Differences in ionomic responses to nutrient deficiencies among plant species under field conditions

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Abstract

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

Keywords: ionomics; maize, soybean, sunflower, wheat

Introduction

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

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

There are also many studies that have combined ionomics and genetic analysis. In particular, the quantitative trait locus (QTL) analysis using the concentration data of each element obtained by ionomics also has been reported. In staple crops, mineral composition in grains is of huge interest towards the reduction of human health risks (e.g., cadmium (Cd), arsenic (As)) and for improving mineral nutritional value (e.g., iron (Fe), zinc (Zn)). In rice, for example, studies have been conducted to find out the QTLs involved in the accumulation of target elements in grains using the concentration data from comprehensive mineral analysis (Norton et al. 2010; Zhang et al. 2014).

In addition to the ionomic variations among different varieties or mutants of one species as described above, research also has been conducted on the ionomic variations among different species and different phylogenetic groups. For example, Watanabe et al. (2007) determined the concentration of 42 elements in leaves of 670 species and 138 families of terrestrial plants including seed and non-seed plants and reported over 25% of the total variation in leaf element concentration occurred at the family level or above.

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

Although some inorganic elements, such as potassium (K), function as an inorganic monomeric ion in the plant tissues, most of essential elements function as components of high-molecular or low-molecular organic compounds. In particular, micronutrients often act as active centers of enzymes and components of cofactors in enzymatic reactions. Thus, the ionome is related closely to the metabolic reactions, and changes in the ionome due to stress may indirectly reflect the metabolic responses to that stress. Previously, we conducted the ionome analysis in leaves of maize cultivated in a field with the long-term fertilizer treatments (complete fertilization, fertilization without nitrogen (N), without P, without K, and no fertilization) to examine plant ionomic responses to nutrient deficiency (Watanabe et al. 2015). As a result, N, P, and K deficiencies greatly altered the ionome of maize leaf, and the effects differed among these three deficient treatments. Under K deficiency, for example, the accumulation of various cationic elements was enhanced, suggesting that these elements might act as alternatives to K in the process of osmoregulation and the counter-action of organic and inorganic anions. By contrast, the accumulation of many elements in leaves decreased as a result of the N deficiency.

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

Materials and method

Cultivation

In 2010, wheat (*Triticum aestivum* L. cv. Haruyokoi), maize (*Zea mays* L. cv. Yumenocorn), sunflower (*Helianthus annuus* L. cv. Summer Sun Rich) and soybean (*Glycine max* (L.) Merr. cv. Toyoharuka) were cultivated in the long-term fertilizer experimental field. This field was established in 1914, and five fertilizer treatments which are complete fertilization (+NPK), without N (−N), without P (−P), without K (−K), and no fertilization (−NPK), have been applied continuously for 96 years. The cultivation history of the field has been described elsewhere (Watanabe et al. 2015). N, P, and K fertilizers were applied as ammonium sulfate (100 kg N), ordinary superphosphate (100 kg P_2O_5), and potassium sulfate (100 kg K_2O ha^{-1}), respectively, once before sowing. Each plot was 5.25 × 18.5 m in size, and the soil type was classified as a brown lowland soil (Haplic Fluvisols). The general properties of the field soils were shown elsewhere (Watanabe et al. 2015). Seeds of each plant species were sown on May 17, except for wheat (May 10). The row and intra-row spacing was at 50 cm × 40 cm in maize and sunflower, and 50 cm × 20 cm in soybean. Wheat seeds were sown under direct drilling with hill spacing of 50 cm.

Plant sampling and analysis

Three replicates of wheat, sunflower, soybean, and maize were sampled on the 28th day of June, 5th, 13th, and 20th day of July respectively (just before the flowering stage for each plant). The three plants for the sunflower, 4 for the soybean, and 2 for the maize were sampled randomly from each plot. The wheat plant samples were randomly sampled from the field area measuring 50 cm × 50 cm of each plot. Plant samples were 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_3 (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_2O_2 (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_3 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_2SO_4 and H_2O_2.

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 5th 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 :

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

Results

Growth

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

Mineral accumulation in leaves, roots, and seeds

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

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

Differences in ionomic response among different species, among different treatments, and among different organs

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

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

Discussion

Growth responses to nutrient deficiency

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

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

Ionomic responses to nutrient deficiency in different plant species

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

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

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

Under K deficient conditions, all plant species showed an increase in accumulation of many elements, particularly in leaves (Figure 2). K is a cation required in large quantities for maintaining the osmotic pressure and pH in plant cells (Hawkesford et al. 2012). It has been reported that Na can replace some of the functions of K in K-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^+ 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.

Conclusion

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

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Figure captions

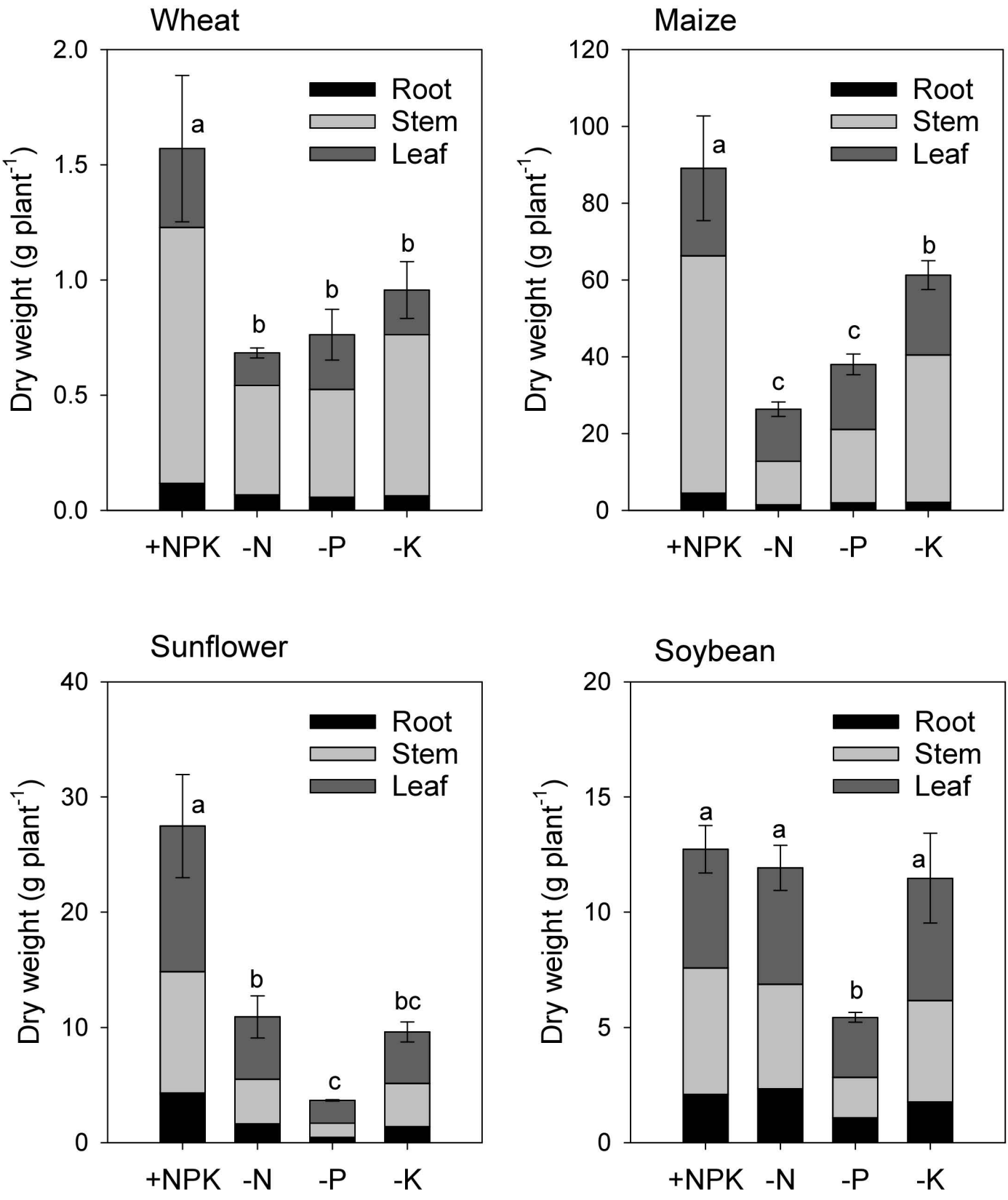
Figure 1. Dry weights of wheat, maize, sunflower, and soybean grown in a long-term fertilizer experimental field at just before flowering stage. +NPK, complete fertilization; −N, fertilization without N; −P, fertilization without P; −K, fertilization without K. Values are means of three replicates, and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

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

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

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

Figure 4. Principal component analysis (PCA) of the relative corrected concentration value to the +NPK treatment in each plant species. PCA was conducted on the data used in the hierarchical clustering of elements in Figure 2, excluding N, P, and K. Scores on the first two components (PC1 and PC2) was plotted. The corresponding loading plot was shown in Figure S2.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Soybean
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Dry weight (g plant-1)
+NPK
-N
-P
-K
a
b
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bc

Figure 1.

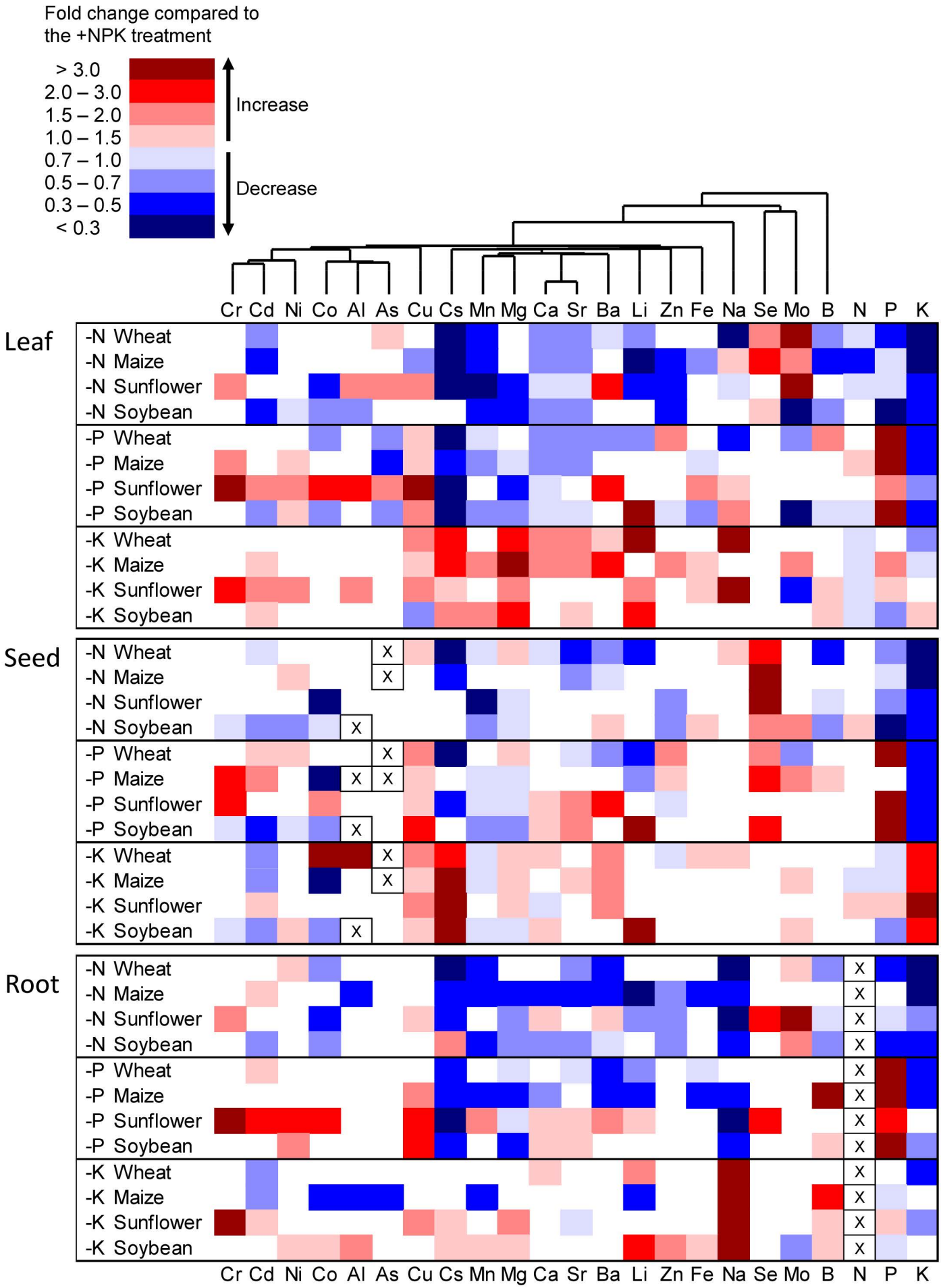
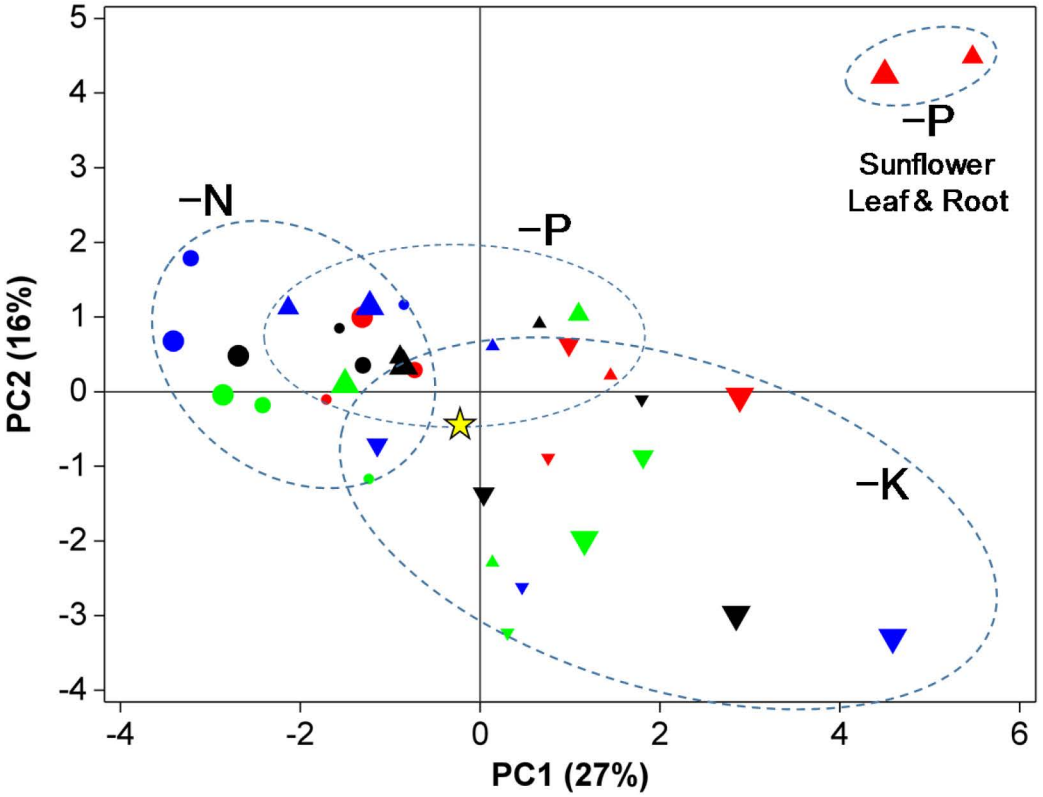

Fold change compared to the +NPK treatment
> 3.0
2.0 – 3.0
1.5 – 2.0
1.0 – 1.5
0.7 – 1.0
0.5 – 0.7
0.3 – 0.5
< 0.3
Increase
Decrease
Cr Cd Ni Co Al As Cu Cs Mn Mg Ca Sr Ba Li Zn Fe Na Se Mo B N P K
Leaf
-N Wheat
-N Maize
-N Sunflower
-N Soybean
-P Wheat
-P Maize
-P Sunflower
-P Soybean
-K Wheat
-K Maize
-K Sunflower
-K Soybean
Seed
Root

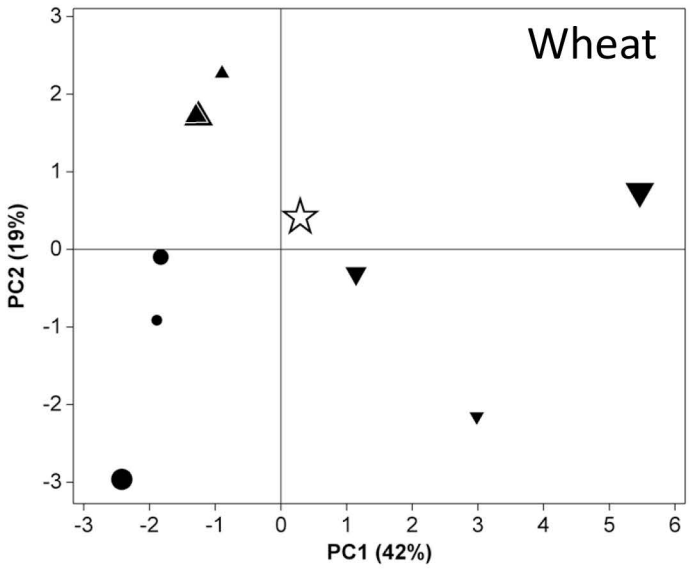
Figure 2.

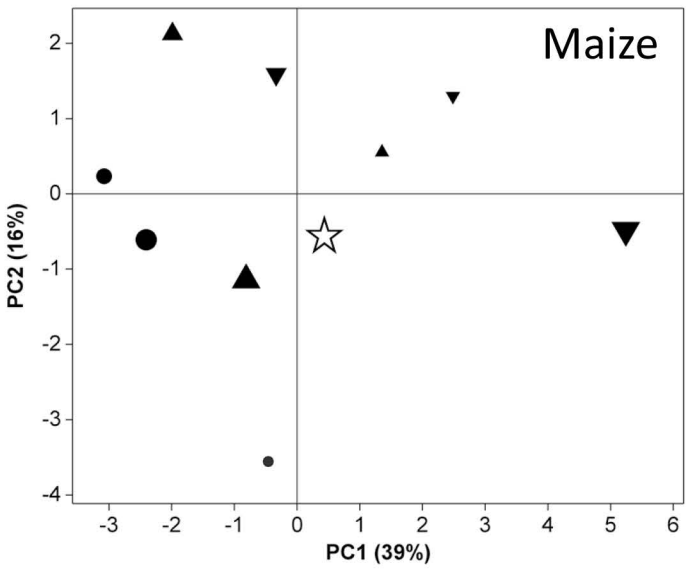


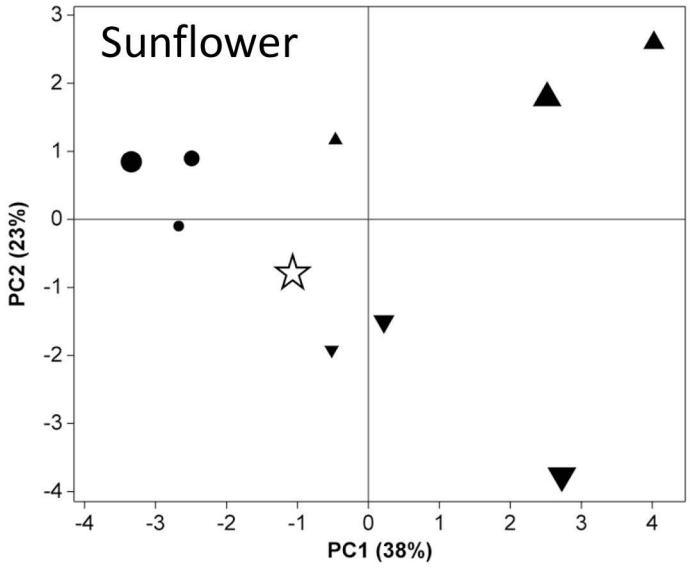
		Wheat	Maize	Sunflower	Soybean
−N	Leaf Root Seed				
−P	Leaf Root Seed				
−K	Leaf Root Seed				

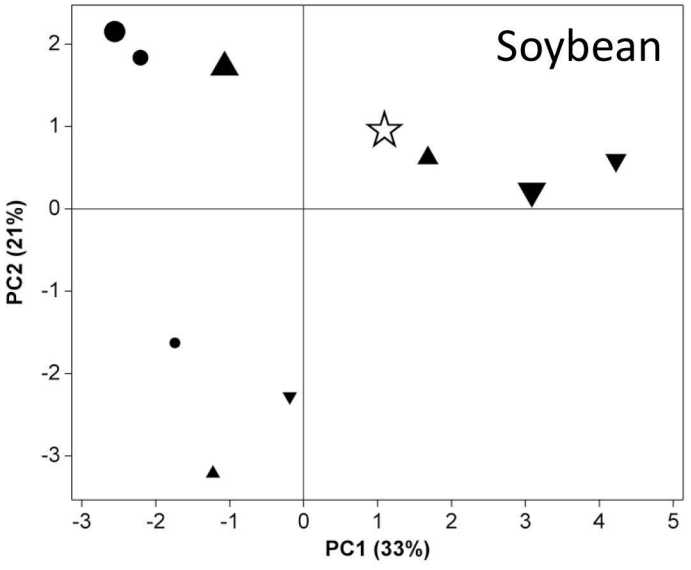
☆ +NPK (relative corrected concentration value in all elements = 1)

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.