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RESEARCH ARTICLE

Unravelling the fate of foliar-applied nickel in soybean: a comprehensive investigation

Jessica Bezerra de Oliveira · Jose Lavres · Peter M. Kopittke · Rufus L. Chaney · Hugh H. Harris · Peter D. Erskine · Daryl L. Howard · André Rodrigues dos Reis · Antony van der Ent

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Abstract

Background and aims Nickel (Ni) deficiency has been reported to occur in soybean (*Glycine max*) grown on leached tropical soils in Brazil. We aimed to determine whether an internal or external Ni supply can compensate for low Ni within the seed by assessing whether the amount of Ni in the seed whether the foliar-application of aqueous $NiSO_4$ influenced the uptake of Ni by the leaf, the nutritional status of the plant, urease activity and growth.

Methods We used Ni-depleted seeds (<0.35 µg Ni per g) and Ni-sufficient seeds (11.1 µg Ni g^{-1}) for hydroponic experiments. Seedlings were grown either with or without an external Ni supply (0 or 0.85 µM Ni in nutrient solution) and either with or without an internal Ni supply (with or cotyledons removed). In addition, we used synchrotron-based micro-X-ray fluorescence analysis to examine the distribution of foliar-applied Ni (50 and 100 mg L^{-1}).

Key results Leaf Ni concentration and urease activity were both enhanced by increasing either the internal (cotyledon seed store) or external (solution) Ni

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J. B. de Oliveira · J. Lavres
Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, Brazil

P. M. Kopittke
School of Agriculture and Food Sustainability, The University of Queensland, St Lucia, Queensland, Australia

R. L. Chaney
Chaney Environmental, Beltsville, MD, USA

H. H. Harris
Department of Chemistry, The University of Adelaide, Adelaide, Australia

P. D. Erskine · A. van der Ent
Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, The University of Queensland, St Lucia, Queensland, Australia

D. L. Howard
Australian Synchrotron, ANSTO, Clayton, Australia

A. R. dos Reis
Department of Biosystems Engineering, São Paulo State University (UNESP), Tupã, SP, Brazil

A. van der Ent
Laboratory of Genetics, Wageningen University and Research, Wageningen, The Netherlands

A. van der Ent (✉)
Université de Lorraine, INRAE, LSE, F-54000 Nancy, France
e-mail: antony.vanderent@wur.nl

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supply. In addition, plants derived from Ni-depleted seed that received external Ni supply had 9.2% higher biomass relative to plants derived from Ni-sufficient seeds which received Ni. When foliar-applied, Ni accumulated in the pedicles of the trichomes within 15 minutes of application, and then moved to the vascular bundles before dispersing further into tissues within 3 hours.

Conclusions Trichomes are an important pathway for foliar Ni absorption in soybean, but there are still major knowledge gaps our understanding of the physiological function of trichomes in the uptake of metal ions from foliar micro-nutrient treatments.

Keywords Foliar nutrition · Leaf ion absorption · Nickel deficiency · Soybean; XFM

Introduction

Nickel (Ni) is a cofactor of the urease enzyme (EC 3.5.1.5, urea amidohydrolase) which contains two Ni atoms at the active site (Ciurli 2001). These Ni atoms are therefore directly involved in nitrogen (N) metabolism and improving Ni nutrition could increase crop yield by upregulating urea decomposition and N-fixing enzyme activity. Urease is responsible for the hydrolysis of urea and ureides, releasing two molecules of ammonia and one of carbon dioxide (Dixon et al. 1975; Eskew et al. 1983; Witte 2011; Polacco et al. 2013; Ohyama et al. 2017; Todd et al. 2006). Subsequent experimentation established the essentiality of Ni for non-legumes grown with ammonium and nitrate supply (Brown et al. 1987). Nickel deficient plants accumulate toxic concentrations of urea in their leaf tips, but Ni addition prevents toxicity (Eskew et al. 1983; Shimada and Ando 1980; Brown et al. 1987; Rutter 2005). Nickel deficiency causes necrosis at the tip of the leaves due to disrupted of ureide catabolism and affects other metabolism process such as amino acid and the citric acid cycle (Bai et al. 2006; Wood 2006; Freitas et al. 2018).

Soybean is one of the most important agricultural crops worldwide, being a major source of cattle feed, products for human consumption and vegetable oil (Food Agriculture Organization of the United Nations 2017). It is often cultivated on leached tropical soils (especially in Brazil) with low Ni availability and with strong Ni adsorption, which can cause a hidden Ni deficiency potentially to occur (Barcelos et al. 2018; Freitas et al. 2018; Macedo et al. 2020). Under such circumstances, plants do not reach their maximum growth potential despite a lack of visible deficiency symptoms (Dalton et al. 1985; Dabkowska-Naskret et al. 2014; Freitas et al. 2018, 2019; Jaworska et al. 2013; Licht et al. 2006; Morrison et al. 2009; Roca et al. 2008; Rodak et al. 2015). In addition, for soybean plants with insufficient Ni, urease activity decreases resulting in the accumulation of urea-related N compounds, causing toxicity and reduced plant growth (Eskew et al. 1983; Brown et al. 1990; Krogmeier et al. 1991; Lavres et al. 2016; Reis et al. 2017). Remediating Ni deficiency in soybean can be achieved by the application of Ni, with the Ni either applied directly on the seeds at planting, or by Ni foliar application of mature plants (Hosseini and Khoshgoftarmanesh 2013), or by soil Ni fertilization (Macedo et al. 2016, 2020; Rodak et al. 2021). In this study, we have a particular interest in examining how Ni is distributed and translocated in soybean leaves in response to foliar application of Ni, which has been shown to increase urease activity in soybean plants (Ojeda-Barrios et al. 2016). Additionally, Ni foliar application can avert toxic urea accumulation in leaf tissue, and glyphosate drift, which are the toxic symptoms caused by the non-hydrolysis of urea (Eskew et al. 1984; Kutman et al. 2013; Kutman et al. 2014). However, despite the importance of foliar fertilization, including for Ni, many questions remain about how micro-nutrients move into the main organs following their foliar application (Fernández et al. 2017; Li et al. 2017). The lack of knowledge of physiological processes involved in the foliar uptake of applied micro-nutrients via the foliage impedes efforts to increase the success of foliar micro-nutrient fertilizers in plants (Fernández et al. 2013; 2021). Furthermore, the coupling of biochemical analyses and imaging coupled assay can be used as complementary techniques to evaluate the nutritional status of plant together with traditional methods of foliar diagnosis (Oliveira et al. 2021).

Understanding the process of Ni absorbtion following its application via the seeds, leaves or roots is the first step to determine effective strategies aiming to improve plant nutrient management (Freitas et al. 2020; Oliveira et al. 2021, 2022). The use of X-ray fluorescence (XRF) techniques is an effective, non-destructive and efficient method for evaluation

of the spatial distribution of elements in plant tissues. Synchrotron-based micro-X-ray fluorescence (μXRF) analysis is a powerful technique that allows for in situ analyses of the tissue-scale spatial distribution of a wide-range of different elements in plants (Kopittke et al. 2018; van der Ent et al. 2018; 2019). There are still only a limited number of investigations that have examined changes in nutritional distribution over time (at spatiotemporal scale) in living plants using X-ray elemental techniques at synchrotron facilities (Scheckel et al. 2004; Lombi et al. 2011; Blamey et al. 2018; Li et al. 2019), a subset of these having focussed on the toxic effects of Mn in soybean, cowpea and sunflower (Blamey et al. 2018; Doolette et al. 2018). Previous experiments examining the foliar application of Zn fertilizers in sunflower have shown the foliar Zn absorption occurs mainly via trichomes, with Zn specifically accumulating within trichomes in ≤15 min, with the stomatal pathway not the only way to nutrient diffusion and further absorption (Li et al. 2019). Furthermore, it has been noted that substantial quantities of fluorescent and ionic tracers can be absorbed and translocated by trichomes, probably because they do not have a cuticle but are surrounded by a pectin-rich cell wall layer (Schreel et al. 2020). However, there is still a lack of information at spatiotemporal scale of Ni absorption and translocation after foliar application and its transference into to the main organs to fulfil the nutritional requirements of plants. Therefore, current advances in plant nutritional status evaluation and experimental methods open the path to unravel the importance and interconnectedness of the processes of micro-nutrient uptake by plants.

The present study aimed to: (i) establish whether an internal or external Ni supply can compensate for low Ni within the seed; (ii) determine the influence of seed stored Ni at tissue-scale range of Ni-concentration, time-scale of leaf applied Ni-distribution and translocation, and upregulation of urease activity.

Materials and methods

Overall experimental design

Three different experiments were carried out to understand the effects of Ni distribution and translocation on soybean growth. The first hydroponics experiment grew soybean plants to maturity in Ni-purified hydroponics culture to produce Ni-depleted seeds. The second experiment used plants grown from Ni-sufficient seeds to examine the lateral distribution of foliar applied Ni (as aqueous $NiSO_4$) in situ within hydrated intact soybean leaves using synchrotron-based μXRF analysis. The third experiment examined the effect of seed Ni concentration, seed Ni internal contribution (based on the presence or absence of cotyledons), and external supply of Ni on biomass, tissue elemental concentrations and urease activity. This third experiment used the original Ni-sufficient seeds (11.1 μg Ni per g) plus the Ni-depleted seeds (<0.35 μg Ni per g) to grow plants which were then dosed with or without Ni at 0.85 μM in solution and on which cotyledons were either kept attached or excised. The concentration of Ni has been quantified in soybean grains through prior studies conducted in the United States, revealing a range from 0.35 to 29 μg Ni per g) (Wolnik et al. 1983).

Experiment 1: Hydroponics culture to generate nickel-depleted seeds

Soybean plants were grown in nutrient solution in controlled conditions at The University of Queensland (St Lucia, Australia) from October 2019 to February 2020. Nickel-sufficient seeds (*Glycine max* (L.) Merr. cv Bunya – containing 11.1 μg Ni per g) were germinated on a moistened fine perlite/vermiculite mixture at 26 °C. After one week, corresponding to the vegetative phenological stage of cotyledons, the seedlings were transferred to four 30 L containers containing nutrient solution. The lids of the containers had four holes in which the seedlings were inserted in foam baskets, with one seedling per hole. The cotyledons were excised upon transfer in order to remove the Ni-store in the seed. The nutrient solution was continuously aerated and kept at room temperature (22 °C) with a relative air humidity of 60–70 RH%, with light provided using high-intensity full-spectrum LED lighting (Black Dog LED, PhytoMAX-2400, 365–750 nm, photon flux density of 1000 μmol m^{-2} s^{-1}) for 12 h d^{-1}. The nutrient solution contained: total 4.5 mM K^+ (as KNO_3 + other K-salts listed), 2.5 mM Ca^{2+} (as $Ca(NO_3)_2$), 1 mM HPO_4^{2-} as K_2HPO_4), 2 mM Mg^{2+} (as $MgSO_4$), 1 μM Cl^- (as KCl), 2 μM B^+ (as H_3BO_3), 1 μM

Mn^{2+} (as $MnSO_4$), 2 µM Zn^{2+} (as $ZnSO_4$), 3 µM Cu^{2+} (as $CuSO_4$), 4 µM MoO_4^{2-} (as Na_2MoO_4), and total 5 mM NO_3^- (from various nitrate salts listed). During the culture period the soybean plants did not develop biological nitrogen fixation (as judged by visual inspection of roots which did not have nodulation). The Fe-source was Fe-diethylenetriaminepentaacetic acid (FeDTPA, 40 µM Fe) as earlier tests with Fe-hydroxybenzyl ethylenediamine (FeHBED) showed that Fe uptake was limited, even at 100 µM Fe. Nutrient solutions were fully changed twice a week and the nutrient solution pH was maintained at pH 5.8 by daily additions of 0.01 M KOH or 0.01 M HNO_3 solution as required. To achieve Ni-limiting conditions in the nutrient solutions, high-purity (AR trace grade) chemical reagents were used, and the solutions were purified as follows: the macro-element concentrate was purified using Chelex 100 resin (Bio Rad iminodiacetate type resin) to remove polyvalent metal ions ($Fe^{2/3+}$, Mn^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}). The micro-nutrient concentrate was purified using a Ni-specific resin (Eichrom Nickel Resin based on dimethylglyoxime, DMG) to selectively remove Ni^{2+} ions. Measured concentrations of Ni in the nutrient solution were < 0.001 mg L^{-1} (this equals to 0.02 µM Ni) as determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The plants were grown to complete physiological maturity after 110 d (which corresponds to the R7 phenological stage). The Ni-depleted seeds were harvested when plants senesced completely, with these Ni-depleted seeds found to contain <0.35 µg Ni per g, compared to the value of 11.1 µg Ni per g for the original Ni-sufficient seeds.

Scanning electron microscopy (SEM)

Plant specimens at the R1 stage (42 d) from Exp #1 were freeze-dried for SEM analysis by rapid freezing against a solid metal block cooled by liquid nitrogen (−196 °C). Then the cooled block with samples was loaded into a lyophilizer (Thermoline), vacuum-pumped and set to −85 °C. The lyophilisation was carried out gradually by increasing in 5 °C steps throughout 3 days until it reached room temperature. Afterward, the specimens were then sputter-coated with carbon (~25 nm) and fixed on stubs. The specimens were imaged using a Hitachi SU3500 instrument at 100–1000× magnification at 4–5 kV with secondary electron (SE) returns.

Experiment 2: Synchrotron-based µXRF analysis

This experiment aimed to examine the distribution of Ni within the plant tissue after foliar Ni application treatments. Plants were grown from (original) Ni-sufficient seeds in Ni-purified nutrient solution, as described for Exp #1, until they reached 14-days old – equivalent to phenological stage V3. The Ni foliar fertilizer was prepared at two concentrations: 50 and 100 mg L^{-1} (i.e., 0.85 and 1.7 mM Ni) from $NiSO_4$. The pH of the solutions ranged from pH 5.4 to pH 5.1 for the Ni treatments of 50 and 100 mg L^{-1}. The solution also contained 0.5 µL of Silwet® L-77 (trisiloxane-based adjuvant) per 10 mL of solution as a non-ionic surfactant/wetting agent. We first conducted a time series experiment to investigate the Ni uptake process using intact, hydrated young leaves (14 d old) for which $NiSO_{4(aq)}$ foliar application was used. Specifically, we investigated several foliar application times: 0 min (control), 45 min. and 3 hrs. for 50 mg L^{-1} of Ni, and 0 min (control), 15 min., 25 min., 45 min. and 3 hrs for 100 mg L^{-1} Ni. The initial Ni application rates were based on preliminary tests, while for the higher application rate, we opted for a more extensive range of time intervals to enhance the precision of information regarding the uptake process. For each of these treatments, the adaxial surface of the soybean leaves received two 5-µL droplets of $NiSO_4$ solution and the entire leaf (whilst still attached to the plant) was placed in a Petri dish containing a hole in the side wall with the stem passing through the hole in the side. To maintain the humidity inside the Petri dish moist filter paper was added to the bottom of the plate. The environment within the Petri dish was maintained at 30 °C and the relative humidity above 98% RH. In the experimental period, the LED lighting was turned on throughout the incubation period and the droplets in the leaf surface were carefully maintained as a liquid (i.e., the droplets did not dry out). Subsequently, the leaves were excised from the plant and rinsed using tap water (Supplementary Fig. 2). For each sample, the washed leaf was carefully blotted dry and sandwiched on a sample holder between two stretched sheets of Ultralene thin-film (4 µm thick). Samples

were then immediately analyzed using μXRF at the X-ray fluorescence microscopy (XFM) beamline of the Australian Synchrotron.

The XFM beamline, with its 384-element Maia detector system in a backscatter geometry, was used to collect X-ray fluorescence emitted by the specimen (Howard et al. 2020). An incident energy of 12.9 keV with a total photon flux of approximately 1.5×10^9 photons s^{-1} was used. First an overview scan was carried out to obtain a comparatively fast map of the leaf to select areas for the detailed scans. The detailed scans were typically approx. 4 × 5 mm in size, used a step size of 10 μm, had a dwell of 5 ms, and took approx. 75 min. to complete. An important concern that needs be considered is that fresh hydrated specimens may be affected by radiation-induced damage from the μXRF analysis. To avoid this type of damage in the hydrated plant tissues, the dose was limited to <4.1 kGy (Jones et al. 2020). The Dynamic Analysis method was used to analyse the synchrotron μXRF event stream (Ryan and Jamieson 1993; Ryan 2000) as implemented in GeoPIXE (Ryan et al. 1990, 1995).

Experiment 3: Nickel dosing to plants grown from Ni-depleted and Ni-sufficient seeds

We used two types of seeds: (i) Ni-depleted seeds described above; and (ii) the original Ni-sufficient seeds. The experiment consisted of a total of eight treatments, with two types of seeds (Ni-depleted and Ni-sufficient seeds), two solution Ni concentrations: 0 and 0.85 μM Ni (supplied in the form of $NiSO_4$) (Reis et al. 2017; De Carvalho Braga Levy et al. 2019), and two cotyledon treatments (cotyledons attached, or cotyledons removed after germination). This experiment aimed to examine the effects of Ni concentration on plants grown from either Ni-sufficient or Ni-depleted seeds and the effect of cotyledon Ni-store removal. Each treatment condition (two types of Ni seeds × cotyledon attached or removed × 0 or 0.85 μM Ni) had four biological replicates. Free metal ion activities ($-\log_{10}$ values) were determined using GEOCHEM-EZ software (Shaff et al. 2010) of the full nutrient solution formulation in the 0.85 μM Ni treatment and the $pNi^{2+} = 11.4$. The two types of seeds were placed on moistened fine perlite/vermiculite mixture for one week. The emerged seedlings were then transferred to 20 L containers filled with the nutrient solution described above, with the lids having six holes for each replicate. The nutrient solution was the same as outlined previously, with solutions changed once per week and maintained at pH 5.8. The soybean plants were harvested at phenological stage V4 (fourth trifoliate; Fehr et al. 1971). The third expanded leaf (from the top) was collected for analysis of urease activity (as described in detail below). At the end of the experiment, roots, tap root, stem, young (fourth and younger trifoliolate leaves) and old (primary and first two trifoliolate leaves) leaves were separately collected for acid digestion and ICP-AES. Wet and dry weights of the various plant fractions were also determined.

Urease activity measurements

The urease activity was analysed in vivo twice (20 d – V3 and 27 d – V4 after germination) by an adaptation of the method described by Hogan et al. (1983). 8 mL of NaH_2PO_4 buffer urea at pH 7.4 was prepared to place the plant tissues from Exp #3 (0.2 g of fresh leaves, cut into slices with a width 1 mm). The plants were incubated in the buffer for 3 h at 30 °C and mixed every 5 min. in a Falcon tube of 15 mL were added 0.5 mL of the extract obtained after incubation, 2.5 mL of Reagent 1 (phenol 0.1 mol L^{-1}, sodium nitroprusside – 50 mg) and 2.5 mL of a solution containing sodium hydroxide 0.125 mol L^{-1}; sodium dihydrogen phosphate dodecahydrate 0.15 mol L^{-1}; and sodium hypochlorite 3% Cl_2. Then, the tubes were closed with lids (so as to not lose the NH_3) and put into a water bath at 37 °C for 35 min. Then, the N-NH_4-concentration was measured using a spectrophotometer at 625 nm (Agilent Technologies, Santa Clara, CA, USA). The amount of ammonium (NH_4^+) produced by the reaction was used to measure urease activity, and the values were compared with a standard curve, determined using NH_4Cl.

Elemental analysis of plant tissues

All combinations of seeds × Ni solution concentration × Ni foliar treatments from plants of Exp #3 were analyzed for the elemental analysis. In addition, seeds, cotyledons, and unripe pods from plants of Exp #1 were analysed. Parts of the plant material samples (old leaves, young leaves, tap root and stem from each treatment) were oven dried at 60 °C for 3 d. The soybean tissues were powdered at 15,000 rpm in an

IKA Tube Mill. The ground samples were weighted using a precision scale (to 100 ± 5 mg) and placed in 6 mL falcon tubes. Subsequently, 2 mL of AR-grade HNO_3 (70%) was added to these samples and left overnight for pre-digestion and the samples were then fully digested in a block heater (Thermo Scientific™ digital dry bath) for 1 hr at 70 °C followed by 1 hr at 125 °C and made to volume (10 mL) with ultrapure water (Millipore 18.2 MΩ·cm at 25 °C). The digestates were finally analysed by ICP-AES (Thermo Scientific iCAP 7400 instrument) focusing on the macro-elements: Na, Mg, Al, P, S, K, Ca, and the trace-elements: Cr, Mn, Fe, Co, Ni, Cu, Zn in radial and axial modes depending on the element and expected analyte concentration. In-line internal addition standardization using yttrium was used to compensate for matrix-based effects. Matrix blanks and SRMs were included (NIST 1515 Apple Leaves). The limit of detection for Ni (on the basis of 100 mg of sample in 10 mL acid digestion solution) was 0.35 µg g^{-1} and values below this were considered as not quantifiable and not used.

Statistical analysis

Data analysis was performed with R Development Core team version 4.3.1 (2023). The data were submitted to analysis of variance by the *F*-test using the package lme4 (Bates et al. 2015), dplyr (Wickham et al. 2022) and tidyr (Wickham et al. 2023). Data were visualized using ggplot2 package (Wickham 2016). When the effect was significant, we applied the Tukey test to compare the effects. In all analysis, the level of significance was 5%. The data were subjected to two-way ANOVA to determine the effects of main effects: type of seeds (Ni depleted and Ni sufficient) and presence or absence of cotyledons and the interaction: type of seeds versus cotyledons factor.

Results

Nickel depletion in hydroponics culture

First, plants were grown to maturity (phenological stage R7-110d) under Ni-limiting conditions in hydroponics to generate Ni deficient seeds for the subsequent Ni dosing experiment (Exp #3). The mature plants in Exp #1 displayed mild Ni deficiency symptoms, including foliar chlorosis, especially at the leaf tips and margins, and some seed pods were infertile (Supplementary Fig. 2). The original seeds contained 11.1 µg g^{-1} Ni, whereas the Ni depleted-seeds produced in Exp #1 contained <0.35 µg g^{-1} Ni (Table 1). We examined the leaves from plants grown in Exp #1 using SEM, with this being important for understanding the foliar absorption of Ni from $NiSO_4.6H_2O$ fertilizer subsequently in Exp #3. Two types of trichomes were found on the adaxial surface of soybean (Fig. 1a): non-glandular trichomes (multicellular, thick walled and non-secretory) and glandular trichomes (multicellular and secretory). The glandular trichomes (Fig. 1b) are comparatively short structures and were highly vacuolated. They typically consisted of an epidermal cell and around four or five stalk cells with one small cell on the apex. The external morphology of the glandular trichomes had a series of strong linear cells separated by periclinal cross walls. The non-glandular trichomes (Fig. 1c) were empty, comprising of a multicellular base and one long stalk cell, namely the pedicle.

Table 1 Elemental concentrations in different plants tissues in soybean plants grown in hydroponics solution culture (Exp #1), except for cotyledons which originate plants grow from Ni-sufficient seeds from #Exp 3. Macronutrients – mg g^{-1} (Ca, Mg, K, P) and micronutrients – µg g^{-1} (Ni, Cu, Mn, Zn, Fe)

	Parts of plant				
	Seeds		Cotyledons		Unripe pods
	Ni depleted seeds	Ni sufficient seeds	Ni depleted seeds	Ni sufficient seeds	Ni depleted seeds
Ni	<0.4	11.1	<0.4	5.3	<0.4
Cu	7.3	13.9	8.5	16.4	8.7
Mn	133	97.4	187	212	188
Zn	71	56.1	70.3	65.2	89.8
Fe	69.6	86.7	71.8	124	75.5
Ca	3.0	2.1	5.3	9.7	12.3
Mg	3.2	2.5	4.2	5.0	4.5
K	23.1	19.8	23.3	31.3	44.9
P	6.2	5.1	7.9	6.2	5.7

*data derived from the analysis of a large bulk sample of the seed stock used in this study ($n = 1$)

Synchrotron µXRF analysis of soybean seeds

The spatial distribution of elements in whole soybean seeds (original stock with 11.1 µg g^{-1} bulk Ni) was analysed by synchrotron-based µXRF. Supplementary

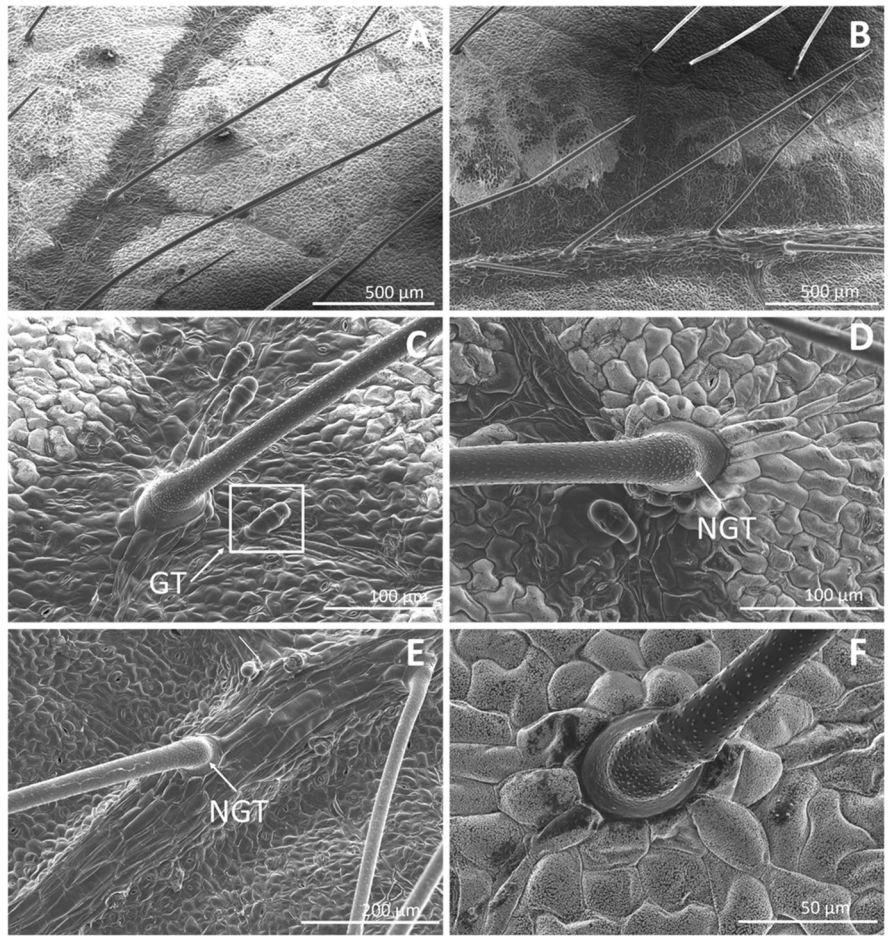

Fig. 1 Scanning electron microscopy (SEM) images of trichomes on the adaxial surface of soybean leaves at the R1 stage from plants originating from Exp #1. In figs. (**A**-**B**), an overview of the soybean leaf surface reveals diverse trichomes types; (**C**-**D**) detailed image of the trichomes: glandular trichomes (GT) and non-glandular trichomes (NGT), as illustrated in figs. **C** and **D**, respectively. Further insight into the trichome is provided in (E-**F**)

Fig. 3 shows the distribution of Ca, K, Fe, Zn, Mn, and Ni in the seeds. Calcium was localised around the seed coat (average of 1.5 µg g^{-1}), with hotspots in parts of the hilum (0.3 µg g^{-1}). Potassium was localised in the endosperm of the seed and was very low in the radicle (0.2 µg g^{-1}), while there were some regions of the seed coat with higher prevailing K concentrations (1.4 µg g^{-1}) (Supplementary Fig. 3). Similar to Ca, Fe was localised in the seed coat (0.15 µg g^{-1}) and in hotspots at the tip of the radicle (0.5 µg g^{-1}). Zinc had a more homogeneous distribution, and it was relatively uniform localised throughout the seed (in the endosperm and seed coat), however in the radicle Zn was more enriched up to 250 µg g^{-1} (Supplementary Fig. 3). Prevailing Mn concentrations were extremely low, although there were some hotspots at the margin of the radicle (up to 200 µg g^{-1}), while other parts of the seed have approximately 50 µg g^{-1} Mn. Nickel was mainly localised in the seed coat, being very low throughout the rest of the seed, with an average of 40 to 20 µg g^{-1}. However, there were areas of hotspots in the seed coat, where the concentration of Ni was around 80 µg g^{-1}.

Synchrotron µXRF analysis of uptake and redistribution of foliar applied nickel

Changes in the spatiotemporal distribution of Ni (and other elements) were examined in situ using fresh hydrated soybean leaf tissues at different stages from Exp #2 following exposure to foliar Ni application at 50 and 100 mg L^{-1} in 5 µL droplets (as $NiSO_4$ solution) after 15 min and after 3 hrs. Rapid survey scans in soybean leaves were used to select the areas of interest, with these demonstrating that within 15 min. the Ni had already relocated across the leaf surface and was present inside the leaf tissues, mainly

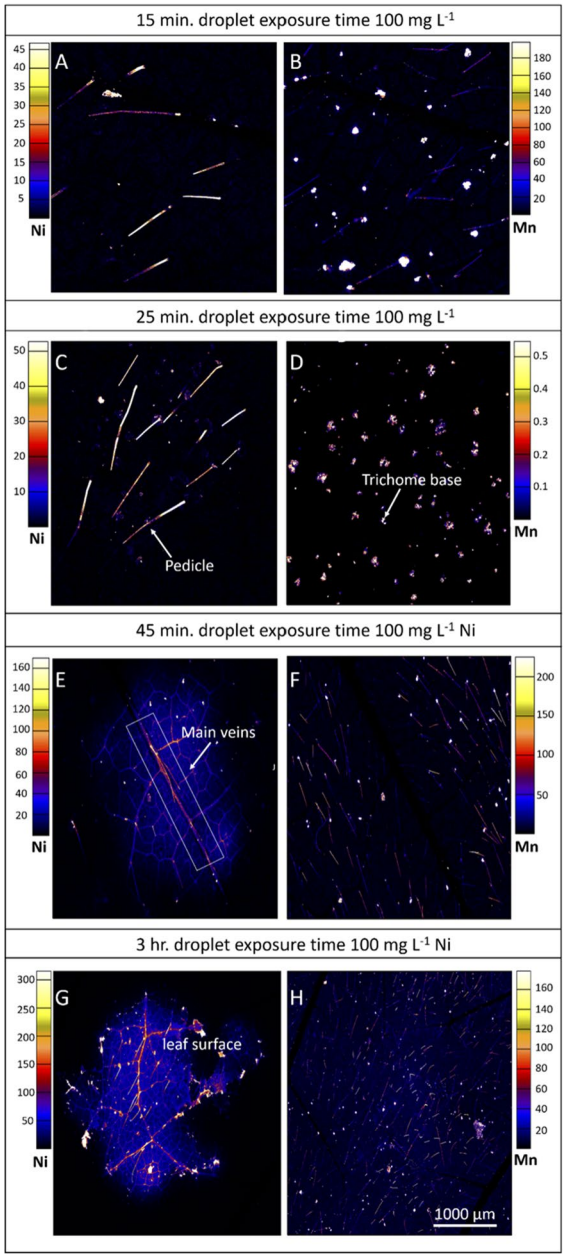

Fig. 2 Synchrotron µXRF elemental maps showing the distribution of Ni and Mn after foliar application of 100 mg L^{-1} at 15, 25, 45 min. and 3 hrs. time intervals in soybean leaf. The concentrations are in wt% for Mn in the second panel and µg g^{-1} for Ni and Mn in all other panels with brighter colours corresponding to higher prevailing elemental concentrations. **A**-**F** have the same colour scale, where brighter colours correspond to higher Ni and Mn concentrations, as shown in the key. The scale bar applies for all the figures. Droplets were left on the left surface for 15 min., 25 min., 45 min. and 3 hrs.

around the trichomes (Fig. 2) and tissue Ni concentrations increased as the exposure time increased. After 15 min., Ni was observed to accumulate in the pedicles of the trichomes with some Ni moving toward the rest of the leaf through the veins; movement continued in the 3-hr study. Nickel was strongly concentrated in the trichome pedicles and Mn was strongly concentrated in the trichome bases, respectively. Figure 2 shows the distribution of Ni and Mn in a hydrated leaf and panels show trichomes after 15- and 25-min. exposure to 100 mg L^{-1} $NiSO_4$ solution; Ni was clearly present in the pedicles of the trichome, whilst Mn was localised in the bases of the trichomes. Figure 2 shows foliar application of Ni at 45 min. and 3 hrs. exposure to $NiSO_4$ solution which suggests that the Ni had rapidly translocated away from the site of application. The overall patterns were very similar after application of 50 mg L^{-1} Ni (Fig. 3) compared to 100 mg L^{-1} Ni. Detailed scans were then used to examine Ni distribution more closely around trichomes after foliar application. High concentrations of Ni accumulated in the pedicles of trichomes, suggesting that the most likely pathway of Ni entry is though the trichomes, as the plants were grown in a culture system devoid of Ni. As such, it unlikely the Ni deposition was delivered by leaf cells or from root uptake to the trichomes (Fig. 3). Using tri-colour maps of Ca-Ni-Mn (Fig. 4) it is evident that Ni and Mn concentrate near the trichomes, whereas Ca is more widespread throughout the tissues. Zinc follows a similar trend as Mn, locating mainly in the trichome bases (Fig. 4). Detailed µXRF scans (Fig. 4) of leaf surfaces after foliar application of Ni on soybean between 25 and 45 min. showed that 15 min. after foliar application, Ni was present only in the pedicles of the trichomes, whilst Mn was concentrated only in the bases of trichomes.

Effect of nickel supply on tissue concentrations and urease activity

Plants were cultured in (Ni-purified) hydroponics solution using the original (Ni-sufficient) and Ni-depleted seeds for a period of three weeks (V4 phenological stage) (Exp #3). Nickel was supplied in solution at 0 (control) or 0.85 µM Ni dose rate, and at the conclusion of the experiment, root and

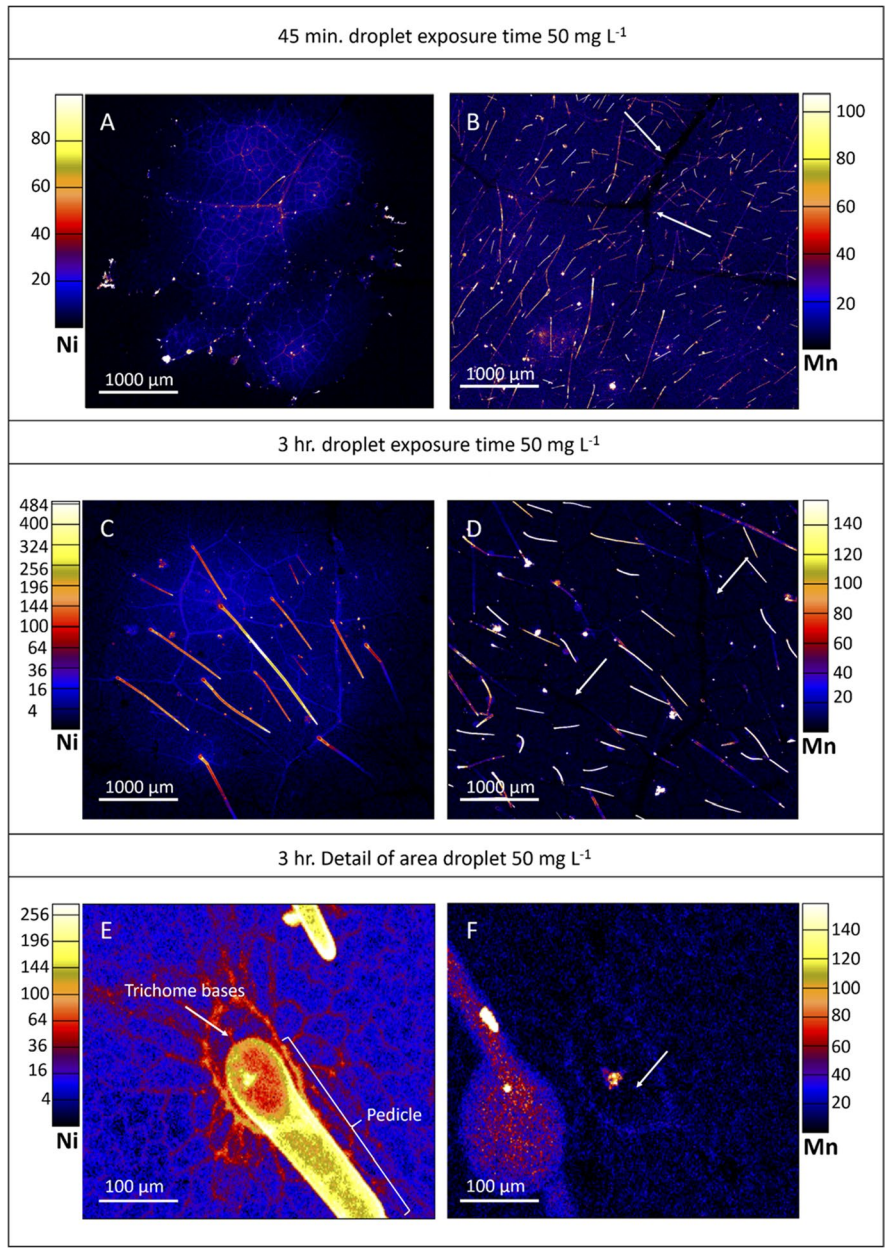

Fig. 3 Synchrotron µXRF elemental maps showing the distribution of Ni and Mn. Images show areas where droplets of 50 mg L^{-1} as $NiSO_4$ were deposited on the leaves of soybean at 45 min. and 3 hrs time intervals. The concentrations are in µg g^{-1} with brighter colours corresponding to higher prevailing elemental concentrations. The figs. **A**-**F** have the same colour scale, where brighter colours correspond to higher Ni and Mn concentrations, as shown in the key. Fig. **E**-**F** shows high resolution of the trichomes structures. The arrows indicate absence of Mn in the presence of Ni

shoot biomass were determined, urease activity measured, and elemental concentrations determined in the different plant parts. The Ni concentration in soybean tissues (root, stem, young, old leaves, all leaves, and whole plant) was compared in plants where the cotyledons were either removed or where they were retained, both without external Ni application (control) or with additional Ni (0.85 µM) (Fig. 5). The presence of the cotyledons, even though they contained very low Ni (<0.35 µg g^{-1} of Ni – Table 1), was sufficient to increase the Ni concentration in soybean tissues compared to the plants that had their cotyledons removed, even without external Ni supply. Plants grown from Ni sufficient seeds stock (Table 1) shows that plants with attached cotyledons had an increase of 2.2-fold of Ni concentration compared to plants with removed cotyledons without external Ni application.

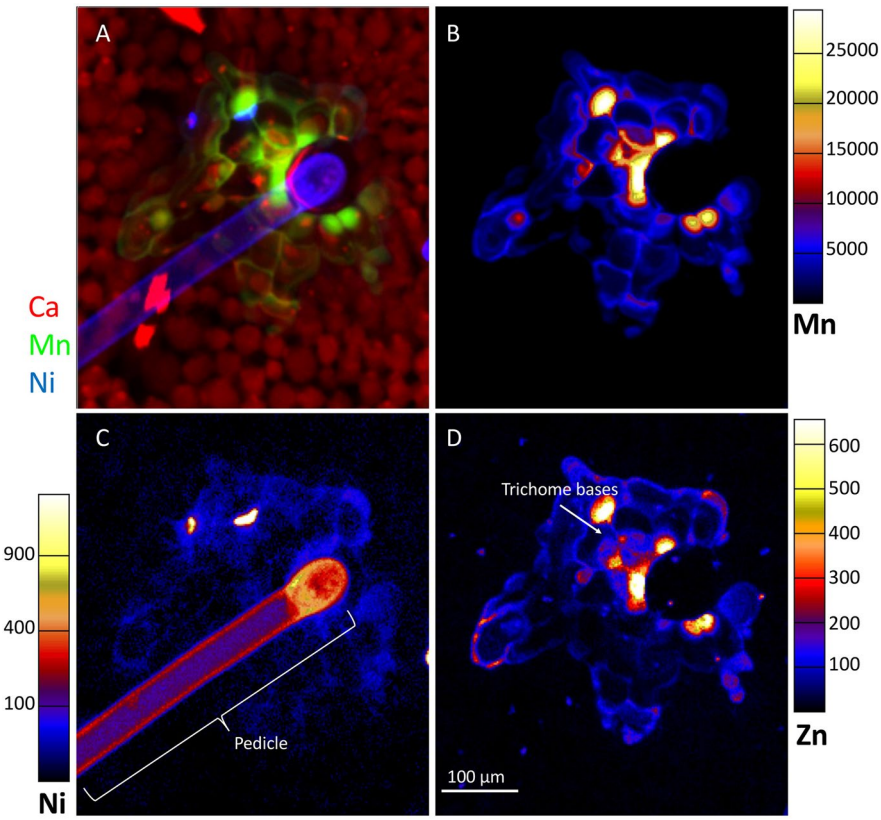

Fig. 4 Synchrotron µXRF elemental maps showing the distribution of Ni, Mn, and Zn. The tricolour image shows Ca in red, Mn in green and Ni in blue. Fig. **A** and **C** show detail of trichomes at foliar Ni application doses of 100 mg L^{-1} at 45 min. and 3 hrs., respectively. Fig. **B** and **D** show the details of trichome base for Mn, and Zn, respectively. The concentrations are in µg g^{-1} where brighter colours corresponding to higher prevailing elemental concentrations

The average tissue Ni concentration was higher when the plants were grown in solutions containing 0.85 µM Ni compared to the control with 0 µM Ni (Fig. 5). The Ni dose increased the Ni concentration in the Ni-depleted seeds 3.12-fold with the cotyledons attached and 3.13-fold in the absence of the cotyledons in the whole plant. For the plants grown from Ni-sufficient seeds, the external supply of Ni increased Ni concentrations 3.78-fold in plants with cotyledons and 7.12-fold in plants without the cotyledons. Plants with cotyledons attached that received Ni grown from Ni sufficient seeds, had a higher concentration of Ni than those grown without cotyledons (Fig. 5), an average of 1.18-fold in the whole plant (stem, roots, old and young leaves) (Fig. 5i). Plants with cotyledons attached grown from Ni-depleted seeds also had a higher Ni concentration (average of 1.09-fold higher) compared to plants with the cotyledon removed from the whole plant. The young leaves from plants grown without cotyledons had an average of 1.61-fold higher Ni compared to those with cotyledons (Fig. 5g).

Figure 6 shows the biomass in different soybean parts: stem, roots, old leaves, and young leaves after different treatments. The plants grown from Ni-sufficient seeds in solution without Ni (0 µM) and those where the cotyledon was attached both had increased biomass compared to the other treatments (Fig. 6k). The plants grown from Ni-depleted seeds whose cotyledons were removed had a lower biomass compared to the plants with cotyledons still attached, being an average of 31% lower. Therefore, seedling development was affected by the presence of cotyledons even in a Ni-free solution. The plants with the cotyledons attached that received Ni (0.85 µM) (Fig. 6) also resulted in an increase of the biomass in the stem and old leaves tissues compared to the plants with cotyledons removed. Additionally, Ni depleted seeds that received Ni had increased biomass (9.2% higher) relative to plants grown from Ni sufficient seeds which received Ni (Fig. 6k).

Urease activity in nickel treated plants

The urease activity (Fig. 7) was influenced by the different seed types (Ni-depleted and Ni-sufficient)

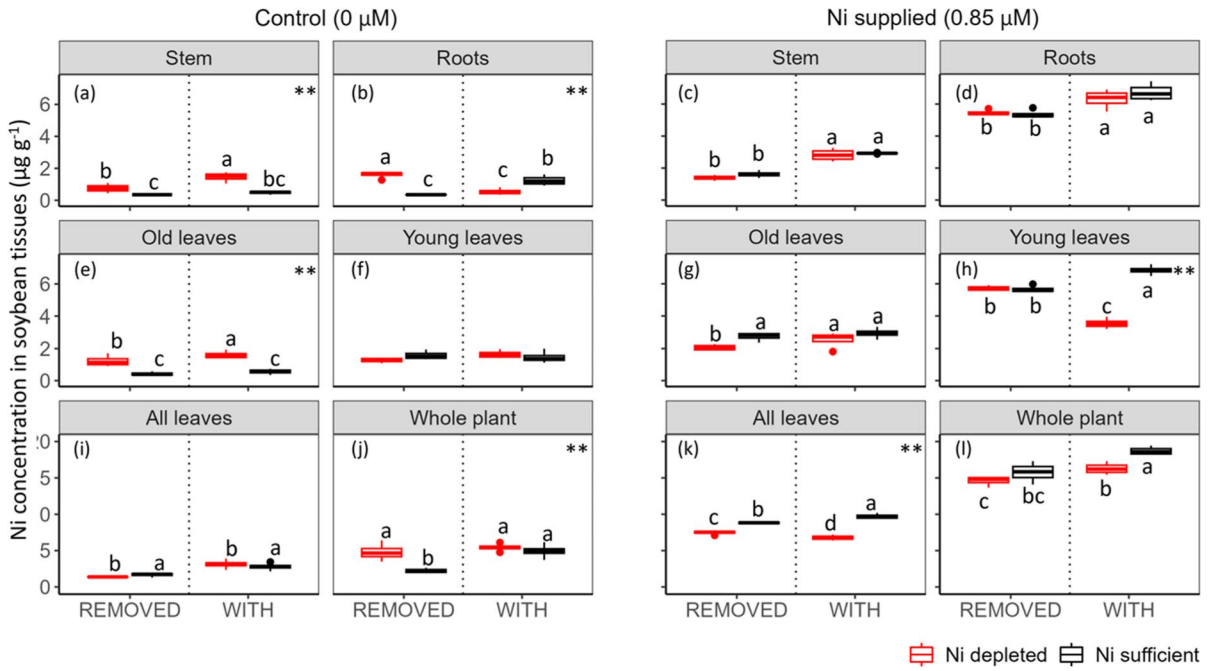

Fig. 5 Box plot of Ni concentrations in organ-tissue scale of the soybean plants grown from two types of seeds (Ni-depleted seeds: red- and Ni-sufficient seeds: black), with ("with") or cotyledon removed ("Removed") and two Ni treatments: control and 0.85 µM Ni in different parts of soybean plant: (**a** and **c**) stem; (**b** and **d**) roots; (**e** and **g**) old leaves; (**f** and **h**) young leaves; (**i** and **k**) all leaves and (**j** and **l**) whole plant. Boxes with different letters indicate significant differences according to least difference (Tukey) test $P < 0.05$. ** indicates that there was interaction between the factors according to least difference (Coty*Seed)

as well as the Ni treatment (0 and 0.85 µM Ni). The addition of 0.85 µM Ni to the solution increased urease activity in the plants grown from both the Ni-depleted and Ni-sufficient seeds. In the plants grown from Ni-depleted seeds, urease activity increased ca. 260% at 0.85 µM Ni. For plants grown from Ni-sufficient seeds, the urease activity tended to be slightly higher than in plants grown from Ni-depleted seeds, especially in the control (0 µM Ni). The presence of the cotyledons in the Ni-sufficient seeds also increased the urease activity even without external Ni supply, increasing from 1.9 (removed cotyledons) to 4.6 N NH_4^+ g^{-1} h^{-1}. Therefore, urease activity was enhanced by higher levels of Ni in the plants.

Elemental concentrations in treated plant tissues

The concentrations of Cu increased in soybean tissues following Ni application. In old leaves of plants which received solution Ni, there was a 65% increase in Cu. Although concentrations of Ca and Mg did not follow a distinct pattern, they tended to increase as Ni concentrations increased (Table 2). Plants grown from Ni-sufficient seeds had higher Mn concentrations in treatment that did not receive external Ni supply compared to those that did receive Ni (0.85 µM). However, for all tissues of plants grown from Ni-sufficient seeds, the average Mn concentration was 10.3% lower compared to the plants from Ni-depleted seeds. For Mn concentration, there were no statistically significant differences for the presence/absence of cotyledons in all tissues, except for the old leaves, regardless of the type of seeds used. Young leaves in plants that received Ni and retained the cotyledons had higher Mn concentrations relative to plants without cotyledons. Manganese concentrations in the stems of plants that did not receive Ni application were higher in plants with the cotyledons, relative to plants where the cotyledons were removed.

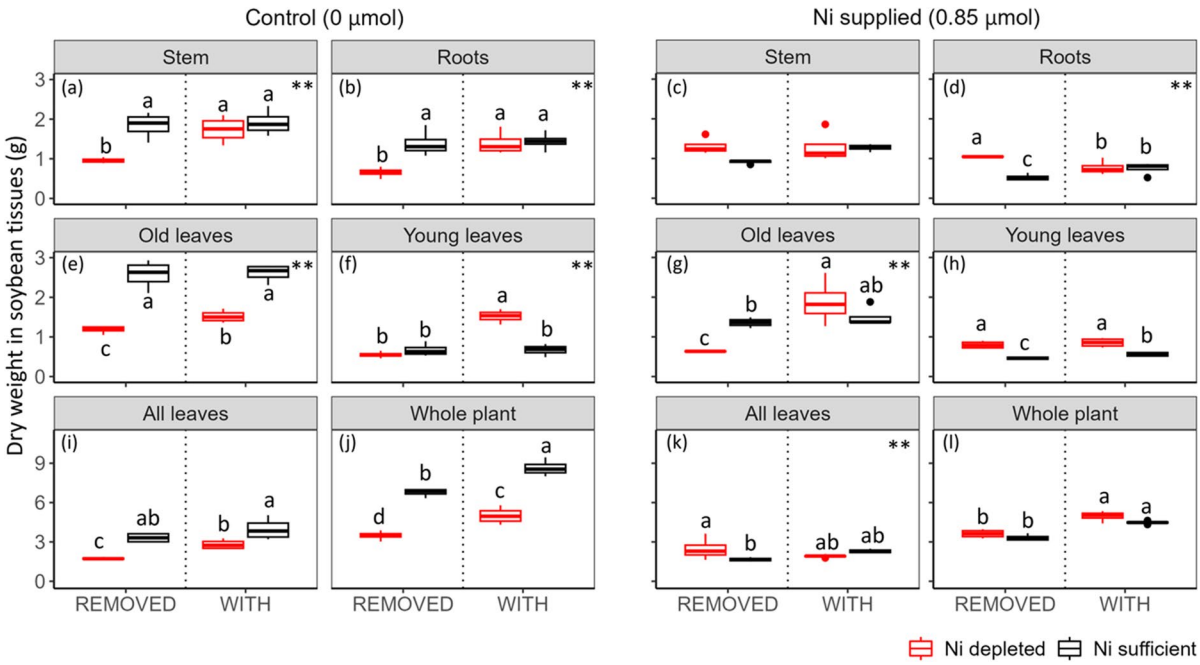

Fig. 6 Box plot of dry weight (g) of soybean parts: stem; roots; old leaves; young leaves; all leaves and whole plant. Plants were grown from two types of seeds (Ni-depleted seeds – red- and Ni-sufficient seeds-black), with ("with") or cotyledon removed ("Removed") and two Ni treatments: control 0 µM Ni and 0.85 µM Ni. Boxes with different letters indicate significant differences according to least difference (Tukey) test $P < 0.05$. ** indicates that there was interaction between the factors according to least difference (Coty*Seed)

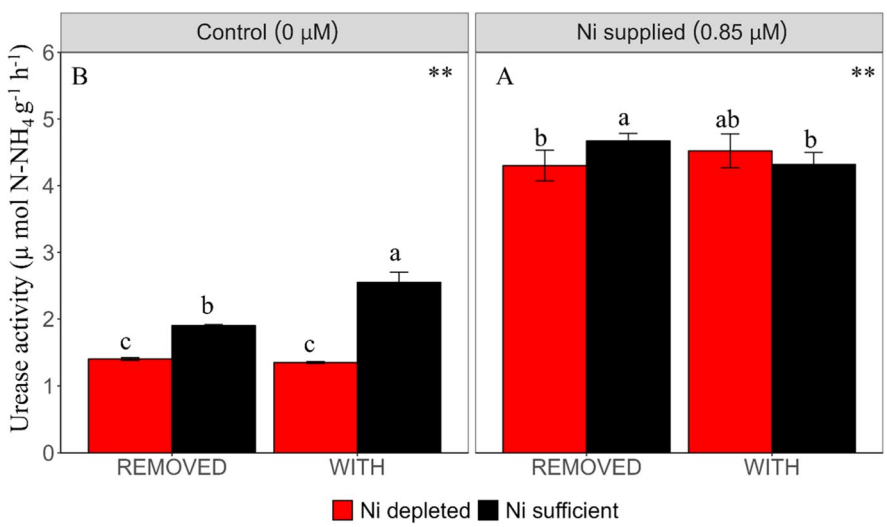

Fig. 7 Urease activity in leaves from plants originating from Exp #3 (grown from Ni depleted seeds and Ni sufficient seeds), with two Ni treatments Ni (control - 0 and 0.85 µM Ni) after 27 days germination). Different letters represent significant differences by Tukey's test ($p < 0.05$); uppercase letter for Ni levels and lowercase letters for seed levels. ** indicates that there was interaction between the factors according to least difference (Coty*Seed)

In contrast, roots had higher Mn concentration in plants that had the cotyledons removed regardless of the Ni application. In general, soybean plants without Ni application and from Ni depleted seeds had higher K concentration compared with plants that received Ni application. Phosphorus concentrations were only statistically different in the stem tissues, whereas plants treated with Ni and without

Table 2 Effect of application of Ni on the concentration of macronutrients – mg g^{-1} (Ca, K, Mg, P) and micronutrients – µg g^{-1} (Cu, Zn, Mn, Fe) in soybean plants grown in hydroponic solution culture (Exp #3)

Old leaves											
Seeds	Treatment	Cotyledon	Ni	Ca	Mg	K	P	Cu	Zn	Mn	Fe
Control	Ni depleted seeds	Attached	1.6 ± 0.2 a	33.3 ± 1.3 a	7.2 ± 0.1 a	37.6 ± 1.7 a	4.3 ± 0.4 ns	5.4 ± 0.5 c	85.0 ± 7.2 a	406.2 ± 25.7 a	144.2 ± 3.7 ns
		Removed	1.2 ± 0.3 b	32.4 ± 1.4 a	7.2 ± 0.2 a	38.3 ± 1.3 a	4.76 ± 0.2 ns	5.9 ± 0.3 bc	89.1 ± 5.0 a	413.7 ± 19.8 a	157.2 ± 8.7 ns
	Ni sufficient seeds	Attached	0.6 ± 0.1 c	24.1 ± 1.2 b	6 ± 0.1 b	37.2 ± 1.1 a	4.3 ± 0.4 ns	6.6 ± 0.6 a	68.2 ± 6.7 a	277.4 ± 17.0 b	147.5 ± 11.4 ns
		Removed	0.4 ± 0.1 c	26.6 ± 2.4 b	5.9 ± 0.2 b	34.4 ± 0.56 b	4.2 ± 0.6 ns	6.6 ± 0.9 ab	86.1 ± 8.9 b	253.8 ± 15.5 b	144.7 ± 20.4 ns
Ni 0.85 µM	Ni depleted seeds	Attached	2.5 ± 0.4 a	33.0 ± 3.3 ns	7.2 ± 0.3 ns	39.8 ± ab	4.9 ± 0.5 ns	9.0 ± 0.8 ns	97.9 ± 2.3 a	340.7 ± 40.9 ns	149.7 ± 7.9 b
		Removed	2.1 ± 0.16 b	31.4 ± 2.7 ns	6.5 ± 1.3 ns	38.9 ± 1.6 b	5.0 ± 0.4 ns	8.4 ± 0.3 ns	88.3 ± 4.4 b	376.5 ± 49.5 ns	167.4 ± 17.0 a
	Ni sufficient seeds	Attached	2.9 ± 0.3 a	28.8 ± 0.9 ns	5.9 ± 0.5 ns	43.9 ± 5.0 a	5.0 ± 0.4 ns	8.9 ± 0.4 ns	87. 9 ± 3.2 b	335.8 ± 11.9 ns	137.7 ± 7.35 b
		Removed	2.8 ± 0.2 a	31.1 ± 1.5 ns	6.2 ± 0.5 ns	37.2 ± 2.6 b	5.4 ± 0.3 ns	9.0 ± 0.3 ns	88.4 ± 4.6 b	360.7 ± 20.1 ns	140.2 ± 6.6 b
Young leaves											
Seeds	Treatment	Cotyledon	Ni	Ca	Mg	K	P	Cu	Zn	Mn	Fe
Control	Ni depleted seeds	Attached	1.6 ± 0.2 ns	15.3 ± 0.5 a	5.6 ± 0.2 a	50.0 ± 5.5 b	7.3 ± 0.4 ns	6.9 ± 0.9 b	81.5 ± 8.0 b	253.5 ± 9.4 a	102.9 ± 4.4 ns
		Removed	1.27 ± 0.12 ns	13.1 ± 0.4 b	5.2 ± 0.2 a	57.5 ± 4.1 a	7.4 ± 1.5 ns	7.7 ± 0.4 ab	90.9 ± 3.7 a	261.8 ± 9.1 a	110.6 ± 7.7 ns
	Ni sufficient seeds	Attached	1.5 ± 0.25 ns	11.3 ± 1.4 b	4.7 ± 0.3 b	49.7 ± 10.6 b	6.7 ± 1.2 ns	8.2 ± 2.1 a	78.5 ± 10.9 b	178.1 ± 13.6 b	99.7 ± 8.7 ns
		Removed	1.6 ± 0.2 ns	11.932 ± 1.1 b	4.3 ± 0.3 b	45.8 ± 3.6 b	7.4 ± 1.1 ns	7.8 ± 1.3 ab	79.1 ± 9.0 b	201.5 ± 34.5 b	94.3 ± 16.3 ns
Ni 0.85 µM	Ni depleted seeds	Attached	3.6 ± 0.3 c	14. 1 ± 2.6 a	4.8 ± 0.4 ns	47.8 ± 6.8 b	8.2 ± 0.9 ns	9.8 ± 1.6 b	83.79 ± 15.1 bc	188.9 ± 21.1 b	107.7 ± 10.0 ns
		Removed	5.7 ± 0.1 b	13.6 ± 0.7 b	4.8 ± 0.1 ns	53.3 ± 5.2 ab	8.4 ± 1.3 ns	11.3 ± 1.2 a	91.7 ± 3.5 a	238.4 ± 1.1 a	107.8 ± 7.1 ns
	Ni sufficient seeds	Attached	6.9 ± 0.3 a	14.9 ± 1.0 a	4.9 ± 0.2 ns	52.5 ± 3.3 ab	7.8 ± 1.9 ns	10.1 ± 0.9 ab	88.9 ± 8.4 ab	241.3 ± 16.0 a	105.4 ± 3.1 ns
		Removed	5.7 ± 0.2 b	11.8 ± 0.5 b	5.0 ± 0.3 ns	54.2 ± 3.6 a	7.9 ± 0.6 ns	11.0 ± 1.3 ab	82.3 ± 12.7 c	237.6 ± 26.37 a	107.1 ± 6.7 ns
Stem											
Seeds	Treatment	Cotyledon	Ni	Ca	Mg	K	P	Cu	Zn	Mn	Fe

Table 2 (continued)

Control	Ni depleted seeds	Attached	1.5 ± 0.3 a	14.3 ± 0.4 a	3.1 ± 0.2 a	74.6 ± 5.5 a	4.2 ± 0.7 ab	5.7 ± 1.0 b	81.7 ± 12.1a	126.2 ± 15.8 a	54.4 ± 6.8 ab
		Removed	0.8 ± 0.2 b	13.2 ± 0.5 ab	3.1 ± 0.2 a	65.9 ± 4.9b	4.3 ± 0.6 a	7.08 ± 1.19 ab	75.6 ± 3.9 b	122.3 ± 6.2 a	59.9 ± 4.3 a
	Ni sufficient seeds	Attached	0.5 ± 0.1 bc	12.9 ± 1.0 b	2.8 ± 0.2 b	64.2 ± 4.2 b	3.7 ± 1.2 ab	7.9 ± 2.0 a	58.6 ± 7.1 c	± 86.0 ± 4.9 c	52.6 ± 4.6 ab
		Removed	0.4 ± 0.0 c	11.9 ± 0.3 b	2.9 ± 0.1 b	64.0 ± 8.7 b	3.4 ± 0.5 b	7.8 ± 1.0 a	59.9 ± 6.1 c	91.5 ± 2.2 b	51.3 ± 2.1 b
Ni 0.85 µM	Ni depleted seeds	Attached	2.8 ± 0.3 a	14.1 ± 0.9 ns	3.4 ± 0.3 ab	65.9 ± 4.8 a	5.2 ± 0.7 a	12.6 ± 1.2 ns	84.0 ± 8.0 a	108.7 ± 12.6 ab	58.3 ± 3.6 ab
		Removed	1.4 ± 0.1 b	13.9 ± 2.1 ns	3.0 ± 0.1 b	67.8 ± 3.9 a	4.3 ± 0.7 b	12.5 ± 1.0 ns	81.9 ± 14.2 a	104.2 ± 15.9 b	68.1 ± 7.2 a
	Ni sufficient seeds	Attached	2.9 ± 0.0 a	13.0 ± 0.5 ns	3.4 ± 0.1 a	59.4 ± 5.3 b	4.7 ± 1.1 ab	11.9 ± 1.6 ns	73.5 ± 14.9 b	104.5 ± 16.0 b	55.7 ± 2.5 b
		Removed	1.6 ± 0.2 b	12.8 ± 0.1 ns	3.1 ± 0.2 ab	65.8 ± 4.3 a	5.3 ± 1.3 a	12.7 ± 1.3 ns	69.5 ± 10.5 b	114.9 ± 18.7 a	66.0 ± 11.7 ab
Roots											
Seeds	Treatment	Cotyledon	Ni	Ca	Mg	K	P	Cu	Zn	Mn	Fe
Control	Ni depleted seeds	Attached	0.5 ± 0.2 c	4.8 ± 0.1 b	16.6 ± 1.4 ns	63.8 ± 2.2 a	4.1 ± 0.6 ns	8.1 ± 1.2 ns	46.7 ± 4.3 b	871.6 ± 77.7 b	366.5 ± 21.3 a
		Removed	1.6 ± 0.2 a	5.3 ± 0.2 a	16.7 ± 1.30 ns	60.3 ± 5.1 a	4.2 ± 0.6 ns	8.9 ± 0.1 ns	49.0 ± 2.6 a	1137.9 ± 105.8 a	274.4 ± 25.2 b
	Ni sufficient seeds	Attached	1.2 ± 0.3 b	4.1 ± 0.1 c	16.8 ± 0.7 ns	49.6 ± 6.5 b	4.1 ± 0.6 ns	8.2 ± 1.0 ns	44.1 ± 5.7 b	862.7 ± 110.3 b	234.3 ± 27.3 b
		Removed	0.4 ± 0.0 c	3.9 ± 0.1 c	16.2 ± 1.0 ns	43.4 ± 3.2 c	4.2 ± 0.9 ns	7.9 ± 0.9 ns	40.4 ± 2.3 c	851.6 ± 109.6 b	248.9 ± 45.7 b
Ni 0.85 µM	Ni depleted seeds	Attached	6.3 ± 0.5 a	5.2 ± 0.4 a	18.1 ± 0.9 ns	54.6 ± 4.2 a	4.4 ± 0.9 ab	14.8 ± 1.5 a	45.8 ± 3.0 b	926.9 ± 52.6 c	768.6 ± 98.1 b
		Removed	5.5 ± 0.2 b	5.4 ± 0.2 a	17.9 ± 1.1 ns	53.7 ± 8.4 a	4.7 ± 1.2 a	14.3 ± 0.5 a	49.1 ± 3.5 a	950.5 ± 83.0 c	615.2 ± 151.7 c
	Ni sufficient seeds	Attached	6.7 ± 0.5 a	4.3 ± 0.1 b	18.9 ± 0.9 ns	53.6 ± 8.8a	4.4 ± 0.7 ab	12.3 ± 1.2 b	43.1 ± 4.6 b	1093.1 ± 86.1 b	648.8 ± 101.5 bc
		Removed	5.4 ± 0.2 b	4.4 ± 0.2 b	17.5 ± 0.8 ns	48.9 ± 0.6 b	3.9 ± 0.5 b	15.2 ± 2.3 a	45.7 ± 4.2 ab	1339.3 ± 151.4 a	1283.8 ± 36.7 a

Values are means and standard deviations of four replicates. Different letters indicate significant differences according to least difference (Tukey) test $P < 0.05$. ns indicates no significant differences

cotyledons had higher P concentrations. Finally, Zn concentrations were higher in plants that did not receive Ni, compared to plants treated with Ni.

Discussion

Trichomes: Pattern of nickel accumulation in leaf tissues after foliar nickel application

The cuticle plays a crucial role in protecting the plant against water loss through transpiration. Additionally, it serves to prevent the loss of organic and inorganic solutes from leaves due to rain. The cuticle contributes significantly to temperature regulation and plays a defensive role against pathogens and diseases (Kerstiens 1996; Riederer and Schreiber 2001; Marschner 2012). However, there are still gaps in our understanding as to how foliar-applied nutrients move across the leaf surface, including the potential role of trichomes (Fernández et al. 2017; Li et al. 2018, 2019). Using synchrotron-based μXRF in fresh leaves of soybean after foliar Ni application with two concentrations, we observed that Ni was concentrated in the pedicles of trichomes, while Mn concentrated in the trichome bases. Manganese localisation in trichomes base has previously been reported for sunflower (Blamey et al. 1986), pumpkin (Iwasaki and Matsumura 1999) and cucumber (Horiguchi 1987). The most likely reason for this is that during the absorption process by the root and possibly during transport to the shoot, Mn and Ni could compete for the same transport sites at the cell membranes (Demidchik et al. 2007). Divalent cations Ca, Mg, Fe, Cu, Ni, Mn, and Zn share characteristics that enable them to compete with one another in tasks like soil binding, root absorption, translocation, and subsequent utilization in the plant system. Another study examining the interaction of Zn and Cd in rice (*Oryza sativa* L.) vs. spinach (*Spinacia oleracea* L.) showed that Ni in shoots of rice and shoots and roots of spinach was significantly reduced by the increase of solution Zn (Wang et al. 2022).

Foliar-applied nickel is distributed quickly thought the trichomes: Synchrotron analysis

Synchrotron-based μXRF analysis of foliar applied Ni revealed that Ni moved quickly through the leaf surface, and was observed in the pedicle of trichomes 15 min. after foliar application (Figs. 2, 3, 4, 5 and 6). Thus, the results showed that trichomes are a primary pathway for the foliar uptake of Ni in soybean. Indeed, Ni appeared to have penetrated through the trichome underneath the Ni droplet, because after the exposure of Ni in soybean leaf there was a pathway following trichomes through the surrounding cells (Figs. 2, 3, 4 and 5). Nickel accumulated first within the trichomes, subsequently moved to the leaf cells, and depending on the time after exposure started, the concentration of Ni increased in the interveinal tissues. It has been reported that sunflower non-glandular trichomes (NGTs) are important for foliar Zn absorption (Li et al. 2019), but trichomes are not part of the primary pathway of foliar-applied Zn uptake in soybean and tomato (Li et al. 2018). Ohrui et al. (2007) showed that specialized trichomes can spread water quickly across the leaf surface by capillary effect, favouring the entire leaf tissue for the absorption of the water. Thus, it is also possible that soybean has a specialized mechanism for the absorption applied nutrient via leaf trichomes, as has already been noted for other plant species during environmental conditions stresses, such as drought (Winkler and Zots 2010; Vitarelli et al. 2016). The processes whereby foliar-applied nickel moves across the leaf surface and is translocated throughout the plant are not well understood (Bickford 2016), and major knowledge gaps in the physiological function of trichomes in taking up metal ions from foliar dosing remain.

Absorption of nickel and iron in soybean plants after nickel fertilization

Our study demonstrates for the first time that foliar-applied Ni (from $NiSO_4$ solution) can be directly absorbed by trichome pedicles before then moving from underneath the Ni droplet toward the epidermal cells and other plant tissues through the veins. When Ni was supplied in the nutrient solution, the Ni was taken up by the roots before moving into the aboveground tissues and shoots (Table 2). The roots in direct contact with the nutrient solution had the highest Ni concentrations compared to other tissues, being 59.3% higher on average than the leaves. It was observed that more Fe was absorbed by the roots following Ni application. In a similar manner, Rahman et al. (2005) found that the supply of 10 μM Ni

increased Fe concentration in roots in barley (Rahman et al. 2005). The Fe concentration can be significantly increased by the presence of Ni in the nutrient solution (Brune and Dietz 1995; Romheld 1991) given that Ni displaces Fe from FeDTPA and causes precipitation of $Fe(OH)_3$ (Chaney 1988). A similar result has been reported by Freitas et al. (2019) in soybean, which demonstrated that Ni-supplied plants had increased Fe in the roots following Ni application. In a similar manner, in the study of Tiffin et al. (1973) which investigated the translocation of Fe from cotyledons, it was observed that the seed coats had significantly higher Fe concentrations, being ca. five-fold higher than the embryos. Furthermore, during the germination process, the radicles contained only 5% of the initial Fe content originally present in the seeds. A decreased concentration of Fe in plant leaves has been reported, which is analogous with the findings in the present study in highly Ni-responsive soybean (Piccini and Malavolta 1992). This phenomenon may be due to the ability of Ni to suppress specific root-to-shoot Fe signalling, which results in a reduction of transport of Fe to leaf tissues. Therefore, in this study, we supplied double the usual Fe concentration in solution to avoid inducing Fe deficiency. However, it appears that the supply of Ni had a negative effect on the development of soybean plants, with dry weight being lower in plants that received Ni fertilization. These results potentially indicate that the highest Ni concentrations can lead to Ni toxicity in the development of the soybean plants, such as interveinal chlorosis, without visual symptoms, compared to plants with normal Ni concentrations (Nagajyoti et al. 2010; Prasad et al. 2005; Reis et al. 2017).

Nickel fertilization and cotyledon absence or presence modulate urease activity

In plants grown from Ni-sufficient seeds which also received Ni treatment, it was observed that the urease activity increased in plants which had elevated Ni concentrations in the tissues that are involved in N storage and transport (Gerendás 1998; Witte 2011). Our findings that the urease activity is higher at elevated Ni concentrations agree with Freitas et al. (2018) and Barcelos et al. (2017, 2018), with these previous studies showing that leaf urease activity is responsive to Ni fertilization. The development of soybean plants was significantly enhanced by the presence of Ni from cotyledons, with the cotyledons being an important storage of Ni (and all nutrients) for soybean.

Seedling development after Ni application and presence of cotyledons

Urease activity was correlated with Ni concentration, as expected, in plants grown from Ni sufficient seeds and with Ni-dosed plants. Plants grown from Ni-sufficient seeds which received 0.85 μM L^{-1} Ni in solution, had a smaller shoot biomass relative to the treatment that did not receive Ni (Fig. 6). In contrast, in the treatment of Ni depleted seeds, the plants which received Ni and did not have the cotyledons removed produced higher biomass relative to the treatment in which the cotyledons were removed and did not receive Ni. Therefore, the Ni level within the cotyledons significantly influences both seedling growth and overall plant development.

Conclusions

Understanding foliar fertilization for enhancing crop micro-nutrition status holds importance. Nonetheless, the mechanism underlying the absorption of micro-nutrients by the leaves remains unclear. The Ni foliar application examined using synchrotron-based μXRF showed that the Ni-absorption occurred mainly by trichomes, specifically via the pedicle, showing that trichomes may be the primary pathway by which foliar applied Ni moves from the trichome pedicles toward the leaf surface and then to other plant tissues. In addition, urease activity, in which Ni plays a central role, was found to be correlated with Ni nutritional tissues status in plants grown from Ni sufficient seeds and with Ni-dosed plants. Even a low Ni seed concentration (<0.35 μg g^{-1} Ni) was enough to raise urease activity compared to the control. The presence of cotyledons resulted in greater biomass for plants that did not receive Ni application, emphasizing the importance of Ni as an essential nutrient for soybean growth but also the importance of cotyledons as nutrient stores. Nevertheless, elevated concentrations of Ni can lead to a reduction in biomass. Thus, determining the optimal Ni dosage is of paramount significance and further studies are required in this

regard. This study deepens our understanding of the ability of plants to accumulate and translocate Ni into plant tissues, especially in well-nourished plants. Given that the leaf surface is covered with a cuticle and cuticular waxes, the underlying process whereby foliar-applied Ni is absorbed through the leaf surface by trichomes, via the pedicles, could contribute to a better understanding of micro-nutrient foliar absorption mechanisms, allowing the development of fertilizers with specific physical-chemical properties that favour greater absorption efficiency and micro-nutrient use by plants.

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Author contributions JBO, JL and AVDE conceived the experiments. RLC advised on the design of the experiments. JBO undertook the hydroponics experiments and chemical analyses under supervision of AVDE. JBO, AVDE, HHH and PDE performed the synchrotron experiments with support from DLH. PMK and ARR helped to interpret the results and contributed to the writing. The manuscript was written by JBO and AVDE with input from all authors.

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