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ARTICLE

Nickel Enhances Soybean Growth and Resilience to Iron Stress by Improving Gas Exchange and Antioxidant Metabolism

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ABSTRACT: Nickel (Ni) is an essential metallic micronutrient for optimal plant growth and development, regulator of essential metabolic processes, but its interaction with other essential nutrients can result in differences in the absorption of these nutrients, which can disrupt the ionic balance. The objective of this research was to evaluate the physiological performance and growth of soybean plants subjected to Ni levels applied via soil under Fe (iron) excess, determining the behavior of redox metabolism, gas exchange, and photosynthetic pigments. The experiment was conducted in a completely randomized design with a factorial 2 × 3, with two Fe levels, defined as control Fe (35.7 μM) and excess Fe (357 μM), and three Ni levels (0.2, 1.0, and 3.0 mg kg^{-1}). Results revealed that Fe toxicity caused significant reductions for leaf dry matter (LDM) and stem dry matter (SDM), but Ni applied to the soil provided increases of 8% and 22% in LDM and SDM. Treatment with toxic Fe caused reductions in photosynthetic pigments in soybean plants. However, 3.0 mg kg^{-1} Ni caused increases ($p < 0.05$) of 10%, 12%, 10%, and 36% for chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids. Plants treated with 3.0 mg kg^{-1} Ni and exposed to Fe stress had boosted the antioxidant system, increasing catalase (14%) and ascorbate peroxidase (16%), while the oxidative damage occasioned by Fe excess in was reduced 6% and 3% in malondialdehyde and hydrogen peroxide, as compared to Fe excess +0.2 mg kg^{-1} Ni. Therefore, the Ni application via soil under experimental conditions was found to be a possible mitigator of the phytotoxic effects caused by Fe excess in soybean plants.

KEYWORDS: Biomass; *Glycine max*; micronutrient; photosynthesis

1 Introduction

Soybean is one of the main oil crops produced and consumed in the world, and Brazil is a biggest producer and exporter of this crop. The 2022/2023 harvest yielded a record production of 155.4 million tons, representing a 3.5% increase compared to the previous harvest [1]. Several factors contribute to this increase in worldwide yield, including favorable climate conditions, the use of cultivars with high vigor, and modern techniques in plant management [2,3].

Optimal development and consequent high yield are the result of extracting nutrients from the soil and proper nutritional management for the plant. Failure to provide nutrients, inadequate absorption, or even excess nutrients can result in mineral and nutritional deficiency [4]. For this reason, mineral nutrition has contributed significantly to crop yield, with the market offering various sources of fertilizers enriched, mainly in microelements [5].

Nickel (Ni) is an essential metallic micronutrient for optimal plant growth and development. Without an adequate supply of Ni, plants cannot complete their life cycle [6]. Currently, studies indicate positive physiological responses under adequate Ni level, benefiting the development of legumes with soil or foliar fertilization [7,8]. There is information in the literature on the direct influence of Ni on seed germination, nitrogen metabolism, root nodulation, enzymatic activities, and grain yield. However, Ni excess can also inhibit dry matter production and chlorophyll biosynthesis [9–11].

Essential metabolic processes are regulated by Ni, but its interaction with other essential nutrients, such as calcium (Ca), zinc (Zn), magnesium (Mg), and iron (Fe) can result in differences in the absorption of these nutrients, which can disrupt the ionic balance [9,12]. The interaction between elements can be synergistic, when the presence of one element in the medium increases the absorption of another, or antagonistic, when the presence of one element reduces the absorption of another [13]. The absorption of large amounts of essential metals, including the Fe, exposes plants to stress and negatively affects them through protein degradation due to the formation of ROS, which are highly phytotoxic [14], causing nutritional disorders, impairs gas exchange and the biosynthesis of photosynthetic pigments [15]. Based on the high physicochemical similarity between Fe and Ni, it is likely that Fe excess may disrupt Ni efficiency [16]. Therefore, it is important that the plant receives Ni adequately, with an optimal concentration and under a safe limit [17].

Fe is essential for photosynthesis, biological nitrogen fixation, respiration and electron transfer processes [18]. However, Fe excess induces significant risks to human health and the environment [19]. Among the metals, Fe is the most abundant, and its availability is highly influenced by soil pH and oxidation-reduction potential. Fe toxicity is frequently observed in flooded environments, where the absence of oxygen favors the conversion of Fe^{3+} to Fe^{2+}, thus increasing its availability and absorption by plants. Clayey soils, considered dystrophic, have high acidity, a condition characterized by high Fe concentrations, for which soil pH correction is an alternative to restrict the element's flow [20]. However, the practice may be insufficient to reduce Fe to non-toxic levels, and may trigger metabolic disorders in plants and compromise their crop yield.

The hypothesis of this research is that Ni supply can reduce the Fe excess, acting as a metallic antagonist [21]. Therefore, the objective of this research was to evaluate the physiological performance and growth of soybean plants subjected to Ni levels applied via soil under Fe toxicity conditions, determining the behavior of antioxidant metabolism, gas exchange, and photosynthetic pigments.

2 Materials and Methods

2.1 Soil, Containers, Plant Material and Fertilization

Universidade Federal Rural da Amazônia, Paragominas, Brazil (2°55′ S, 47°34′ W), hosted the experiment. Minimum, maximum, and median temperatures were 22, 30, and 26.1°C and relative humidity ranged from 60% to 80% [22]. The soil was dystrophic yellow Latosol [23]. The soil in 4L pots was adjusted to pH 6.0 by adding 1.76 g of $CaCO_3$ kg^{-1} and 0.76 g of $MgCO_3$ kg^{-1} and incubated for 30 days. This experiment used soybean TMG 2158, a genotype known for combining technologies such as Intacta RR2 PRO (resistance to caterpillars and tolerance to glyphosate) and INOX technology (resistance to Asian soybean rust), with a super-early cycle and good performance under water-stress conditions. Previous research has shown that genotype TMG2158 soybeans respond well to Ni fertilization [24]. The soil received macro and micronutrients (excluding N, Fe, and Ni) at the following concentrations: 150 mg of P kg^{-1} ($Ca[H_2PO_4]_2$), 50 mg of P kg^{-1} + 100 mg of K kg^{-1} (KH_2PO_4), 50 mg of S kg^{-1} ($MgSO_4{\cdot}7H_2O$), 4.0 mg of Cl kg^{-1} ($MnCl_2{\cdot}4H_2O$), 4.0 mg of Mn kg^{-1} ($MnSO_4{\cdot}H_2O$), 3.0 mg of Zn kg^{-1} ($ZnSO_4{\cdot}7H_2O$), 1.0 mg of B kg^{-1}

(H_3BO_3), 2.0 mg of Cu kg^{-1} ($CuSO_4 \cdot 5H_2O$), and 0.5 mg of Mo kg^{-1} ($[NH_4]6Mo_7O_{24} \cdot 4H_2O$). Table 1 shows soil physical and chemical properties after pH correction and fertilizer.

Table 1: Physical and chemical characteristics of the clayey yellow Oxisol before experiment, after incubation with limestone and after experiment.

Before Experiment											
Sand	Silt %	Clay	Fe mg dm^{-3}	Ni μg dm^{-3}	pH ($CaCl_2$)	P mg dm^{-3}	Ca	Mg	K $cmol_c$ dm^{-3}	Al	H+Al
15	13	72	48.9	3.49	5.1	14.8	81.7	21.5	2.7	0.2	28.5
After Soil Incubation											
			Fe	Ni	pH	P	Ca	Mg	K	Al	H+Al
			8.10	1.12	5.7	25.1	92.2	27.1	4.2	0.0	19.8
After Experiment											
Fe Supply	Ni Supply (mg kg^{-1})		Fe	Ni	pH	P	Ca	Mg	K	Al	H+Al
Control	0.2		2.50	0.80	4.7	0.4	16.3	6.2	0.4	0.2	29.0
Control	1.0		3.12	2.71	4.5	0.6	15.3	5.9	0.6	0.2	28.3
Control	3.0		2.81	10.31	4.6	0.8	14.3	5.6	0.8	0.2	27.6
Excess	0.2		3.10	1.32	4.6	1.0	13.3	5.3	1.0	0.2	26.9
Excess	1.0		3.25	9.49	4.5	1.2	12.3	5.0	1.2	0.2	26.2
Excess	3.0		3.45	12.28	4.4	1.4	12.5	4.7	1.4	0.2	25.5

The data shown come from one sample per time (before experiment, after soil incubation, and after experiment). n = 1.

2.2 Design and Treatments

The experiment used a randomized design with a factorial 2 × 3, two Fe levels (control Fe: 35.7 μM/×1 Fe) and excess Fe: 357 μM/×10 Fe), and three Ni levels (0.2, 1.0, 3.0 mg kg^{-1}). A prior study [25] set Fe concentrations [25], while another set Ni values [24]. To eliminate Ni and Fe contamination, solutions were cleansed [26]. This research had 30 experimental units, with one plant per pot, for a total of 6 treatments.

2.3 Fe and Ni Treatments

Directly into the pot went soybean seeds. On the fourth day following seeding, each plant received 1 mL of a *Bradyrhizobium japonicum* containing 8×10^8 cells·mL^{-1}. Fe and Ni were added to the soil before seeding. $FeSO_4 \cdot 7H_2O$ was utilized at 35.7 and 357 μM Fe concentrations. Ni supplies, $NiCl_2$ was utilized at 0.2, 1.0, and 3.0 mg kg^{-1} Ni. With deionized water only, the pots' soil water content was regulated daily to about 70% of field capacity by weighing to a consistent weight. On the 45th day of the experiment, all plants' physiological and morphological characteristics were examined, and leaf tissue was harvested for biochemical examination at stages R1-R2 during flowering [27].

2.4 Analysis Soil and Plants

Mehlich solution was used to measure extractable Ni in soil samples after plant culture. Samples were shaken for 10 min at 200 rpm and left for 16 h to obtain the extract [28]. ICP-OES measured nickel concentration. The dry mass of leaves, stem, and roots was calculated by collecting one plant from each container and oven drying at 65°C for 72 h. ICP-OES was used to measure macronutrient and micronutrient concentrations in leaves, stem, and root to assess plant nutrition [29].

The gas exchange was evaluated following the calibration procedures [30]. Stress indicators (hydrogen peroxide [H_2O_2] and malondialdehyde [MDA]) were extracted [31]. H_2O_2 was detected [32]. MDA was

measured [33]. Electrolyte leakage (EL) was evaluated [34]. The chlorophyll and carotenoid determination were performed with 40 mg of leaf tissue grounded in liquid nitrogen and homogenized in the dark with 8 mL of 90% methanol. Pigments were quantified using a spectrophotometer (model UV-M51; Bel Photonics) [35]. Catalase (CAT), ascorbate peroxidase (APX), and soluble proteins were extracted [36]. Total soluble proteins [37]. The CAT activity was evaluated [38], with activity expressed in μmol H_2O_2 mg^{-1} protein min^{-1}. The APX assay was done using the protocol [39], with APX activity expressed in μmol as AsA mg^{-1} protein min^{-1}.

2.5 Data Analysis

Statistical analysis was performed using SAS System for Windows 6.11 [40]. ANOVA was used depending on the significance level in the F test ($p < 0.05$). To find significant treatment differences, factorial ANOVA and Scott-Knott test were used.

3 Results

3.1 Ni Stimulated the Biomass in Plants Exposed to Fe Excess

The effect of Fe isolated caused significant reductions for LDM and SDM in soybean plants (Table 2). However, the application of 3.0 mg kg^{-1} Ni provided increases of 8% and 22% in LDM and SDM, respectively, compared to 0.2 mg kg^{-1} Ni soil, for plants exposed to Fe excess. Plants in the Fe control condition also exhibited increases in LDM and SDM with increasing levels of Ni. Fe excess reduced ($p > 0.05$) the RDM and TDM. However, the large of Ni level again increased in 10% and 14% the same variables, compared to 0.2 mg kg^{-1} Ni, in the plants toxic Fe condition.

Table 2: Biomass of soybean plants exposed to Fe excess and different Ni supplies.

Fe Supply	Ni Supply (mg kg^{-1})	LDM	SDM	RDM	TDM
Control	0.2	4.00 ± 0.06 Ba	3.53 ± 0.09 Ba	2.73 ± 0.12 Ba	10.26 ± 0.26 Ba
Control	1.0	4.11 ± 0.06 Ba	3.61 ± 0.11 Ba	2.99 ± 0.16 Ba	10.71 ± 0.28 Ba
Control	3.0	4.33 ± 0.09 Aa	3.90 ± 0.14 Aa	3.66 ± 0.17 Aa	11.89 ± 0.34 Aa
Excess	0.2	3.94 ± 0.05 Ba	3.24 ± 0.05 Bb	2.24 ± 0.15 Cb	9.42 ± 0.13 Cb
Excess	1.0	4.06 ± 0.07 Ba	3.36 ± 0.08 Bb	2.63 ± 0.11 Bb	10.05 ± 0.19 Bb
Excess	3.0	4.25 ± 0.10 Aa	3.58 ± 0.10 Ab	3.28 ± 0.15 Ab	11.11 ± 0.21 Ab

LDM = leaf dry matter; SDM = stem dry matter; RDM = root dry matter; TDM = total dry matter. Columns with different uppercase letters between Ni supplies (0.2, 1.0, and 3.0 mg kg^{-1} under equal Fe supply) and lowercase letters between Fe supplies (control and Fe excess under equal Ni supply) indicate significant differences from the Scott-Knott test ($p < 0.05$). Means ± SD. n = 5.

3.2 Chloroplastic Pigments and Gas Exchange Were Improved with Ni Treatment

Fe excess caused decreases in chloroplastic pigments (Table 3). However, 3.0 mg kg^{-1} Ni caused increases ($p < 0.05$) of 10%, 12%, 10% and 36% for Chl *a*, Chl *b*, Total Chl and Car, respectively, compared to treatment with 0.2 mg kg^{-1} Ni and Fe excess. Chl *a*/Chl *b* and Total Chl/Car were significantly increased after Fe excess, but reduced by 9% and 2%, respectively, comparing with equal Fe treatment without Ni. To gas exchange, Fe toxicity provoked significant reductions (Table 4). Ni supplies mitigated the deleterious effects in plants under Fe excess. Plants exposed to Fe excess and treated with 3.0 mg kg^{-1} Ni presented increases in P_N, g_s, E, WUE and P_N/C_i by 22%, 26%, 15%, 3% and 33%, respectively, when compared with equal treatment without Ni.

Table 3: Photosynthetic pigments of soybean plants exposed to Fe excess and different Ni supplies.

Fe Supply	Ni Supply (mg kg^{-1})	Chl *a* (mg g^{-1} FM)	Chl *b* (mg g^{-1} FM)	Total Chl (mg g^{-1} FM)	Car (mg g^{-1} FM)	Ratio Chl *a*/ Chl *b*	Ratio Total Chl/Car
Control	0.2	7.54 ± 0.13 Ca	2.10 ± 0.05 Ca	9.64 ± 0.19 Ca	1.41 ± 0.11 Ca	3.59 ± 0.08 Ab	6.84 ± 0.21 Ab
Control	1.0	8.27 ± 0.21 Ba	2.33 ± 0.07 Ba	10.60 ± 0.22 Ba	2.30 ± 0.12 Ba	3.55 ± 0.08 Ab	4.61 ± 0.18 Bb
Control	3.0	8.77 ± 0.23 Aa	2.53 ± 0.08 Aa	11.30 ± 0.25 Aa	2.65 ± 0.14 Aa	3.47 ± 0.06 Ab	4.26 ± 0.13 Cb
Excess	0.2	7.27 ± 0.06 Cb	1.60 ± 0.03 Cb	8.87 ± 0.12 Cb	1.12 ± 0.08 Cb	4.54 ± 0.07 Aa	7.92 ± 0.28 Aa
Excess	1.0	7.73 ± 0.09 Bb	1.71 ± 0.04 Bb	9.44 ± 0.15 Bb	1.34 ± 0.09 Bb	4.52 ± 0.07 Aa	7.04 ± 0.23 Ba
Excess	3.0	8.03 ± 0.11 Ab	1.80 ± 0.04 Ab	9.83 ± 0.19 Ab	1.57 ± 0.10 Ab	4.46 ± 0.08 Aa	6.26 ± 0.22 Ca

Chl *a* = chlorophyll *a*; Chl *b* = chlorophyll *b*; Total chl = total chlorophyll; Car = carotenoids. Columns with different uppercase letters between Ni supplies (0.2, 1.0, and 3.0 mg kg^{-1} under equal Fe supply) and lowercase letters between Fe supplies (control and Fe excess under equal Ni supply) indicate significant differences from the Scott-Knott test ($p < 0.05$). Means ± SD. n = 5.

Table 4: Gas exchange of soybean plants exposed to Fe excess and different Ni supplies.

Fe Supply	Ni Supply (mg kg^{-1})	P_N (μmol m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	*E* (mol m^{-2} s^{-1})	C_i (μmol mol^{-1})	WUE (μmol $mmol^{-1}$)	P_N/C_i (μmol m^{-2} s^{-1} Pa^{-1})
Control	0.2	10.64 ± 0.33 Ba	0.18 ± 0.01 Ba	2.47 ± 0.17 Aa	266 ± 2 Ab	4.31 ± 0.09 Ba	0.040 ± 0.001 Ca
Control	1.0	11.30 ± 0.40 Ba	0.20 ± 0.01 Ba	2.57 ± 0.19 Aa	259 ± 4 Ba	4.40 ± 0.15 Ba	0.044 ± 0.002 Ba
Control	3.0	12.78 ± 0.54 Aa	0.25 ± 0.02 Aa	2.68 ± 0.13 Aa	244 ± 3 Cb	4.77 ± 0.18 Aa	0.052 ± 0.004 Aa
Excess	0.2	7.44 ± 0.26 Cb	0.15 ± 0.01 Bb	2.21 ± 0.14 Aa	282 ± 9 Aa	3.37 ± 0.05 Cb	0.026 ± 0.001 Cb
Excess	1.0	8.19 ± 0.29 Bb	0.17 ± 0.01 Bb	2.31 ± 0.18 Aa	264 ± 6 Ba	3.55 ± 0.07 Bb	0.031 ± 0.002 Bb
Excess	3.0	9.50 ± 0.34 Ab	0.21 ± 0.01 Ab	2.44 ± 0.16 Aa	266 ± 5 Ba	3.89 ± 0.14 Ab	0.036 ± 0.002 Ab

P_N = net photosynthetic rate; g_s = stomatal conductance; *E* = transpiration rate; C_i = intercellular CO_2 concentration; WUE = water-use efficiency; P_N/C_i = carboxylation instantaneous efficiency. Columns with different uppercase letters between Ni supplies (0.2, 1.0, and 3.0 mg kg^{-1} under equal Fe supply) and lowercase letters between Fe supplies (control and Fe excess under equal Ni supply) indicate significant differences from the Scott-Knott test ($p < 0.05$). Means ± SD. n = 5.

3.3 Ni Mitigates Oxidative Stress Caused by Fe Excess

Treatment with Ni reduced the oxidative damage occasioned by Fe excess in soybean plants. However, there were reductions ($p < 0.05$) of 6% and 3% in MDA and H_2O_2, as compared to Fe excess +0.2 mg kg^{-1} Ni (Fig. 1). For antioxidant metabolism (Fig. 2), application of 3.0 mg kg^{-1} Ni caused increases in APX (16%) and CAT (14%), when compared to Fe excess without Ni applied.

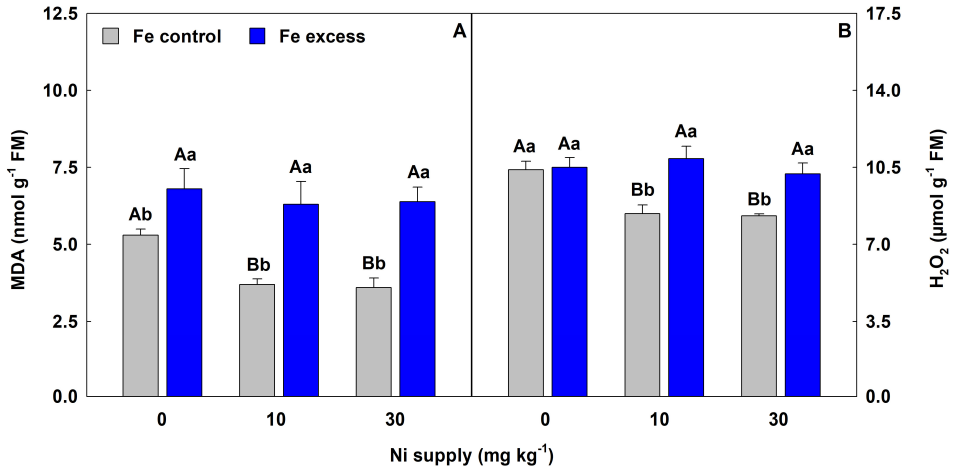

Figure 1: Malondialdehyde (MDA, A) and hydrogen peroxide (H_2O_2, B) in soybean plants exposed to Fe excess and different Ni supplies. Columns with different uppercase letters between Ni supplies (0.2, 1.0, and 3.0 mg kg^{-1} under equal Fe supply) and lowercase letters between Fe supplies (control and Fe excess under equal Ni supply) indicate significant differences from the Scott-Knott test ($p < 0.05$). Means ± SD, n = 5.

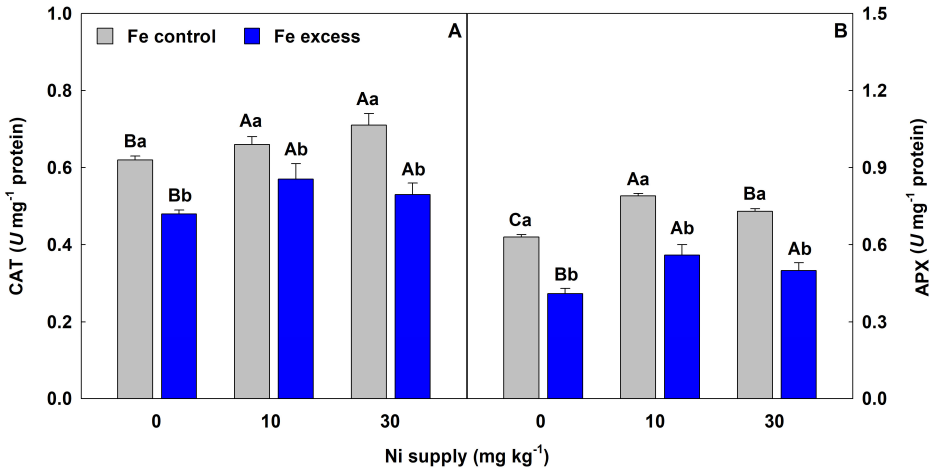

Figure 2: Catalase (CAT, A) and ascorbate peroxidase (APX, B) in soybean plants exposed to Fe excess and different Ni supplies. Columns with different uppercase letters between Ni supplies (0.2, 1.0, and 3.0 mg kg^{-1} under equal Fe supply) and lowercase letters between Fe supplies (control and Fe excess under equal Ni supply) indicate significant differences from the Scott-Knott test ($p < 0.05$). Means ± SD, n = 5.

4 Discussion

Fe excess caused reductions in the biomass accumulation of soybean plants; however, the application of 3.0 mg kg^{-1} Ni mitigated the damage to growth. Ni acts as a cofactor of the enzyme urease, which decomposes urea into CO_2 and NH_3, playing a crucial role in nitrogen metabolism in legumes [41]. The improvement in growth may also be related to the presence of Ni as a structural component in biological nitrogen fixation, which facilitates the development of the root system, enhances nutritional status, promotes biological N_2 fixation, and regulates N metabolism [42,43]. Ni assimilation can be associated with lower Fe uptake by root cells, mitigating the harmful effects of excess Fe on plant growth [44], because both micronutrients are antagonistic and absorbed by the same transporter, called IRT1 (iron-related transporter 1) [45]. Corroborating this research, linear increases in the dry matter of common bean plants and a maximum increase of 28% with Ni (60 g ha^{-1}) [46]. In a study evaluating the effects of different levels of Ni (0, 7.5, 15, 30, and 60 mg L^{-1}) on growth of sweet potato, observed that biomass was improved with the Ni treatment, increasing LDM (14% and 56%) and RDM (12% and 35%) with treatments using 7.5 and 15 mg L^{-1} Ni, respectively [47].

Fe excess caused reductions in photosynthetic pigments of soybean plants, with negative impacts on chlorophyll biosynthesis [48] and essential reactions in photosynthetic machinery [49] due to ROS overproduction [50]. The decreases in Chl *a*, Chl *b*, and Total Chl are related to the increase in MDA content in Fe-stressed plants, given that this is an indicator of lipid peroxidation in cells in the presence of free radicals [51,52]. Oxidative damage resulting from toxic levels of Fe and its effects on photosynthetic variables in rice cultivars, detecting a reduction in pigment indices in the BR IRGA 409 cultivar, after seven days of application of the treatments [53].

Soybean plants treated with 3.0 mg kg^{-1} Ni had increases in pigment levels in the Fe excess condition, which shows that Ni attenuated further changes in chloroplasts. Ni plays an essential role in nitrogen metabolism and biological nitrogen fixation in soybean plants [52], participating in numerous enzymatic metabolic activities, such as urease, which facilitates the assimilation and metabolism of nitrogen [53], a constituent of the chlorophyll molecule. Similar to our results, different nickel concentrations (0, 0.5, 1, 2, and 3 mg kg^{-1}) on nodulation and biological nitrogen fixation in cowpea plants resulted in increases in

the contents of Chl *a*, Chl *b*, and Total Chl [54]. Low concentrations of Ni^{2+} in *Spirulina platensis* plants resulted in a significant increase in Chl *a* and Car values, whereas higher concentrations of Ni^{2+} suppressed the levels of these pigments [55].

Fe is an essential element to the electron transport during the photosynthesis process, but is also responsible for its toxic effects when in excess [55], resulting from degradation of the cell membrane [56] due to the presence of free radicals and excessive generation of reactive oxygen species (ROS) [56]. In rice cultivation, low stomatal conductance has been reported under Fe toxic conditions [57] and near-zero transpiration rates [58]. Reductions in P_N and decreased g_s were detected, being explained by oxidative damage from Fe toxicity on the photosynthetic apparatus [59]. Increased Ni supplementation provided benefits for gas exchange and mitigated the phytotoxic effect of Fe overexposure. In conditions of Fe excess and Ni increase, an adequate dose can benefit the higher activity of the photosynthetic apparatus, specifically photosystem II, which contributes to the reaction center of oxygenic photosynthesis [24]. Physiological performance of cotton plants exposed to different concentrations of Ni (0, 15, 30, 45, 60, 75, and 90 mg dm^{-3}) in the soil to varying stages of development was studied, being obtained the maximum point for P_N, g_s, and E obtained in the treatment with 45 mg dm^{-3} [60].

The increase in MDA and H_2O_2, with excess Fe, is a natural plant response to stress. H_2O_2 can produce hydroxyl radicals (OH^-) in the presence of Fe^{2+}, which is catalyzed by Fe itself, promoting a reaction that contributes to lipid peroxidation [61]. Increases in MDA concentrations have also been detected in rice exposed to toxic levels of Fe [62]. Ni application minimized free radical production and lipid peroxidation. High MDA levels are primary indicators of damage associated with ROS, resulting from the destabilization of membrane integrity and functionality, with subsequent adverse effects on cytoplasmic ion balance [6]. Increases in CAT and APX activities and reduction in MDA and H_2O_2 contents, when salicylic acid was applied simultaneously with Ni ions in *Brassica napus*, attributing the reversal of oxidative stress to the Ni molecule [63]. Similar results, a study evaluating the effect of foliar Ni supply in alleviating cellular damage and photosynthesis impairment resulting from infection of soybean plants by *P. pachyrhizi,* in which the accumulation of H_2O_2 and MDA was less pronounced when Ni applied in plants, compared to plants without Ni, indicating less cellular damage induced by Ni [64].

Fe stress caused reductions in enzyme activities. Fe excess induces oxidative stress and lipid peroxidation in plant cells [65]. As a strategy to regulate the redox system, plants detoxify ROS by enhancing enzyme activities [66]. However, treatment with 3.0 mg kg^{-1} Ni reestablished the redox balance, improving the activity of CAT and APX enzymes. Ni participates in several physiological, biochemical, and morphological processes in plants, among which it acts indirectly on the activity of antioxidant enzymes [67]. In plants subjected to stress levels, CAT and APX act in the detoxification of ROS, which are highly toxic substances for plant metabolism [68,69]. H_2O_2, a substrate of CAT and APX, is converted into H_2O and O_2. Thus, enzymatic activity varies as a function of H_2O_2 levels [15,70]. Similar results, research with two tomato genotypes, pretreated with Ni (15 and 30 mg L^{-1}) and under salt stress, significantly increased the activities of antioxidant enzymes (CAT and APX) [70].

5 Conclusion

This study showed that Fe excess reduced the gas exchange, mainly photosynthesis, of soybean plants. However, the application of 3.0 mg kg^{-1} Ni was able to attenuate the negative effects, reflecting in the biomass. Stress indicators (hydrogen peroxide and malondialdehyde) were drastically reduced by Ni applied in plants under Fe excess. Fe stress caused destruction and delayed biosynthesis of chloroplastic pigments, and reduced the biomass, more specifically of leaf and stem. However, treatment with Ni clearly activated

plant defense mechanisms, inducing positive responses on growth, as validated by the increments in catalase and ascorbate peroxidase activities. The Ni application via soil under experimental conditions was promoted as a possible attenuator for the phytotoxic effects provoked by Fe excess in soybean plants.

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Author Contributions: Elaine Maria Silva Guedes Lobato and Allan Klynger da Silva Lobato were advisors for this project, planned all phases of the research, and critically revised the manuscript. Elizeu Monteiro Pereira Júnior, Lorena de Souza Cunha, Andreza Sousa Carmo, Ana Clara Lucarini and Ynglety Cascaes Pereira Matos conducted the experiment in the greenhouse and performed physiological, biochemical, nutritional and morphological determinations, as well as it wrote and edited the manuscript. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: The data that support the findings of this study are available from the Corresponding Author, Elaine Maria Silva Guedes Lobato, upon reasonable request.

Ethics Approval: Not applicable.

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

Abbreviations

APX	Ascorbate peroxidase
CAR	Carotenoids
CAT	Catalase
Chl *a*	Chlorophyll a
Chl *b*	Chlorophyll b
C_i	Intercellular CO_2 concentration
CO_2	Carbon dioxide
E	Transpiration rate
Fe	Iron
g_s	Stomatal conductance
H_2O_2	Hydrogen peroxide
LDM	Leaf dry matter
MDA	Malondialdehyde
P_N	Net photosynthetic rate
P_N/C_i	Instantaneous carboxylation efficiency
RDM	Root dry matter
ROS	Reactive oxygen species
SDM	Stem dry matter
TDM	Total dry matter
Total Chl	Total chlorophyll
WUE	Water-use efficiency

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