in (D) roots, (E) shoots, and (F) seeds of soybean treated with n-NiO. (G) Form composition (ionic form and particulate form) of Ni in different parts of soybean. Data are average of four replicates ± standard deviations (n = 4).

(Figure 4F). Total shoot carbon was unaffected at 50 mg kg^{-1} Ni-based materials, which resulted in a decrease in the C/N ratio in the shoots. The C/N ratio of a plant is often regarded as a convenient indicator of growth and quality, as well as a reliable indicator of C allocation to defense-related metabolites.[40] The influence of NBMs on the C/N was different in the root and the shoot. In the shoot, all treatments reduced the C/N ratio significantly, except for n-NiO at 50 mg kg^{-1}. However, in the root, (Figure 4G), n-NiO and b-NiO at 50 mg kg^{-1} increased the C/N ratio by 17 and 5%, respectively. At 200 mg kg^{-1}, n-NiO did not change the C/N ratio, while b-NiO and $NiSO_4$ significantly reduced the C/N ratio by 9 and 7%, respectively. These results suggested that n-NiO showed overall enhancement of C/N metabolism, while the effects of b-NiO and $NiSO_4$ were dose-dependent. Since Ni is present in the active site of UE, supplementation of appropriate amounts of Ni could enhance UE activity. NR activity can reflect the intensity of protein metabolism and is closely related to photosynthetic C metabolism.[41] The increase in chlorophyll content in the shoots and the observed enhancement of net photosynthesis indirectly elevated NR activity. These changes then indirectly affected the GS-GOGAT cycle, accelerating the

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N assimilation and amination processes, and subsequently reducing urea accumulation and possible NH^{4+} cytotoxicity.[42] This increase in photosynthetic efficiency also promoted N metabolism, and the resulting balance in C and N metabolism subsequently led to enhanced plant growth.

Balanced mineral homeostasis and sufficient nutrient supply are also crucial at the R5 phase so as to ensure maximum seed yield and nutrition.[43] A detailed discussion of this topic can be found in Texts S14, S15. Taken together, the above findings suggest that the differential modulation of nitrogen metabolism and mineral homeostasis induced by NBM exposure led to overt changes in plant phenotype and performance.

3.3. In-Planta Accumulation of the Particulate and Ionic Ni. The increased soybean yield may be attributed to fertilizer effects,[44] possibly through the controlled release of Ni^{2+} from n-NiO, as this provides a more controlled and sustainable supply of nutrients than $NiSO_4$. Jessica et al. recently reported that n-NiO led to more effective delivery of Ni to the radicle and seed of soybean, and consequently promoted seedling growth.[45] Notably, b-NiO has a greater positive (10−13%) effect on nodule weight than that of n-NiO and $NiSO_4$; this effect may be a function of the slowest release of Ni^{2+} (Figure S13). Again, the high concentration (200 mg kg^{-1}) of NBMs resulted in a reduction in the biomass and length, with $NiSO_4$ exerting the greatest toxicity (Figure S3). This might be due to the instant release of excessive Ni^{2+}, which can damage plant growth or lead to death. However, n-NiO may release Ni^{2+} slowly in a sustainable way that can promote the activity of various enzymes related to nitrogen metabolism and plant growth. To understand the release of Ni^{2+}, the dissolution kinetics of n-NiO and b-NiO was examined in vitro in various media, including pure water, soil pore water (no plant existed in the soil), plant pore water (plant existed in the soil), root exudates, and simulate xylem sap (Figure S13). The dissolution was fast in the first 2 days. The dissolution rate was highly related to the media, with the highest being found in soil pore water. Nearly, 10% of n-NiO dissolved after 2 months in soil pore water, while only 4% dissolved in the xylem sap. The dissolution of b-NiO was much slower, with only 1.5% dissolution in soil pore water after 2 months. This is consistent with the uptake of Ni in plant tissues; the overall total Ni content in plant tissue and seeds followed the order $NiSO_4$ > n-NiO > b-NiO (Figure 5A−C). The root Ni content upon exposure to 200 mg kg^{-1} $NiSO_4$ was 2.1 and 2.6-fold higher than n-NiO and b-NiO, respectively (Figure 5A). The degree of inhibition of soybean growth with NBMs is closely correlated to the actual amount of Ni uptake. At concentrations greater than 50 mg kg^{-1}, the Ni content in roots, shoots, and seeds showed a notable increase, and the corresponding seed yield and soybean biomass began to decline. At higher concentrations (≥100 mg kg^{-1}), more toxic Ni^{2+} was released, leading to the most severe growth inhibition with $NiSO_4$. This suggests that the toxicity is determined by the actual Ni accumulation. Although the dissolution rate of b-NiO was slower than that of n-NiO (Figure S13), the difference in the actual uptake of Ni in plant tissues and seeds is not substantial between the two treatments (Figure 5A,B). This is likely due to the influence of soil properties, as well as the agglomeration and aggregation of the particles. In soil, nanoparticles tend to aggregate or agglomerate with soil particles, thereby reducing the differences in dissolution potency of b-NiO and n-NiO that were observed in pure solutions (Figure S13). Indeed, the extractable soil Ni (dissolved over 140 days in real soil) in n-NiO and b-NiO treatments was similar, but still significantly lower than that in the $NiSO_4$ treatment (Figure S14).

The total Ni and extractable Ni in the initial soil were 22.45 and 0.083 mg kg^{-1} (Table S2), respectively. The soil is classified as mildly nickel-deficient soil. The extractable Ni content in the rhizosphere soil after n-NiO and b-NiO treatment was significantly lower than with $NiSO_4$ (Figure S14A), and in addition, the extractable Ni content in the rhizosphere soil was closely correlated with the Ni content in soybean tissues (Figure S14B−D). Excessive Ni supply is clearly detrimental to plants; exposure to 87.8 and 110 mg kg^{-1} n-NiO significantly inhibited the growth and development of barley,[46,47] while soybean exposed to 9 mg kg^{-1} Ni^{2+} did not show any effect on yield.[16] Based on our findings related to the slow release of Ni^{2+} from n-NiO and b-NiO, differences in treatments in the current work can largely be attributed to the kinetics of Ni^{2+} release as a function of time.

This study demonstrates that the potential ecotoxicity of n-NiO is much lower than $NiSO_4$, given that the extractable Ni largely determines acute toxicity. In addition, related studies have shown that nanoparticles can be adsorbed by plant roots and transported to the aboveground through the plant xylem.[48,49] n-NiO that may directly enter plants has the potential to provide trace nutrients in a sustained and controlled fashion, avoiding the acute toxicity triggered by a rapidly available large supply of Ni^{2+}.

To further understand the uptake and translocation of n-NiO and Ni^{2+} in the plant, sp-ICP-MS was used to identify n-NiO in different soybean tissues. Overall, the content of n-NiO decreased with increasing transport distance from the roots (Figure 5D−F). A higher number of NPs observed in the roots than in shoots was partially due to the absorption of NPs on the plant root surface. An increase in the mean particle size of n-NiO was observed in the root and seed as the applied concentration of n-NiO increased. The average particle size in the root system increased from 99 ± 8 to 118 ± 20 nm (Figure 5D), whereas the particle size in the seeds increased significantly from 68 ± 9 to 83 ± 12 nm (Figure 5F). Plant cells are surrounded by a cell wall with pore sizes ranging from 5 to 30 nm;[50] only very small amounts of NPs could theoretically enter the plant through the cell wall pores. The presence of large particles was detected in both soybean tissues. Since the root system was the primary site of n-NiO exposure, it is likely that the increase in concentration resulted in greater root secretions to block uptake by the plant.[51] Furthermore, the potential for agglomeration may increase with increasing NP concentrations, which decreases bioavailability.[25] For the seeds, it is possible that the production of a protein corona led to the increase of agglomerated particles.[52] Since the kinetics of n-NiO absorption, dissolution, and reduction is highly dynamic, it is also possible that Ni ions were accumulated and transferred to the seeds, where they were reduced and the n-NiO particles that happen to reform were large than those initially applied.

The concentration of the Ni in the particulate form is calculated by subtracting the ionic Ni^{2+} measured by sp-ICP-MS from the total Ni.[53] The data demonstrate for the first time that n-NiO could be transferred to soybean seeds as particulate form at both 50 and 200 mg kg^{-1} n-NiO exposure (Figure 5F,G). Moreover, the percentage of particulate form in seeds after 200 mg kg^{-1} n-NiO treatment (33.55%) was higher than 50 mg kg^{-1} (27.59%). Transformation processes occurring in

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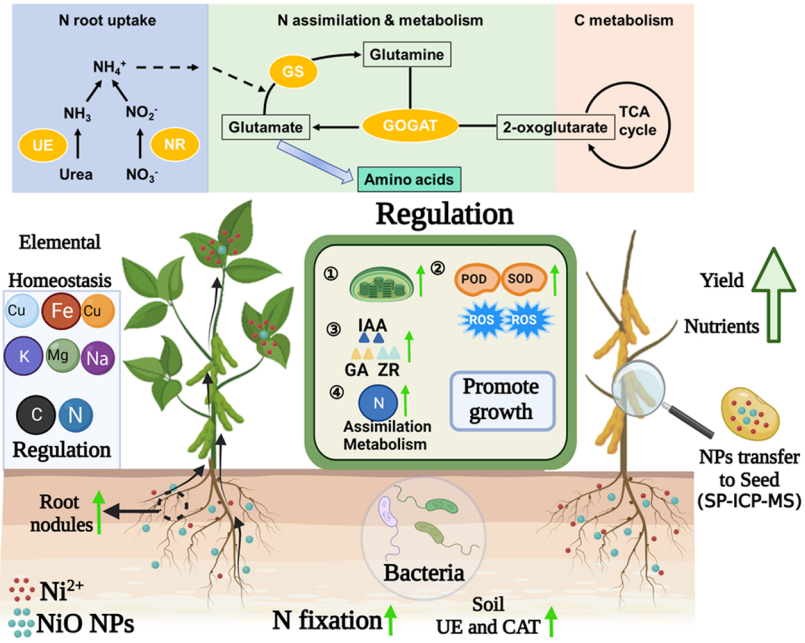

Figure 6. Potential n-NiO-based strategies for promoting soybean yield and nutrition. The figure was created using BioRender with a license obtained.

the root system (ionic form accounts for 8.57−8.58%) are much more extensive than in the shoot (ionic form accounts for 5.68−7.16%) probably due to the nutrient acquisition effect of root secretions (Figure 5G). Since particulate n-NiO was found in seeds, we evaluated the safety of soybean plants exposed to different NBMs using the maximum allowable daily intake (ADI) and the hazard quotient (HQ) to assess the risk of Ni ingestion through the food chain (Text S16). The highest HQ was only 0.28 (exposed at 200 mg kg^{-1} $NiSO_4$), with all values being much less than 1 (Table S6). For 50 mg kg^{-1} n-NiO, the HQ was 0.05 and 0.02 for children and adults, respectively. Therefore, the levels of total Ni found in soy grains in this study are considered safe when the direct consumption of the grains is taken into account. However, this is only for the consumption of Ni-containing soybeans alone, and further evaluation is needed regarding the intake of other Ni-containing foods and the potential risk from small quantities of n-NiO contained in seeds.

3.4. Environmental Implications. This study compares the long-term biological implications and environmental impacts of three NBMs (n-NiO, b-NiO and $NiSO_4$) in soybean−soil−microbial systems. Our study demonstrates for the first time that n-NiO can be directly accumulated by plants and transferred to soybean seeds in particulate form. This study deepens our understanding of the ability of plants to accumulate and translocate three different forms of Ni into different plant tissues. n-NiO at environmentally relevant concentrations does not produce significant phytotoxicity. Compared to $NiSO_4$, n-NiO can maintain a more constant source of Ni^{2+} over a longer period of time, thereby reducing the toxicity associated with rapidly available large doses. n-NiO at appropriate concentrations (50 mg kg^{-1}) can effectively improve soybean yield and nutritional quality by enhancing plant photosynthesis and modulating nitrogen metabolism and hormone levels (Figure 6). In addition, this study adds to our understanding of the long-term fate of n-NiO, b-NiO, and $NiSO_4$ in agricultural soils. However, additional research should be conducted on n-NiO retained in different types of soil for long time periods to examine their safety, particularly across longitudinal soil profiles. The accumulation of particulate NiO in seeds should be also examined in long-term field conditions to ensure food safety. The beneficial dose in this specific study was 50 mg kg^{-1}. However, to optimize the dose for other types of soils as well as plant species, further examination is required. This can be achieved through experiments with computational modeling as a prescreening tool. Additionally, the foliar application can be explored as an alternative approach for application, given its higher absorption efficiency. However, it needs to be tested to examine whether Ni can reach the root, which is particularly important for legume plants. Strategies such as surface modification or coating can be explored to enhance the delivery efficiency and control the release of Ni.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c00959.

Details of NM characterization (Figure S1), phenotypic data (Figure S2 and S3), seed nutrients (Figure S4-S7), antioxidant system (Figure S8), phytohormone (Figure S9), nodule nitrogenase activity (Figure S10), inorganic nutrients (Figure S11), soil enzyme activity (Figure S12), dissolution kinetics (Figure S13), extractable Ni (Figure S14), and sp-ICP-MS results (Figure S15) (PDF)

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Notes

The authors declare no competing financial interest.

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