

Nitrogen ionome dynamics on leafy vegetables in tropical climate

Itamar Shenhar^{1,2,3}, Aravind Harikumar^{1,3}, Miguel Raul Pebes Trujillo^{1,3}, Zhitong Zhao^{1,3}, Qin Lin^{1,3}, Magdiel Ingrid Setyawati^{1,3}, David Tan^{3,4}, Jie He^{3,5}, Ittai Herrmann^{2,3}, Kee Woei Ng^{1,3,6}, Matan Gavish^{1,3,7*}, Menachem Moshelion^{2,3*}

¹ School of Materials Science and Engineering, Nanyang Technological University, Singapore

² The Robert H Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel

³ Singapore-HUJ Alliance for Research and Enterprise (SHARE), Singapore

⁴ Netatech Engineering Pte Ltd, Singapore

⁵ National Institute of Education - Natural Sciences & Science Education, Nanyang Technological University, Singapore

⁶ Nanyang Environment and Water Research Institute (NEWRI), Singapore

⁷ School of Computer Science and Engineering, Hebrew University of Jerusalem, Jerusalem, Israel

*Corresponding authors: matan.gavish@mail.huji.ac.il; Menachem.moshelion@mail.huji.ac.il

ORCID's:

Itamar Shenhar: <https://orcid.org/0009-0008-4081-8831>

Matan Gavish: <https://orcid.org/0000-0001-6747-6888>

Menachem Moshelion: <https://orcid.org/0000-0003-0156-2884>

32 **Abstract**

33 Nitrogen is known to be a critical macro-nutrient influencing plant physiology, growth, and mineral
34 composition. In tropical conditions, which are challenging for leafy vegetable farming, the nitrogen
35 delivery effect is unclear. In this study, we aimed to investigate the effect of nitrogen application on
36 key physiological traits and the mineral composition of the plants, the plant ionome. Experiments
37 were conducted under tropical conditions greenhouse with varying levels of nitrogen supply to
38 examine the effect on plant transpiration, yield, use efficiency of water and nitrogen, and nutrient
39 uptake dynamics followed by cross-correlation analysis, trying to understand the physiological
40 behavior-uptake dynamics relationships. The results demonstrated that transpiration, yield and WUE
41 theoretic optimum curve, which peaking in nitrogen concentration of 120 mg/L for Chinese spinach
42 and 200 mg/L for Chinese broccoli. Conversely, NUE reduce significantly with increasing nitrogen
43 delivery which reflected on antagonistic increase of excess nitrogen.

44 In terms of mineral composition, nitrogen application resulted in an increase I nitrogen content in the
45 plant leaf tissue, while concentration of certain macronutrients and micronutrients were affected,
46 including potassium, phosphorus, calcium, magnesium, iron, zinc, and molybdenum. Part of the
47 minerals exhibited decreasing pattern due to potential competitive uptake mechanism, iron revealed
48 increasing pattern that correlated with nitrogen delivery, and some minerals correlated with the
49 measured physiological parameters. These results underscore the importance of optimizing nitrogen
50 fertilization to balance plant growth, physiological processes, and plant nutrient homeostasis. The
51 study offers valuable insights for sustainable nitrogen management in agricultural systems aimed at
52 maximizing crop yield while maintaining nutritional quality.

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54 **Keywords:** Nitrogen application, plant ionome, nutrient uptake dynamics, plant physiology,
55 sustainable agriculture.

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71 Introduction

72 Over the past decade, plant ionome have become a significant research scope in plant science. Plant
73 ionome is determined as the composition of seventeen known essential mineral nutrients and trace
74 elements used for plant growth and development. As nutrient availability is highly dependent on the
75 soil physiochemical properties of the soil (Singh et al., 2022; Hartemink and Barrow, 2023). Plants
76 evolved to maintain mineral homeostasis (Williams and Salt, 2009) and allow physiological resilience
77 under nutrient-changing conditions in order to sustain optimal physiological development under
78 deficiency or excess conditions. Recent studies suggest that the plant ionome has a dynamics role in the
79 crosstalk between different mineral uptake pathways (Fan et al., 2021; Kumar et al., 2021). Ionome
80 crosstalk refers to the change of the mineral composition following a change in mineral
81 supplementation, considering how a change in a mineral concentration can affect and interact with
82 different mineral uptake mechanisms. Most of the studies have been done on a scope of two specific
83 minerals on major crop plants or genetic studies that aim to understand the genetic machinery behind
84 two minerals crosstalk (Lin et al., 2013). For example, a wide mineral dynamics study that On Rapeseed
85 (*Brassica napus* L.), revealed eighteen change situations of minerals uptake following specific mineral
86 deficiency were detected (Maillard et al., 2016) reflecting the biochemical homeostasis dynamics within
87 the plant. Although of those studies, the effect on the crop production, quality, and his connection to the
88 ionome crosstalk is unclear.

89 In changing environments, mineral uptake is known to limit plant growth (Sinclair, 1992). After carbon
90 (C), nitrogen (N) is the most essential element for plants physiological and morphological development
91 (Hawkesford et al., 2012) as it takes a significant role in the synthesis of proteins, nucleic acids,
92 chlorophyll, cell walls, membranes, and many more primary and secondary metabolites (Marschner,
93 2011; Mu and Chen, 2021a). Hence, N fertilization can significantly affect the plant physiological
94 properties. Recent studies showed that deficiency and excess in nitrogen delivery reduce plant growth
95 and WUE, which followed by reduced in crop yield (Uhart and Andrade, 1995; Niu et al., 2007; Wang
96 et al., 2011; Mu and Chen, 2021b). Despite the physiological effect, the NUE is increasing in deficiency
97 conditions (Ngosong et al., 2019) and reduce as the concentration increases resulting a negative
98 relationship emphasizing the plant adaptation to the changing conditions.

99 N availability serves as a fundamental macro element that significantly affect the ionome dynamics in
100 the plant tissues. Previous studies showed that NO_3^- signaling can regulate the Phosphate (P) starvation
101 response in *Arabidopsis thaliana* and demonstrate conserved phenomena response in rice (*Oryza sativa*)
102 and Wheat (*Triticum aestivum*) (Medici et al., 2015; Medici et al., 2019a). Furthermore, on bean
103 (*Phaseolus vulgaris* L.) P starvation led to a decrease in inorganic P concentration in the roots, and NO_3^-
104 simultaneously (Gniazdowska and Rychter, 2000). Ammonium (NH_4^+) was also shown to enhance P
105 transport in Corn (*Zea mays* L.) roots (Smith and Jackson, 1987). Additionally, NH_4^+ and Potassium
106 (K^+) were reported to have a competitive relationship in Barley (*Hordeum vulgare* L.) and *Arabidopsis*
107 *thaliana* roots (ten Hoopen et al., 2010), considered to be due to NH_4^+ permeability through voltage-
108 dependent K^+ channels at the root's plasma membrane (White, 1996). Zinc (Zn) and nitrogen are
109 suggested to share a unique interaction. Cakmak and Marschner, 1990 suggest that Zn deficiency
110 conditions depressed the net uptake of NO_3^- on Cotton (*Gossypium* L.), Sunflower (*Helianthus annuus*),
111 and Buckwheat (*Fagopyrum esculentum*). Furthermore, On Rice (*Oryza sativa*), Zn and N have positive
112 synergetic effects on the root-to-shoot translocation (Ji et al., 2022). However, a more comprehensive
113 perspective study is needed to clarify the effect of N application on crop yield and ionome so that more
114 species-specific nitrogen application decisions can be made to maximize production and quality.

115 The impotence of N fertilizer resulted in the overuse of N and a highlighted environmental impact on
116 the soil and the groundwater N contamination (Zhao et al., 2012; Yu et al., 2019). Moreover,
117 manufacturing N fertilizer is a heavy energy consumer that uses fossil fuels and contributes to
118 greenhouse gas emissions (Zhang et al., 2019). Thus, reducing fertilizer use to the actual minimal-

119 optimal level aligns with sustainable agriculture objectives, particularly in regions with a pronounceable
120 environmental footprint from agricultural practice.

121 In tropical climates, the impact of N supply is unclear. Most of the experiments have been done under
122 chambers with controlled ambient conditions, which can alter the results from common farm conditions
123 and not include a deep analysis of the ionome of the plant, considering mainly the growth and yield,
124 which lack understanding of the crop's nutritional value (Lee et al., 2024). A study should include
125 physiological yield properties and nutritional outcomes to understand the broad effect of N
126 supplementation on the crop level. Additionally, ionome dynamics can help us understand physiological
127 phenomena that can be difficult to define by standard practice.

128 Hence, the main objective of this study is to reveal the effect of N supply on the physiological, ionome
129 dynamics, and mineral cross-relationship of leafy vegetables in tropical conditions. To do so, we
130 established an experimental platform that delivers eight fertilizer solution treatments that differ in N
131 concentration and monitor the physiological behavior of the plants as well as their ambient
132 environmental conditions throughout the season. In addition, an ionome analysis was done to
133 understand the crosstalk between N supplementation and ionome dynamics. Our hypotheses were: 1.
134 based on the previous work, is that application of N will have a positive relationship with P and a
135 negative relationship with K; 2. Macro-nutrients will be correlated with the plant's transpiration and
136 growth traits. 3. An optimum curve will be revealed on the physiological parameters. This study will
137 benefit farmers in tropical conditions by maximizing the yield and quality of production and providing
138 crucial insights into the balanced nutrient management necessary for optimal growth and mineral-dense
139 crops.

140

141 **Materials and methods**

142 The study was conducted between September 2023 and November 2023 in a commercial farm
143 greenhouse at Oasis Living Lab, Singapore (1°41'36.0"N, 103°71'95.1"E). We used two commercial
144 leafy vegetable cultivars: *Brassica oleracea* (Alboglabra Group; Chinese broccoli) and *Amaranthus*
145 *tricolor* L. (Chinese spinach; Supplied by Netatech Ltd., Singapore) in two independent experiments
146 (Fig. 1, A-B). The plants were sewed in seedling trays for 3-4 weeks before being transplanted to 4L
147 pots ("18", Tefen Ltd, Nahsholim, Israel) filled with coarse sand ("F2", Rock and Sand industries Ltd,
148 Singapore) for the Chinese spinach and coco-peat ("250", Riococo Ltd, Sri Lanka) for the Chinese
149 broccoli. The plants grew for 4-5 weeks in a semi-controlled ambient condition greenhouse (Netafim
150 Ltd, Israel), including fans, roof vents, and mesh walls for ventilation.

151 This study is a spatial and temporal multilevel, multifactorial experiment. Therefore, we used one main
152 meteorological station ("Watchdog 2745", Spectrum technologies Ltd, Bridgend, Wales) joined with
153 eight smaller meteorological stations ("VP4", Meter, Washington, USA) to measure the temperature,
154 relative humidity, and vapor pressure deficit (Fig. S1). In addition, we used the main photosynthetic
155 active radiation (PAR) sensor ("#3668", Spectrum Technologies Ltd, Bridgend, Wales) joined with eight
156 PAR sensors ("SQ-512", Meter, Washington, United States). Those sensors were connected to the
157 PlantArray system (Plant-Ditech, Yavne, Israel) to maintain a dimensional and continuous
158 measurement. In addition, plants were positioned in a block-randomized design in each experiment to
159 reduce the effect.

160 Treatments were applied to the plants using pumps ("Shurflo 5050-1311-H011", Pentair, Minnesota,
161 United States) and controlled by the PlantArray system (Plant-Ditech, Yavne, Israel). Eight different
162 nitrogen concentrations especially suited to the experimental demands were calculated and measured
163 for the study (Table. S1). Continuous EC ("ES-2", Meter, Washington, United States) and PH
164 ("PHEHT", Ponsel-Aqualabo, Champigny-sur-Marne, France) were measured during the experiment

165 to monitor the treatment application. The study was conducted on a functional phenotyping platform,
166 PlantArray (Plant-Ditech, Yavne, Israel), to measure continuous physiological parameters. Daily
167 transpiration and cumulative transpiration were measured as described by Halperin et al. 2017 using the
168 experimental protocol described in Dalal et al., 2020. Irrigation was conducted on the plants
169 proportionally according to the measured plant's transpiration, following four short irrigation events
170 each night to reach soil field capacity and reduce the effect of diverse soil water content on the results.

171 Plant shoots were harvested and separated manually into leaves and stems. The shoots were dried in a
172 60°C oven ("DHG-9920A", Bluepard instrument, Shanghai, China) for one week and measured for dry
173 shoot weight. For mineral analysis, dried leaf tissues of 0.2 g were digested in 4 ml of 70% nitric acid
174 using UltraWAVE single reaction chamber microwave digestion system (Milestone, Sorisole, Italy).
175 The digested solution was diluted with deionized water to a final volume of 25 ml. The Inductively
176 coupled plasma optical emission spectrometer (ICP-OES; "Avio 200", PerkinElmer Ltd, Massachusetts,
177 United States) and Syngistix software (PerkinElmer Ltd, Massachusetts, United States) were used to
178 measure and calculate the concentrations of minerals (P, K, Ca, Mg, Na, Mn, Cu, Zn, Mo, Fe, B). For
179 the measurement of nitrate concentration, dried leaf samples (0.01 g) were grounded with 10 ml of
180 deionized water and incubated at 37°C for two hours. Sample turbidity was then removed by vacuum
181 filtering the mixture through a 0.45 µm-pore-diameter membrane. The final volume was made to 50 ml.
182 The nitrate concentration of the leaf tissues was determined using the Flow Injection Analyser (Model
183 Quikchem 8000, Lachat Instruments Inc., Milwaukee, USA). Total reduced nitrogen (TRN) content was
184 determined by Kjeldahl digestion of 0.05 g of dried leaf tissues and a Kjeldahl tablet in 5 ml of
185 concentrated sulphuric acid for 60 min at 350°C. After digestion, TRN concentration was quantified by
186 a Kjeltex 8400 analyzer (Foss Tecator AB, Höganäs, Sweden) through titration.

187 Agronomic transpiration use efficiency (water user efficiency; Agronomic WUE), the ratio between
188 crop yield and transpiration was calculated according to:

$$189 \quad \text{Agronomic WUE} = \frac{\text{Shoot dry weight (g)}}{\text{Cumulative transpiration (g)}}$$

190 Nitrogen efficiency and balance parameters were calculated according to Congreves et al., 2021:

191 Nitrogen use efficiency (NUE_{crop}) is the ratio of the dry biomass to the supplied N throughout the
192 experiment. It is calculated by:

$$193 \quad \text{NUE}_{\text{crop}} = \frac{\text{Shoot dry weight (g)}}{\text{Supplied N (g)}}$$

194 Partial nitrogen balance (PNB) is the ratio of the calculated shoot N to the supplied N throughout the
195 experiment. It is calculated by:

$$196 \quad \text{PNB} = \frac{\text{plant shoot N (g)}}{\text{Supplied N (g)}}$$

197 Excess N is the amount of N supplied to the plant and not detected in the plant shoot. It was calculated
198 by:

$$199 \quad \text{Excess N} = \text{supplied N (g)} - \text{Plant shoot N (g)}$$

200 Plant shoot nitrogen was calculated by multiplying the shoot dry weight with the nitrogen concentration.

201

202 Data analysis was performed using Microsoft Office 365 Excel (Microsoft Ltd, Washington, United
203 States) and OriginPro 2023 (OriginLab Ltd, Massachusetts, United States) for graph plotting and JMP
204 17 (SAS Institute Inc., North Carolina, United States) for the statistical analysis. In all multiple

205 comparison tests, data was checked for normal distribution using Shapiro-Wilk's test and homogeneity
206 of variance using Levene's test. A Tukey honest significant difference ANOVA test was applied if both
207 tests were satisfied. If the normality or homogeneity of variance criteria were violated,
208 Wilcoxon/Kruskal-Wallis's nonparametric ANOVA test was used. All tests were done at a p-value<0.05
209 significance. Data mean values for all the results presented with \pm standard error (SE). The squared
210 value of the Pearson correlation coefficient was calculated in a regression fit analysis, and p-values
211 were calculated according to the multivariate coefficient probability test.

212

213 **Results**

214 **N alters the physiological behavior of *Amaranthus tricolor L.* and *Brassica*** 215 ***oleracea***

216 Experiments were designed to examine the physiological traits and ionome dynamics of *Amaranthus*
217 *tricolor L.* (Chinese Spinach) and *Brassica oleracea* (Chinese Broccoli) on different concentration of N.
218 We recognized a significant appearance difference in the other treatments on Chinese Spinach (Fig. 1,
219 C) and Chinese Broccoli (Fig. 1, D). Correspondingly, plants' daily transpiration throughout the
220 experiments were affected by the N treatments. Treatments 120, 160, and 200ppm showed a
221 significantly higher daily transpiration already 7 days after the treatment start (Fig. 2 A) and maintained
222 this pattern throughout the experiment as revealed in the end of the experiment (Fig. 2B) and the
223 cumulative transpiration (Fig. 2C). Shoot dry weight of these higher transpiring treatments was also
224 significantly higher compared to 20, 40, 80, 400, and 800 treatments (Fig. 2D). An optimum curve was
225 revealed for both cultivars whereas on Chinese spinach 120, 160, and 200 transpired significantly more
226 than other treatments on both cultivars. The plant transpiration-use-efficiency (agronomic WUE, see
227 materials and methods) of treatment range 120-400 exhibited significant higher WUE throughout the
228 experiment duration (Fig. 2E). We further investigate the nitrogen use efficiency (NUE_{crop}) and
229 discovered a significant exponential decline pattern of NUE_{crop}, where the lower N concentration
230 treatments revealed the highest efficiency (Fig. 2 F). The partial nitrogen balance (PNB) and the excess
231 N revealed NUE_{crop} antagonistic pattern from the PNB (Fig.2, G). Similar results repeated in the Chinese
232 broccoli with the differences on the optimum treatment concentrations of 200ppm exhibited the highest
233 cumulative transpiration and shoot dry weight (Fig. 3, C-D).

234 **N-Ionome dynamics of *Amaranthus tricolor L.* and *Brassica oleracea***

235 Mineral analysis was performed to the harvested plant leaf tissue to clarify the effect of the N
236 treatment on the plant ionome. The NO₃ and TRN concentrations in the plant tissue exhibited direct
237 correlation with the supplied treatment (Fig. 4, A-B) on both cultivars. This relationship is expected due
238 to the supplied N gradient. Nevertheless, the relationship between the supplied N treatments and other
239 minerals, revealed several different relationship patterns. K showed a negative correlation to N, in
240 particular at the physiological zone (80 – 200 ppm) (Fig. 4 C and D) which reflected on the relationship
241 with leaf N concentration on both cultivars ($R^2=0.787$, P-value<0.0001 and $R^2=0.8138$, P-
242 value=0.0022; Fig. 6). P concentration revealed non-significant pattern under physiological
243 concentrations of N (80- 200ppm; Fig. 4, E and F). Both Ca and Mg displayed similar concentration
244 patterns across N treatments in Chinese spinach and Chinese broccoli. Lower concentrations of both
245 were noted at N concentrations of 20, 40, and 80, with increases observed from 120 to 200. The highest
246 N treatments (400 and 800) significantly reduced concentrations, suggesting a potential toxicity effect.
247 (Fig. 4, G-J). The resembling results between the Ca and the Mg revealed high positive correlation
248 between them on both cultivars (Fig. 6).

249 Fe concentrations in both cultivars increased with N (Fig. 5 A and B), demonstrating strong positive
250 correlations with N ($R^2=0.943$ and $R^2=0.84$; Fig. 6). The other microelements, Zn, Mn, Cu, and B

251 exhibited non-significant patterns with N at physiological concentrations of 80-200 in both Chinese
252 spinach and Chinese broccoli (Fig. 5, C-J).

253

254 Discussion

255 Mineral nutrition balance is essential for growth, development, and disease protection in plants
256 (Hawkesford et al., 2012; Tripathi et al., 2022). As a suggested definition by Arnon, 1950, an essential
257 mineral is not replaceable by other element and has direct or indirect action in plant metabolism. Hence,
258 the ability of the plant to keep mineral homeostasis under changing conditions is crucial to successful
259 crop production. Nitrate and Ammonium uptake is affected by soil PH and soil water content, which
260 results in significant yield reduction (Ruan et al., 2007; Dijkstra and Cheng, 2008). Yet, the effect of
261 lack of or excessive nitrogen on the uptake of other mineral elements is still being determined, especially
262 in terms of practical crop production and quality. In the current study, we clarify the practical impact on
263 physiological and ionome crosstalk dynamics under a wide range of nitrogen supply for leafy greens.
264 Our results reveal the actual optimum response of key physiological parameters as well as on the effect
265 on the PNB and excess nitrogen runoff.

266 Plant physiological traits has been affected significantly by the nitrogen treatment. Daily transpiration
267 revealed gradual increase in significance between the N treatments throughout the experiments (Fig. 2,
268 A-C and Fig. 3, A-C respectively). This cumulative transpiration response has been reported before, for
269 example, the response of the transpiration of *S. bicolor* (Sorghum) to salinity stress over time (Dewi et
270 al., 2023). Transpiration is known to correlate with crop yield in Chinese Spinach (Liu and Stützel,
271 2004), and indeed, the agronomic WUE reveals that the 120, 160, and 200 are also more efficient in
272 using the transpired water to increase dry biomass (Fig. 2, D-E and Fig. 3, D-E). Moreover, N supply is
273 related to positive feedback of plant growth, N supply showed to increase chlorophyll a and b (Mu et
274 al., 2016; Mu and Chen, 2021b). Our results support recent studies that suggest that optimal N supply
275 can enhance plant productivity by three leading causes: 1. Regulation of stomatal conductance without
276 impacting the assimilation rate reduces water loss; 2. Increased N investment in the photosynthetic
277 apparatus increases the assimilation rate; 3. There is a moderate increase in the assimilation rate with a
278 slight decrease in stomatal conductance (Plett et al., 2020). Thus, we suggest that under low N supply
279 transpiration is reduced even if water is not limited factor as N limitation prevent the use of
280 photosynthetic produced sugars. Furthermore, plants may invest more N to bioenergetic processes to
281 support a high electron transport rate during the photosynthesis process during N starvation stress (Mu
282 and Chen, 2021b), which might create additional starvation stress on the pre-stress plants. The
283 improvement of NUE_{crop} under low N concentrations (Fig 2, F and Fig. 3, F) suggest the ability of the
284 plant to cope with nitrogen stress by maximizing its physiological traits per N molecules. A similar
285 observation of NUE_{crop} reduction concerning the N supply was found in Rice (*Oryza Sativa*; Nguyen et
286 al., 2014). In opposition to the starvation response, we suggest that the plant minimize the N toxicity
287 by reducing NUE_{crop} to overcome the N abundance toxicity symptoms when transpiration and
288 photosynthesis decreasing, and plant N requirements reduce. Similar NUE response to N delivery had
289 been shown in previous study on *Cannabis Sativa L.* (Saloner and Bernstein, 2020)

290 Excess N is one of the most negatively impacting elements on soil health in modern agriculture practice
291 (McLauchlan, 2007). Our results indicate that low N concentrations are associated with elevated PNB
292 and reduced excess nitrogen, whereas high nitrogen concentrations correlate with decreased PNB and
293 increased excess nitrogen (Fig. 2, G and Fig. 3, G). Thus using lower nitrogen concentrations in the
294 fertilizers will facilitate a more environmentally sustainable approach, without significantly impacting
295 key physiological traits and yield, and enhancing ionomic crosstalk balance. Furthermore, discovering
296 the different relationships between minerals under stress conditions could reveal a dedicated
297 compensation mechanism within the plant to extend our knowledge of the ionome-physiological

298 processes within the plant. One of the interesting results is the decrease in K absorbance to the plant
299 along the nitrogen treatment (Fig. 4, C-D). We suggest that this N-K trade-off might occur due to the
300 competition between increasing NH_4^+ along with the N concentration treatment increase and K
301 transport to the cells, which might explain our results (White, 1996; Hoopen et al., 2010). P response
302 crosstalk with N was much less substantial than K, where only in the Chinese broccoli some optimum
303 curve revealed around N concentration of 80 ppm (Fig. 4, E-F). Recent studies reported mutual N-P
304 interaction, as N showed to regulate P starvation response suggested by mediating degradation of
305 plasma membrane P transporters (Lin et al., 2013; Medici et al., 2019b), and can suggest N can affect
306 the P concentration by regulating the P transporters. Yet, the exact interaction is still unclear and need
307 further investigation on different crops to understand the relationship. Ca and Mg optimum curve
308 patterns were similar, with moderate positively correlation with N (Fig. 4, I-J, and Fig. 6). Mg has been
309 proven beneficial to growth and yield (Yousaf et al., 2021) and have crucial part in the chlorophyll a
310 molecule (Lincoln Taiz, Eduardo Zeiger, Ian M. Møller, 2015). Fe (Lee et al., 2024) was highly
311 correlated to the N treatment (Fig. 5, A-B; Fig 6; Fig S2). We suggest two mechanisms that might
312 explain that. First, is the fact that Fe is an immobile nutrient in the plant, thus, as faster the plant grow
313 due to ample N conditions the higher the need for new Fe to be absorbed and transformed to the new
314 tissues. Secondly, we suggest that as Fe is a fundamental factor in several Nitrate and ammonium
315 assimilation pathway enzymes (e.g. nitrate reductase, Nitrite reductase, and Glutamate synthase;(Berges
316 and Mulholland, 2008; Heldt and Piechulla, 2011), higher N concentration will result an increase in the
317 nitrogen assimilation pathway, following enzymatic activity increase, and higher demand for Fe within
318 the cell. A cultivar-specific effect was shown on the Zn concentration, whereas the Chinese Broccoli
319 concentration was induced on higher N treatments, while on Chinese Spinach, no significant pattern
320 was revealed. Mn concentration reduces the physiological toxic concentration, which might serve as an
321 indicator for N toxicity in plants.

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326 **Competing interest**

327 The authors declare that they have no conflict of interest.

328 **Author contribution**

329 I.T played a pivotal role in the planning and formulation of the hypothesis, conducted all the
330 experiments, performed statistical analysis, managed plant growth and post-harvest analysis and was
331 primary contributed to the writing of this manuscript. A.H was part of the harvest processing. M.R.P.T
332 was part of the harvest processing and consulted on statistical analysis. Z.Z was part of the harvest
333 processing. Q.L was actively involved in the leaf mineral analysis procedure. M.I.S managed fertilizer
334 preparation. D.T supported the plant growth. H.J revised the manuscript. I.H revised the manuscript.
335 K.W revised the manuscript. M.G revised the manuscript. M.M as the principal investigator, managed
336 the experiments, contributed to hypothesis generation, experimental design, data analysis, writing and
337 revising the manuscript.

338 **Data availability**

339 The data supporting this study's findings are available from the corresponding authors upon reasonable
340 request. Additionally, some of the data are located in this article's supplementary material.

341

342 **Reference**

- 343 **Berges JA, Mulholland MR** (2008) Enzymes and Nitrogen Cycling. Nitrogen in the Marine
344 Environment 1385–1444
- 345 **Cakmak I, Marschner H** (1990) Decrease in nitrate uptake and increase in proton release in zinc
346 deficient cotton, sunflower and buckwheat plants. Plant Soil **129**: 261–268
- 347 **Congreves KA, Otchere O, Ferland D, Farzadfar S, Williams S, Arcand MM** (2021) Nitrogen
348 Use Efficiency Definitions of Today and Tomorrow. Front Plant Sci **12**: 637108
- 349 **Dalal A, Shenhar I, Bourstein R, Mayo A, Grunwald Y, Averbuch N, Attia Z, Wallach R,**
350 **Moshelion M** (2020) A Telemetric, Gravimetric Platform for Real-Time Physiological
351 Phenotyping of Plant–Environment Interactions. JoVE (Journal of Visualized Experiments)
352 **2020**: e61280
- 353 **Dewi ES, Abdulai I, Bracho-Mujica G, Appiah M, Rötter RP** (2023) Agronomic and Physiological
354 Traits Response of Three Tropical Sorghum (*Sorghum bicolor* L.) Cultivars to Drought and
355 Salinity. Agronomy **13**: 2788
- 356 **Dijkstra FA, Cheng W** (2008) Increased soil moisture content increases plant N uptake and the
357 abundance of ¹⁵N in plant biomass. Plant Soil **302**: 263–271
- 358 **Fan X, Zhou X, Chen H, Tang M, Xie X** (2021) Cross-Talks Between Macro- and Micronutrient
359 Uptake and Signaling in Plants. Front Plant Sci **12**: 663477
- 360 **Gniazdowska A, Rychter AM** (2000) Nitrate uptake by bean (*Phaseolus vulgaris* L.) roots under
361 phosphate deficiency. Plant Soil **226**: 79–85
- 362 **Hartemink AE, Barrow NJ** (2023) Soil pH - nutrient relationships: the diagram. Plant Soil **486**:
363 209–215
- 364 **Hawkesford M, Horst W, Kichey T, Lambers H, Schjoerring J, Møller IS, White P** (2012)
365 Functions of Macronutrients. Marschner’s Mineral Nutrition of Higher Plants: Third Edition
366 135–189
- 367 **Heldt H-W, Piechulla B** (2011) Nitrate assimilation is essential for the synthesis of organic matter.
368 Plant Biochemistry 273–305
- 369 **Hoopen F Ten, Cuin TA, Pedas P, Hegelund JN, Shabala S, Schjoerring JK, Jahn TP** (2010)
370 Competition between uptake of ammonium and potassium in barley and Arabidopsis roots:
371 molecular mechanisms and physiological consequences. J Exp Bot **61**: 2303–2315
- 372 **Ji C, Li J, Jiang C, Zhang L, Shi L, Xu F, Cai H** (2022) Zinc and nitrogen synergistic act on root-
373 to-shoot translocation and preferential distribution in rice. J Adv Res **35**: 187–198
- 374 **Kumar S, Kumar S, Mohapatra T** (2021) Interaction Between Macro- and Micro-Nutrients in
375 Plants. Front Plant Sci **12**: 665583
- 376 **Lee HW, Bi X, Henry CJ** (2024) Comparative evaluation of minerals content of common green leafy
377 vegetables consumed by the Asian populations in Singapore. Journal of Food Composition and
378 Analysis **125**: 105787
- 379 **Lin WY, Huang TK, Chiou TJ** (2013) NITROGEN LIMITATION ADAPTATION, a Target of
380 MicroRNA827, Mediates Degradation of Plasma Membrane–Localized Phosphate Transporters
381 to Maintain Phosphate Homeostasis in Arabidopsis. Plant Cell **25**: 4061–4074

- 382 **Lincoln Taiz, Eduardo Zeiger, Ian M. Møller AM** (2015) *Plant Physiology* (Fourth Edition).
383 Accumulation and Partitioning of Photosynthates—Starch and Sucrose 230–237
- 384 **Liu F, Stützel H** (2004) Biomass partitioning, specific leaf area, and water use efficiency of vegetable
385 amaranth (*Amaranthus* spp.) in response to drought stress. *Sci Hortic* **102**: 15–27
- 386 **Maillard A, Etienne P, Diquélou S, Trouverie J, Billard V, Yvin JC, Ourry A** (2016) Nutrient
387 deficiencies modify the ionic composition of plant tissues: a focus on cross-talk between
388 molybdenum and other nutrients in *Brassica napus*. *J Exp Bot* **67**: 5631–5641
- 389 **Marschner H** (2011) *Mineral Nutrition of Higher Plants* 3rd Edition eBook ISBN: 9780123849069.
390 eBook ISBN: 9780123849069
- 391 **McLauchlan K** (2007) The Nature and Longevity of Agricultural Impacts on Soil Carbon and
392 Nutrients: A Review. *Ecosystems* 2007 9:8 **9**: 1364–1382
- 393 **Medici A, Marshall-Colon A, Ronzier E, Szponarski W, Wang R, Gojon A, Crawford NM,**
394 **Ruffel S, Coruzzi GM, Krouk G** (2015) AtNIGT1/HRS1 integrates nitrate and phosphate
395 signals at the Arabidopsis root tip. *Nature Communications* 2015 6:1 **6**: 1–11
- 396 **Medici A, Szponarski W, Dangeville P, Safi A, Dissanayake IM, Saenchai C, Emanuel A, Rubio**
397 **V, Lacombe B, Ruffel S, et al** (2019a) Identification of Molecular Integrators Shows that
398 Nitrogen Actively Controls the Phosphate Starvation Response in Plants. *Plant Cell* **31**: 1171–
399 1184
- 400 **Medici A, Szponarski W, Dangeville P, Safi A, Dissanayake IM, Saenchai C, Emanuel A, Rubio**
401 **V, Lacombe B, Ruffel S, et al** (2019b) Identification of Molecular Integrators Shows that
402 Nitrogen Actively Controls the Phosphate Starvation Response in Plants. *Plant Cell* **31**: 1171–
403 1184
- 404 **Mu X, Chen Q, Chen F, Yuan L, Mi G** (2016) Within-leaf nitrogen allocation in adaptation to low
405 nitrogen supply in maize during grain-filling stage. *Front Plant Sci* **7**: 194737
- 406 **Mu X, Chen Y** (2021a) The physiological response of photosynthesis to nitrogen deficiency. *Plant*
407 *Physiology and Biochemistry* **158**: 76–82
- 408 **Mu X, Chen Y** (2021b) The physiological response of photosynthesis to nitrogen deficiency. *Plant*
409 *Physiology and Biochemistry* **158**: 76–82
- 410 **Mu X, Chen Y** (2021c) The physiological response of photosynthesis to nitrogen deficiency. *Plant*
411 *Physiology and Biochemistry* **158**: 76–82
- 412 **Ngosong C, Bongkisheru V, Tanyi CB, Nanganoa LT, Tening AS** (2019) Optimizing Nitrogen
413 Fertilization Regimes for Sustainable Maize (*Zea mays* L.) Production on the Volcanic Soils of
414 Buea Cameroon. *Advances in Agriculture* **2019**: 4681825
- 415 **Nguyen HTT, Van Pham C, Bertin P** (2014) The effect of nitrogen concentration on nitrogen use
416 efficiency and related parameters in cultivated rices (*Oryza sativa* L. subsp. indica and japonica
417 and *O. glaberrima* Steud.) in hydroponics. *Euphytica* **198**: 137–151
- 418 **Niu J, Chen F, Mi G, Li C, Zhang F** (2007) Transpiration, and Nitrogen Uptake and Flow in Two
419 Maize (*Zea mays* L.) Inbred Lines as Affected by Nitrogen Supply. *Ann Bot* **99**: 153–160
- 420 **Plett DC, Ranathunge K, Melino VJ, Kuya N, Uga Y, Kronzucker HJ** (2020) The intersection of
421 nitrogen nutrition and water use in plants: new paths toward improved crop productivity. *J Exp*
422 *Bot* **71**: 4452–4468

- 423 **Ruan J, Jo' J, Gerenda's J, Gerenda's G, Ha'rdter R, Ha'rdter H, Sattelmacher B** (2007) Effect
424 of Nitrogen Form and Root-zone pH on Growth and Nitrogen Uptake of Tea (*Camellia sinensis*
425) Plants. *Ann Bot* **99**: 301–310
- 426 **Saloner A, Bernstein N** (2020) Response of Medical Cannabis (*Cannabis sativa* L.) to Nitrogen
427 Supply Under Long Photoperiod. *Front Plant Sci* **11**: 572293
- 428 **Singh H, Northup BK, Rice CW, Prasad PVV** (2022) Biochar applications influence soil physical
429 and chemical properties, microbial diversity, and crop productivity: a meta-analysis. *Biochar* **4**:
430 1–17
- 431 **Smith FW, Jackson WA** (1987) Nitrogen Enhancement of Phosphate Transport in Roots of *Zea mays*
432 L: II. Kinetic and Inhibitor Studies. *Plant Physiol* **84**: 1319–1324
- 433 **Tripathi R, Tewari R, Singh KP, Keswani C, Minkina T, Srivastava AK, De Corato U,**
434 **Sansinenea E** (2022) Plant mineral nutrition and disease resistance: A significant linkage for
435 sustainable crop protection. *Front Plant Sci* **13**: 883970
- 436 **Uhart SA, Andrade FH** (1995) Nitrogen Deficiency in Maize: II. Carbon-Nitrogen Interaction
437 Effects on Kernel Number and Grain Yield. *Crop Sci* **35**: 1384–1389
- 438 **Wang D, Xu Z, Zhao J, Wang Y, Yu Z** (2011) Excessive nitrogen application decreases grain yield
439 and increases nitrogen loss in a wheat–soil system. *Acta Agric Scand B Soil Plant Sci* **61**: 681–
440 692
- 441 **White PJ** (1996) The permeation of ammonium through a voltage-independent K⁺ channel in the
442 plasma membrane of rye roots. *Journal of Membrane Biology* **152**: 89–99
- 443 **Williams L, Salt DE** (2009) The plant ionome coming into focus. *Curr Opin Plant Biol* **12**: 247
- 444 **Yu CQ, Huang X, Chen H, Godfray HCJ, Wright JS, Hall JW, Gong P, Ni SQ, Qiao SC, Huang**
445 **GR, et al** (2019) Managing nitrogen to restore water quality in China. *Nature* 2019 567:7749
446 **567**: 516–520
- 447 **Zhang S, Zhao Y, Shi R, Waterhouse GIN, Zhang T** (2019) Photocatalytic ammonia synthesis:
448 Recent progress and future. *EnergyChem* **1**: 100013
- 449 **Zhao X, Zhou Y, Min J, Wang S, Shi W, Xing G** (2012) Nitrogen runoff dominates water nitrogen
450 pollution from rice-wheat rotation in the Taihu Lake region of China. *Agric Ecosyst Environ*
451 **156**: 1–11
- 452

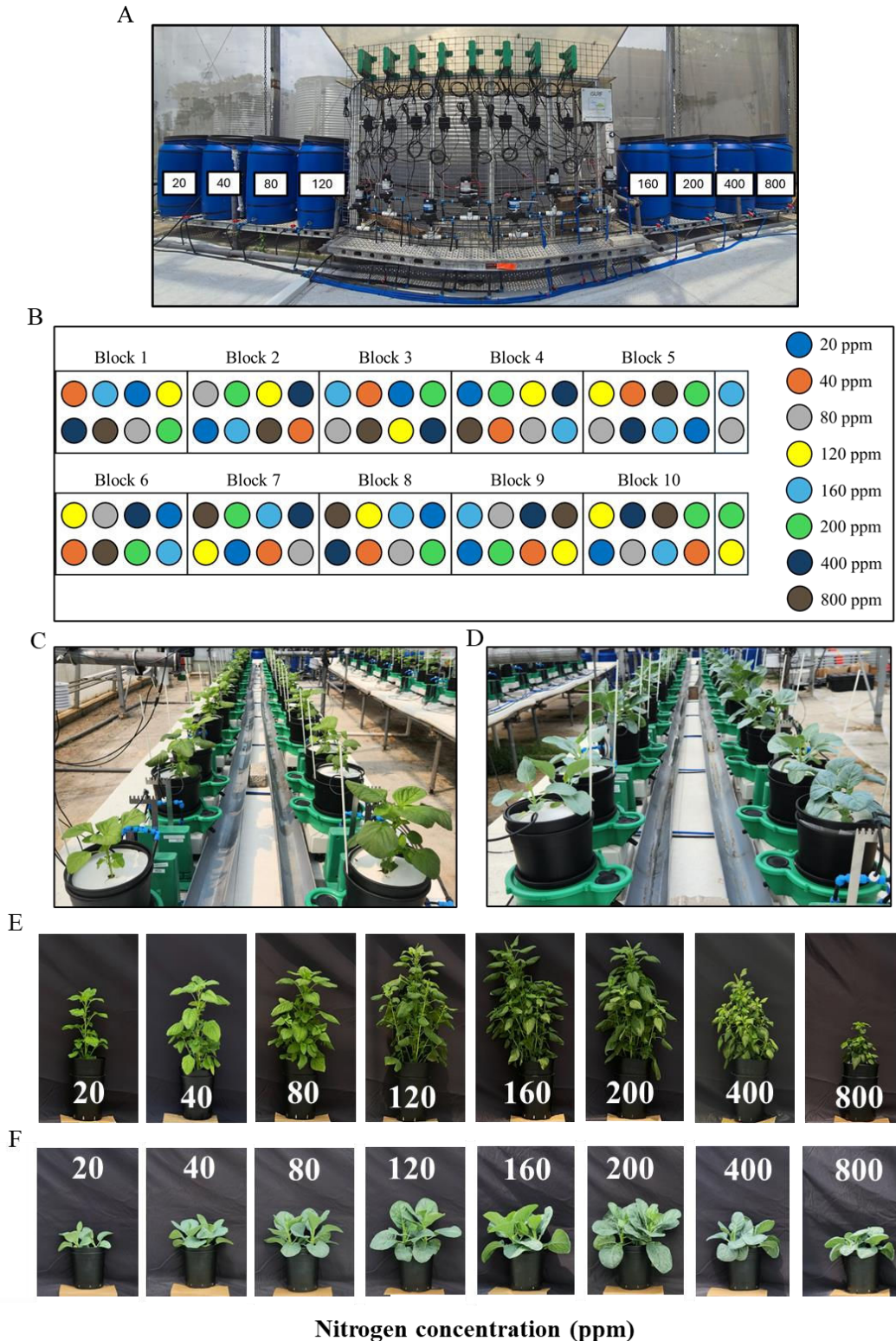


Fig. 1 The experimental platform and harvested plants. (A) Panoramic picture of fertilizer solution tanks and automated pump-controller system. (B) Block randomization experiment design of Chinese spinach experiment. Each experimental block includes one repetition from every treatment (C) Picture in the 17 day of the Chinese Spinach experiment. (D) picture on the 27 day of the Chinese Broccoli experiment. Harvest photos of representative plants from the Chinese Spinach (day 45) and Chinese Broccoli (day 30) experiments (E and F respectively).

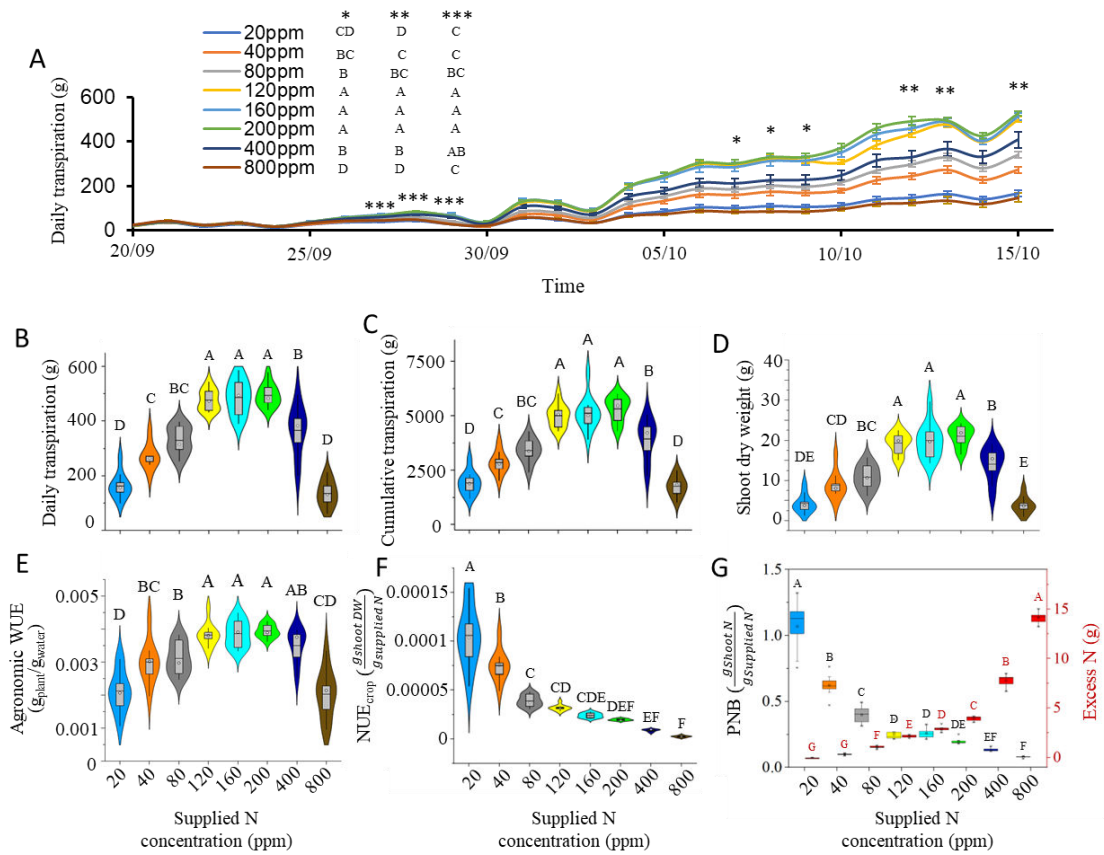


Fig. 2 Physiological response of Chinese Spinach to eight nitrogen treatment. (A) Daily transpiration throughout the experiment, (B) Daily transpiration on the 13.10.24, (C) Cumulative transpiration all over the experiment, (D) Shoot dry weight, (E) Agronomic WUE, (F) Nitrogen use efficiency (NUE_{crop}), and (G) partial nitrogen balance (PNB) and Excess nitrogen during the experiment. Different letters represents significant differences using Tukey honest significant difference test (P-value<0.05, 9≤N≤11).

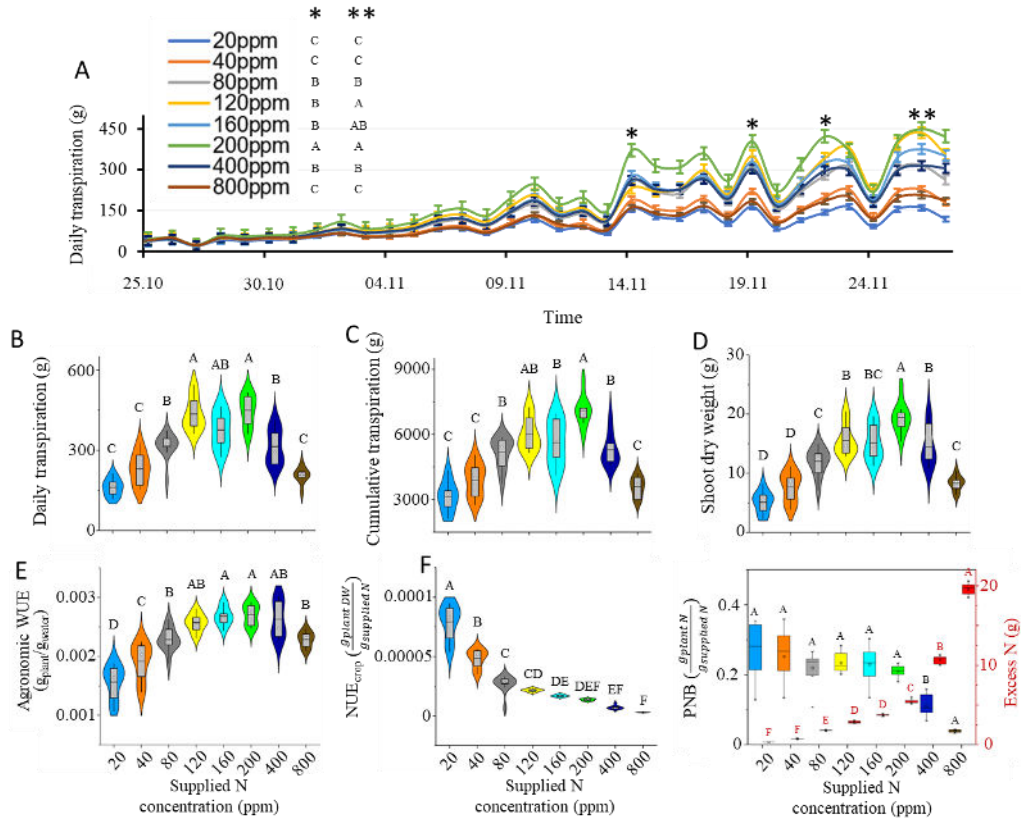


Fig. 3 Physiological response of Chinese Broccoli to eight nitrogen treatment. (A) Daily transpiration throughout the experiment. (B) Daily transpiration on the 27.11.24, (C) Cumulative transpiration all over the experiment, (D) Shoot dry weight, (E) Agronomic WUE, (F) Nitrogen use efficiency (NUE_{crop}), and (G) Partial nitrogen balance (PNB) and Excess nitrogen during the experiment. Different letters represents significant differences using Tukey honest significant difference test ($P\text{-value} < 0.05$, $9 \leq N \leq 11$).

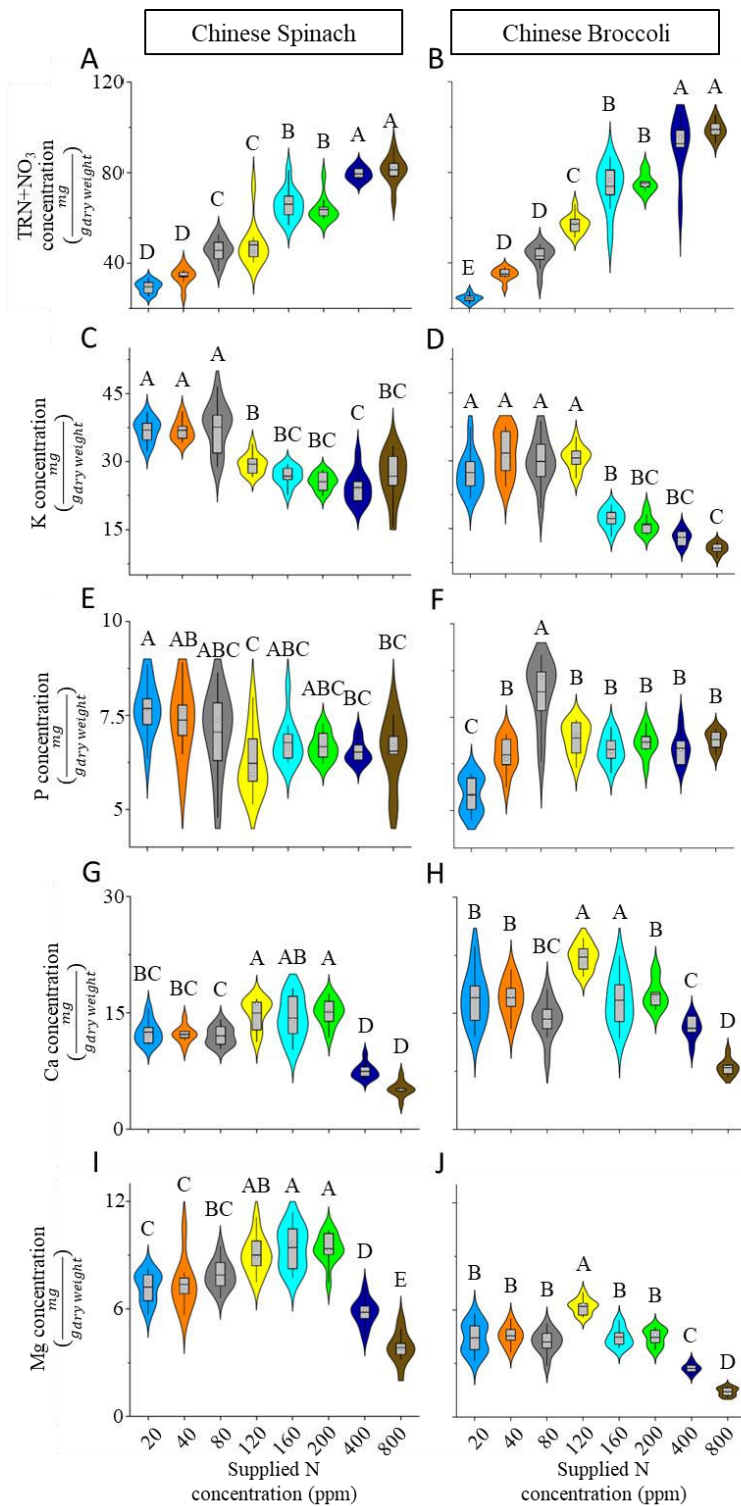


Fig. 4 Macro-nutrient effect of the nitrogen treatments of leaf tissue. The macro-nutrients concentrations: (A and B) Nitrogen, (C and D) Potassium, (E and F) Phosphorus, (G and H) Calcium, and (I and J) Magnesium on harvested leaves of Chinese Spinach and Chinese Broccoli respectively. Different letters represents significant differences using Tukey honest significant difference test (P -value <0.05 , $9 \leq N \leq 11$).

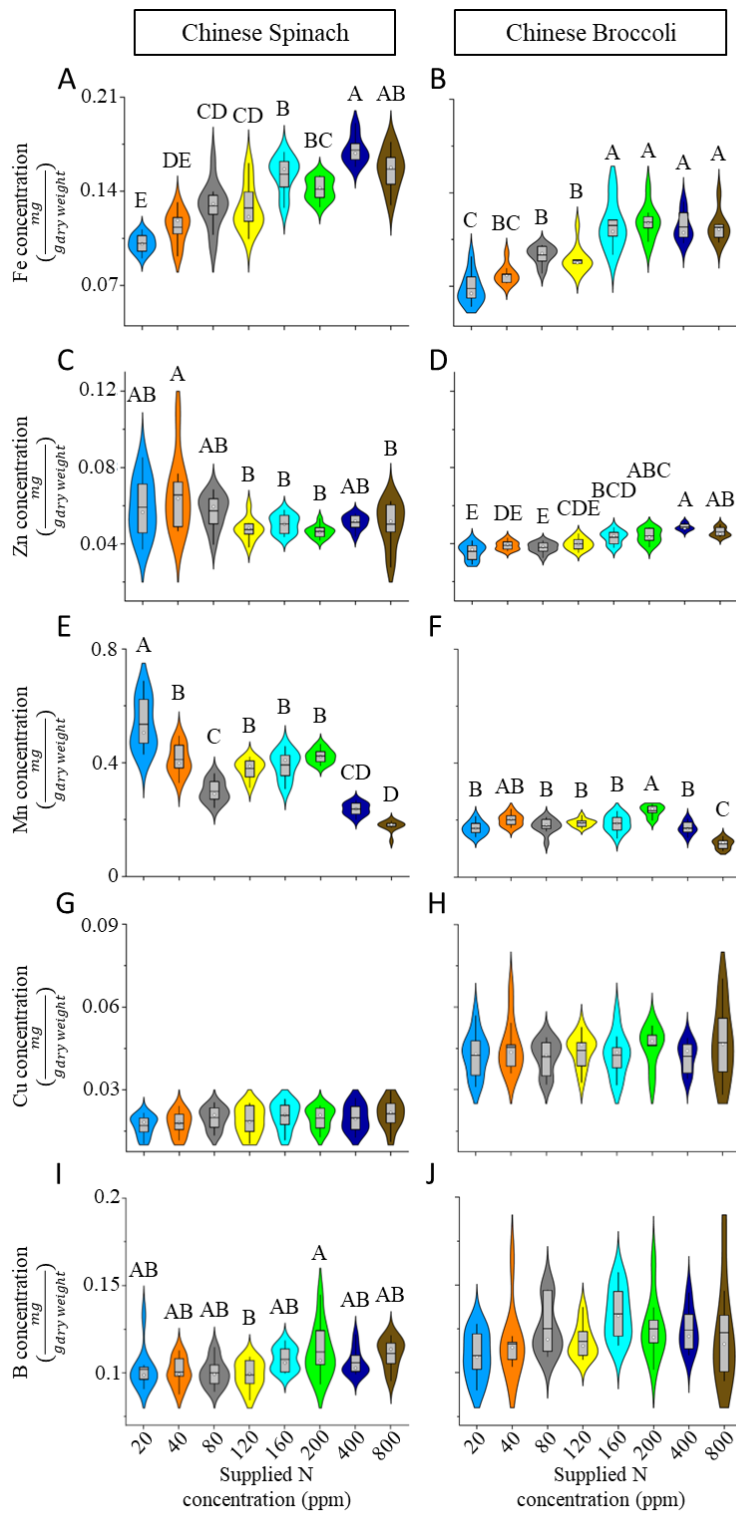


Fig. 5 Micro-nutrient effect of the nitrogen treatments of leaf tissue. The macro-nutrients concentrations: (A and B) Iron, (C and D) Zinc, (E and F) Manganese, (G and H) Copper, and (I and J) Boron on harvested leaves of Chinese Spinach and Chinese Broccoli respectively. Different letters represents significant differences using Tukey honest significant difference test (P -value <0.05 , $9 \leq N \leq 11$).

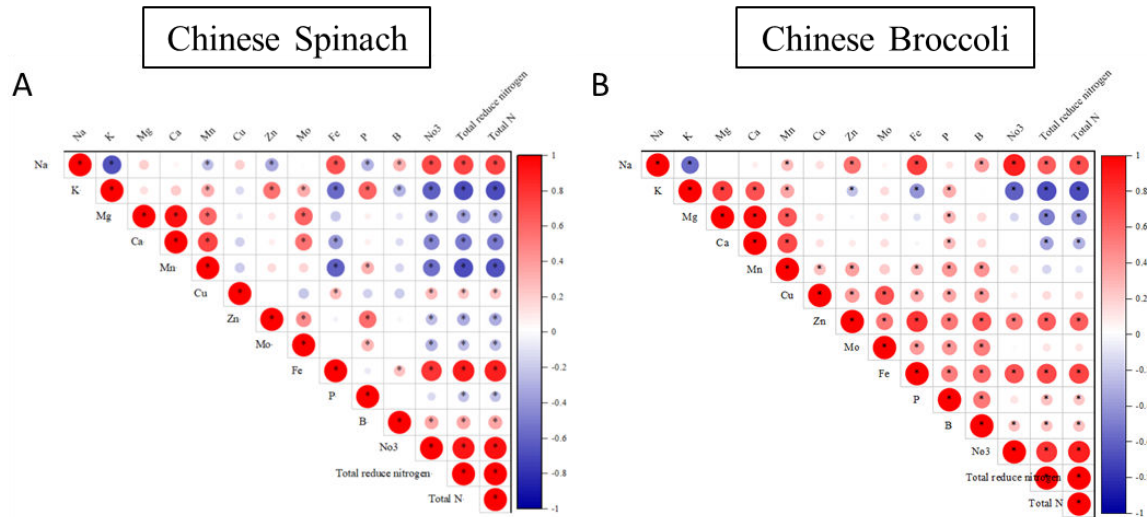


Fig. 6 Correlation matrix of leaves mineral concentration. Pearson linear correlation matrix on harvested leaves mineral concentration on (A) Chinese Spinach and (B) Chinese Broccoli. Colors and circle size represents the Pearson correlation R2 value according to the color bar. Asterisk represent significance difference using Pearson correlation coefficient ($P\text{-value} \leq 0.05$, $9 \leq N \leq 11$).