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## **Differences in ionic responses to nutrient deficiencies among plant species under field conditions**

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## 1 **Abstract**

2 The ionome is defined as the mineral element composition of an organism or tissue and  
3 is genetically and environmentally influenced. Although nutrient deficiency changes the  
4 plant ionome, the differences among different species have not been elucidated fully. In  
5 the present study, we examined the ionic responses to nutrient deficiency in wheat  
6 (*Triticum aestivum* L.), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), and  
7 soybean (*Glycine max* (L.) Merr.) to understand the differences in response to nutrient  
8 deficiency among different plant species. The plants were cultivated in fields with four  
9 fertilizer treatments: complete fertilization, without nitrogen, without phosphorus, and  
10 without potassium. Each plant species was sampled just before the flowering stage, and  
11 mineral concentrations of each organ were analyzed. The species-specific and  
12 nonspecific changes in accumulation by the treatment were observed in different  
13 elements. Under phosphorus deficiency, sunflower showed a different variation in the  
14 ionome profile than the other species, particularly in the increased accumulation of  
15 many metal elements. Increased accumulation of many elements was observed in all  
16 plant species under potassium deficiency. Under nitrogen deficiency, leaf molybdenum  
17 accumulation was increased in non-leguminous plants. An unknown role of  
18 molybdenum in the metabolic responses to the nitrogen deficiency was predicted. To  
19 carry out further research based on the results of such ionomics studies may reveal the  
20 unknown function of essential elements in metabolic responses in plants.

21 **Keywords:** ionomics; maize, soybean, sunflower, wheat

## 22 **Introduction**

23 The ionome is defined as all metals, metalloids, and nonmetals present in an organism  
24 irrespective of their essentiality (Salt 2004; Lahner et al. 2003). Study of ionome, called

25 ionomics, has the advantage in revealing the network among different mineral elements  
26 in an organism. Ionomics can be applied to various types of plant research. One of the  
27 most common ionomic studies is the screening of mutants with different mineral  
28 accumulation properties. Chen et al. (2009) identified 31 mutants which had altered  
29 elemental profiles from approximately 2000 ethyl methanesulfonate-mutagenized M2  
30 population of the model legume *Lotus japonicus* MG20. They suggested that the  
31 elements magnesium (Mg), nickel (Ni), phosphorus (P), and cobalt (Co) form the ion  
32 homeostasis network in *L. japonicus*, as this network has also been seen in yeast (Eide  
33 et al. 2005). Duan et al. (2017) further characterized a high-affinity molybdenum (Mo)  
34 transporter (LjMOT1) in *L. japonicus* using the low Mo-accumulating mutant for this  
35 ionomic screening.

36 There are also many studies that have combined ionomics and genetic analysis. In  
37 particular, the quantitative trait locus (QTL) analysis using the concentration data of  
38 each element obtained by ionomics also has been reported. In staple crops, mineral  
39 composition in grains is of huge interest towards the reduction of human health risks  
40 (e.g., cadmium (Cd), arsenic (As)) and for improving mineral nutritional value (e.g.,  
41 iron (Fe), zinc (Zn)). In rice, for example, studies have been conducted to find out the  
42 QTLs involved in the accumulation of target elements in grains using the concentration  
43 data from comprehensive mineral analysis (Norton et al. 2010; Zhang et al. 2014).  
44 In addition to the ionomic variations among different varieties or mutants of one species  
45 as described above, research also has been conducted on the ionomic variations among  
46 different species and different phylogenetic groups. For example, Watanabe et al.  
47 (2007) determined the concentration of 42 elements in leaves of 670 species and 138  
48 families of terrestrial plants including seed and non-seed plants and reported over 25%  
49 of the total variation in leaf element concentration occurred at the family level or above.

50 By contrast, Neugebauer et al. (2020) hydroponically cultivated 14 native Brazilian  
51 species and characterized their ionomes in roots, stems, and leaves and indicated that  
52 the ionic differences among the 14 species did not reflect their phylogenetic  
53 relationships or successional ecology.

54 Although some inorganic elements, such as potassium (K), function as an inorganic  
55 monomeric ion in the plant tissues, most of essential elements function as components  
56 of high-molecular or low-molecular organic compounds. In particular, micronutrients  
57 often act as active centers of enzymes and components of cofactors in enzymatic  
58 reactions. Thus, the ionome is related closely to the metabolic reactions, and changes in  
59 the ionome due to stress may indirectly reflect the metabolic responses to that stress.

60 Previously, we conducted the ionome analysis in leaves of maize cultivated in a field  
61 with the long-term fertilizer treatments (complete fertilization, fertilization without  
62 nitrogen (N), without P, without K, and no fertilization) to examine plant ionic  
63 responses to nutrient deficiency (Watanabe et al. 2015). As a result, N, P, and K  
64 deficiencies greatly altered the ionome of maize leaf, and the effects differed among  
65 these three deficient treatments. Under K deficiency, for example, the accumulation of  
66 various cationic elements was enhanced, suggesting that these elements might act as  
67 alternatives to K in the process of osmoregulation and the counter-action of organic and  
68 inorganic anions. By contrast, the accumulation of many elements in leaves decreased  
69 as a result of the N deficiency.

70 Meanwhile, it is reasonable to say that there are differences in ionic responses to  
71 nutrient deficiency among plant species since different species have different adaptation  
72 to nutrient deficiency. In the present study, therefore, we investigated and compared the  
73 differences in ionic response to nutrient deficiency among plant species cultivated  
74 under field conditions.

## 75 **Materials and method**

### 76 ***Cultivation***

77 In 2010, wheat (*Triticum aestivum* L. cv. Haruyokoi), maize (*Zea mays* L. cv.  
78 Yumenocorn), sunflower (*Helianthus annuus* L. cv. Summer Sun Rich) and soybean  
79 (*Glycine max* (L.) Merr. cv. Toyoharuka) were cultivated in the long-term fertilizer  
80 experimental field. This field was established in 1914, and five fertilizer treatments  
81 which are complete fertilization (+NPK), without N (-N), without P (-P), without K  
82 (-K), and no fertilization (-NPK), have been applied continuously for 96 years. The  
83 cultivation history of the field has been described elsewhere (Watanabe et al. 2015). N,  
84 P, and K fertilizers were applied as ammonium sulfate (100 kg N), ordinary  
85 superphosphate (100 kg P<sub>2</sub>O<sub>5</sub>), and potassium sulfate (100 kg K<sub>2</sub>O ha<sup>-1</sup>), respectively,  
86 once before sowing. Each plot was 5.25 × 18.5 m in size, and the soil type was  
87 classified as a brown lowland soil (Haplic Fluvisols). The general properties of the field  
88 soils were shown elsewhere (Watanabe et al. 2015). Seeds of each plant species were  
89 sown on May 17, except for wheat (May 10). The row and intra-row spacing was at 50  
90 cm × 40 cm in maize and sunflower, and 50 cm × 20 cm in soybean. Wheat seeds were  
91 sown under direct drilling with hill spacing of 50 cm.

### 92 ***Plant sampling and analysis***

93 Three replicates of wheat, sunflower, soybean, and maize were sampled on the 28<sup>th</sup> day  
94 of June, 5<sup>th</sup>, 13<sup>th</sup>, and 20<sup>th</sup> day of July respectively (just before the flowering stage for  
95 each plant). The three plants for the sunflower, 4 for the soybean, and 2 for the maize  
96 were sampled randomly from each plot. The wheat plant samples were randomly  
97 sampled from the field area measuring 50 cm × 50 cm of each plot. Plant samples were  
98 separated into leaves, stems, and roots, washed with de-ionized water; the mature seeds

99 also were sampled at harvesting stage for each plant species. After determining the fresh  
100 weight of each plant sample, a part of each sample was then reweighed and lyophilized.  
101 Dry weight of each lyophilized sample was determined, and each sample was stored at -  
102 20°C before mineral analysis. Plant samples were ground and digested in 2 ml of 61 %  
103 (w/v) HNO<sub>3</sub> (EL grade; Kanto Chemical, Tokyo, Japan) at a temperature of 110°C in a  
104 DigiPREP apparatus (SCP Science, Canada) for approximately 2 h until the solution  
105 had almost disappeared. When the samples had cooled, 0.5 ml of H<sub>2</sub>O<sub>2</sub> (semiconductor  
106 grade; Santoku Chemical, Tokyo, Japan) was added, and the samples were heated at  
107 110°C for another 20 min. As soon as the process of digestion was complete, the tubes  
108 were cooled and filled to 10 ml with 2 % (w/v) HNO<sub>3</sub> in ultrapure water. The  
109 concentrations of lithium (Li), boron (B), sodium (Na), Mg, aluminum (Al), P, K,  
110 calcium (Ca), chromium (Cr), manganese (Mn), Fe, Co, Ni, copper (Cu), Zn, As,  
111 selenium (Se), strontium (Sr), Mo, Cd, cesium (Cs), and barium (Ba) were determined  
112 using an inductively coupled plasma-mass spectrometry (ICP-MS; ELAN DRC-e,  
113 Perkin Elmer, Waltham, MA, USA) according to the instruction manual provided by the  
114 manufacturer. External calibration standard containing these elements were measured  
115 every 10 samples. Nitrogen concentration contained in the plant samples was  
116 determined by the Kjeldahl method after wet digestion with H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>.

### 117 *Soil sampling and analysis*

118 For soil sampling, three replicates of bulk soils (between the rows of each plant species,  
119 0–25 cm) were collected on the 5<sup>th</sup> day of July. Soils in three randomly selected points  
120 in each replicate were sampled and mixed well. Fresh soil samples were then allowed to  
121 dry for a period of 14 days under room temperature. The dried-soil samples were passed  
122 through a 2-mm sieve for chemical analysis. The total N concentration was determined  
123 by the Kjeldahl method as described above. Water- and 0.1 M HCl-extractable (soil :

124 water or 0.1M HCl = 1 : 2.5, w/v) concentrations of Li, B, Na, Mg, Al, P, K, Ca, Cr,  
125 Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Cs, and Ba in soils were determined by  
126 ICP-MS or ICP atomic emission spectroscopy (ICPS-7000, Shimadzu, Kyoto, Japan).

## 127 **Results**

### 128 ***Growth***

129 The dry weight of each plant at just before flowering stage is shown in Figure 1. The  
130 growth of all plant species was affected negatively by the nutrient deficiency treatments,  
131 but the trend was different depending on the plant species. The gramineous species,  
132 wheat and maize, showed the greatest suppression in growth due to N deficiency. In  
133 sunflower, P deficiency severely limited growth, and N and K deficiencies also limited  
134 growth. By contrast, significant growth suppression in soybean was observed only with  
135 P deficiency.

### 136 ***Mineral accumulation in leaves, roots, and seeds***

137 A comprehensive mineral analysis in leaves and roots at just before flowering stage and  
138 seeds at harvest stage were conducted. Likewise, bulk soil at around flowering stage  
139 was extracted with 0.1 M HCl and water, and mineral concentrations in the extracts  
140 were analyzed. All results are summarized in Tables S1 and S2. Because of the long-  
141 term fertilizer treatment with cultivation of different plant species for 96 years, soils in  
142 different treatments have different available mineral profile (Watanabe et al. 2015).  
143 Therefore, the effect of treatment on mineral accumulation was evaluated by the  
144 concentration in plant corrected by the extractable concentration in soil for each element  
145 (corrected concentration value), which is the ratio of concentration in each plant organ  
146 ( $\text{mg g}^{-1}$  plant) to the extractable (0.1 M HCl- or water-extractable) concentration in soil  
147 ( $\text{mg g}^{-1}$  soil) (Watanabe et al. 2015). Results are shown as a heatmap in Figure 2.



148 Hierarchical clustering was used to arrange the elements based on their relative value of  
149 the corrected concentration value with respect to the +NPK treatment excluding N, P,  
150 and K. In seeds, except for some nonessential elements such as Cd, Co, Cs, and Se, the  
151 effect of nutrient deficiency treatment on element accumulation was negligible. In  
152 contrast, in leaves and roots, -N and -K treatments decreased and increased the  
153 accumulation of many mineral elements, respectively. Under P deficiency conditions,  
154 accumulation of some metal elements such as Cr, Cd, Ni, Co, Al, Cu, and Fe increased  
155 in leaves of sunflower, but these increases were not observed in other species. In  
156 element-specific responses, Cs accumulation tended to decrease by N and P deficiencies  
157 and increased by K deficiency whereas the trend was slightly different depending on the  
158 plant species and plant organ. The behaviour of Ca and Sr accumulations was very  
159 similar, particularly in leaves. The accumulation of Mo in leaves of non-leguminous  
160 species (wheat, maize, sunflower) remarkably increased under N deficiency. In contrast,  
161 Mo accumulation in soybean, a leguminous species, showed decrease in leaves and  
162 increase in roots under N deficiency. The Se accumulation also tended to increase under  
163 N deficiency, but particularly in seeds regardless of plant species.

164 ***Differences in ionic response among different species, among different treatments,***  
165 ***and among different organs***

166 Principal component analysis (PCA) was performed based on the relative corrected  
167 concentration value with respect to the +NPK treatment used in the hierarchical  
168 clustering of elements in Figure 2 to give an overview of the difference in ionic  
169 response among different species, among treatments, and organs. The data of N, P, and  
170 K were not used for PCA. First, in order to see the general tendency, PCA was  
171 performed using all the relative corrected concentration values. The score plot of PCA  
172 showed the rough separation of the PC scores among different treatments except for the

173 leaf and root of sunflower in -P treatment (Figure 3). Next, PCA was performed in each  
174 plant species (Figure 4). The ionome variation in leaves due to the treatment was large  
175 in all plant species. The ionome variation in roots was relatively small in wheat and  
176 maize, but large in sunflower and soybean. Conversely, the ionome variation in seeds  
177 was large in wheat and maize, but small in sunflower and soybean.

178

## 179 **Discussion**

### 180 *Growth responses to nutrient deficiency*

181 Ionomics has potential for various applications in plant science. One of them is to  
182 indirectly capture metabolic responses for various environmental changes. In the present  
183 study, we performed a comparative analysis of four plant species in ionic responses  
184 to N, P, and K deficiencies. Of the four species used in the experiment, sunflower has  
185 the greatest growth suppression by N, P, and K deficiencies, particularly by P  
186 deficiency (Figure 1). Wheat and maize, both belonging to the Poaceae, showed similar  
187 trends in growth response to N, P, and K deficiencies, and their growth limited in the  
188 order of  $-K > -P > -N$ . Soybean, a leguminous species, did not show growth  
189 suppression except for in the -P treatment. In soybean under N deficiency, N  
190 acquisition from rhizobial  $N_2$ -fixation could contribute greatly to its N nutrition. Thus,  
191 the four species used in this study differed in tolerance to N, P, and K deficiencies.  
192 These differences in tolerance to nutrient deficiencies may themselves affect the plant  
193 ionome. The growth suppression can reduce root activity and uniformly decrease the  
194 concentration of various elements in plant (Al-Ithawi, Deibert, and Olson 1980).  
195 Conversely, extreme growth suppression can also increase the elemental concentration  
196 in plant. Osaki, Watanabe, and Tadano (1997) reported that concentrations of Ca and

197 Mg, whose uptake is normally antagonistically decreased by Al application, were rather  
198 high in leaves of barley whose growth was severely suppressed by Al stress in the  
199 medium. They speculated that this contradictory result was caused by the Ca and Mg  
200 enrichment in leaves due to excessive growth suppression. These indirect effects of  
201 nutrient deficiencies should also be considered when discussing the results in this study.

### 202 *Ionic responses to nutrient deficiency in different plant species*

203 The effects of treatments on the accumulation of elements in each organ were evaluated  
204 by heatmap, which illustrated significant increase or decrease compared with +NPK  
205 treatment, using the corrected concentration value (the concentration in leaves relative  
206 to the soluble concentration in soils) for each element (Figure 2). Furthermore, PCA  
207 was performed using the relative corrected concentration values (Figure 3). In the score  
208 plot of PCA for all organs of all plant species, the leaf and root of sunflower in -P  
209 treatment clearly separated from others, indicating that the leaves and roots of sunflower  
210 have large ionome responses due to P deficiency. As shown in the heatmap, sunflower  
211 shows trends for the increase of accumulation in many metal elements such as Cr, Cd,  
212 Ni, Co, Al, Cu, and Fe under -P treatment in the leaf and root, which was not observed  
213 in other species (Figure 2). This result might be considered to be partly due to the poor  
214 growth of sunflower under P deficiency, concentrating these elements in the plant.  
215 However, because the degree of decline in leaf P concentration in the -P treatment  
216 compared to +NPK treatment was the smallest in sunflower (Table S1), it is also  
217 possible that the remarkable growth decline in sunflower under -P treatment was  
218 caused by its low P use efficiency, and the P-acquisition ability was rather superior in  
219 sunflower than to other species. Secreted organic acids and pH decline in the  
220 rhizosphere play major roles in the increase of the availability of insoluble phosphate in  
221 rhizosphere (Neumann and Römheld 2012). Meanwhile, these organic acid secretion

222 and pH decline induced by plant roots can also solubilize many metal elements  
223 (Gobran, Wenzel, and Lombi 2000). Therefore, these possible responses to P deficiency  
224 in sunflower could result in increase of leaf accumulation of these elements. Moreover,  
225 mycorrhizal symbiosis is an important strategy for sunflower to acquire P in P-deficient  
226 soils (Chandrashekara, Patil, and Sreenivasa 1995). It has been reported that  
227 mycorrhizal infection increases absorption of heavy metals such as Cr (Davies et al.  
228 2001), Cd (de Andrade et al. 2008), and Cu (Hassan, Hijri, and St-Arnaud 2013) as well  
229 as P absorption.

230 Under N deficient conditions, the accumulation of many elements in leaves and roots  
231 decreased, while leaf Mo accumulation increased in common for non-leguminous  
232 species (Figure 2). We previously reported in maize that N deficiency increases Mo  
233 accumulation in its leaves (Watanabe et al. 2015). This study suggests that this  
234 relationship between N deficiency and Mo accumulation is common in other non-  
235 leguminous species. Well-known roles of Mo in plants are its involvement in N  
236 metabolism, such as a component of nitrate reductase. So, plants with Mo deficiency  
237 tend to exhibit N deficiency-like symptoms (Broadley et al. 2012). Meanwhile, Mo is  
238 also a component of nitrogenase in N-fixing microorganisms. In the present study, a  
239 significant increase in Mo accumulation in roots of soybean was observed under N  
240 deficient conditions in the field (Figure 2), presumably due to its higher accumulation in  
241 root nodules (Chu et al. 2016).

242 Under K deficient conditions, all plant species showed an increase in accumulation of  
243 many elements, particularly in leaves (Figure 2). K is a cation required in large  
244 quantities for maintaining the osmotic pressure and pH in plant cells (Hawkesford et al.  
245 2012). It has been reported that Na can replace some of the functions of K in K-  
246 deficient plants (Wakeel et al. 2011). Plants under K deficiency may have a mechanism

247 to increase the absorption of other cations as well as Na to complement insufficient K  
248 concentration. The relationship between K deficiency and Cs uptake has also been well  
249 studied. In *Arabidopsis*, K deficiency induces the expression of *AtHAK5*, encoding a  
250 high-affinity K<sup>+</sup> transporter (Gierth, Mäser, and Schroeder 2005), which is localized on  
251 the plasma membrane of root cells. As this transporter can also transport Cs efficiently,  
252 plants accumulate more Cs under K deficiency (Qi et al. 2008).

253 PCA was performed using the relative corrected concentration values on each plant  
254 species to compare the effects of nutrient deficiency on ionomes among different  
255 organs. Ionome variation in plants under different nutrient deficiencies was small in  
256 roots in wheat and maize, and in seeds in sunflower and soybean (Figure 4). The roots  
257 and seeds are sink organs, and phloem transport is considered to be more involved in  
258 their accumulation of mineral elements. Therefore, it is suggested that the effects of  
259 nutrient deficiency on the transport of mineral elements by phloem are different  
260 between graminaceous species (wheat and corn) and dicotyledonous species (sunflower  
261 and soybean). Since the root/shoot ratio of biomass in wheat and maize was lower than  
262 that in sunflower and soybean (Figure 1), wheat and maize may have a high ability to  
263 maintain mineral homeostasis in roots to maintain their root function with low biomass  
264 distribution under nutrient deficient stress. By contrast, the small effect of nutrient  
265 deficiency treatment on the ionome profile of seeds in sunflower and soybean may be  
266 due to their high ability to maintain the seed mineral homeostasis in order to maintain  
267 the initial growth of their next generation. In fact, it was reported that saline stress  
268 induced a greater reduction in number of seeds per plant than seed weight per seed,  
269 while wheat showed the opposite trends (Ghassemi-Golezani et al., 2018; Dikgwatlhe et  
270 al., 2008), suggesting that soybean have a high ability to produce healthy seeds even  
271 under environmental stress.

272 **Conclusion**

273 In conclusion, this study revealed the common and different ionic responses to N, P,  
274 and K deficiencies among different crop species. N, P, and K deficiencies altered  
275 ionome profile in each species considerably, and it could be related closely to their  
276 metabolic responses to these deficiencies. The results obtained in this study may trigger  
277 the unknown function of essential elements in plants. For example, concentration of Mo  
278 in leaves remarkably increased under nitrogen deficiency in wheat, maize, and  
279 sunflower (Figure 2). Although more than 80 years have passed since Mo was noticed  
280 as an essential element (Arnon & Stout, 1939), its function might not be fully elucidated  
281 because Mo is contained in only a very small amount in plants. Based on the results  
282 obtained in this study, it is suggested that unknown function of Mo may be involved in  
283 the adaptation mechanism to N deficiency in the non-leguminous species. Further  
284 research is expected to elucidate new physiological roles of Mo in plants.

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288 **Disclosure statement**

289 No potential conflict of interest was reported by the authors.

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### 375 **Figure captions**

376 **Figure 1.** Dry weights of wheat, maize, sunflower, and soybean grown in a long-term  
377 fertilizer experimental field at just before flowering stage. +NPK, complete fertilization;  
378 -N, fertilization without N; -P, fertilization without P; -K, fertilization without K.  
379 Values are means of three replicates, and bars indicate  $\pm$  standard errors of total dry  
380 weight. Different letters indicate statistically significant difference in total dry weight ( $P$   
381  $< 0.05$ ) using Tukey's multiple comparison test following a one-way ANOVA.

382 **Figure 2.** Heatmap analysis of the corrected concentration value of each element in  
383 leaves, seeds, and roots of each plant species grown under different nutrient deficiency  
384 treatments compared to +NPK treatment (complete fertilization). -N, fertilization  
385 without N; -P, fertilization without P; -K, fertilization without K. The corrected  
386 concentration value, which is the ratio of concentration in each plant organ ( $\text{mg g}^{-1}$   
387 plant) to the extractable (0.1 M HCl- or water-extractable) concentration in soil ( $\text{mg g}^{-1}$   
388 soil), was used to correct for differences in soil element concentrations among  
389 treatments. For element concentration in the soil, 0.1 M HCl-extractable concentrations,  
390 except those of N, P, Cr, Co, Se, and Cs, were used. For soil P, Cr, Co, Se, and Cs  
391 concentrations, the water-extractable concentrations were used. For soil N  
392 concentration, the total N concentration was used. The dendrogram represents  
393 relationships between elements using hierarchical clustering based on the relative value  
394 of the corrected concentration value with respect to the +NPK treatment. N, P, and K

395 were not included in the hierarchical clustering. In each treatment in each plant species,  
396 when a significant difference (Student's t-test,  $P < 0.05$ ) in the corrected concentration  
397 value of each element compared to the +NPK treatment was found, the cell in the figure  
398 was color-coded according to the difference (fold change). X: no data. Blank cell: no  
399 significant difference.

400 **Figure 3.** Principal component analysis (PCA) of the relative correlated concentration  
401 value to the +NPK treatment in all plant species and organs. PCA was conducted on the  
402 data used in the hierarchical clustering of elements in Figure 2, excluding N, P, and K.  
403 Scores on the first two components (PC1 and PC2) was plotted. The corresponding  
404 loading plot was shown in Figure S1.

405 **Figure 4.** Principal component analysis (PCA) of the relative corrected concentration  
406 value to the +NPK treatment in each plant species. PCA was conducted on the data used  
407 in the hierarchical clustering of elements in Figure 2, excluding N, P, and K. Scores on  
408 the first two components (PC1 and PC2) was plotted. The corresponding loading plot  
409 was shown in Figure S2.

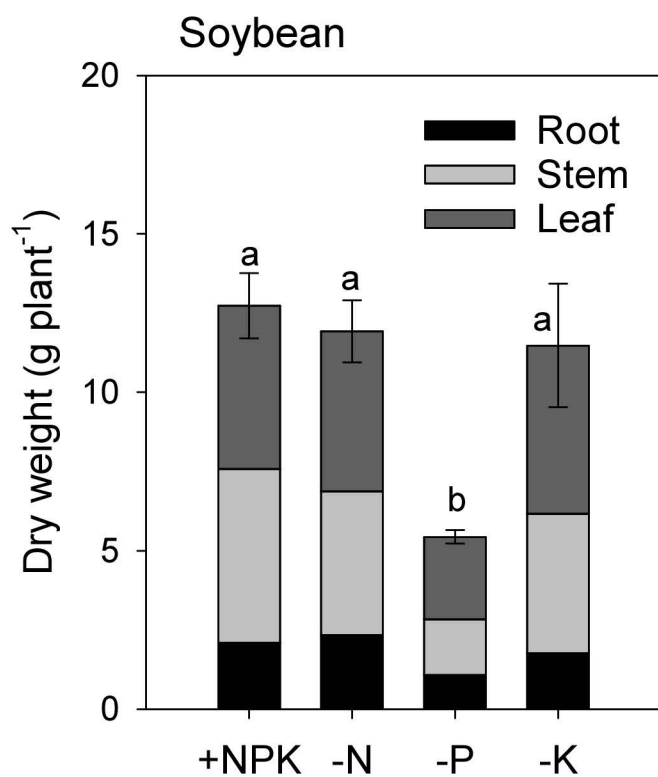
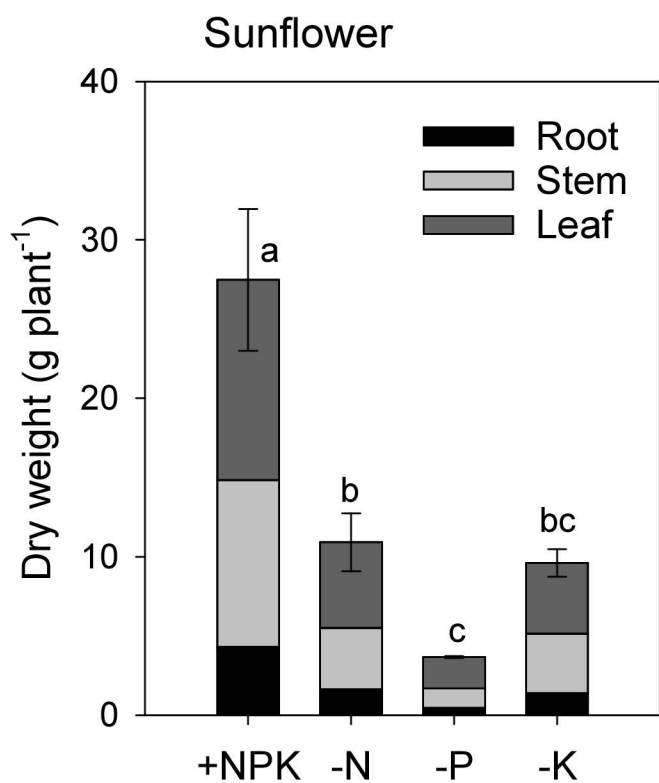
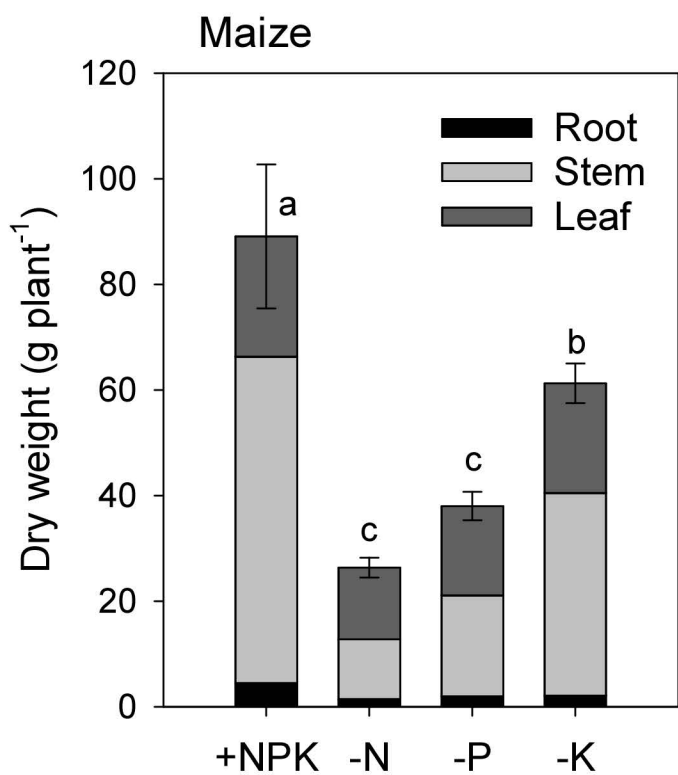
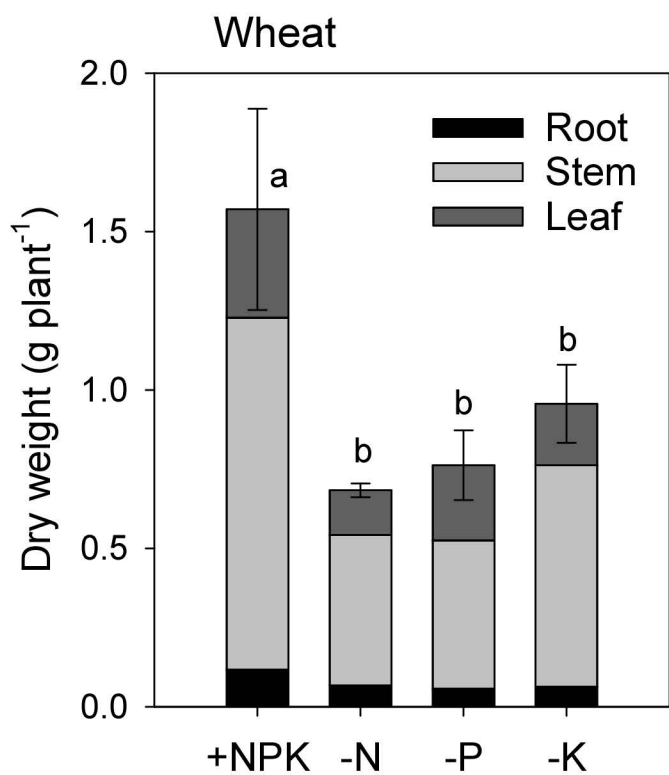


Figure 1.

Fold change compared to the +NPK treatment

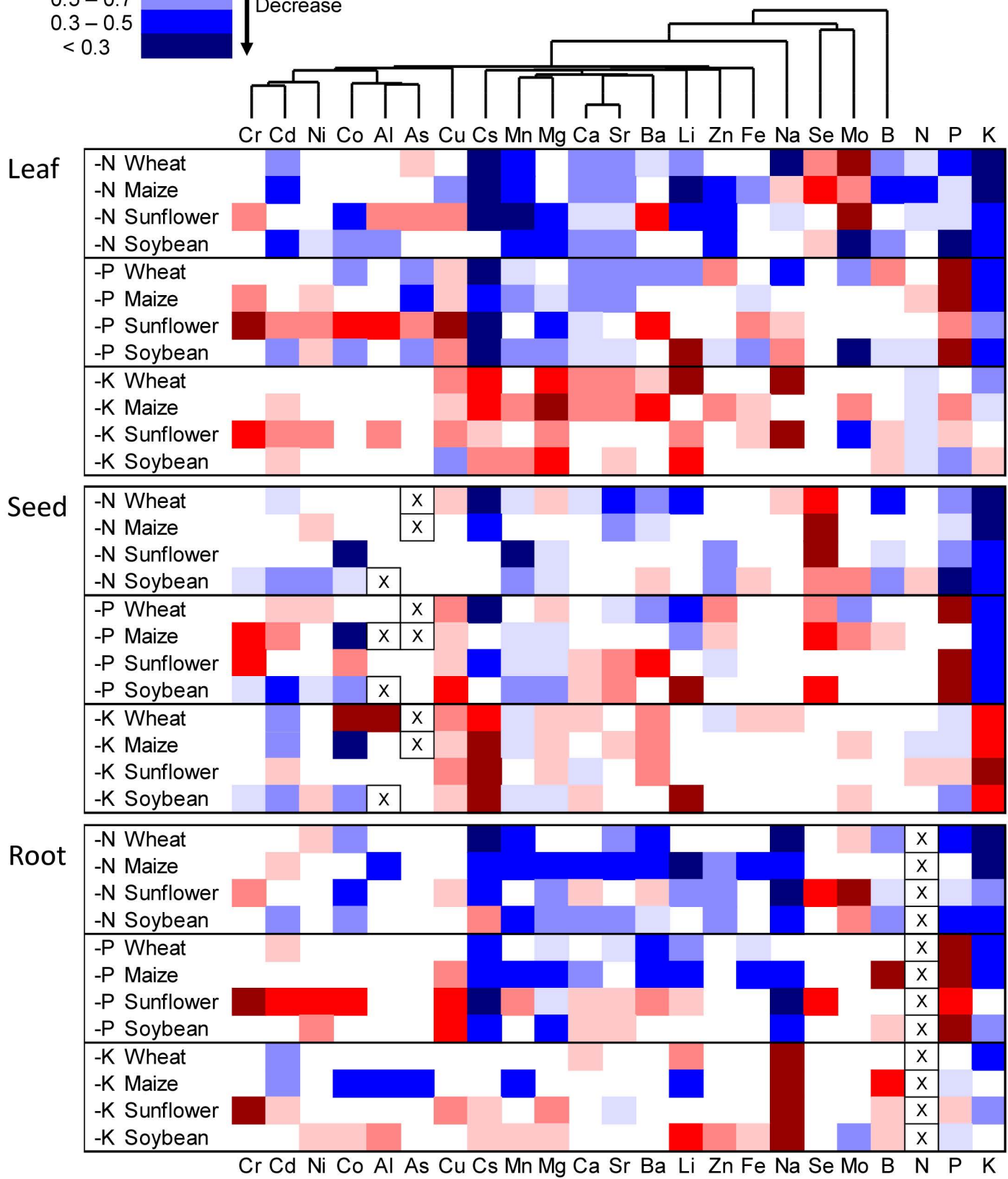
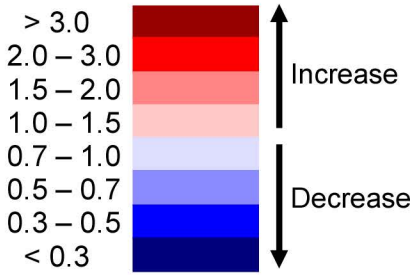
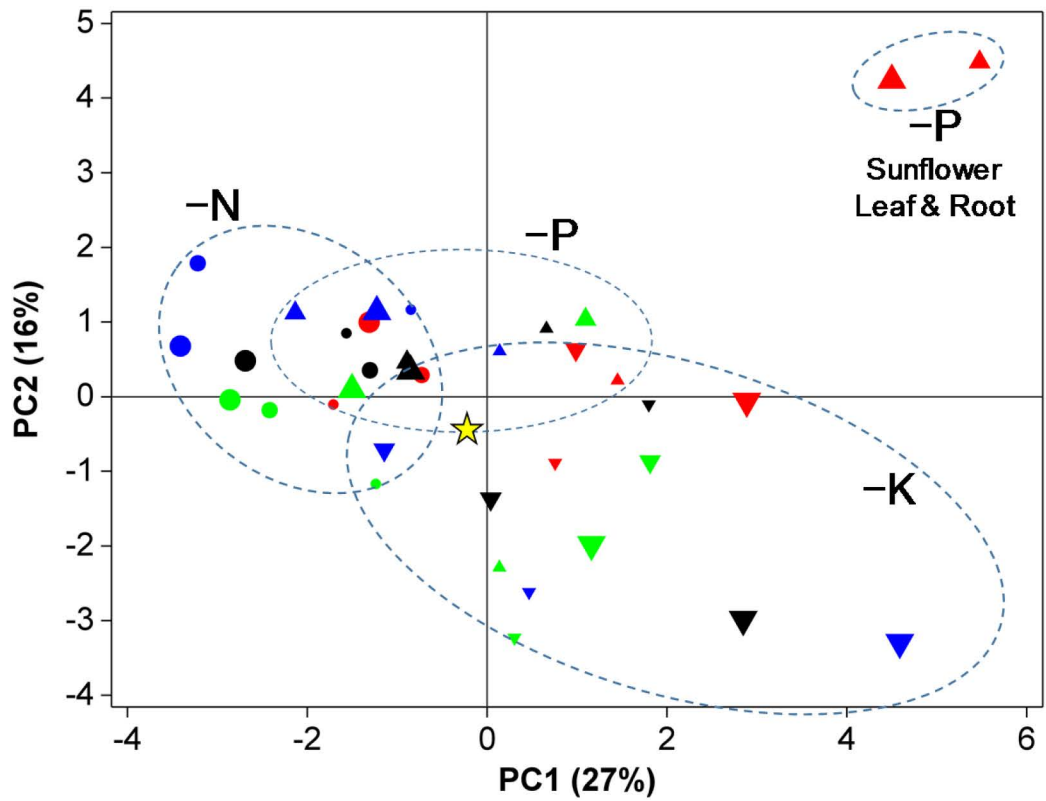


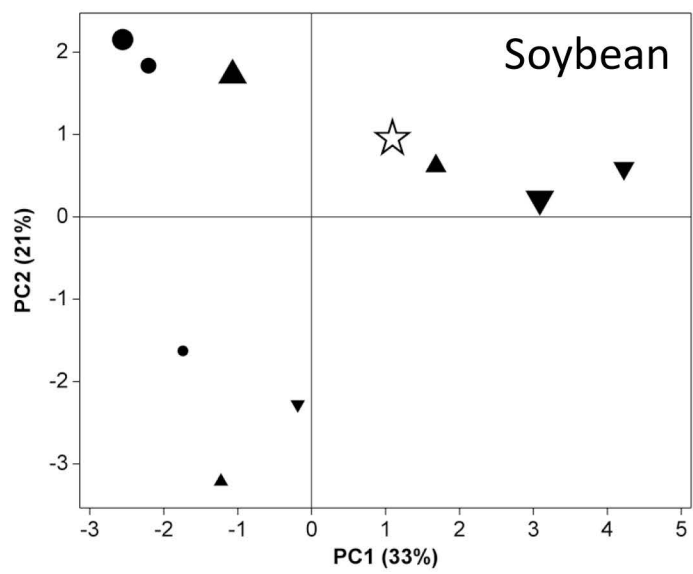
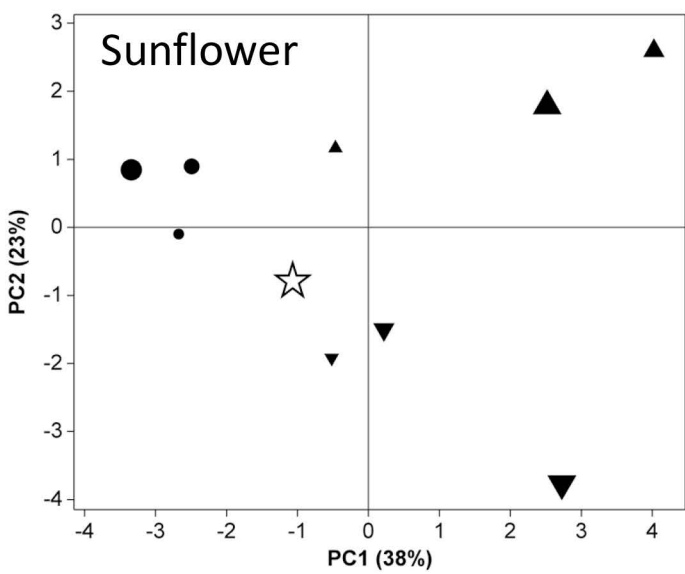
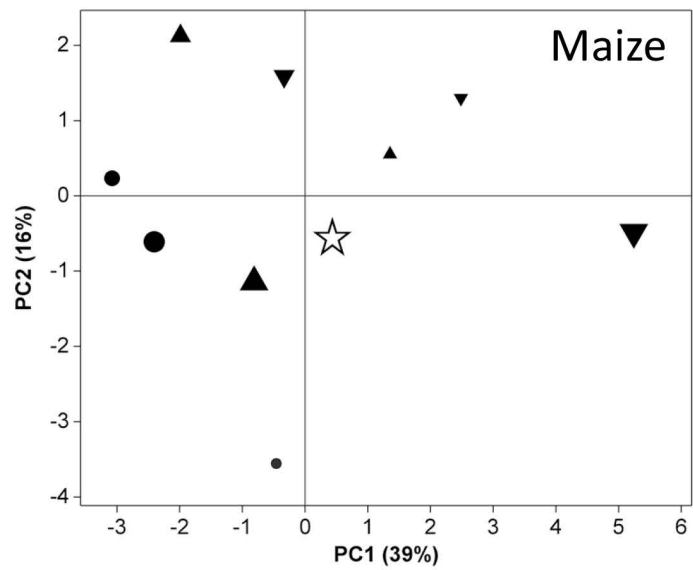
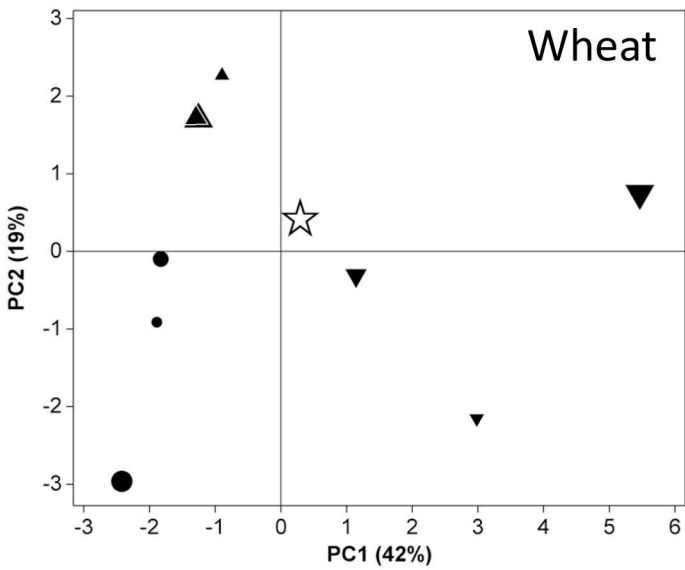
Figure 2.



		Wheat	Maize	Sunflower	Soybean
-N	Leaf	●	●	●	●
	Root	●	●	●	●
	Seed	●	●	●	●
-P	Leaf	▲	▲	▲	▲
	Root	▲	▲	▲	▲
	Seed	▲	▲	▲	▲
-K	Leaf	▼	▼	▼	▼
	Root	▼	▼	▼	▼
	Seed	▼	▼	▼	▼

★ +NPK (relative corrected concentration value in all elements = 1)

Figure 3.



-N	Leaf	●
	Root	●
	Seed	●
-P	Leaf	▲
	Root	▲
	Seed	▲
-K	Leaf	▼
	Root	▼
	Seed	▼

☆ +NPK (relative corrected concentration value in all elements = 1)

Figure 4.