

Thriving on half: low nutritional demand in *Stephanopodium engleri* involves adjustments in photosynthetic apparatus functionality

Evandro Alves Vieira

Instituto Tecnológico Vale - Desenvolvimento Sustentável

Luiz Palhares Neto

Universidade Estadual do Sudoeste da Bahia

Joana Serrão Filgueira

Instituto Tecnológico Vale - Desenvolvimento Sustentável

Marina Ludmila Conon Salles

Instituto Tecnológico Vale - Desenvolvimento Sustentável

Rafael Valadares

Instituto Tecnológico Vale - Desenvolvimento Sustentável

Silvio Ramos

Instituto Tecnológico Vale - Desenvolvimento Sustentável

markus Gastauer

Instituto Tecnológico Vale - Desenvolvimento Sustentável

Cecilio Frois Caldeira

cecilio.caldeira@itv.org

Instituto Tecnológico Vale - Desenvolvimento Sustentável

Research Article

Keywords: endangered species, growth, nutritional sources, proteome, Quadrilátero Ferrífero

Posted Date: June 7th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4450944/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Cultivating threatened plant species is vital for conservation, safeguarding against extinction by ensuring propagation and maintaining genetic diversity. In this study, we conducted an integrative morphological, physiological, and quantitative proteomic analysis of *Stephanopodium engleri* plants grown under different substrates and nutritional sources. *Stephanopodium engleri* is an endangered and range-restricted species endemic to the Quadrilátero Ferrífero region, a historical hub for mining activities in Brazil. Seedlings of *S. engleri* were cultivated in three different substrates: organic substrate (Carolina Soil® - CS), dystrophic soil (DS), and a mixture of both substrates (1:1). Additionally, we evaluated nutrient availability levels, including complete fertilization and half of the nutrients in the DS, as well as treatments with complete fertilization excluding nitrogen (N), phosphorus (P), potassium (K), and micronutrients.

Results

Different nutritional sources resulted in changes in the growth of *S. engleri*, with nitrogen (N), phosphorus (P), and micronutrients identified as the most limiting nutrients. The species exhibited maximum growth with half of the applied fertilization, suggesting a low nutritional requirement. The highest growth was strongly related to nutritional availability, coupled with adjustments in the photosynthetic apparatus and protein regulation, with no significant differences in morpho-anatomical traits. The most representative proteins displayed a diverse array of metabolic functions, particularly related to iron (Fe) and zinc (Zn) homeostasis, participation in photosynthesis and energy pathways, reactive oxygen species (ROS) signaling, and the preservation of functional structures.

Conclusions

Our findings are crucial to support field planting, as adequate fertilization is essential to produce high-quality seedlings, thereby facilitating plans to reintroduce the species into its native environment. Additionally, our results reveal insights into *S. engleri* physiology and adaptations. Our data can further support management and conservation plans through the reinforcement of natural populations, introduction of new populations, maintenance of germplasm banks, and living collections.

Background

Several soil types often have mineral nutrient imbalances compared to the plant nutritional requirements. Adequate nutrient supply ensures plant growth, development and yield by keeping the multiple physiological processes in optimal conditions [1]. Essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K) are required in higher quantities and play crucial roles in plant metabolism [2].

They support processes like photosynthesis, nutrient uptake, and overall plant health [3]. Balancing these nutrients in the soil is vital to maximize crop production and maintain the health of natural ecosystems [4]. Inadequate supply of NPK usually exhibits stunted growth by limiting pigments content and quantum yield, thereby reducing carbon assimilation and distribution, and lipid peroxidation in chloroplasts [5]. Together with macronutrients, micronutrients availability can be also decisive for plant growth and development. Elements such as iron (Fe) and manganese (Mn) play important roles in many physiological processes, such as respiration, chlorophyll biosynthesis, and photosynthesis, while copper (Cu) and zinc (Zn) are important activator and cofactor of various plant enzymes [6, 7].

Plants developed adaptations to deal with adverse or favorable soil conditions, and several species can acclimate to a range of soils. This acclimatization involves physiological mechanisms, such as excretion of Fe-chelating molecules, reabsorption of elements from senescent leaves; anatomical changes, with the formation of more or longer root hairs when P supply is short; and morphological adjustments leading to root: shoot mass ratio increment when N or P are limiting plant growth [8]. An essential physiological response in plants dealing with nutrient unbalancing involves safeguarding their photosynthetic machinery. Elevated N availability enhances N metabolism and bolster stress resilience within the photosynthetic apparatus by mitigating cell membrane damage and enhancing both energy dissipation and reactive oxygen species (ROS) scavenging [9, 10]. Furthermore, the crosstalk between Fe and Cu has been documented as critical cofactors for components of the electron transport chain in the mitochondria and the chloroplast [11]. Likewise, Fe interferes with Zn translocation and reduces the rate of its absorption [12, 13], as also interfere remarkably in the complex cross-talks between N, P, calcium (Ca), magnesium (Mg) and sulfur (S), altering the plants nutritional homeostasis [3].

The proteomic approaches may help to elucidate the mechanisms used by species to deal with nutrient excess or deficiency. Root ferric-chelate reductase activity is a reliable biomarker for plant Fe uptake activity, and thus an indicator of Fe sufficiency/deficiency status [11]. Increased Cu results in rapid synthesis of Cu proteins, which replaces the Fe proteins, thus reducing the Fe demand and generating a feedback inhibition of ferric-chelate reductase activity [11, 14]. Another Fe chelator, nicotinamide (NA), demonstrated the ability to bind both Fe and Cu facilitating Fe transport in the phloem [15]. The chelating function is important for maintaining redox homeostasis and CO₂ assimilation rates. Different proteins are involved in redox regulation, photosynthesis (RubisCO), ATP-dependent carboxylation and chaperone [16]. Rubisco activase (RCA) enables the release of the inhibitory sugar phosphates from the active site of Rubisco, thereby sustaining Rubisco in its active state and continuing ribulose-1,5-bisphosphate substrate regeneration, which is a critical factor in photosynthesis [17]. Under N-deficient conditions, senescence of leaves is accelerated mainly due to degradation of Rubisco proteins [18]. Indeed, studies on wheat have demonstrated that Mg sources promote RCA activity, thereby stabilizing Rubisco activation [7].

The interplay between various plant nutrients indicates that they influence each other's uptake, transport, and assimilation. However, the complex interactions between these nutrients remain poorly understood, necessitating further studies to elucidate the multi-level dynamics involved. Such research is crucial for

gaining a deeper understanding of the sensing and signaling pathways activated in response to fluctuations in the availability of these essential elements [19]. Despite the unfavorable nutritional characteristics for the growth of many species, semi-deciduous forests have high plant biodiversity, with a large number of rare and endemic species. Allied to the edaphoclimatic characteristics, these ecosystems are characterized by the fragmentation of the landscape due to the strong anthropization, which threatens the maintenance of its flora [20]. *Stephanopodium engleri* Baill. (Dichapetalaceae) is an endemic species of the underlying areas of Quadrilátero Ferrífero in Minas Gerais state, Brazil. It is a small tree species considered extinct until 2010s and currently recognized as an “endangered” species due to the restriction of its occurrence and area of occupation [21]. The lack of information on the biology of the species, combined with the increasing environmental degradation of its habitat, compromises its survival and requires the development of research aimed at its propagation and conservation.

In this study, we conducted an integrative morphological, physiological and quantitative proteomic analysis in plants of *S. engleri* grown under differential substrates and nutritional sources, aiming to provide a more in-depth and comprehensive understanding of its edaphic requirements, morphological and physiological adjustments, focusing on the roles of the proteome in the regulation of assimilatory metabolism. Considering that the species has recalcitrant seeds, and that propagation studies were carried out aiming at reintroduction plans, understanding the nutritional requirements of *S. engleri* can contribute to the production of seedlings with high physiological quality to guide management and conservation plans. Therefore, we hypothesize: (i) the balance of macro and micronutrients availability influences the net rates of CO₂ assimilation and plant growth; (ii) Metal chelating proteins, ion transporters and proteins linked to the assimilatory metabolism and energy pathway are up-regulated according to the nutritional source; (iii) changes in some structural attributes would provide better absorption or retention of nutrients.

Methods

Plant material

Seeds of *S. engleri* were removed from fresh fruits harvested from areas of natural occurrence near Belo Horizonte, Brazil. Access to the plants at these locations was kindly granted by Vale S.A. and Gerdau S.A. Vouchers from these plants were previously deposited in the herbaria BHZB (11303), FLOR (78441), and CVRD (15967). The seeds were sterilized (ethanol solution 70% for one minute and sodium hypochlorite 4% during five minutes) and placed to germinate in Gerbox® dishes. After germination, the seedlings were transferred to trays with conical cells filled with a mixture of commercial organic substrate (Carolina soil®) and sterile sand. At this stage, the seedlings were cultivated in growth chamber (SGC 120, Weiss Technik, UK) under constant relative humidity (60%), photoperiod of 12:12h for day: night and density of photon flux of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and day: night temperature of 28: 22 °C.

Plant growth conditions

Substrates analysis and preparation

After 30 days of cultivation, seedlings of uniform size were transferred to individual PVC pots of 2.3 dm^{-3} containing different substrates and nutrients availability. We selected three substrates: a commercial organic substrate (Carolina soil® - CS), a dystrophic Yellow Latosol (control), and a mixture of both substrates (1:1 ratio). To evaluate the nutrients requirement, we used dystrophic soil. Treatments consisted of complete fertilization and half of these nutrients and, complete fertilization excluding nitrogen (N), phosphorus (P), potassium (K), or micronutrients.

The physical and chemical properties of the substrates were determined after plants cultivation. Soil samples were homogenized and sieved through a 4 mm mesh (Table S1); the pH was determined by a pH electrode in a 1:2.5 soil-to-water ratio, and the organic carbon was determined using the potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) method. The available P, K, B, Zn, Fe, Mn, and Cu contents were determined using the Mehlich-1 method ($0.05 \text{ mol L}^{-1} \text{ HCl} + 0.0125 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$), where P was determined by colorimetry, K was determined by flame photometry and the other elements were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Exchangeable Ca, Mg and Al were measured using atomic absorption spectrophotometry on 1 M KCl extracts with the addition of lanthanum oxide. The soil texture was determined as described by Kettler et al. [22].

Due to the high acidity and reduced base saturation, 30 days before seedlings planting, the soil of all treatments using the dystrophic soil (except control) was treated with dolomitic limestone (Dimy®) to increase the base saturation to 60% (3.82 g of limestone per dm^{-3} of soil). Treatments with complete fertilization received 100 mg N dm^{-3} ($\text{CH}_4\text{N}_2\text{O}$, Sigma Aldrich), 200 mg P dm^{-3} ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, Sigma Aldrich) and 100 mg K dm^{-3} (K_2SO_4 , Sigma Aldrich). The micronutrients manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), boron (B) and molybdenum (Mo) were applied in amounts of 10 mg Mn dm^{-3} (MnCl_2 , Sigma Aldrich), 5 mg Fe dm^{-3} (6% FeEDDHA, Sigma Aldrich), 5 mg Zn dm^{-3} ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, Sigma Aldrich), $1.5 \text{ mg Cu dm}^{-3}$ ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Sigma Aldrich), 0.5 mg B dm^{-3} (H_3BO_3 , Sigma Aldrich) and $0.15 \text{ mg Mo dm}^{-3}$ (Na_2MoO_4 , Sigma Aldrich). Chemically, the studied latosol is desaturated with a dystrophic character, however, correction with limestone increased base saturation above 70%. The low cation exchange capacity corroborates the lower proportion of clay detected, while the pH is in the normal range. In general, the soil's nutritional concentration is low, with emphasis on the reduced values of nitrogen, sodium and potassium, and the low organic matter content.

Environmental conditions

The plants (12 plants in each treatment) were grown for 120 days in greenhouse conditions with temperature and relative air humidity monitored every 15 minutes with a thermocouple connected to a datalogger (RHT10, Extech Instruments, Boston, USA). The daily air temperature varied between 25 and 37°C , and the vapor pressure deficit varied between 0.4 and 2.5 kPa. The water availability was held at

70% of the soil water retention capacity by replacing water loss from evapotranspiration with distilled water after pot weights monitoring every two days.

Plant measurements

Growth and biomass accumulation

Plant height and shoot diameter parameters were recorded monthly. Root and shoot biomass, gas exchange, foliar nutrients, anatomical traits, and proteomic profiling were performed at the end of the experiment. The shoot length (cm), stem diameter (mm), shoot dry mass (g), root dry mass (g), root/shoot ratio, specific leaf area (m^2) and relative growth rate (RGR) were evaluated monthly in 10 plants between 0 and 120 days. To calculate root and shoot dry mass, samples were oven-dried at 65 °C for 72 h. Relative growth rate (RGR) was calculated according to the equation: $(\log P2 - \log P1) / (t2 - t1)$, where P2 and P1 correspond to the plant height between t2 and t1.

Gas exchange

Instantaneous measurements of net carbon assimilation rates (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mmol m}^{-2} \text{ s}^{-1}$), transpiration (E , $\text{mmol m}^{-2} \text{ s}^{-1}$), and the quantum yield of photosystem II (QY) were taken between 08:00 and 11:00 A.M., using an infrared gas analyzer (IRGA) model Li-6400 (Li-Cor-Nebraska, USA), under controlled conditions of CO_2 concentration (400 ppm), PPFD of 500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and chamber temperature of 25°C. During the experimental period, measurements were conducted in the middle portion of the third fully expanded leaf, as described above. Intrinsic water use efficiency (A/g_s), the ratio of intercellular to ambient CO_2 concentration (C_i/C_a) and instantaneous carboxylation rate (A/C_i) were also estimated.

Leaf nutrients content

Leaf nutrients content was determined in eight plants aleatory sampled from each treatment. The content of N, P, K, Ca, Mg, S, Cu, Fe, Mn, B, Zn were determined according to Gai et al. [12]. Nitrogen content was determined after sulfuric acid digestion by Kjeldahl's method. The P, K, Ca, Mg, S, Cu, Fe, Mn and Zn contents were assessed by the nitric perchloric acid digestion method; P was dosed by the metavanadate colorimetric method; K was quantified by flame photometry (600 plus Fento, SP, Brazil); Ca and Mg were quantified by atomic absorption spectrophotometry (2380, Perkin-Elmer, Norwalk, CT USA); S was assessed by turbidimetry; Cu, Fe, Mn and Zn were analyzed by atomic absorption spectrophotometry and B by colorimetry (Azomethine-H)

Anatomical traits

Samples were collected from the middle region of the leaf blades of fully expanded leaves and roots 5 cm from the root apex were fixed in FAA (formalin, acetic acid, 70% alcohol) for 24 h. The material was isolated, dehydrated in an ethyl series, embedded in methacrylate (Historesin, Leica®) and sectioned in a rotary microtome (Leica RM 2245, Leica® Biosystems, Heidelberg Germany). The cross sections (5 μm

thick) were stained with toluidine blue, pH 4.0 [23]. For stomatal characterization, the epidermal impression method was used according to Segatto et al. (2004). The samples were observed and photomicrographed under an optical microscope (DM750, Leica® Microsystems, Switzerland) coupled to a digital camera (Leica CH-9134, Leica® Microsystems, Switzerland). The images were analyzed with Image J software, previously calibrated. The root anatomical parameters evaluated were as follows the root periderm thickness (RPT), root cortex thickness (RCT) and root cortex diameter (RCD). In the leaf blade, the epidermis thickness from adaxial leaf side (ETAd), the epidermis thickness from the abaxial leaf side (ETAb), palisade parenchyma thickness (PPT) and spongy parenchyma thickness (SPT) were measured. The stomatal density (SD) of abaxial surface was calculated as the number of stomata per mm² and the stomatal functionality.

Protein isolation and proteome analysis

Leaves from three individual plants were sampled aleatory from each treatment and fast frozen in liquid nitrogen. Before protein isolation, samples were kept in deep freezer at -80°C. Protein isolation, determination of protein concentrations, and further processing were performed according to Nascimento et al. [24]. The identification and quantification of proteins were performed in a nanoACQUITY UPLC® ultra-performance liquid chromatography (Milford, MA, USA), configured for fractionation in two dimensions as reported in Herrera et al. [25]. The chromatograph was coupled to a NanoLock ESI-Q-ToF SYNAPT G2-S (Waters) mass spectrometer. The acquisition ranged from 50 to 2000 Da, in MSE mode (data independent analysis) at a scan rate of 0.5 s and an interscan delay of 0.1 s.

The data were processed using the Progenesis QI software (Waters) for identification and quantification, using the Viridiplantae database from UniProt (UniProtKB/swiss-prot, uniprot.org). Protein identification was accepted if the probability of identifying peptides was greater than 90% and proteins with 95%. The significance levels of the differential abundances of proteins were determined by applying the ANOVA test ($p < 0.05$). The functional annotation of proteins was performed using the OmicsBox v1.2.4 (bioBam) and Uniprot (UniProtKB/swiss-prot, uniprot.org). Kyoto Encyclopedia of Gene and Genome (KEGG) pathway enrichment analysis for proteins was performed using the KOBAS software v3.0. (kobas.cbi.pku.edu.cn) with *Arabidopsis thaliana* as the background species for the analysis. The R package ggplot2 was used for the enriched KEGG pathways visualization. The PPI networks were predicted based on functional analysis using the software STRING (research tool for recovering genes/proteins in interaction) v11.0 (<http://string-db.org/>), using homologous proteins from the *Arabidopsis thaliana* as the background species.

Data analysis

The soil-type with different nutritional supplementation was considered a factor (independent variable), and the physiological, morphological, and biochemical changes were response variables (dependent variable). A completely randomized design was used, and all data were subjected to a One-way ANOVA ($p < 0.05$) and compared by Tukey's test using R software (R Core Team 2018; <https://www.R->

project.org). The main correlations between assimilation and growth rates, as well as the interactions between macro and micronutrients were demonstrated by the Debiased Sparse Partial Correlation (DSPC) algorithm analysis using the software MetaboAnalyst 6.0 (<https://www.metaboanalyst.ca>). Proteomic data matrix was subjected to hierarchical clustering heatmap analysis, principal component analysis (PCA), and enrichment analysis using the Heatmapper online program (<http://heatmapper.ca/expression>).

Results

Nutrients and plant growth

Differential soil fertilization promoted changes in the initial growth of *S. engleri* (Fig. 1). The positive effect of treatments complete fertilization, half fertilization and absence of K increased respectively 112, 156 and 158% for shoot length, 37, 47 and 33% for stem diameter, 442, 379 and 419% for shoot dry mass and 229, 293 and 266% for root dry mass (Figs. 1A, B, C, D). While plants from the commercial substrate and mix of substrates treatments exhibited intermediate growth for most parameters, the control (C), absence of micronutrients and absence of P treatments were the least promising for the *S. engleri* growth. Higher values of SLA were observed in plants grown in the absence of micronutrients (Fig. 1G).

Gas exchanges

The treatments with the highest growth, namely complete fertilization, half fertilization, and absence of K, were strongly correlated with carbon assimilation. Notably, these treatments showed significant changes in several parameters, including a 28%, 59%, and 47% increment in photosynthetic rate (A), a 152%, 87%, and 157% increase in the ratio of A/g_s , and a 73%, 86%, and 93% rise in A to intercellular CO_2 concentration (A/C_i), respectively. These treatments exhibited higher quantum yield of photosystem II (QY), with respective increases of 38%, 26%, and 19% (Figs. 2A, D, E, F). Conversely, treatments with lower growth rates showed reduced assimilation rates, notably the complete fertilization without micronutrients. Also, plants grown in absence of N, P, or K exhibited lower stomatal conductance (g_s) and transpiration rate (E) (Figs. 2B, C).

Leaf nutrients content

Leaf N concentration did not change significantly between treatments, even with fertilization (Fig. 3A). While the use of CS contributed to high P concentrations and organic matter, plants grown on absence of micronutrients, absence of N and absence of K NP treatments showed similar concentrations of this nutrient in their leaves (Fig. 3B). For the K concentration, only the control treatment showed a significant difference with lower values (Fig. 3C). Higher Ca accumulation was detected in commercial substrate, absence of micronutrients and absence of P treatments, while the lowest concentrations were observed in treatments with higher RGR (Fig. 3D), with possible dilution effect due to greater accumulated biomass. Magnesium also accumulated more in the control treatment, followed by complete fertilization

and commercial substrate (Fig. 3E), while S concentration did not change significantly between treatments (Fig. 3F).

Micronutrient concentrations exhibited distinct patterns across various fertilization treatments. Specially, B concentration significantly increased in treatments with complete fertilization but without N or P (Fig. 3G). Higher Cu accumulation was observed in the absence of P, showing significant differences from other treatments (Fig. 3H), while Fe values remained consistent across all fertilization treatments (Fig. 3I). Leaf Mn concentrations were highest in commercial substrate and without N treatments and lowest in half fertilization and without N treatments (Fig. 3J). Plants subjected to P omission and control treatments exhibited the highest Zn concentrations, while those in the complete fertilization treatment showed the lowest levels (Fig. 3K). Debiased Sparse Partial Correlation (DSPC) analysis revealed several notable correlations (Fig. 4), including positive associations between shoot dry mass and A ($r = 0.74$), shoot dry mass and A/gs ($r = 0.74$), Ca and Mg ($r = 0.89$), Ca and Zn ($r = 0.73$), Zn and S ($r = 0.76$), Zn and Fe ($r = 0.74$), S and Fe ($r = 0.78$), and Fe and Mn ($r = 0.72$) (See Supplementary Material for further details).

Anatomical changes in roots and leaves

Root periderm thickness (RPT) and root cortex thickness (RCT) was more developed in absence of N and absence of micronutrients treatments (Figs. 5A, B), while root cortex diameter (RCD) was also more expanded in absence of N, commercial substrate, and absence of P (Fig. 5C). While palisade parenchyma thickness (PPT) was higher in control, half fertilization and absence of P, the treatments commercial substrate and absence of N exhibited larger spongy parenchyma thickness (SPT) (Figs. 5D, E). Conversely, the control and absence of micronutrients treatments showed a negative effect for ETAd, PPT and SPT. Regarding the stomatal density (SD), higher values were observed in micronutrient absence, together with higher SLA and gs and lower $iWUE$ (Fig. 5F). See Figure S1.

Leaf proteome profiling

The proteomic analysis detected 3969 proteins in *S. engleri* leaves, and 1499 were initially quantified by the Progenesis Q1 software. From that, 52 proteins were present in all eight replicates of the nine treatments and quantified with at least 1 peptide (Fig. S2). Only these protein species were considered in this study, and identified proteins were analyzed by placing them in appropriate functional pathways. Based on biological function, the identified proteins were categorized into nine groups, with a predominant focus on biological and metabolic processes, particularly photosynthesis and energy pathways (Fig. 6A). Regarding molecular functions and localization of these proteins, the most prominent functions were DNA and nucleotide binding, with localization observed within organelles, cellular compartments, and the cell (Fig. 6B).

The partial least squares-discriminant analysis (PLS-DA) revealed that proteins with high variable importance in projection correlated with treatments fostering greater plant growth and the absence of P and micronutrients (Fig. S3). Treatments such as complete fertilization, half fertilization, absence of N

and K exhibited up-regulation of proteins associated with important nutrients like Ca and Zn and maintenance of Fe homeostasis (Q9SYP3, Q9ZQV9). These proteins also played roles in assimilatory metabolism and energy pathway (Q40073, P12860, O49213, Q67VU7, Q94B74), plant ROS signaling (Q9M9W9, Q9C5R8), nitrogen metabolism (Q6ZLB0), as well as in lipid transport (Q84M24) in mix of substrates (Fig. 6B). Conversely, treatments with lower assimilation and growth rates (control, absence of micronutrients, and absence of P) exhibited down-regulation of these proteins. Additionally, proteins linked to increased cellular expansion (Q7XR91), photosystem I activity and Mg transport (Q1ACL4), carbon uptake (A2T345), Zn and Cu binding, and methionine metabolism (Q9SRV5), and triterpenoid biosynthetic process (Q2XPU6) were up regulated in these treatments. Notably, the absence of micronutrients treatment demonstrated an increase in the catabolism of ubiquitin-dependent proteins (Q9XIE8, Fig. 6B).

Discussion

Thriving on half: plants of *S. engleri* show efficient growth with less nutrients input

The growth responses observed in our study indicate that *S. engleri* responds positively to different levels of fertilization and substrates, even when grown in sandy soil with lower ability to adsorb nutrients. When all nutrients were provided in the dystrophic soil (in complete and half fertilization), we could observe an improvement in nutritional status, biomass production and allocation that resulted in better growth results. We also observed that half of the fertilization was enough to *S. engleri* reach the maximum growth, showing no differences when compared to complete fertilization. Reduced nutrients requirement has also been observed in native species that have not experienced selection for cultivation, despite their gain in biomass and growth with partial fertilization [26–28]. Then, in the context of fertilization requirement to obtain healthy *S. engleri* plants and increase the potential success in the field, using lesser amount of nutrients can favor the management, reducing costs and avoiding nutrients leaching and pollution, as well as minimizing the invasion of ruderal and weed species [29].

The addition of CS to the control soil added a greater diversity of nutrients and organic matter, and this mix provided greater growth and biomass gain for *S. engleri*. According to Nagase and Dunnett [30], organic matter maintains a good soil structure; improves water retention, increases the cation exchange capacity, thereby reducing the loss by leaching of elements such as P, Ca and Mg. Thus, the addition of CS promoted significant growth of *S. engleri*, due to its low nutritional requirement. Otherwise, the absence of N reduced both the growth and biomass allocation to the shoot in *S. engleri*, stemming from decreased CO₂ fixation and carbon redistribution, likely directed towards the roots. Similarly, the growth deficit observed without P indicates a significant impact on assimilation and energy metabolism. Phosphorus is required in photosynthesis, synthesis and carbohydrates breakdown and energy transfer within plant [31], and its absence severely affects the growth [32].

The low K requirement by *S. engleri* plants indicates that the exposure time without this nutrient was not sufficient to affect growth. Several studies indicate that K deficiency affects photosynthetic metabolism

and growth [33–35]. We propose that upregulation of the potassium/proton antiporter CemA (A2T345) may have maintained proton homeostasis in the chloroplasts of *S. engleri*, thus minimizing the absence of K in plastidial regulation. According to Marchand et al. [36], CemA protein acts in the light-induced H⁺ extrusion from the chloroplast stroma, keeping the alkaline stromal pH for functional carbon concentrating mechanism in chloroplasts. Similarly, the reduced growth of *S. engleri* in the absence of micronutrients confirms their importance for various metabolic functions. Micronutrients deficiency reduces plant growth, as these participate in different enzymatic activities and are involved in different metabolic processes, such as photosynthesis and respiration [37]. Thus, our findings confirm that even in low quantities, if unavailable, micronutrients can reduce energy metabolism and render growth unfeasible.

Regarding growth and nutritional concentration in leaves, the absence of N and K did not influence the concentration of these nutrients in the leaves of *S. engleri*, showing a dilution effect, where plants that grew more also absorbed more nutrients. The degradation nutritional quality is usually associated with the inability of the plants to absorb nutrients in sync with dry matter accumulation. As previously reported, there is a direct relationship between CO₂ assimilation rates, stomatal conductance, and the dilution effect on nutrient concentration in tissues [38–41]. Therefore, it is possible to consider that the concentration of certain nutrients in *S. engleri* plants was influenced by this effect.

Assimilatory metabolism depends on nutrient supply

Proper nutritional supplementation works on the development of root structure and increases the flow rate of root xylem because of enhanced water absorption and the provision of essential nutrient elements. This leads to differences in assimilatory metabolism and growth rates, compared to plants not treated with fertilizers or lacking certain elements [42]. The highest values of carbon assimilation, *iWUE*, *A/Ci* and QY on plants receiving all nutrients indicate that adequate supplementation improves the development of the species, while the absence of some nutrients causes the opposite effect. As observed here, nitrogen fertilization consistently enhances carbon assimilation in plants, likely attributable to heightened photosynthetic pigment contents [43–45], thus, emphasizing the pivotal role of nitrogen in bolstering photosynthetic capacity. Otherwise, the decrease in growth in the absence of P was certainly associated with the lower *A* rate, *gs*, *A/gs*, *A/Ci* and QY. Phosphorus is another key element for growth, making up nucleic acids and phospholipids of cell membranes, is involved in essential metabolism processes such as energy transfer, carbon assimilation, and sugar transformation [46].

Potassium fertilization is recognized to significantly enhance gross primary production (GPP) in plants by reducing stomatal and mesophyll resistance to carbon dioxide (CO₂) diffusion and boosting the photosynthetic capacity within the leaves [47, 48]. In this sense, the greater growth registered in *S. engleri* fertilized with complete fertilization, half fertilization and absence of K would also have been directly related to an improvement in key steps of the plant carbon assimilation and energy transfer efficiency. Furthermore, the fact that the absence of K did not reduce gas exchange and QY and its concentration in the leaves in our study, it could be a result of their higher concentration in the soil. The

decrease in the values of A rate, A/g_s and A/C_i in the absence of micronutrients treatment may be related to the low levels of Zn and Mn. The important role of Fe, Zn, and Mn has already been reported for the electron transport chain, in which the reduction in these microelements decreases efficiency of electron acceptors in the electron transport chain which causes decline in transported electrons between PSI and PSII [49].

Accumulation of Ca and Mg enhances responses to nutritional imbalances

The foliar Ca accumulation in absence of micronutrients detected in this study suggests its role in signaling for micronutrient deficiency. According to Bang et al. [50], Ca^{2+} plays a central role in nutrient sensing and in adaptation to changes in nutrient status and the link between external nutrient availability and transport activities is constituted by the Ca^{2+} -dependent CBL–CIPK pathway. For NK, increased Ca levels may be associated with P deficiency, leading to changes in membrane properties. High levels of Ca^{2+} promote the signaling and stabilization of cell walls, as well as stabilize cell membranes via the formation of bridges with phosphate and carboxylate groups in lipids and proteins [50, 51]. In the control treatment, the higher concentration of foliar Mg may be related to attenuating the CO_2 assimilation deficit and intensified catalytic activity to obtain energy. When RuBisCo binds to Mg^{2+} , carboxylation is favored many times faster than oxygenation. The ability of Mg^{2+} to perform ligand exchange is essential for the coupling of ATP with substrates and enzymes in phosphorylation processes, energy-demanding transport processes and ATP synthesis [50, 52]. Thus, in nutrient-poor soil, the accumulation of Mg would improve assimilation rates and attenuate the energy deficit in *S. engleri*. Additionally, the increments of Fe, Zn, Cu and Mn especially in treatment without P (NK) are related to the antagonism of these elements with P, as already mentioned. Low P content causes Fe increment in the soil, which once increased, can induce Zn and Mn accumulation.

Chelating proteins regulate the levels of Fe and Zn

The proteomic approach used in this study has allowed for the reliable identification and quantification of a large set of protein in leaves, with 90.3% of the proteins changing significantly in at least one treatment. Our findings suggested that most of the identified and quantified proteins likely originated intracellularly, reflecting adjustments within the cytoplasm and organelles in response to varying nutritional source. Notably, the nutritional source provided to the plants of *S. engleri* appeared to be effectively regulated, as evidenced by the up regulation of specific proteins. While Putative F-box/kelch-repeat protein (Q9SYP3) displayed remarkable increases in relative abundance in five of the nine treatments tested, nicotianamine synthase 1 (Q9ZQV9) was up-regulated in absence of micronutrients and absence of K. Q9SYP3 is recognized as a component of an E3 ubiquitin ligase complex, playing a crucial role in preventing toxic Fe overload by acting as negative regulators of Fe homeostasis. According to Rodríguez-Celma et al. [54], the activity of E3 ubiquitin ligases is modulated by Fe binding to the N-terminal hemerythrin motifs. FBXL5 comprises an N-terminal hemerythrin (Hr) motif, which binds a

redox-active di-iron center, and an F-box domain that constitutes part of a multi-subunit E3 ubiquitin ligase, thus regulating Fe homeostasis. When Fe binds, the Hr domain folds, enabling the FBXL5-E3 ligase complex to interact with IRP2, leading to its degradation. The upregulation of this protein may be associated with greater Fe availability in the commercial substrate, complete fertilization, and half fertilization treatments. Due to the species' low nutritional requirement, Fe may have accumulated, triggering this control mechanism.

Nicotianamine (NA) is the major chelator facilitating Fe transport in phloem and either loss-of-function of NA synthases or Fe-NA transporters results in developmental and Fe homeostasis defects of sink organs [15]. Furthermore, NA acts to chelates Zn^{2+} and other divalent cations. The up regulation of these proteins suggests an efficient adjustment of Fe and Zn levels in *S. engleri* leaves, corroborated by the concentration of these nutrients in the complete fertilization and absence of K treatments. Interestingly, the downregulation of 5-methyltetrahydropteroyltrimethylglutamate-homocysteine methyltransferase 2 (Q9SRV5), which catalyzes the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine resulting in methionine, in several treatments in our study suggests a catalytic process for nicotianamine formation. Homocysteine is crucial for *de novo* methionine synthesis and S-adenosylmethionine recycling, acting as a precursor of nicotianamine, polyamines and ethylene [53]. However, the increase in relative abundance of this protein in absence of P signals an increase in Fe, Zn, Cu and Mn. As already mentioned, the increase in transferase activity favors the production of nicotianamine, which facilitates transport and acts as a chelator of divalent cations. For treatments with higher micronutrient availability, the conversion of nicotianamine may have maintained the homeostasis of these cations in *S. engleri*, without causing detrimental effects to assimilatory metabolism, thus preventing oxidative damage and ensuring growth.

The increase of chlorophyll-protein complexes maintains photosynthesis and energy regulation

Regarding of assimilatory metabolism, the up-regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase activase A (RCA, Q40073) in plants grown in mix of substrates and half fertilization reinforces the better performance in CO_2 assimilation and biomass allocation of *S. engleri* in these treatments. RCA participates in the RuBisCO activation by involving ATP-dependent carboxylation that is activated substantially by the cooperative binding of a Mg^{2+} . According to Hazra et al. [54], Mg^{2+} concentrations are known to vary in the stroma as a function of proton pumping across the thylakoid membranes. As the pH of the stroma rises, Mg^{2+} increases to provide electrostatic compensation, thereby upregulating RCA hydrolytic activity to reactivate Rubisco for CO_2 fixation. The RCA increases in plants grown in absence of micronutrients indicate an attempt to maintain CO_2 fixation and attenuation of photoinhibition due to the absence of some nutrients. Carbon assimilation homeostasis is maintained by the electron transport chain, which is enriched with Fe containing metalloproteins [55]. In the absence of micronutrients, cells must perform some sort of resource allocation, manifesting in increase or degradation of selected polypeptides to adapt to unfavorable conditions [56]. The increases in the

catalytic activity of GDP-L-fucose synthase 1 (O49213) in the complete fertilization treatment reveal that Golgi-localized nucleotide sugar transporter was active. According to Rautengarten et al. [57], the transport of GDP-Fuc is essential for the fucosylation of xyloglucan and is indispensable for plant growth and development, since plants with significantly reduced GFT1 expression are severely dwarfed and do not grow normally. The regulation of these proteins undoubtedly targets sugar import for the cell wall structuring, ensuring growth in *S. engleri*.

The enhanced activity of nudix hydrolase 2 (NUDT2, Q94B74) in the commercial substrate, complete fertilization and half fertilization treatments indicates its function as a regulator of oxidative stress induced by nutrient excess in *S. engleri*. As reported to Ogawa and Yoshimura [58], NUDT2 asymmetrically hydrolyzes di-adenosine tetraphosphate (Ap4A) into ATP and AMP. This process may have important roles in critical physiological events in chloroplasts such as oxidative stress, apoptosis, DNA repair, transcription, and immune responses. Indeed, the specificity of phosphoprotein phosphatases as oxidative stress signaling has already been reported [59]. Our data show a remarkable increase in relative abundance of probable protein phosphatase 2C 34 (PP2C type, Q9M9W9) in commercial substrate, complete fertilization, half fertilization, and absence of K, which certainly was involved in the maintenance of redox homeostasis. Máthé et al. [60] report that PP2C act scavenging the ROS generated via plasma membrane-associated NADPH oxidases (respiratory burst oxidase homologs, RBOHs) as the endpoint of intracellular signaling, and/or ROS arising from other cells of tissue during systemic oxidative stress that triggers the production of excess $^1\text{O}_2$, $\text{O}_2^{\cdot-}$ and H_2O_2 in chloroplasts. Thus, the higher rates of CO_2 assimilation and growth observed in these treatments were certainly maintained, in part, by ROS control.

Interestingly, most of the up-regulated proteins described above exhibited decreases in relative abundance in the treatments with reduced growth, i.e., control, absence of micronutrients and absence of P. These findings reinforce the relevant role of different nutritional sources for the development of *S. engleri*. Indeed, unfavorable nutritional conditions increased the abundance of COBRA-like protein 7 (COBL 7, Q7XR91), a type of growth-related protein, especially in control, absence of micronutrients and absence of N. Previous studies report that COBL family proteins are strongly involved in cellulose biosynthesis and cell expansion regulation [61–63]. The reduced growth reported here is certainly a result of the lower QY and A rate observed in control and absence of micronutrients, which is corroborated by the up-regulation of photosystem I P700 chlorophyll apoprotein A1 (PSAA, Q1ACL4) and Glyceraldehyde-3-phosphate dehydrogenase B (G3PB, P12860). Zhang et al. [64] related that when the expression levels of photosystem I P700 chlorophyll A apoprotein A1, photosystem II Qb protein D1, and ribulose biphosphate carboxylase are remarkably repressed, photosynthetic rates are impaired. We thus suggest that the up regulation of PSAA and G3PB in this treatment is associated with improved electron transfer activity into photosystem I of *S. engleri*, as a mechanism to maintain minimum rates of CO_2 assimilation. Likewise, the increased activity of cycloartenol synthase (CAS1, Q2XPU6) observed in the control plants of our study indicates the increased biosynthesis of cycloartenol, a sterol that maintains the stability of chlorophyll–protein complexes. As reported to Babiychuk et al. [65], the biogenesis of

thylakoids requires extensive exchange of lipid precursors between the chloroplast and the endoplasmic reticulum (RE). These cellular compartments develop contact sites between the outer plastid envelope and the sterol-rich ER membranes. Unbalanced or depletion of sterol composition compromise ER–plastid physical contacts, altering the number of chloroplasts and consequently photosynthesis. The protein adjustment mechanisms of the photosynthetic apparatus appear to be essential for the minimal maintenance of its functionality, thus allowing *S. engleri* plants to remain energetically active under unfavorable nutritional conditions.

Conclusions

Although different nutritional sources caused changes in gas exchanges and growth rates, *S. engleri* plants grew substantially under favorable nutritional conditions and exhibited acclimation to nutritional deficit. The absence of some macronutrients and micronutrients limits the development of biometric variables and the production of dry mass, where P, Ca, Mg and micronutrients are the most limiting. Most of the common proteins increasing in abundance may have an intracellular origin and thus reflect cytoplasmic and organelle adjustments. These proteins have a diverse array of metabolic functions, especially related to Fe and Zn homeostasis, participation in photosynthesis and energy pathway, ROS signaling and preservation of functional structures. Our findings indicate that *S. engleri* plants maintain a tight adjustment of the photosynthetic apparatus functionality to cope with nutrients shortages. Half of the fertilization is enough for the species to reach maximum growth. The application of Ca, Mg and micronutrients are essential for the development of *S. engleri*. Our findings are crucial to support field planting, as adequate fertilization is essential to produce high-quality seedlings, thereby facilitating plans to reintroduce the species into its native environment. Furthermore, *ex situ* conservation strategies, such as captive breeding for reintroduction and population augmentation, are considered useful tools to help recover endangered species.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Declaration of Competing Interest

The author has no relevant financial or non-financial interests to disclose.

Funding

This research was funded by Instituto Tecnológico Vale project “Plantas do QF” (grant number R100603.EQ.01).

Authors’ contribution

Designed research: EAV, LPN and CFC; Performed research: EAV, LPN, JFS, MLC, RV and CFC; Analyzed Data: EAV, MLC and CFC; Wrote the paper: EAV, LPN, JFS, MLC, RV, SJR, MG and CFC.

Acknowledgments

The authors are grateful to Vale S/A and Gerdau S/A for granting access to sites where seeds and fruits were harvested. CFC and MG acknowledge support from CNPq productivity scholarship (grant number: 311637/2022-1 and 310865/2022-0, respectively).

References

1. Lombardo S, Pandino G, Mauromicale G. Minerals profile of two globe artichoke cultivars as affected by NPK fertilizer regimes. *Food Research International*. 2017;100:95–9.
2. Khalofah A, Ghramh HA, Al-Qthanin RN, L’taief B. The impact of NPK fertilizer on growth and nutrient accumulation in juniper (*Juniperus procera*) trees grown on fire-damaged and intact soils. *PLoS ONE*. 2022;17:e0262685.
3. Fan X, Zhou X, Chen H, Tang M, Xie X. Cross-Talks Between Macro- and Micronutrient Uptake and Signaling in Plants. *Front Plant Sci*. 2021;12:663477.
4. Karthika KS, Rashmi I, Parvathi MS. Biological Functions, Uptake and Transport of Essential Nutrients in Relation to Plant Growth. In: Hasanuzzaman M, Fujita M, Oku H, Nahar K, Hawrylak-Nowak B, editors. *Plant Nutrients and Abiotic Stress Tolerance*. Singapore: Springer Singapore; 2018. p. 1–49.
5. Soares MB, De Mello Prado R, Tenesaca LFL, Lúcio JCB, Carvalho RF. Tomato phytochromes B1 and B2 are part of the responses to the nutritional stress induced by NPK deficiency. *Physiologia Plantarum*. 2021;173:2238–47.
6. Liu H, Wang R, Lü X-T, Cai J, Feng X, Yang G, et al. Effects of nitrogen addition on plant-soil micronutrients vary with nitrogen form and mowing management in a meadow steppe. *Environmental Pollution*. 2021;289:117969.

7. Shao Y, Li S, Gao L, Sun C, Hu J, Ullah A, et al. Magnesium Application Promotes Rubisco Activation and Contributes to High-Temperature Stress Alleviation in Wheat During the Grain Filling. *Front Plant Sci.* 2021;12:675582.
8. Lambers H, Oliveira RS. Mineral Nutrition. In: *Plant Physiological Ecology*. Cham: Springer International Publishing; 2019. p. 301–84.
9. Zhong C, Cao X, Bai Z, Zhang J, Zhu L, Huang J, et al. Nitrogen metabolism correlates with the acclimation of photosynthesis to short-term water stress in rice (*Oryza sativa* L.). *Plant Physiology and Biochemistry.* 2018;125:52–62.
10. Kartseva T, Dobrikova A, Kocheva K, Alexandrov V, Georgiev G, Brestič M, et al. Optimal Nitrogen Supply Ameliorates the Performance of Wheat Seedlings under Osmotic Stress in Genotype-Specific Manner. *Plants.* 2021;10:493.
11. Rai S, Singh PK, Mankotia S, Swain J, Satbhai SB. Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese. *Plant Stress.* 2021;1:100008.
12. Gai APC, Dos Santos DS, Vieira EA. Effects of zinc excess on antioxidant metabolism, mineral content and initial growth of *Handroanthus impetiginosus* (Mart. ex DC.) Mattos and *Tabebuia roseoalba* (Ridl.) Sandwith. *Environmental and Experimental Botany.* 2017;144:88–99.
13. Chen C, Cui Y, Cui M, Zhou W, Wu H, Ling H. A FIT -binding protein is involved in modulating iron and zinc homeostasis in *Arabidopsis*. *Plant Cell & Environment.* 2018;41:1698–714.
14. Waters BM, Armbrust LC. Optimal copper supply is required for normal plant iron deficiency responses. *Plant Signaling & Behavior.* 2013;8:e26611.
15. Chao Z, Chao D. Similarities and differences in iron homeostasis strategies between graminaceous and nongraminaceous plants. *New Phytologist.* 2022;236:1655–60.
16. Ahmad P, Abdel Latef AAH, Rasool S, Akram NA, Ashraf M, Gucel S. Role of Proteomics in Crop Stress Tolerance. *Front Plant Sci.* 2016;7.
17. Turek I, Gehring C, Irving H. Arabidopsis Plant Natriuretic Peptide Is a Novel Interactor of Rubisco Activase. *Life.* 2020;11:21.
18. Feller U, Anders I, Mae T. Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. *Journal of Experimental Botany.* 2007;59:1615–24.
19. Kumar S, Kumar S, Mohapatra T. Interaction Between Macro- and Micro-Nutrients in Plants. *Front Plant Sci.* 2021;12:665583.
20. Do Carmo FF, Jacobi CM. Diversity and plant trait-soil relationships among rock outcrops in the Brazilian Atlantic rainforest. *Plant Soil.* 2016;403:7–20.
21. Ferreira MTM, Meyer PB, Mendes MS, Rezende SG, Junqueira PA, Giomi VT, et al. Contribuição ao conhecimento da ocorrência e distribuição da espécie *Stephanopodium engleri* Baill. (Dichapetalaceae) em Minas Gerais, Brasil. *MGBIOTA.* 2022;14:6–19.
22. Kettler TA, Doran JW, Gilbert TL. Simplified Method for Soil Particle-Size Determination to Accompany Soil-Quality Analyses. *Soil Science Society of America Journal.* 2001;65:849–52.

23. O'Brien TP, Feder N, McCully ME. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma*. 1964;59:368–73.
24. Nascimento SV do, Magalhães MM, Cunha RL, Costa PH de O, Alves RC de O, Oliveira GC de, et al. Differential accumulation of proteins in oil palms affected by fatal yellowing disease. *PLoS ONE*. 2018;13:e0195538.
25. Herrera H, Valadares R, Oliveira G, Fuentes A, Almonacid L, do Nascimento SV, et al. Adaptation and tolerance mechanisms developed by mycorrhizal *Bipinnula fimbriata* plantlets (Orchidaceae) in a heavy metal-polluted ecosystem. *Mycorrhiza*. 2018;28:651–63.
26. Holdo RM. Revisiting the Two-Layer Hypothesis: Coexistence of Alternative Functional Rooting Strategies in Savannas. *PLoS ONE*. 2013;8:e69625.
27. Ramos SJ, Gastauer M, Mitre SK, Caldeira CF, Silva JR, Furtini Neto AE, et al. Plant growth and nutrient use efficiency of two native Fabaceae species for mineland revegetation in the eastern Amazon. *J For Res*. 2020;31:2287–93.
28. Caldeira CF, Lima MO, Ramos SJ, Gastauer M. Native Amazonian Canga Grasses Show Distinct Nitrogen Growth Responses in Iron Mining Substrates. *Plants*. 2021;10:849.
29. Booth BD, Murphy SD, Swanton CJ. Invasive Ecology of Weeds in Agricultural Systems. In: Inderjit, editor. *Weed Biology and Management*. Dordrecht: Springer Netherlands; 2004. p. 29–45.
30. Nagase A, Dunnett N. The relationship between percentage of organic matter in substrate and plant growth in extensive green roofs. *Landscape and Urban Planning*. 2011;103:230–6.
31. Srivastava AK. Integrated Nutrient Management in Citrus. In: Srivastava AK, editor. *Advances in Citrus Nutrition*. Dordrecht: Springer Netherlands; 2012. p. 369–89.
32. Moura J, Vieira EA. Responses of young plants of *Vachellia farnesiana* to drought. *Aust J Bot*. 2020;68:587.
33. Pettigrew WT. Potassium influences on yield and quality production for maize, wheat, soybean and cotton. *Physiologia Plantarum*. 2008;133:670–81.
34. Lu Z, Ren T, Pan Y, Li X, Cong R, Lu J. Differences on photosynthetic limitations between leaf margins and leaf centers under potassium deficiency for *Brassica napus* L. *Sci Rep*. 2016;6:21725.
35. Hu W, Gu H, Wang K, Lu Z, Li X, Cong R, et al. Potassium deficiency stress reduces Rubisco activity in *Brassica napus* leaves by subcellular acidification decreasing photosynthetic rate. *Plant Physiology and Biochemistry*. 2023;201:107912.
36. Marchand J, Heydarizadeh P, Schoefs B, Spetea C. Chloroplast Ion and Metabolite Transport in Algae. In: Larkum AWD, Grossman AR, Raven JA, editors. *Photosynthesis in Algae: Biochemical and Physiological Mechanisms*. Cham: Springer International Publishing; 2020. p. 107–39.
37. Magalhães DDS, Viegas IDJM, Barata HDS, Costa MG, Silva BCD, Mera WYWDL. Deficiencies of nitrogen, calcium, and micronutrients are the most limiting factors for growth and yield of small pepper plants. *Rev Ceres*. 2023;70:125–35.

38. Högy P, Fangmeier A. Atmospheric CO₂ enrichment affects potatoes: 2. Tuber quality traits. *European Journal of Agronomy*. 2009;30:85–94.
39. Loladze I. Hidden shift of the ionome of plants exposed to elevated CO₂ depletes minerals at the base of human nutrition. *eLife*. 2014;3:e02245.
40. Myers SS, Zanutti A, Kloog I, Huybers P, Leakey ADB, Bloom AJ, et al. Increasing CO₂ threatens human nutrition. *Nature*. 2014;510:139–42.
41. Dong J, Gruda N, Lam SK, Li X, Duan Z. Effects of Elevated CO₂ on Nutritional Quality of Vegetables: A Review. *Front Plant Sci*. 2018;9:924.
42. Almadiy AA, Shaban AE, Ibrahim AM, Balhareth SM, El-Gioushy SF, Khater E-SG. Partially substituting chemical NPK fertilizers and their impact on Eureka lemon trees (*Citrus limon* L. Burm) productivity and fruit quality. *Sci Rep*. 2023;13:10506.
43. Martin KC, Bruhn D, Lovelock CE, Feller IC, Evans JR, Ball MC. Nitrogen fertilization enhances water-use efficiency in a saline environment. *Plant, Cell & Environment*. 2010;33:344–57.
44. Wang L, Xue C, Pan X, Chen F, Liu Y. Application of Controlled-Release Urea Enhances Grain Yield and Nitrogen Use Efficiency in Irrigated Rice in the Yangtze River Basin, China. *Front Plant Sci*. 2018;9:999.
45. Kubar MS, Alshallash KS, Asghar MA, Feng M, Raza A, Wang C, et al. Improving Winter Wheat Photosynthesis, Nitrogen Use Efficiency, and Yield by Optimizing Nitrogen Fertilization. *Life*. 2022;12:1478.
46. Valle-Romero P, García-López JV, Redondo-Gómez S, Flores-Duarte NJ, Rodríguez-Llorente ID, Idaszkin YL, et al. Biofertilization with PGP Bacteria Improve Strawberry Plant Performance under Sub-Optimum Phosphorus Fertilization. *Agronomy*. 2023;13:335.
47. Christina M, Le Maire G, Battie-Laclau P, Nouvellon Y, Bouillet J, Jourdan C, et al. Measured and modeled interactive effects of potassium deficiency and water deficit on gross primary productivity and light-use efficiency in *Eucalyptus grandis* plantations. *Global Change Biology*. 2015;21:2022–39.
48. Battie-Laclau P, Delgado-Rojas JS, Christina M, Nouvellon Y, Bouillet J-P, Piccolo MDC, et al. Potassium fertilization increases water-use efficiency for stem biomass production without affecting intrinsic water-use efficiency in *Eucalyptus grandis* plantations. *Forest Ecology and Management*. 2016;364:77–89.
49. Roosta HR, Estaji A, Niknam F. Effect of iron, zinc and manganese shortage-induced change on photosynthetic pigments, some osmoregulators and chlorophyll fluorescence parameters in lettuce. *Photosynth*. 2018;56:606–15.
50. De Bang TC, Husted S, Laursen KH, Persson DP, Schjoerring JK. The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. *New Phytologist*. 2021;229:2446–69.
51. Kölling M, Kumari P, Bürstenbinder K. Calcium- and calmodulin-regulated microtubule-associated proteins as signal-integration hubs at the plasma membrane–cytoskeleton nexus. *Journal of*

- Experimental Botany. 2019;70:387–96.
52. Tränkner M, Tavakol E, Jákli B. Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. *Physiologia Plantarum*. 2018;163:414–31.
 53. Sobieszczuk-Nowicka E, Arasimowicz-Jelonek M, Tanwar UK, Floryszak-Wieczorek J. Plant homocysteine, a methionine precursor and plant's hallmark of metabolic disorders. *Front Plant Sci*. 2022;13:1044944.
 54. Hazra S, Henderson JN, Liles K, Hilton MT, Wachter RM. Regulation of Ribulose-1,5-bisphosphate Carboxylase/Oxygenase (Rubisco) Activase. *Journal of Biological Chemistry*. 2015;290:24222–36.
 55. Sebastian A, Prasad MNV. Operative photo assimilation associated proteome modulations are critical for iron-dependent cadmium tolerance in *Oryza sativa* L. *Protoplasma*. 2015;252:1375–86.
 56. Kolesinski P, Rydzy M, Szczepaniak A. Is RAF1 protein from *Synechocystis* sp. PCC 6803 really needed in the cyanobacterial Rubisco assembly process? *Photosynth Res*. 2017;132:135–48.
 57. Rautengarten C, Ebert B, Liu L, Stonebloom S, Smith-Moritz AM, Pauly M, et al. The Arabidopsis Golgi-localized GDP-L-fucose transporter is required for plant development. *Nat Commun*. 2016;7:12119.
 58. Ogawa T, Yoshimura K. Modulation of the subcellular levels of redox cofactors by Nudix hydrolases in chloroplasts. *Environmental and Experimental Botany*. 2019;161:57–66.
 59. Neill S. Hydrogen peroxide signalling. *Current Opinion in Plant Biology*. 2002;5:388–95.
 60. Máthé C, Garda T, Freytag C, M-Hamvas M. The Role of Serine-Threonine Protein Phosphatase PP2A in Plant Oxidative Stress Signaling—Facts and Hypotheses. *IJMS*. 2019;20:3028.
 61. Sato K, Ito S, Fujii T, Suzuki R, Takenouchi S, Nakaba S, et al. The carbohydrate-binding module (CBM)-like sequence is crucial for rice CWA1/BC1 function in proper assembly of secondary cell wall materials. *Plant Signaling & Behavior*. 2010;5:1433–6.
 62. Liu L, Shang-Guan K, Zhang B, Liu X, Yan M, Zhang L, et al. Brittle Culm1, a COBRA-Like Protein, Functions in Cellulose Assembly through Binding Cellulose Microfibrils. *PLoS Genet*. 2013;9:e1003704.
 63. Sangi S, Araújo PM, Coelho FS, Gazara RK, Almeida-Silva F, Venancio TM, et al. Genome-Wide Analysis of the COBRA-Like Gene Family Supports Gene Expansion through Whole-Genome Duplication in Soybean (*Glycine max*). *Plants*. 2021;10:167.
 64. Zhang J-Y, Wang T, Jia Z-H, Guo Z-R, Liu Y-Z, Wang G. Photosystem Disorder Could be the Key Cause for the Formation of Albino Leaf Phenotype in Pecan. *IJMS*. 2020;21:6137.
 65. Babiychuk E, Bouvier-Navé P, Compagnon V, Suzuki M, Muranaka T, Van Montagu M, et al. Allelic mutant series reveal distinct functions for *Arabidopsis* cycloartenol synthase 1 in cell viability and plastid biogenesis. *Proc Natl Acad Sci USA*. 2008;105:3163–8.

Figures

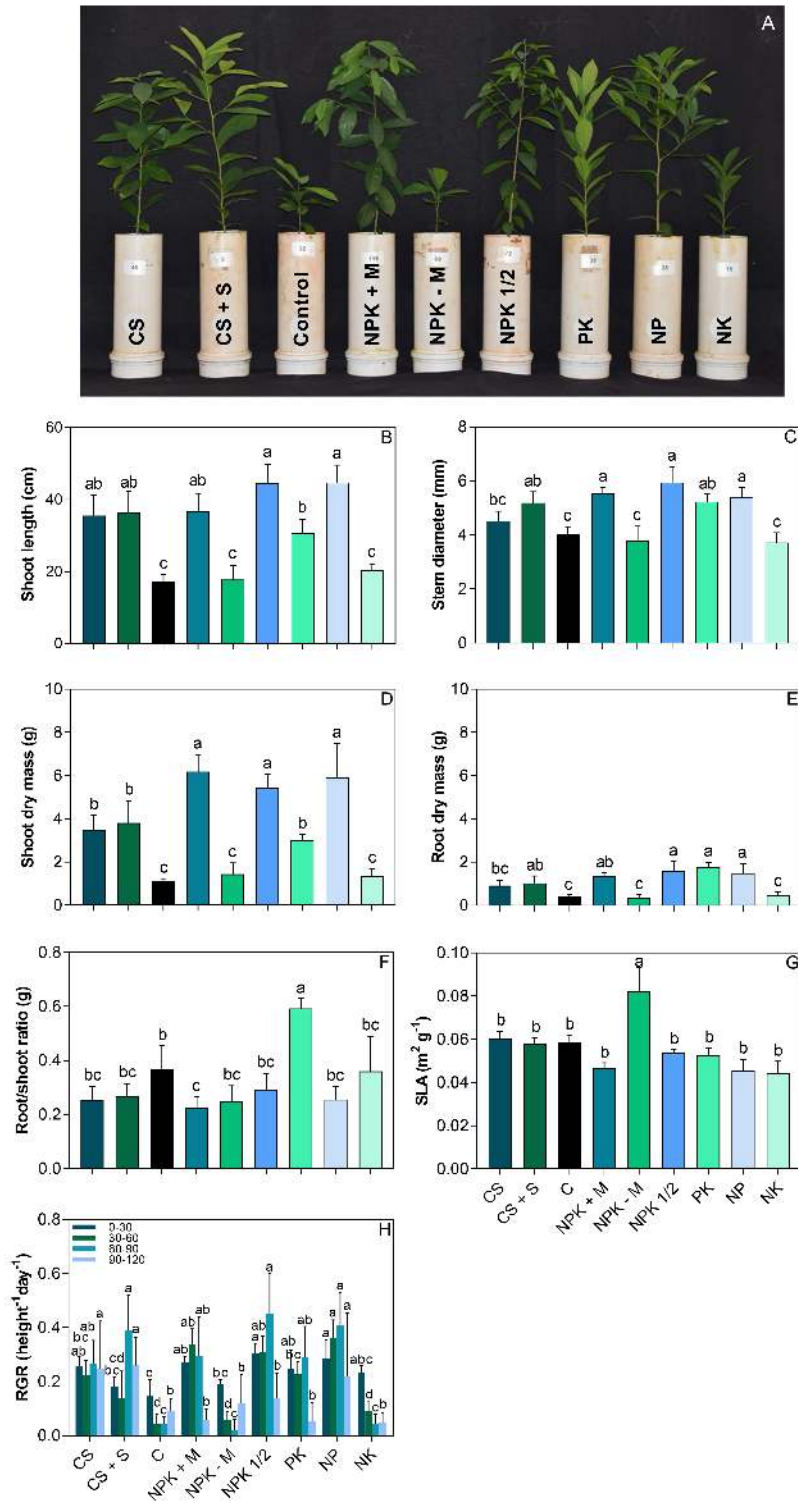


Figure 1

Growth parameters of *Stephanopodium engleri* under different nutritional sources for 120 days. CS = commercial substrate, CS+S = commercial substrate + soil, C = control, NPK+M = NPK solution with micronutrients, NPK-M = NPK solution without micronutrients, NPK 1/2 = NPK half-strength solution, PK = nitrogen-free solution, NP = potassium-free solution and NK = phosphorus-free solution. Different

letters indicate statistically significant differences between means by the Tukey's test ($P < 0.05$). Bars show mean standard deviation ($n = 8$ per treatment).

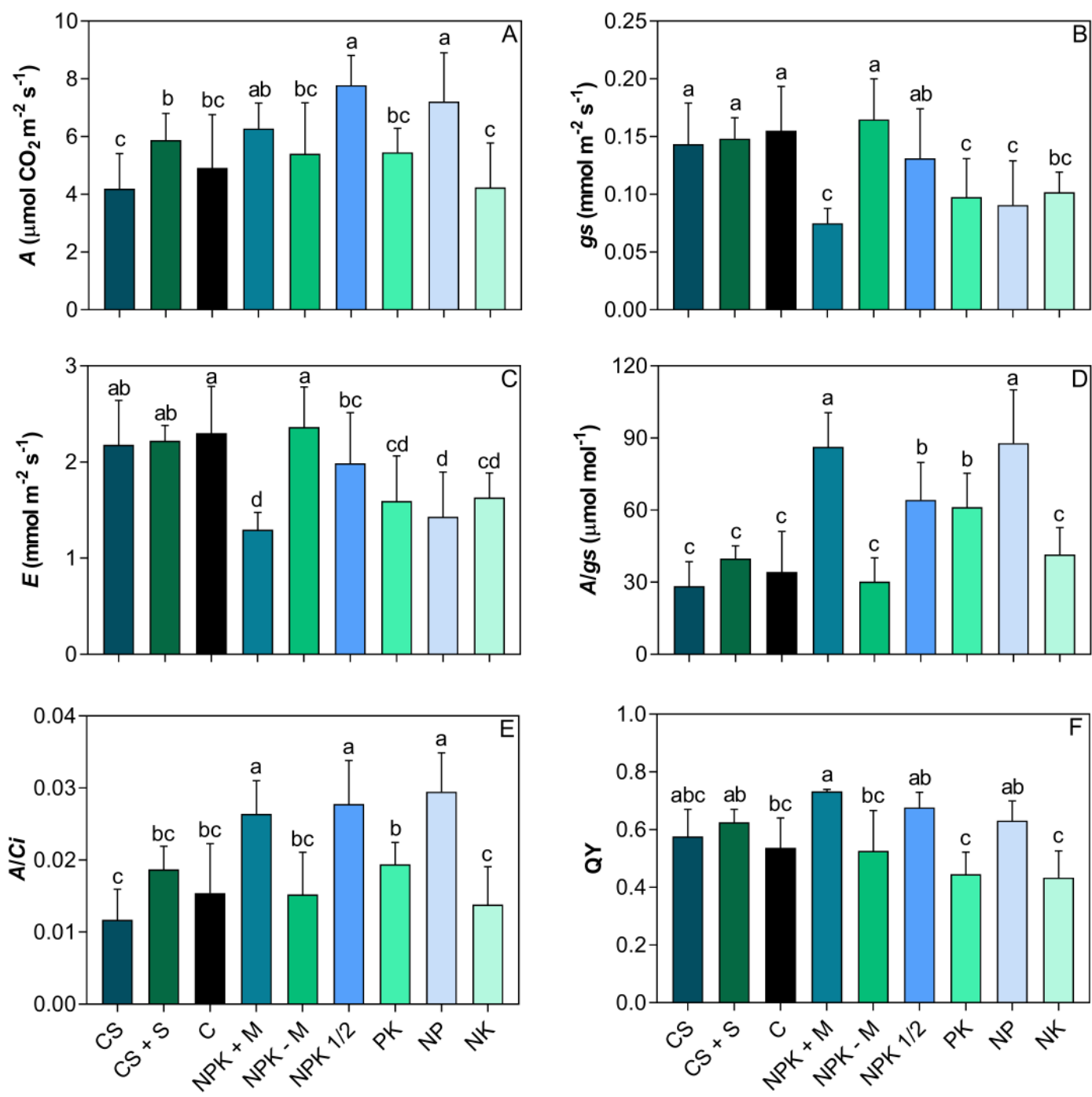


Figure 2

Gas exchange of *Stephanopodium engleri* under different nutritional sources for 120 days. (A) Liquid carbon assimilation (A $\mu\text{mol m}^{-2} \text{ s}^{-1}$); (B) stomatal conductance (gs $\text{mmol m}^{-2} \text{ s}^{-1}$); (C) transpiration rate (E $\text{mmol m}^{-2} \text{ s}^{-1}$); (D) intrinsic efficiency of water use (A/gs); (E) instantaneous carboxylation rate (A/Ci); (F) ratio of intercellular to ambient CO_2 concentration (Ci/Ca); (G) quantum yield of photosystem II

(QY); and (H) leaf temperature (°C). CS = commercial substrate, CS+S = commercial substrate + soil, C = control, NPK+M = NPK solution with micronutrients, NPK–M = NPK solution without micronutrients, NPK 1/2 = NPK half-strength solution, PK = nitrogen-free solution, NP = potassium-free solution and NK = phosphorus-free solution. Different letters indicate statistically significant differences between means by the Tukey’s test ($P < 0.05$). Bars show mean standard deviation ($n = 8$ per treatment).

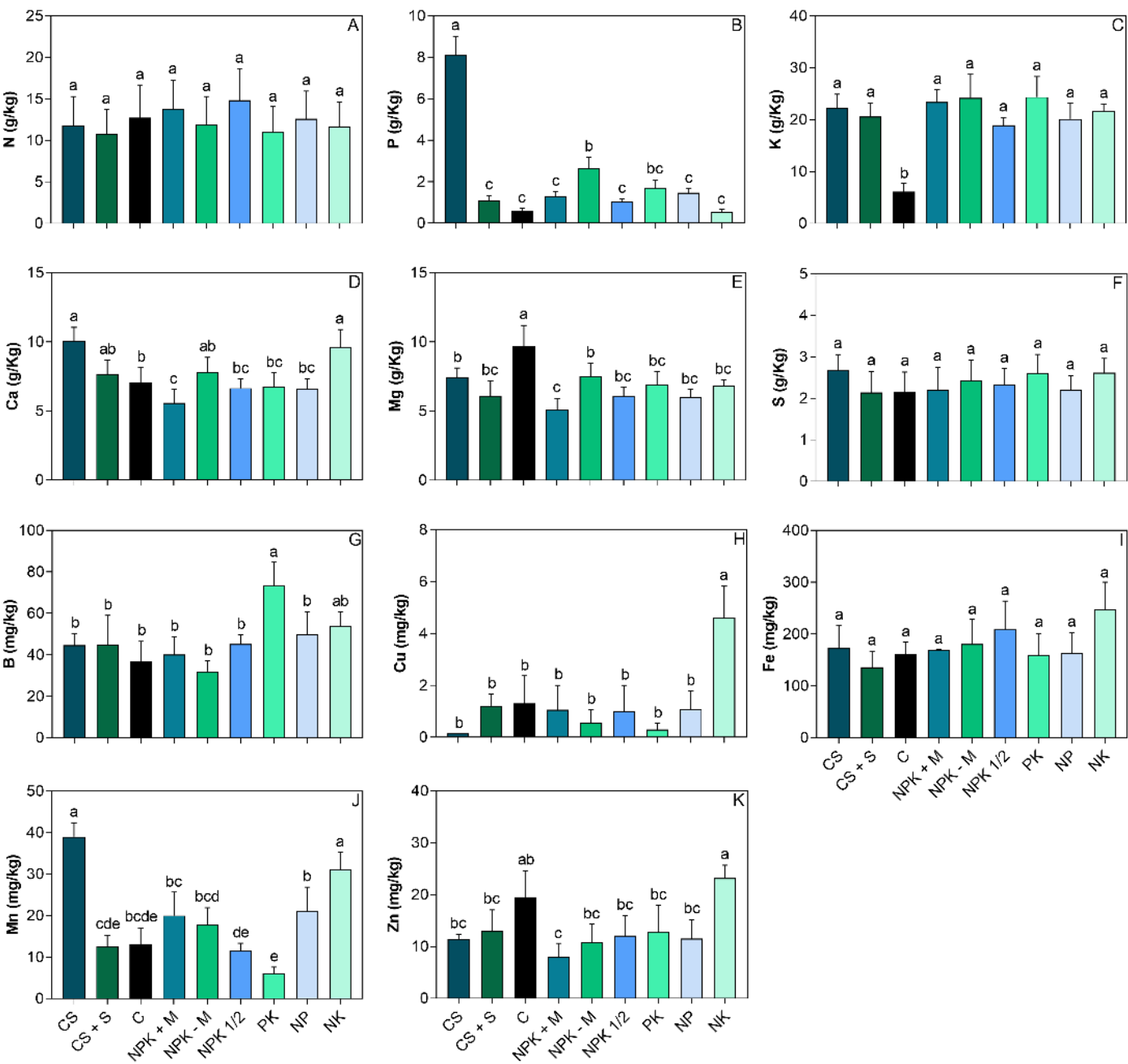


Figure 3

Macronutrients and micronutrients concentration in leaves of *Stephanopodium engleri* under different nutritional sources for 120 days. CS = commercial substrate, CS+S = commercial substrate + soil, C = control, NPK+M = NPK solution with micronutrients, NPK–M = NPK solution without micronutrients, NPK

1/2 = NPK half-strength solution, PK = nitrogen-free solution, NP = potassium-free solution and NK = phosphorus-free solution. Different letters indicate statistically significant differences between means by the Tukey's test ($P < 0.05$). Bars show mean standard deviation ($n = 8$ per treatment).

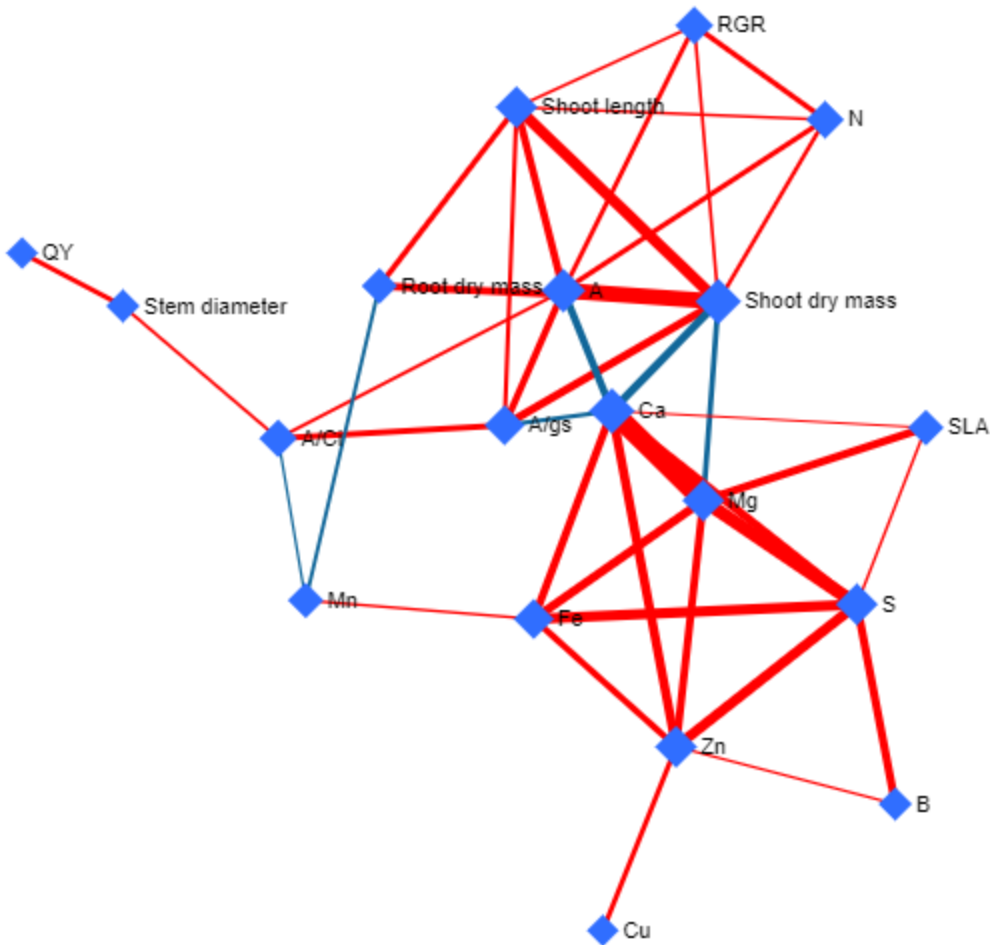


Figure 4

Network building between growth parameters, gas exchanges and nutritional composition based on Debiased Sparse Partial Correlation algorithm (DSPC) analysis of *Stephanopodium engleri* under different nutritional sources for 120 days. Nodes demonstrate reconstructing correlation-based networks, and the edge thickness is proportional to the strength of the shown correlation. Red lines indicate positive correlations, while blue lines show negative correlations.

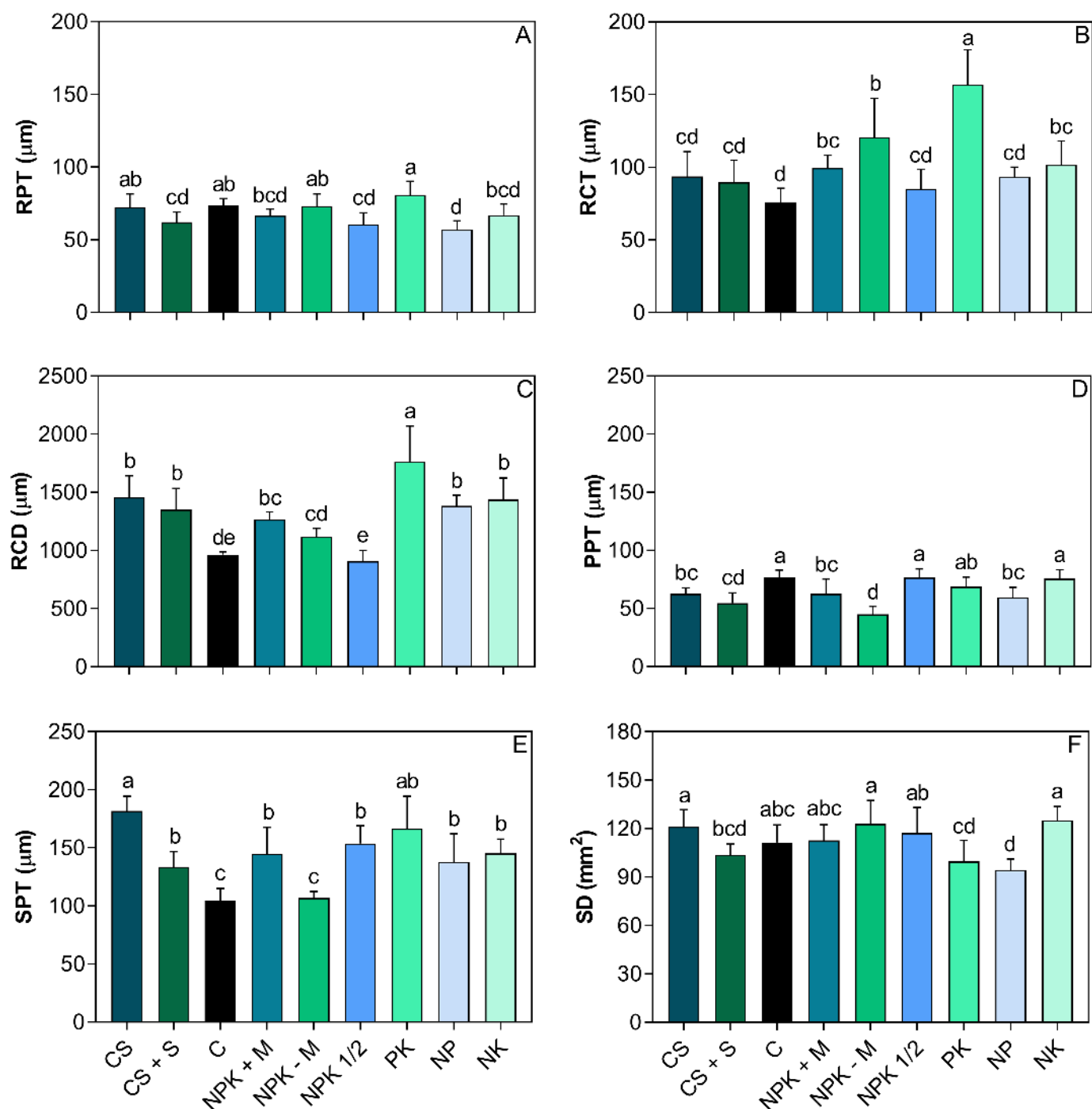


Figure 5

Anatomical traits on roots and leaves of *Stephanopodium engleri* under different nutritional sources for 120 days. (A) root periderm thickness (RPT); (B) root cortex thickness (RCT); (C) root cortex diameter (RCD); (D) palisade parenchyma thickness (PPT); (E) spongy parenchyma thickness (SPT); and (F) stomatal density (SD). CS = commercial substrate, CS+S = commercial substrate + soil, C = control, NPK+M = NPK solution with micronutrients, NPK-M = NPK solution without micronutrients, NPK 1/2 = NPK half-strength solution, PK = nitrogen-free solution, NP = potassium-free solution and NK =

the mean of each treatment. Colors represent abundance (red, significantly higher than control; blue, significantly lower than control; lighter, no significant changes) by Tukey's test ($p < 0.05$). Red dotted arrows indicate nutrient binding proteins. Undetected proteins are indicated in gray (See Figure S1). CS = commercial substrate, CS+S = commercial substrate + soil, C = control, NPK+M = NPK solution with micronutrients, NPK–M = NPK solution without micronutrients, NPK 1/2 = NPK half-strength solution, PK = nitrogen-free solution, NP = potassium-free solution and NK = phosphorus-free solution.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigS1Anato.jpg](#)
- [FigS2Heatmapproteome.jpg](#)
- [FigS3VIPscoresproteome.tif](#)
- [SuppTable1Sengleri.pdf](#)