Ionomics study of soybean

（ダイズのイオノミクス研究）

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General introduction

Soybean (*Glycine max (L.) Merr*.) is one of the most important crop plants in the world, for its seed protein (about 40%) and oil contents (about 20%) to be feed and food products, and for its capacity to fix atmospheric nitrogen through symbioses with soil-borne microorganisms (Schmutz et al., 2010, Drinkwater et al., 1998). Besides, the elemental composition of soybean is an important component of its overall nutritional value as well (Messina, 1999). Soybeans are rich in iron, zinc, calcium and other mineral nutrients required for human and animal nutrition (Sale and Campbell, 1980). Therefore, cultivation of soybean was spread to many countries from Northeastern China, and the production of soybean continues to expand since the enormous economic value was realized day by day (Bilyeu et al., 2010). The high demand of soybean comes from the increasing use for human consumption, and for animal feed in both developed and developing countries where the animal production are high. Recently, the elemental component, physiological and genetic networks controlling an element uptake, transport and accumulation are of interest to researchers, and the combination study of soybean and mineral elements have been the subject of intense study.

Mineral elements in plant were of interest to people when the “Mineral element theory” was set up by Liebig (1803-1873), among which only N, P, K, Mg, Si, Na and Fe were studied. Followed extensive investigations on the mineral composition of different plant species growing on various soils, gave the criterion for essential elements (Aronon and Stout, 1939). Except essential elements, plants may accumulate nonessential metals such as cadmium (Cd) and lead (Pb), when they are present in the environment (Rautaray et al., 2003). It was reported that soybean absorb Cd more easily than other crops, and soybean cultivars show significant differences in Cd concentrations (Sugiyama et al., 2007, Sugiyama et al., 2010). With the in-deep study on the combination of plant nutrition, plant chemistry and plant physiology, the term metallome and metallomics were proposed (Outten and O'Halloran, 2001, Oldiges et al., 2007). The metallome refer to the entirety of individual metal species in a cell and encompass the inorganic element content and the ensemble of its complexes with biomolecules, and especially with proteins (Szpunar, 2004, Szpunar, 2005). At the same time, the ability to explore the information content of an organism's metabolite profile became truly with the advent of small-molecule profiling technologies such as Gas Chromatography-Mass Spectrometer (GC-MS) (Benkeblia et al., 2007, Jeong et al., 2004, Okazaki et al., 2008). Moreover, the technologies to simultaneously analyze all the significant inorganic components of plants or tissues were developed rapidly: inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), neutron activation analysis (NAA) and etc.(Sucharova and Suchara, 2006, Ohata et al., 2009). The fusion of ideas of metabolomics and the development of high-throughput analysis platforms gave rise to the concepts of the ionome. Ionome is defined as "the mineral nutrient and trace element composition of an organism, representing the inorganic component of cellular and organismal systems" (Salt et al., 2008). Plant ionome is termed as the inorganic component in plant tissues, which is a consequence of complex plant processes and plant-environment interactions, including 17 mineral elements which plant required to complete their life-cycles (Marschner, 1995), and some non-essential elements such as Cd, Al and Cs when these minerals are present in the environment (Baxter et al., 2007). It is a dynamic network of elements that are controlled by the physiology and biochemistry of the plant, and ultimately controlled by the genome, in response to the environment. With the advantages of low cost, high-through output compare to the proteomics and metabolomics (Baxter, 2010), ionomics became an excellent tool for detecting alterations in a plant's physiology or its environment (Baxter, 2009).

Ionomics study is a powerful approach to build connections not only with genome, metabolome, and physiological processes of plant, but also the connections with environment, and ecology. Thus, lots of researches could be benefit from ionomics study as follow:

(1) Identifying genes

Ionomics has been used to identify mutants and natural alleles in model plants grown in controlled environments (Lahner et al., 2003, Chen et al., 2009a, Chen et al., 2009b), which identified mutants with single elements altered, and mutants with multi-element phenotypes. Following studies led to the discovery of genes of ESB1 and TSC10a in *Arabidopsis thaliana*, which have functions to affect suberin deposition in the root endodermis and therefore have a strong effect on the leaf ionome (Chao et al., 2011).

(2) Engineering plants with enhanced nutritional value and phytoremediation

Insights into how plant ionome offers the potential for developing strategies for fortifying the essential mineral nutrient composition or decreasing the harmful non-essential elements of crops (Zhao and McGrath, 2009). Producing nutritious and safe foods sufficiently and sustainably is the ultimate goal of modern agriculture. Deficiencies of the mineral micronutrients Fe, Zn, Se, and I affect more than half of the world population, at the same time, some non-essential elements, such as metalloid As and heavy metal Cd have no demonstrated biological function in plant, and are highly toxic and of major concern with respect to their accumulation in soils, in the food-chain or in drinking water. Therefore, biofortification and phytoremediation were of interests to lots of scientists. Biofortification, which aims to increase micronutrient concentrations in the edible parts of plants through breeding or the use of biotechnology, is a cost-effective way to alleviate micronutrient malnutrition in the developing countries where the problem is most prevalent (Zhao and McGrath, 2009). Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and destroy contaminants in the soil and groundwater. Heavy metals and radioactive elements existed in the environment are the main problems which need urgent solutions. Understanding how plants regulate mineral elements also has the potential of yielding new phytoremediation capabilities. However, the study of biofortification and phytoremediation were limited to single element or several elements, such as Cd in rice (Ueno et al., 2009b, Uraguchi et al., 2009) and Zn in wheat (Tarighi, 2011). It was obviously that there is homeostasis among the elements in plant, and soil is a major source for multiple elements uptake by plant. Plant cell homeostasis for the elements involves strategies for obtaining them from soil, intracellular compartmentalization in to organelles, coordinating transport mechanisms through different plant tissues and the sensing mechanisms providing a link between environmental changes and cellular responses, and there are co-transport and competition between elements undoubtedly (Tejada-Jimenez et al., 2009). Therefore it is important to induce the ionomic study to the biofortification and phytoremediation in the future study.

(3) Broad and Ecologic survey

To date, broad survey of ionome in a large number of plant species was conducted by Watanabe et al. and Han et al.(Watanabe et al., 2007, Han et al., 2011). In the first study, up to 42 elements were detected in 670 species and 138 families of terrestrial plants, and the results showed different species and families may have different elemental uptake mechanisms. The latter investigated the biogeography pattern and variability of 11 elements in 1900 plant species, and gave the raise to the "Stability of Limiting Elements Hypothesis", which is defined that elements with high physiological requirements, high average concentrations and most frequently limiting in nature would be more stable and less sensitive to environmental gradients.

(4) Environmental control

Inomics lies in its ability to precisely capture information about the functional state of an organism under different conditions. These conditions may either be driven by genetic differences, developmental differences, as well as the environmental effects including biotic and abiotic factors. Except carbon and oxygen, most of the other elemental composition of plant is obtained from soil (Baxter and Dilkes, 2012). Any alterations related to soil will finally lead to the variation in plant ionome. Most of the initial ionomic studies ignored the influence of environmental parameters until Baxter found that either P deficient conditions produced significantly impact on the concentrations of Mn, Co, Zn, Mo and Cd, whilst P deficient may affect the concentrations of B, Zn, As, Cu and Co (Baxter et al., 2008). Later, it was found that ionome of *Lotus japonicas* altered under different root-zone temperature (Quadir et al., 2011), and fertilizer application has significant on the shoot ionomes of angiosperm (White et al., 2012).

Extending the ionomics study from model plant and controlled growth systems to field-grown crop plants is important for producing nutritious and safe foods, understanding the ionome of plant and environmental control, as well as the sustainable modern agriculture. Although hydrology and topology of the field soil have large effects on the elements that are available for uptake, plants with altered elemental profiles in a field condition have the potential to shed light on soil and plant interactions which are not visible in controlled growth environments. In this thesis, three independent field experiments were carried to reveal the environmental control of ionome in soybean.

Firstly, we investigate the impacts of fertilizer applications on the concentration of 21 mineral elements including essential elements N, P, K, Mg, Ca, Mn, Fe, Cu, Zn, Ni, Co, B, Se and non-essential elements Sr, Ba, Na, Li, Cr, V, Cd, and Cs of soybean shoot with 44 varieties. In the second experiment, the distribution of mineral elements in soybean tissues during successive growth stages were detected by growing soybean (cv. Tsurumusume) in the field which previous cropped with arbuscular mycorrhizal (AM) host crop with different P application levels. Finally, the ionome of soybean seed affected by previous cropping with mycorrhizal plant and manure application were studied.

Accordingly, this study offers detailed information of the elemental distribution and interactions in soybean, reveals genotypic and environmental control of the ionome of soybean, and the study of ionome of soybean seeds has the potential to improve yield, quality and our knowledge of soybean-soil interactions. The study develops the understanding of the correlations among the ionomic study, agricultural practice, and food safety.

CHAPTER 1

Profiling the ionome of soybean shoots: A study of the effects of fertilizer application on different genotypes

1.1 Introduction

Many studies indicate that the concentrations of mineral elements in shoot differ among plant species and plant genotypes because of their own physiological factors and affected by widely environment factors such as temperature, soil properties, and fertilization (White et al., 2012, Watanabe et al., 2007, Chen et al., 2009a, Quadir et al., 2011). Vice versa, plant ionome could be used as a multivariable system to detect a plant's physiological status and reflect plant adaptions to the environment (Baxter et al., 2008, Baxter and Dilkes, 2012). To date, ionomics approach has been applied extensively for forward and reverse genetics, and screening diversity panels in plant, including model plant *Arabidopsis thaliana* (Baxter et al., 2008, Lahner et al., 2003), *Lotus japonicas* (Chen et al., 2009b) and food crops rice (Norton et al., 2009), soybean (Sha et al., 2012), and maize (Menkir, 2008).

Fertilization is fundamental agronomic measures used to improve growth and yield in soybean cultivation (Mandal et al., 2009), it is also an important environmental factor which may affect the ionome of soybean plant by altering the elemental resources and soil properties. Chemical fertilizers, rich in the three major nutrients needed for plant growth, are widely used in agriculture since the “Green revolution”, whereas it caused soil acidification after long term use (Guo et al., 2010). Manure can supply soil with diverse mineral elements directly, and improve mineral availability indirectly by changing the pH and biological activity in soil, but it also provides some stable humic substances which account for the fixation of some minerals (Amiri and Fallahi, 2011). Many studies have demonstrated that unexpected mineral elements availability were affected by the application of fertilizers: Cd and Zn by P fertilizer (Bogdanovic et al., 1999), Cs and Cd by K fertilizer (Zhu and Smolders, 2000, Rautaray et al., 2003) and Zn and Fe by manure (Mekki and G.Ahmed, 2005). Knowledge of the genetic variation in the concentrations of mineral elements in shoots, and the plant-fertilizer interactions can be used both in agriculture to choose the best genotype plant adapt to the environment and optimize the application of fertilizer to crops, as well as the food quality for human consumption and feed for animals. Till now, little studies have done about the entire network of elements in soybean influenced by genotype × environment (fertilizer application) in the field conditions. In this experiment, 21 mineral elements contents were examined in 44 soybean lines with 3 different fertilizer treatments to compare the effect of genotype × fertilizer on the ionome characteristics of the soybean shoot.

1.2 Materials and methods

Experimental field

Field experiment was conducted to investigate the mineral characteristics in 44 soybean lines with 3 fertilizer treatments on a volcanic ash soil, which is classified as a Melanudands under the Classification of U.S. Soil Taxonomy, at the National Agriculture and Food Research Organization, Hokkaido Agricultural Research Center, northern Japan (42°53’ N, 143°03’E) in 2009. The mean air temperature and precipitation during the study period (May to October) were 15.5℃ and 71.9 mm $month^{-1}$, respectively. The chemical properties of manure, forty four lines used in present study, and chemical properties of tested soil in 0-20cm soil profile in plots before planting are shown in tables 1.1, 1.2 and 1.3, respectively. Experiment includes three fertilizer treatments, manure, chemical fertilizer and only potassium fertilizer, and forty four soybean lines provided by Hokkaido Research Organization, Agriculture Research Department, Tokachi Agricultural Experiment Station, and NARO Institute of crop science. For soybean cultivation, cattle farmyard manure in the M treatment was applied into the plot at the rates of 20 t/ha according to the equivalent amount for N (20kg N/ha) in April, which was 1 month before soybean planting. C treatment received N (20 kg/ha), P_2O_5 (80 kg/ha), and K_2O (80 kg/ha) in forms of ammonium sulfate, superphosphate, and potassium sulfate respectively. O treatment received only 80 kg K_2O/ha in the forms of potassium sulfate. All of the chemical fertilizers were applied into the field in late May when the soybeans were sown. Then the fields were covered with a nonwoven fabric sheet (Paopao 90, MKVDREAM, Tokyo, Japan) for 3 weeks to retain heat temperature and avoid damage by wildlife. Soybean plants were harvested for determination of mineral elements after 2 months growth. The plot area of each treatment was $10.5\times16.8m^2$.

Analysis of sample

Sixty days after sowing, soybean shoots were sampled, dried in the oven at 80℃ for 72 hours, and grounded. About 50 mg dried samples were digested with 2 ml of 61% HNO_3 (EL grade; Kanto Chemical, Tokyo, Japan) at 110 ℃ in a DigiPREP apparatus for 2h, then 0.5ml of H_2O_2 (semiconductor grade, Santoku Chemical, Tokyo) was added and heated for another 30 min until the organic matter was decomposed completely and the solution became clear. The tubes were cooled to room temperature and diluted to 15ml with 2% HNO_3. Nineteen elements K, Ca, Mg, Mn, Fe, Zn, Cu, Na, Sr, Co, Se, Ni, Cr, Cd, B, V, Ba, Li, and Cs were simultaneously analyzed using ICP-MS (ELAN; Perkin Elmer, Waltham, MA, USA). The N and P concentration were determined by using auto analyzer (QuAAtro 2-HR, BLTEQ, Tokyo, Japan).

The total mineral elements in soil were analyzed according to the same method as was used for plants, but the solution was filtered after dilution to omit impurities that would damage the machine. To determine the available minerals in the soil, 2 g of air-dried soil was extracted by 40 ml of 1 M ammonium acetate, and 5 ml of filtered extract was concentrated in the DigiPREP apparatus until it almost disappeared.

Subsequently, 2 ml of 61% HNO_3 was added and the extract was digested again using the same procedure as was used for the plant tissue. Finally the tube was filled to 10 ml with 2% HNO_3 for ICP-MS analysis.

Statistics

To visualize the differences among the 21 mineral elements in 44 soybean shoots with 3 treatments, principal component analysis (PCA) was employed using Minitab 15 (Minitab, State College, PA, USA). Estimates of variation in concentrations of elements in shoots were assigned to genotype, fertilizer treatment and genotypes-treatment interactions using residual maximum likelihood (REML) procedures. Difference of each element between chemical fertilizer treatment and manure treatment in each soybean line was revealed by the student's t-test. Spearman rank correlation analysis was performed to investigate the similarity of the shoot concentration of certain mineral elements response to fertilizer treatments. REML, correlation analysis and student's t-test were conducted using SAS 9.1.

1.3 Results

Statistic and variation of mineral element contents in soybean shoots with different genotypes and fertilizer treatments

In order to get an impression of the 21 element concentrations, 44 lines of soybean were analyzed. The mean concentrations of the elements in soybean shoots vary greatly – from 27.9 mg g^{-1} for N to 0.048 µg g^{-1} for Cd , in the order N > K > Ca > Mg > P > Fe > Na > Sr > Mn > B > Ba > Zn > Cr > Ni > Cu > V > Li > Co > Se > Cs > Cd (Fig. 1.1 and Table 1.4), with ratios of N : K : Ca : Mg : P : Fe : Na : Sr : Mn : B : Ba : Zn : Cr : Ni : Cu : V : Li : Co : Se : Cs : Cd = 100 : 72.1 : 40.7 : 14.2 : 6.8 : 1.6 : 0.4 : 0.3 : 0.21 : 0.1 : 0.07 : 0.06 : 0.04 : 0.03 : 0.02 : 0.004 : 0.002 : 0.001 : 0.0004 : 0.0002 : 0.0001. Remarkable differences in mineral element contents were found in soybean shoots studied (Table 1.4). The contents of macro essential elements N, P, K, Mn, Mg, and Ca varied less than or equal to 20, with CV in shoots of 11, 20, 17, 20, 13, and 14 respectively. For the minor essential elements, Fe, Co, Cu, Zn and B contents showed larger relative differences and Fe had a largest CV with a ratio of the maximum/minimum content with 10 fold (Table 1.4). For non-essential elements, the rank of CV is Ba (74), V (69), Cr (67), Cs (61), Cd (60), Li (56), Ni (52), Se (49), Na (34) and Sr (29). The differences in mineral element contents might be due to different genetic resources and fertilizer application involved.

In order to quantify the sources of variation affecting each mineral element, a residual estimated maximum likelihood (REML) analysis, with fertilizer treatments and genotypes as random effects, was performed (Table 1.5). Most of the variance in shoot N, P, K, B, Sr, Ba and Cd can be attributed to a combination of genotype and treatment effects, with relatively little residual variation. Most of the variance in shoot P, K, B, Sr and Ba can be attributed to fertilizer treatments, whereas most of the variance in shoot N and Cd can be attributed to characteristic differences between soybean genotypes. By contrast, a large proportion of the variation in shoot concentrations of other elements attributed to residual variation, which includes genotype × fertilizer interactions. For several elements, such as N, Mg, Ca, and Cd, the variances attributed to characteristic differences between genotypes are higher than that attributed to fertilizer treatments, whereas the variance attributed to fertilizer treatments is greater than that attributed to differences between genotypes for the other elements.

Effect of fertilizer application on the mineral concentration in soybean shoots

Twenty one mineral elements detected in the shoots of 44 soybean lines with three fertilizer treatments were subjected to multivariate analysis. Mineral profiles in soybean shoots were strongly influenced by the different fertilizer applications, which were revealed by the plots of the principle component score: PC1 accounted for 37.2% of the total variances and clearly discriminated between the manure and chemical fertilizer treatments (Fig. 1.2a). The contributions of each compound were characterized in terms of response indexes representing the values of the PC1 and

PC2 compound loading scores (Fig. 1.2a). Co, Fe, Ni, V, Cr and Cs (Fig. 1.2a, 1.2b) showed higher negative values of PC1 loadings, indicating that the variation in their concentrations was strongly depend on chemical fertilizer application, whereas P, K, Zn, Sr, Ba and Se in soybean shoots were increased by manure application (Fig. 1.2b).

In order to further investigate the elemental profiles response to manure, K applications and chemical fertilizer treatments in certain soybean line, student's t-test was employed to compare the difference of each element in each line between the two treatments (Fig. 1.3, table 1.6), and data was summarized in Fig. 1.4. 16%, 68%, 57%, 36%, 20%, 32%, 77%, 39% and 68% of 44 soybean lines have significantly higher N, P, K, Mn, Ni, Zn, Sr, Ba and Cd concentrations in their shoots in manure treatment compared with chemical fertilizer treatments, whereas 5%, 18%, 22%, 14% and 43% of 44 lines have significantly lower concentrations of Fe, Cr, V, Co and Cs in manure treatments. While when compared the K treatment and chemical fertilizer treatment, it was found that 18%, 16%, 23%, 20%, 11%, 59%, 25%, 50% and 65% of 44 soybean lines have significantly higher N, P, K, Ca, Mn, Sr, Ba Cd and B concentrations in their shoots in K treatment compared with chemical fertilizer treatments, whereas 10% and 18% of 44 lines have significantly lower concentrations of Mg and Na in the only K treatment.

Effect of soybean genotypes on the mineral elements of shoots and identification of accumulators

Spearman rank correlation analysis was performed in order to investigate the similarity of the shoot concentration of certain mineral elements response to fertilizer treatments in all soybean lines (Table 1.7). Pairwise Spearman rank correlations of soybean genotypes between fertilizer treatments were significant for the shoot concentrations of N, P, K, Mg, Ca, Mn, B, Na, Sr, Cd, indicating that the shoot concentration of these elements behaved similarly in response to fertilizer treatments imposed, and implying that the relative concentration of these elements in the shoots of soybeans growing in the same environment is determined, to an extent, by the genotypes to which they belong. Fewer pairwise Spearman Rank Correlations were significant for shoot concentrations of Fe, Cu, Zn, Co, Ba, Ni, V, Cr, Li, Se and Cs, suggesting significant genotype × fertilizer interactions for these elements in some circumstances, which means the concentrations of elements in the latter group in soybeans shoots were affected by interaction effect of genotype and fertilizer.

The profiling of N, P, K, Mg, Ca, Mn, Fe, Ni, Cu, Zn, Na, Sr, Ba, Cr, V, Cd, Co, Se, Li, B and Cs in 44 soybean lines with 3 treatments was described by z-score for the identification of certain element accumulator from 44 lines according to methods of Lahner (Lahner et al., 2003) and Chen (Chen et al., 2009b). Most of the data fall within 3 standard deviations (s.d.) above or below the mean, plant was identified as accumulator if it contained elements in amounts that were more than 3 s.d. from the mean in either direction in all of the three treatments. Using this criterion, “Horosoy” and “Labrador” were identified among all of the lines, which showing higher Cd concentrations independent of the treatments (Data not shown).

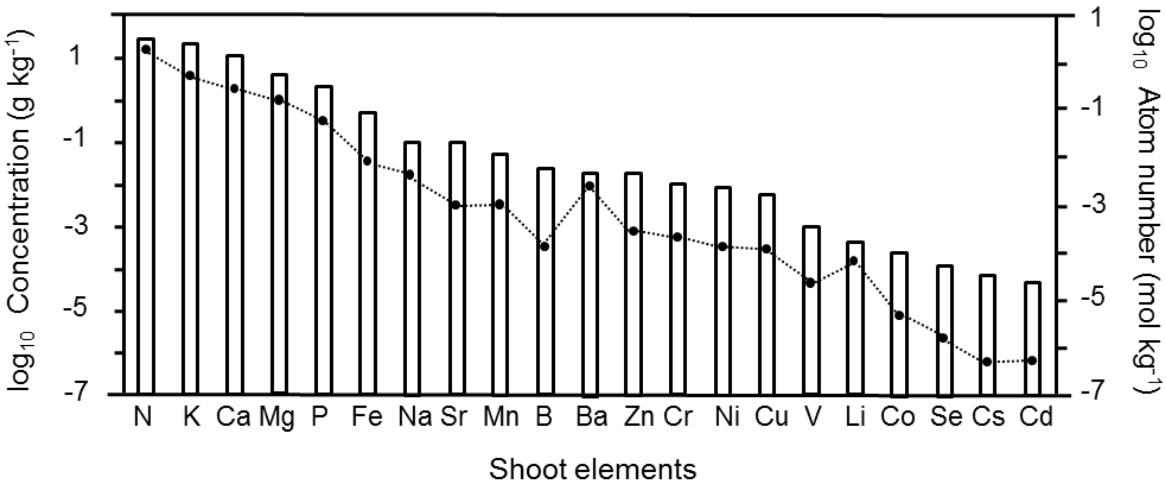


Fig. 1.1. Average concentration of 21 minerals in soybean shoots (n=396). Bars and solid circles are the means on mass and atom basis, respectively.

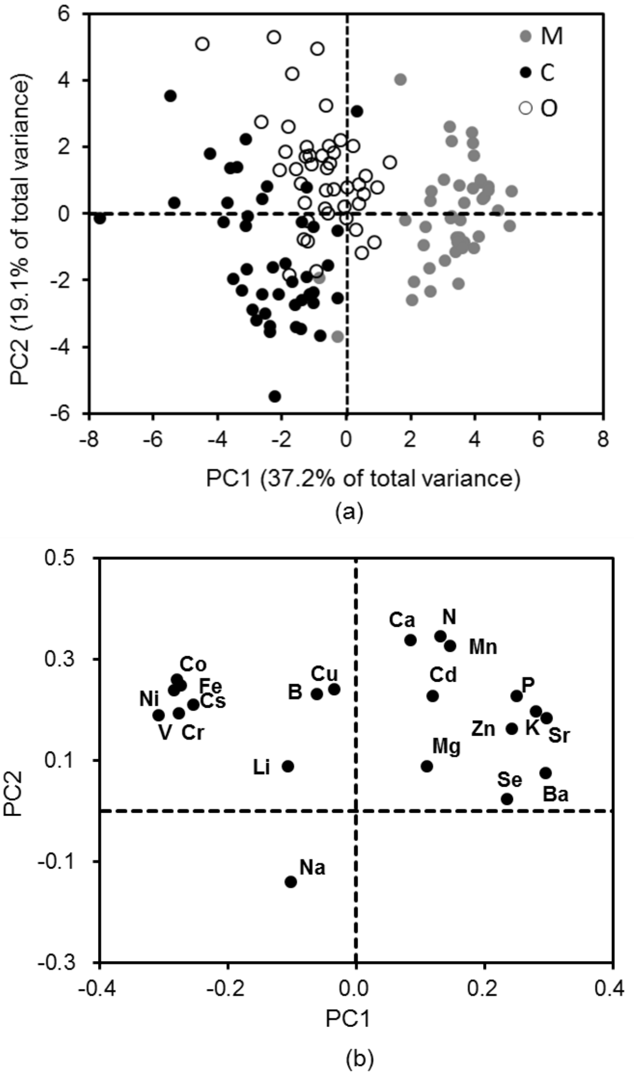


Fig. 1.2a. Sample scores for the first (PC1) and second (PC2) principal components draw from the PCA for 21 mineral elements. M, C and O indicate manure, chemical fertilizer, minus N, P treatments, respectively. **Fig. 1.2b.** Values of the compound loadings scores for the first (PC1) and second (PC2).

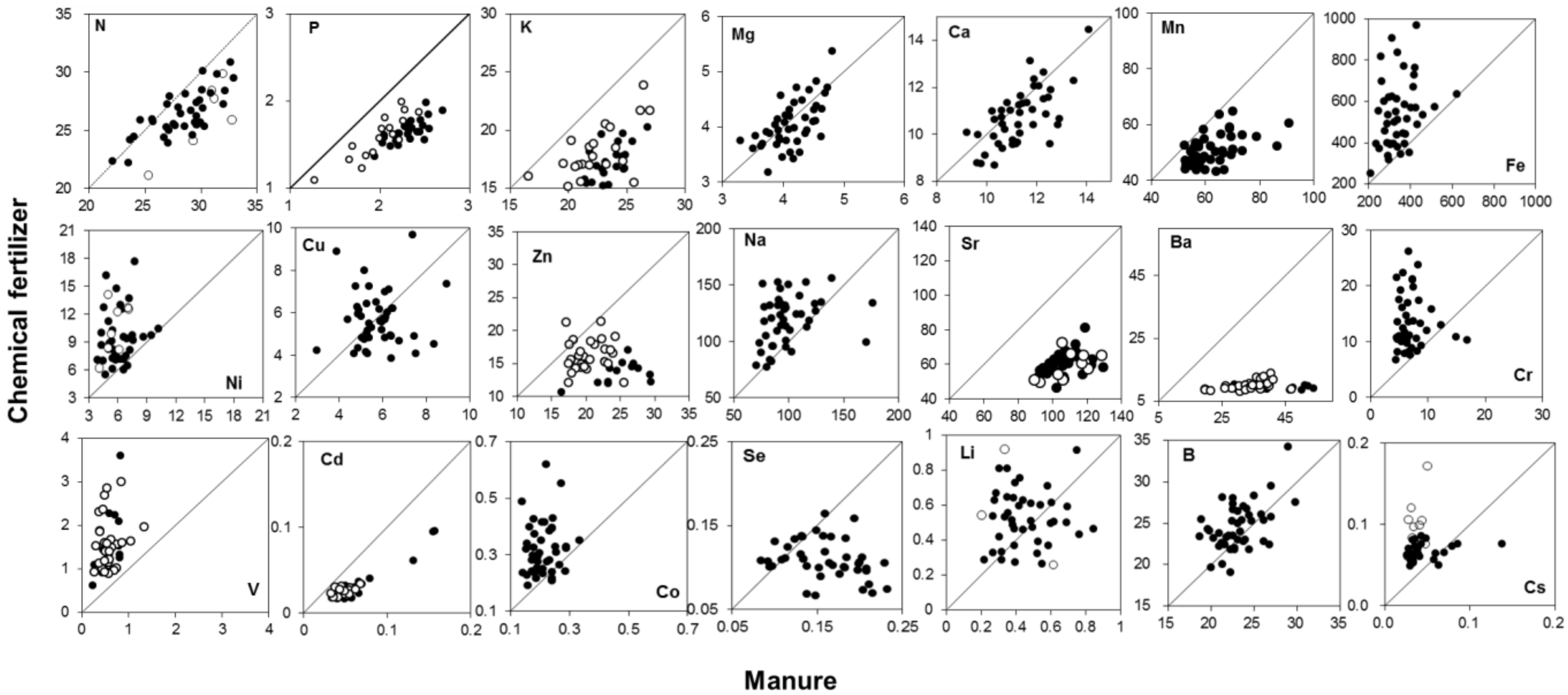
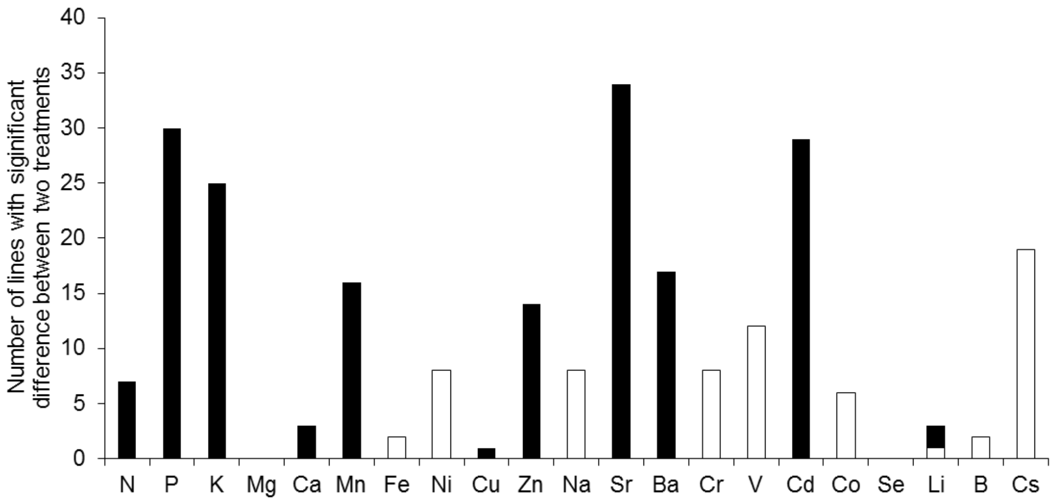
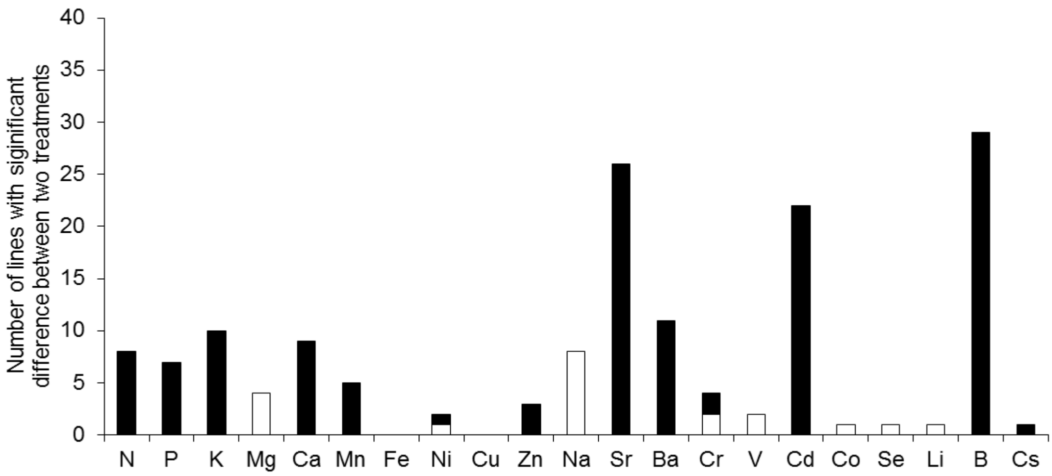


Fig.1.3. Elements concentrations of 44 soybean shoots in manure and chemical fertilizer treatments.

Each dot indicates the concentration of an element in the shoot of an individual line in two treatments, which X-axis and Y-axis indicate manure treatment and chemical fertilizer treatment, respectively. White circles and solid points indicate there were significant differences or no significant differences between two treatments in one line by student's t-test. Data are expressed as mg g^{-1} DM for N, P, K, Mg and Ca, and as μg g^{-1} for others.



(a)



(b)

Fig. 1.4. 44 soybean lines summarized by the particular elements that significantly decreased (open bars) and increased (solid bars) in shoots induced by manure application compare with chemical fertilizer treatments (a) and K application compare with chemical fertilizer treatment (b).

Table 1.1. Total mineral elements in manure.

P	K	Mg	Ca	Mn	Fe	Cu	Zn	B
		g kg^{-1} DM[a]					mg kg^{-1} DM	
7.72	25.87	10.85	17.55	0.40	3.7	65.6	200.04	13.84
Co	Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
				mg kg^{-1} DM				
3.76	5700	62.09	62.72	48.31	0.19	9.13	0.71	0.17

DM[a], dry matter; water content, 32.5%.

Table 1.2. Soybean lines tested in this study.

Num.	Name	Num.	Name	Num.	Name
1	Tokei803gou	16	Tokei952gou	31	Miciiun
2	Tokei805gou	17	Tokei978gou	32	Hokkaihadaka
3	Tokei806gou	18	Hakutsurunoko	33	Kariyutaka
4	Toiku112gou	19	Karikachi	34	Jack
5	Toiku113gou	20	Kimusumeigelaki1gou	35	Hayahikari
6	Toiku130gou	21	Tachisuzunari	36	Toyokomachi
7	Toiku738gou	22	Miyagishirome	37	Uien
8	Toiku769gou	23	Tsurumusume	38	Sapporomidoli
9	Toiku793gou	24	Yukihomare	39	Yukimusume
10	Toiku834gou	25	Harosoy	40	Sayamusume
11	Toiku853gou	26	Kitamusume	41	Ajitaalou
12	Toiku867gou	27	Suzuhime	42	Blackmoon
13	Toiku937gou	28	Wasekogane	43	Sayanishiki
14	Toiku238gou	29	Labrador	44	America guline
15	Kulakakeedamane	30	Toyoharuka		

Table 1.3. Chemical properties of soil in experiment site.

pH	Available P_2O_5	NO_3-N	NH_4-N	Total N	Exchangeable cations ($cmol_c$ kg^{-1})		
(H_2O)	(mg kg^{-1}) [a]	(mg kg^{-1})	(mg kg^{-1})	(g kg^{-1})	Ca	Mg	K
5.8	28	6.3	5.0	2.7	5.74	1.23	0.54

[a] P_2O_5 was determined by the Truog method.

Table 1.4. Elemental concentrations in soybean shoots.

	N	P	K	Mg	Ca	Mn	Fe	Cu	Zn	B	Co
Mean (mg g^{-1})	27.0	1.89	20.2	3.97	11.4	0.058	0.457	0.006	0.018	0.027	0.0003
Geo. Mean (mol kg^{-1})	2.0	0.06	0.5	0.17	0.3	0.001	0.008	0.0001	0.0003	0.0001	4.6E-06
SE (mg g^{-1})	0.2	0.02	0.2	0.03	0.1	0.001	0.011	0.0001	0.0003	0.0003	5.9E-06
CV (%)	11	20	17	13	14	20	47	37	28	24	43

	Na	Sr	Ba	Ni	Cd	V	Cr	Li	Se	Cs
Mean (μg g^{-1})	100.7	85.6	19.4	8.31	0.048	1.14	10.94	0.46	0.13	0.065
Geo. Mean (mol g^{-1})	4.4	1.0	2.5	0.14	5.4E-04	0.02	0.21	0.07	1.6E-03	4.8E-04
SE (μg g^{-1})	1.8	1.3	0.7	0.22	0.001	0.04	0.37	0.01	0.003	0.002
CV (%)	34	29	74	52	60	69	67	56	49	61

Geo. mean are the geometric mass. SE is standard error. CV, coefficient of variation.

Table 1.5. Variances obtained from a residual maximum likelihood (REML) analysis of 132 observations for concentrations of each of 21 mineral elements in shoots of soybean lines sampled from 3 treatments.

	N	P	K	Mg	Ca	Mn	Fe	Cu	Zn	B	Co
Genotypes (%)	36.7	22.4	14.7	25.0	25.5	18.8	8.6	0.9	0	5.4	6.0
Treatment (%)	20.3	47.5	50.7	17.4	15.7	28.2	19.4	18.3	39.2	68.6	20.4
Residual (%)	43.0	30.1	34.6	57.6	58.8	53.0	72.0	80.8	60.8	26.0	73.6

	Na	Sr	Ba	Ni	Cd	V	Cr	Li	Se	Cs
Genotypes (%)	18.0	1.7	0.2	5.7	51.5	5.8	2.6	2.9	0	0.5
Treatment (%)	22.8	72.0	71.0	19.3	29.4	34.6	19.3	6.9	21.5	23.1
Residual (%)	59.2	26.3	28.8	75.0	19.1	59.6	78.1	90.2	78.5	76.4

Variances are portioned as percentages of the total variance attributable to genotypes, fertilizer treatment and residual components. The residual component includes genotypes ×fertilizer interactions and other affects.

Table 1.6.a. Statistically significant changes in the elemental profile of manure treatment compared to chemical fertilizer treatment.

	Significant mean percentage change compared to chemical fertilizer treatment																					
Line	N	P	K	Mg	Ca	Mn	Fe	Ni	Cu	Zn	Na	Sr	Ba	Cr	V	Cd	Co	Se	Li	B	Cs	Total
1	22	47				23				95		67			-53	75			137			8
2		49	33									85			-76	94	-39				-54	7
3		52	42		24					121		94	431		-36	94					-53	9
4		44	54		14							88	418									5
5		44				29										190					-57	4
6		43	38							65		93	503			94						6
7		33	66							85		119				148	-71				-70	7
8		41						-51		54		58	249	-62	-74	80	-48				-65	10
9		27	39				-32	-34		67		66	320	-42		100						9
10			49					-33		92	-33	70	127		-63						-44	8
11	12	33										80		-66		57						5
12			25								-40	58	189	-57		99					-61	7
13												63									-49	2
14			42		31	41				141		107	343			112						7
15		48	43			38						105	410			105					-41	7
16		32	66							81		76				115					-58	6
17			29			48				80		117				209						5
18			33									93	492		-39	127						5
19												75				69						2
20								-46			-21	46			-68		-45		-63			6
21	16											74										2
22		38	35									86				120						4
23		40				42	-45	-44			-24	95	306	-62	-63	104	-39				-63	12
24		38	34										445								-52	4
25			28			25					-33	65				65						5
26		50											131								-60	3
27	9	43	40									69				110					-44	6
28								-43			-41	78			-68	127					-50	6
29											-27	69		-65		65			-64			5
30						37				54		72	132			128						5

Table 1.6.a (Continued).

	Significant mean percentage change compared to chemical fertilizer treatment																					
Line	N	P	K	Mg	Ca	Mn	Fe	Ni	Cu	Zn	Na	Sr	Ba	Cr	V	Cd	Co	Se	Li	B	Cs	Total
31	12	43	17			19				48		60	331		-61	87						9
32		36	49			37									-77	184						5
33	26	61	34			34										115					-51	6
34		37	51			31				77		58	279			79						6
35	20	42	40						13													4
36		30				30		-38				62									-56	4
37			43								-37	78		-46		81					-59	6
38		36				39						122		-79		232						5
39		27				53						102									-40	4
40		38						-65				62				63						4
41		39	20																			2
42		43											244		-48					-24		4
43		33								27			165				-54					4
44	22	47	36			22														-18		6
Total	7	30	25		3	16	2	8	1	14	8	34	17	8	12	29	6		3	2	19	

Table 1.6.b. Statistically significant changes in the elemental profile of only K treatment compared to chemical fertilizer treatment.

	Significant mean percentage change compared to chemical fertilizer treatment																					
Line	N	P	K	Mg	Ca	Mn	Fe	Ni	Cu	Zn	Na	Sr	Ba	Cr	V	Cd	Co	Se	Li	B	Cs	Total
1						33				55			46			146				38		5
2		19											65			174				59		3
3			22								-35	61	45			175				49		6
4			35		17							53				121						4
5												48	42			141				32		4
6				-16								53	54			126				31		5
7	19	21	49		27							72	55			198				82		6
8				-20								27										2
9														72		158						2
10										31	-37	53	59			223				66		6
11												68				63						2
12			18								-51									49		3
13	16																			48		1
14					23							59	39			112				52		5
15												43				133				33		3

Table 1.6.b (Continued).

	Significant mean percentage change compared to chemical fertilizer treatment																					
Line	N	P	K	Mg	Ca	Mn	Fe	Ni	Cu	Zn	Na	Sr	Ba	Cr	V	Cd	Co	Se	Li	B	Cs	Total
16			34									38				111				52		4
17								30					71	28		208				46		5
18																177						1
19																						
20								-36						-43								2
21																						
22																127				42	23	3
23												43					-24		-44	60		4
24				-21								45	42							31		4
25						42					-38									41		3
26	11											57								60		2
27				-19								26										2
28											-53	51								46		3
29											-33	39										2
30	19		31		28	31						51				124				65		6
31						23										88		-27		57		4
32		27	33		21							49			-74	166				60		6
33	17				23							41								42		3
34					21	25						48								37		4
35		23	38		28					44		66								44		5
36	22	27										59	56			102				41		4
37			18								-28	51								54		4
38												56		-62		204				68		4
39	9	36																				
40	14																					
41			20								-47											2
42		20													-36	108						2
43																						
44					31							57								22		3
Total	8	7	10	4	9	5		2		3	8	26	11	4	2	22	1	1	1	29	1	

Data represents the mean percentage change of element concentration that were statistically significantly different in the manure treatment (1.6.a) and K treatment (1.6.b) compared to the chemical fertilizer treatment ($P \leq 0.05$, $n = 3$).

Table 1.7. Significance of the Spearman rank correlations in 3 pairwise comparisons of the rankings of shoot concentrations of each mineral element in 44 soybean genotypes with three treatments.

	N	P	K	Mg	Ca	Mn	Fe	Cu	Zn	B	Co
M-C	****	***	****	****	****	**	*	ns	ns	*	ns
M-O	****	****	****	****	****	****	ns	ns	ns	***	ns
C-O	****	***	****	***	**	**	ns	ns	ns	ns	ns

	Na	Sr	Ba	Ni	Cd	V	Cr	Li	Se	Cs
M-C	*	**	*	ns	****	*	ns	ns	*	ns
M-O	***	*	**	ns	****	ns	ns	ns	ns	ns
C-O	**	**	*	ns	**	ns	ns	*	ns	*

Notes: ****, p<0.0001; ***, p<0.001; **, p<0.01; *, p<0.05; ns, no significant difference.

1.4 Discussion

Comprehensive view of ionome and its variation in soybean shoots

Previous studies of the variability of ionome focused on smaller sets of elements and mostly in the model plants under controlled growth conditions (Lahner et al., 2003, Chen et al., 2009b). Our studies measured 21 elements including essential elements and trace elements as Li, Cd, and Cs in the soil condition with fertilizer treatments, which giving more direct information for the agricultural practices. In this study, mineral concentrations in soybean shoots varied by nine orders of magnitude, from the most (N) to the least (Cd), and decreased as a function of atom number except for Ba, Li and Cd (Fig.1). The concentrations of essential elements are generally within the normal range for the healthy growth of plants (Marschner, 1995), and the nonessential elements is consistent with the results of terrestrial plant studied by Watanabe et al. (2007) and *Lotus japonicas* reported by Chen (Chen et al., 2009a), suggesting that although environment factors influence ionome profoundly, the rank order in many mineral concentrations is generally consistent. The mineral elements in plant are determined by the multiple processes of plant-environment interaction, especially the elemental composition of the environment and plant characteristics that affect the uptake of elements (Baxter and Dilkes, 2012), therefore the stability and sensitivity of each element response to environment must be different. It was reported that elements with high physiological requirements, high average concentrations and most frequently limiting in nature would be more stable and less sensitive to environmental gradients (the Stability of Limiting Element Hypothesis (Han et al., 2011). In the present study, the significant negative correlation ($p < 0.001$) was found between the CV of shoot element and mineral concentrations in shoots (Fig. 1.5). Moreover, the patterns of CV of shoot minerals are consistent with the Stability of Limiting Elements Hypothesis: N, P, K, Mg, and Ca (the essential macronutrients) had the lowest CV values (11, 20, 17, 13 and 14, respectively), while Ba, V, Cr, Cs, Cd (the non-essential toxic elements) the highest (74, 69, 67, 61, and 60, respectively), suggesting that the more important to the requirement of plant growth, the more stable the concentration of an element should be.

Most mineral elements found in plant come exclusively from the soil (Baxter and Dilkes, 2012), but only part of the minerals in soil could be absorbed by plants, named available minerals. Transport of the mineral elements from soil to plant depends on their solubility in the growth media and their binding strength to soil particles (Zhong et al., 2007). The performances of each element in soybean shoots and soil, soybean shoots and ammonium acetate (NH_4-AC) extracted soil solution are shown in Fig. 6a, b, respectively, as well as their transportability from soil to plant. It was observed that there were significant correlations between the elements in soybean shoots and that in soil, either the available forms or total, and the higher the concentration of elements in soil, the higher uptake by plants. K, Mg, and Ca were present at relatively high concentrations in both soybean shoot and soil, reflecting their natural abundance and their ease of transport within the plant compare to other elements. In well agreement with these observations, Olayinka et al. showed that the relationship between the concentrations of Cd, Cr, Pb and Zn in vegetable plants and in soil is intimately (Olayinka et al., 2011). Most of the mineral concentrations in soybean shoots were higher than that in the extracted soil solution, but lower than that in soil (total mineral elements), which indicate that comparing to the total mineral elements in soil, available minerals showed higher transportability to the plant shoots (Fig. 1.6a b). However, Cr and Li, existed in soil, were found in soybean shoots although they couldn't extracted by the ammonium acetate; Ba, and Cs have lower transportability from soil to soybean plants even they were in the available forms, indicates that they might be absorbed by plant in other forms (Zhong et al., 2007). It has been established that the total concentrations of elements in soil does not provide information about its bioavailability, and most of the elements are found in soil solution either in the form of inorganic or organic salts or ions, which may exist in either in free state or bound to clay particles. The chemical forms which these elements present in soil, as well as the physical and chemical characteristics of the environment can affect mobility, reactivity and availability for the plant absorption. A number of extractants have been applied for the single extraction or sequential extraction to evaluate the different elements forms in soil and the relationship between soil element forms and plant uptake (Yafa and Farmer, 2006, Sherene, 2010, Hsu and Lo, 2001, Bystrzejewska-Piotrowska and Urban, 2004), but all of the them just focus on one or several elements. Using NH_4-AC as the soil extractant, it is possible to determine most of the elements in our study in the available forms, which may give us direct information for the plant uptake. However, the chemical composition of plant tissues does not reflect the availability of some elements in the soil solution, as Cr and Li, and Fe and Ni with high transportability in present study, which the difference may results from active uptake and complicated transport by the root system (Mendoza-Cozatl et al., 2011). Therefore it is important to estimate the concentration of different forms of elements which present in soils, their changes during the plant growth and correlating this to element uptake mechanisms in plant in future.

Ionome of soybean shoot response to the fertilizer and genetic factors

According to PCA (Fig. 1.2), over 37% of the total variation in shoot ionome could be assigned to the fertilizer treatments. The remaining variation corresponded to differences between genotypes of soybean, to differences between different tissues in shoot, and to other factors during the experiment (Baxter, 2009). In present study, P, K, Zn, Sr and Ba showed higher positive values of PC1 loadings, with 0.25, 0.28, 0.25, 0.29, 0.29, respectively, indicating that the variation in their concentrations was strongly dependent on manure application. However, Co, Fe, Ni, V, Cr and Cs in the soybean shoots showed very high PC1 negative loadings, with the value of -0.28, -0.27, -0.28, -0.30, -0.27 and -0.25, indicating that these elements were responsive to changes in chemical fertilizer application. Significantly, soybean shoots with chemical fertilizer treatments containing 1.50, 1.56, 1.63, 2.70, 1.91 and 1.77 times more Co, Fe, Ni, V, Cr and Cs than that with manure treatments, respectively. Whilst manure treatment can increase 0.26, 0.23, 0.29, 0.45, 0.49, 0.73 and 0.31 times P, K, Zn, Sr, Cd, Ba and Se in soybean shoots than the chemical fertilizer treatment, respectively. These results were consistent with previous studies in soybean seeds indicating that ionome in both soybean shoots and seeds were sensitive to fertilizer treatment, the variation of these elements above were robust to the manure perturbation, and even more, the transport of these elements from root to shoot directly determine the composition in soybean and seeds (Sha et al., 2012, Citak and Sonmez, 2010, Citak and Sonmez, 2009, Baxter et al., 2012). Cs, Fe and Ni have been reported to display low mobility in the soil profile due to adsorption on organic matter (Rautaray et al., 2003, Narwal and Singh, 1998), which might be the reason for low concentrations of Cs, Fe and Ni in soybean shoot with manure treatment. Moreover, interactions of fertilizer application and the microbial activities were studied for a long time (Ai et al., 2013, Gadd, 2004, Mendoza et al., 2006), although their mutual effect and the mechanism of elements transport by microbes to plant were still not clear, there are reports showed that some elements, like P, Zn and Cs could be transport by AM fungi (Declerck et al., 2003, Broadley et al., 2007). Aspects of the ionome have previously been observed to differentiate plant families (White et al., 2012) and geographical origins of rice (Li et al., 2013), the PCA analysis reported in this study separated fertilizer treatments on the basis of soybean shoot ionome, which confirms its potential use as a tool of traceability of agricultural products.

RELM analysis was used to determine phylogenetic and environmental effects on mineral concentration of plant in many studies (Watanabe et al., 2007, White et al., 2012, Broadley et al., 2004). In present study, RELM analysis revealed the sources of variation related to individual element. 47.5%, 50.7%, 72.0%, 71.0% and 68.6% of the total variance of P, K, Sr, Ba and B attributed to fertilizer treatments, 57.6%, 80.8%, 53.0%, 72.0%, 80.8%, 60.8%, 73.6%,59.2%, 75.0%, 59.6%, 78.1%, 90.2%, 78.5%, 76.4% of total variance of Mg, Ca, Mn, Fe, Cu, Zn, Co, Na, Ni, V, Cr, Li, Se and Cs attributed to residual component including the interactions between fertilizer and genotypes, respectively, among which the results of P, K, Mg, Cu, Na, Ni, Na and Fe were consistent with White et al. s' study(White et al., 2012). Notably, the variance of Cd is significantly different from other elements, with the genotypes contributing 51.5% to the total variance, indicating that the Cd performed a unique character in soybean. However, Cd in rice was wildly studied in recent years (Ueno et al., 2009a, Ueno et al., 2009b).

The uptake of elements by plants varies not only among plant species but also among varieties (Arao et al., 2003). The genetic differences in the element uptake and accumulation have been found in diverse plants, e.g. maize (Kurz，H. et al., 1999), wheat (Zhang et al., 2002), and rice (Liu et al., 2003a, Liu et al., 2003b).Genetic variation occurring within species is important for the plant to regulate the elemental composition of their tissues to balance the mineral nutrient and trace element bioavailability in the soil with the requirements of the plant for growth and development (Salt et al., 2008, Buescher et al., 2012). Therefore, the biodiversity that exists within a species can be utilized to investigate how regulatory mechanisms of individual elements interact and to identify genes important for these processes (Baxter et al., 2012). Pairwise Spearman rank correlation analysis indicated that soybean lines responded similarly in their shoot concentrations of N, P, K, Mg, Ca, Mn, B, Na, Sr and Cd to the fertilizer treatments in previous experimental condition, although some genotype ×fertilizer interactions were existed for the elements Fe, Cu, Zn, Co, Ba, Ni, V, Cr, Li, Se and Cs. These results consistent with the RELM analysis, a smaller variation in shoot concentrations of N, P, K, Mg, Ca, Mn, B, Na, Sr and Cd were attributed to the residual variation, whilst the Fe, Cu, Zn, Co, Ba, Ni, V, Cr, Li, Se and Cs were mostly caused by the residual variation, which includes genotype × fertilizer interaction. The similar genotype × fertilizer interaction results were also found for the for Cd, As, Ni, lead and Cr in rice grain (Cheng et al., 2006, Zeng et al., 2008), Cd in maize (Kovacevic and Vragolovic, 2011), and uptake of Cs in lettuce and radish (Melnitchouck and Hodson, 2004). Other studies have reported genotype × fertilizer interactions for root biomass and root depth of wheat (Botwright Acuña and Wade, 2012). This is the first paper, to our knowledge, that reports on genotype × fertilizer interaction for the ionome of soybean shoots in field conditions. It is obviously the different genotypes of plant response differently to the environmental stimulation. The genotype × fertilizer interactions identified within the soybean lines have implications for attempt at breeding target elements' traits for diverse environments, e.g. the organic agricultural system, although it is notoriously difficult. More researches should be done for completely understanding the interactions between elements uptake and genotype × fertilizer control. However, in present study, "Harosoy" as a Cd accumulator was substantiated with showing high Cd concentrations in all of the three treatments of the experimental conditions, consist with the other studies (Arao et al., 2003, Sugiyama et al., 2010). Another line "Labrador", showing the same behavior as "Horosoy", which may have the potential accumulate ability for Cd was found in our study.

Interaction between elements

Pearson correlation analysis of large number of data from samples in different lines and fertilizer treatments make it possible to reduce the random connections of elements in an accession and emphasize the relationships among those elements strongly connected through metal-homeostasis genes and independent of environmental factors. In present study, we identified amount of positively and negatively correlated elements (Fig. 1.7, Table 1.8), suggesting the complicated regulation of elements in soybean shoots. There are significant synergistic actions among multiple elements studied: N and P ($r = 0.6$), N and Ca ($r = 0.6$), N and Mn ($r = 0.6$), P and K ($r = 0.7$), P and Zn($r = 0.7$), P and Sr ($r = 0.7$), P and Ba ($r = 0.7$), which may be as a function between cations and anions to balance the ion charge in plant tissues (Han et al., 2011). It was reported that elements belonging to the same group in the periodic table are not distinguishable by the transporters in plant or distinguishable from ions with the same charge because of their chemical similarity and functional replace ability (Tejada-Jimenez et al., 2009). In this study, we just find the positive correlations between Li and Na in the first group ($r = 0.5$), and strong positive correlations ($r > 0.7$) in the group of K, Ba, Sr and Zn, which is not consistent with the results of previous study in soybean seeds (Sha et al., 2012). Plant genotypes and tissues differ in their uptake, translocation, accumulation, and use of elements (Clark, 1983, Gong et al., 2003, Mendoza-Cozatl et al., 2011, Michael and Grausak, 2002, Puig and Penarrubia, 2009, Waters and Sankaran, 2011), therefore at least partly the differences in our studies were caused by the different contribution of element transporter in different tissues. Interestingly, additional strong positive correlations ($r > 0.7$) were found in the group of Fe, Ni, Cr, V, Cs and Co. Interactions between Mg and Ni, Fe and Cd have been found in Arabidopsis and other plants (Hermans et al., 2011, Siedlecka and Krupa, 1999), but there are few studies on the interactions among the Fe, Ni, and no essential elements Cr, V, Cs and Co, which need further study.

In conclusion, fertilizer application and soybean genotypes have impacts on the ionome of soybean, especially the increase of P, K, Sr, Zn, Cd and Ba and decrease Co, Fe, Cs, Ni, V and Cr in manure treatment compared with chemical fertilizer treatment. Therefore, this chapter revealed the genotypic and environmental control of the ionome of soybean.

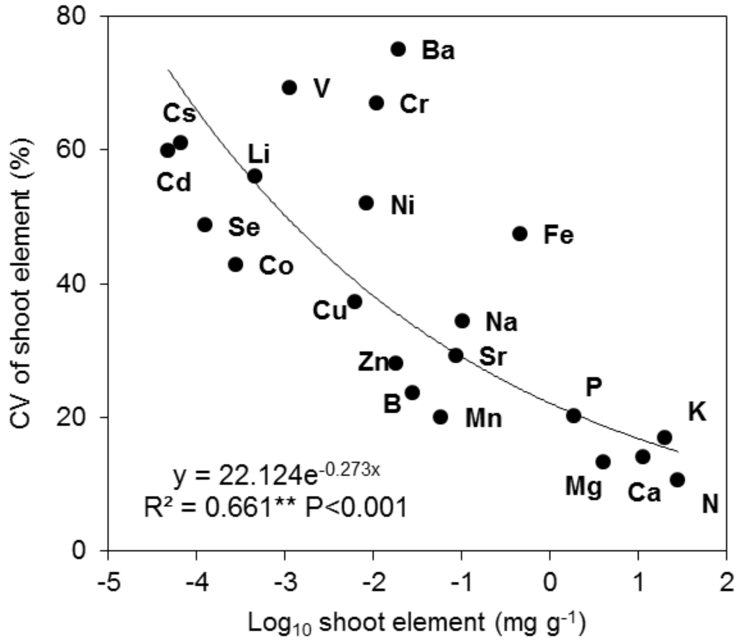


Fig. 1.5. Relationships between mean leaf elemental concentration and its coefficient of variation.

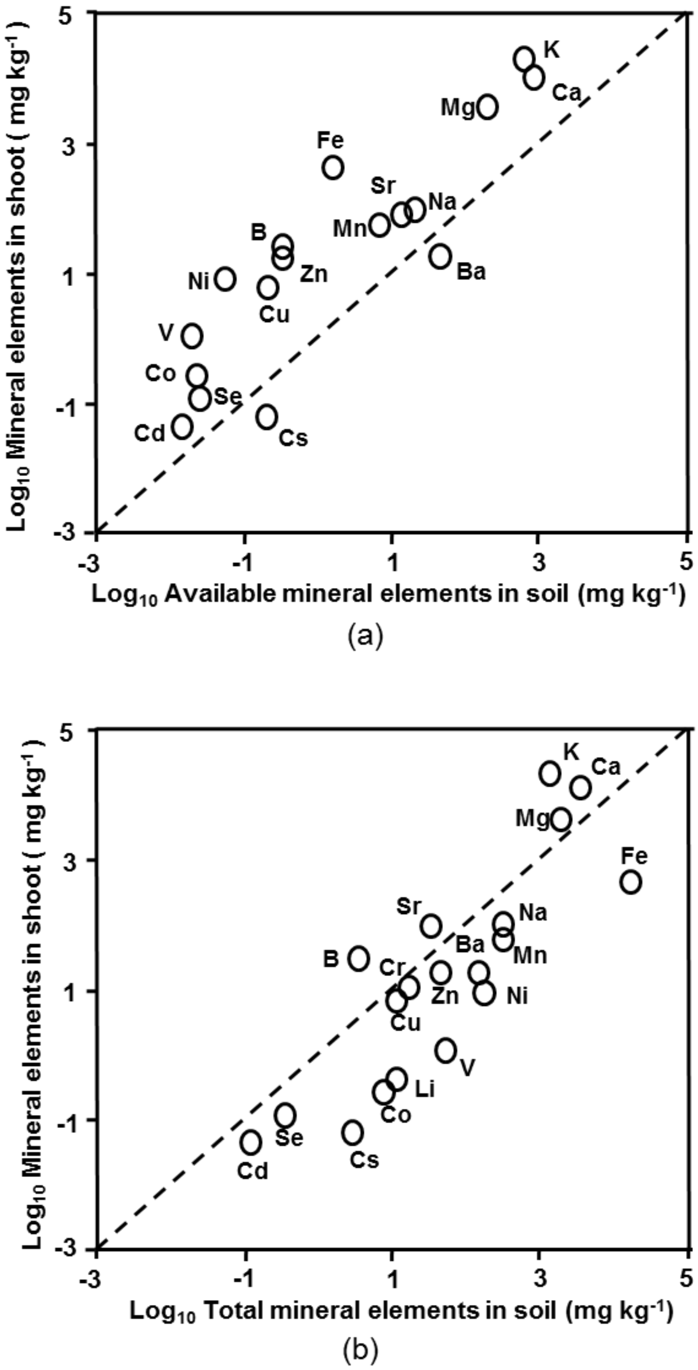


Fig. 1.6a, b. Transportability of available and total mineral element from soil to plant shoot.

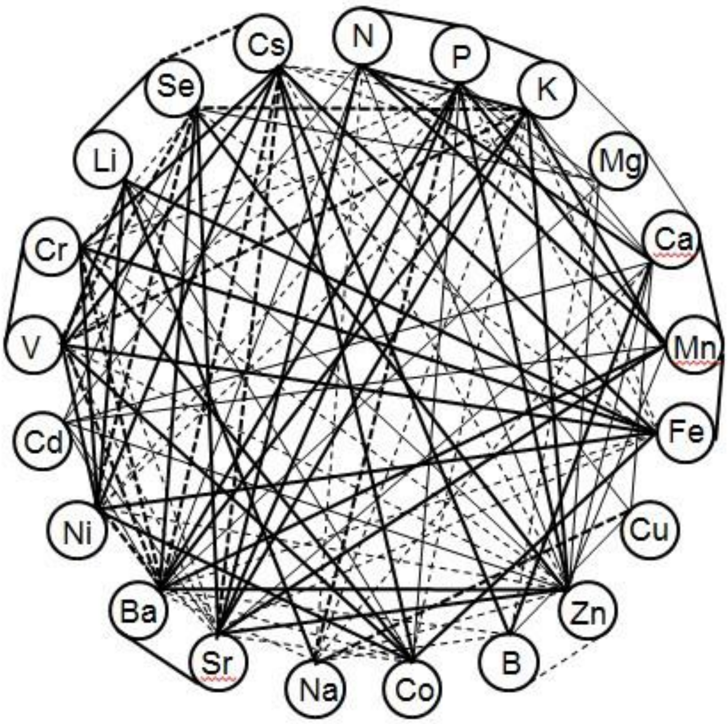


Fig. 1.7. Correlations wheels with significant correlations between elements.

Only significant correlations ($p < 0.01$) are displayed on the wheel. Positive correlations are denoted by solid lines, negative correlations are denoted by dashed lines. Thick lines indicate $r > 0.5$, thin lines indicate $0.3 < r < 0.5$.

Table 1.8 Correlation efficient between elements in soybean shoots.

	N	P	K	Mg	Ca	Mn	Fe	Ni	Cu	Zn	Sr	Cd	Ba	Cr	V	Na	Co	Se	Li	B
P	0.6**																			
K	0.6**	0.7**																		
Mg	0.1	0.3**	0.3**																	
Ca	0.6**	0.3**	0.4**	0.3**																
Mn	0.6**	0.5**	0.5**	0.2*	0.5**															
Fe	0.0	-0.2**	-0.4**	0.0	0.1	0.0														
Ni	-0.1	-0.3**	-0.4**	0.1	0.0	0.0	0.9**													
Cu	0.2*	0.1	0.0	-0.1	0.3**	0.2*	0.2*	0.2*												
Zn	0.4**	0.7**	0.7**	0.4**	0.3**	0.4**	-0.2**	-0.3**	0.0											
Sr	0.5**	0.7**	0.7**	0.2*	0.4**	0.6**	-0.4**	-0.4**	0.1	0.7**										
Cd	0.4**	0.3**	0.4**	0.0	0.3**	0.4**	-0.1	0.0	0.2*	0.3**	0.4**									
Ba	0.3**	0.7**	0.7**	0.3**	0.2	0.5**	-0.4**	-0.5**	-0.2*	0.7**	0.8**	0.3**								
Cr	-0.1	-0.3**	-0.4**	0.0	0.0	0.0	0.8**	0.9**	0.1	-0.3**	-0.5**	-0.1	-0.5**							
V	-0.1	-0.4**	-0.5**	-0.1	0.0	-0.1	0.9**	0.9**	0.2*	-0.4**	-0.6**	-0.1	-0.6**	0.8**						
Na	-0.3**	-0.5**	-0.3**	0.1	-0.3**	-0.2	0.2	0.2*	-0.5**	-0.1	-0.3**	-0.2*	0.0	0.1	0.2*					
Co	0.0	0.4**	-0.4**	-0.1	0.1	0.0	0.9**	0.9**	0.2*	-0.3**	-0.4**	0.0	-0.4*	0.9**	0.9**	0.2*				
Se	0.1	-0.1	0.5**	0.4**	0.1	0.2*	-0.4**	-0.5**	-0.1	0.7**	0.5**	0.3**	0.6**	-0.4**	-0.5**	0.0	-0.4**			
Li	-0.1	-0.1	-0.1	0.2*	-0.1	-0.1	0.5**	0.5**	-0.1	0.3**	-0.2*	0.0	0.0	0.4**	0.5**	0.5**	0.4**	0.2		
B	0.3*	-0.2	0.1	-0.3**	0.5**	0.2*	0.1	0.1	0.5**	-0.3**	0.0	0.2*	-0.4**	0.1	0.2	-0.3**	0.1	-0.2**	-0.2**	
Cs	0.1	-0.3**	-0.4**	-0.3**	0.2*	-0.1	0.7**	0.7**	0.3**	-0.4**	-0.5**	0.0	-0.6**	0.6**	0.7**	0.0	0.7**	-0.5**	0.1	0.5**

CHAPTER 2

Ionome distribution of soybean during vegetative growth stage affected by previous cropping with mycorrhizal plant and P applications

2.1 Introduction

In recent years, there has been increasing interest in determining the ionome of various plant species and identifying the genes that dominate the composition of ions in the plant (Baxter and Dilkes, 2012, Salt et al., 2008). Understanding how plants regulate element composition of tissues is critical for agriculture, the environment, and human and animal health. Uptake of mineral elements from soil is an important pathway for the entry of essential and non-essential elements into the human food chain. Uptake, distribution and storage of mineral elements in plant involve multiple molecular components including transporters, channels, and the genes that encode and regulate them. At the same time, this process can be affected by the physiological properties such as root architecture and transpiration (Chao et al., 2011), as well as the environmental factors such as temperature, pH, and nutrient stress (Quadir et al., 2011, Watanabe et al., 2007, Chen et al., 2009a). Differences in soil–plant transfer abilities have been reported for plant species grown on the same soils (Staunton et al., 2003), but there are few studies attempted to investigate the ionome of soil-to-root, root-to-shoot and shoot-to-seed translocations , as well as their response to the farmer’s practices such as crop rotation and fertilizer application.

Arbuscular mycorrhizal (AM) fungi are ubiquitous in terrestrial ecosystems and form mutualistic associations with most land plants. The main benefit of the symbiosis for plants is facilitated uptake of P (Cheng et al., 2012). It has been well documented that the production of soybean could be maintained with reduced phosphorus application by previous cropping with mycorrhizal plants, indicating the AM associations make significant contributions to agricultural production via improving P nutrition of soybean (Oka et al., 2010). At the same time, it is noteworthy that the colonization of AM fungi improves host plant uptake of the mineral elements N, Zn, Na, S, Cd, Se, Cs, Fe and Mn (Gyuricza et al., 2010, Cacagnaro et al., 2006).

P is a non-renewable and expensive fertilizer for subsistence farmers, and difficult to utilize by crops, leading to runoff and cause severe environmental problems. Furthermore, a widely studied problem of agriculture soils is the low P availability caused by intense calcium phosphate precipitation (Diez et al., 1992). Sustainable agriculture requires growing crops with efficient usage of fertilizer resources and satisfying human food. Plants comprise the major portion of the human and animal diet, and improving their elemental nutrient content can greatly affect human health. However, efforts directed at a single element can have unforeseen deleterious effects. For example, limiting Fe or P can lead to increased accumulation of the toxic elements Cd and As (Baxter et al., 2008).

Here we would like to investigate the distribution of ionome in soybean organs during vegetative stages affected by previous cropping with mycorrhizal plant and P application, to track the ionome in soybean plant and improve our knowledge of ionome distribution and sustainable agricultural control.

2.2 Materials and methods

Field experiments were conducted on a volcanic ash soil, which is classified as a Melanudands under the Classification of US soil Taxonomy, at the National Agricultural Research Center for Hokkaido Region, Sapporo, Hokkaido. The experiment took two years, with sunflower (*Helianthus annuus* L., AM-host crop) and mustard (*Capsella bursa-pastos* Medicus, non-AM-host crop) grown in the fields in the first year as the main effects, and soybean (*Glycine max* L. Merr. cv. Tsurumusume) cultivated in the same fields in the following year and treated with P fertilizer (0, 50, and 150 kg/ha) as split plots. Experiment was conducted during 2012–2013, with the mean temperature of 15.8 ℃/day and precipitation of 1.8 mm/day during soybean growth periods (May–October) in 2013. Chemical properties of the soils and manure from samples taken before the experiments began are shown in Table 2.1. Experiments were arranged in randomized complete blocks in a split-plot arrangement with four replications. Each replication contained first year crop plots and manure plots.

For soybean cultivation, each first-year plot had sub-plots with 3 P levels: 0, 50 and 150 kg P_2O_5/ha. According to available soil P contents of the fields (Table 2.1) and the Fertilization Guide in Hokkaido (Hokkaido Government Department of

Agriculture 2002), the standard P application rate for soybean was 150 kg ha^{-1}. The level of 0 and 50 kg/ha were set based on the interaction of AM Fungi colonization and P uptake. N and K were applied as ammonium sulfate at a rate of 20 kg N/ha and potassium sulfate at the rate of 80 kg K_2O/ha in late May before soybean was sown. The subplot was 4.2 m × 4.2 m, interplant distance was 20 cm and row width 60 cm. After sowing, field were covered with non-woven fabric sheet (Paopao 90, MKV DREAM, Tokyo, Japan) for three weeks to increase temperature and avoid damage by wildlife.

Plant and soil analysis

Soybean plants were sampled at 39 d (seedling), 73 d (late flowering), 100 d (mid-mature) and 130 d (mature) after sowing. After sampling, plants were move to lab to separate into leaf, stem, root, pod and seed, washed with tap water and deionized water as soon as possible. Separated tissues were dried in oven for 3 days at 80 ℃ and milled by ball-miller (Vibrating Sample Mill, T1-100, Tokyo, Japan). Soil sample were taken from the field at seedling stage and after harvest. Ionome in plant and soil was analyzed according to the method in Chapter 1.

Statistics

The results were subjected to analysis of variance (ANOVA) of a split-plot analysis using SAS 9.1 to reveal significant differences among treatments.

2.3 Results

General aspects of the elemental distribution affected by previous crop cultivation and P application

Statistical differences in the mineral element concentrations in plant tissues affected by previous crop and P application were clarified by ANOVA analysis (Table 2.2a, 2.2b, 2.2c, 2.2d). In seedling stages, when previously cropped with sunflower, there were significant increase in the concentration of Ba and Cs ($P<0.01$) in leaf, Mn and Na in stem, Cd in root, Mg, K, Ca and Co in leaf and stem, and Cu in the whole plant compared to previous cropping with mustard which indicate active transport of Mg, K, Ca and Co from root to stem and leaf, but inactive transport of Sr from root to shoot. In the late flowering stage, when previous cropping with sunflower, Co and Na increased, while Ca, Sr and Cd decreased in leaf; K and Mn increased in stem; Fe decreased in root; Co is increased in both pod and stem, Rb is decreased in both leaf and root, Cu is increased in pod, leaf and root, and Mo is decreased in pod, stem and root. In mid-mature, when previous cropping with Sunflower, Li is increased but Mg is decreased in seed; Zn is increased in pod; Cd is decreased in leaf; Co increased in stem; Mg is decreased both in seed and root, Rb is decreased both in pod and stem, and Cu in increased in seed, leaf and pod. In mature, previous cropping with mycorrizal plant significantly increase the Ni and Co, decrease the Fe and Cd in pod, and increase the Cu and Zn in both pod and seed.

In seedling stage, with the increasing of P application, Cd is significantly increased but Zn is decreased, and Ni and Rb in the treatment of P 50 have the highest concentration in leaf. Cs is decreased with the increasing of P application, while Ca, Fe, Ni, Co, Sr and Ba have the highest concentration in P 50 treatment in root. Moreover, we found that the concentrations of K, Fe, Ni and Rb in leaf, Ca, Cu, Na and Rb in stem were significant affected by the interaction of mycorrhizal plants and P applications. In late flowering stage, Rb in leaf has the highest concentration in P 50 treatment, while Fe in root is increased with the increasing application of P. The interaction effect was found in the Cu in leaf, Mg and Mn in stem, and Ca and Zn in root. In mid mature, P 50 treatment has the lowest Mg and highest Rb concentrations in pod, while K in pod is increased with the increasing or P fertilizer. Fe is the highest in treatment P 50 in stem. In mature, Sr and Ba in leaf is significantly decreased with the increasing of P application. Fe and Ni in pod are decreased with the increasing of P application, and we find the interaction effect on the Ni concentration in pod.

Changes of soil available elements

Table 2.3 shows the available elements in soil measured in the seedling stage and after harvest by using 1 M NH_4-Ac. Paired t-test for differences between available mineral elements at seedling stage and after harvest showed that available essential and beneficial elements: K, Mn, Cu, Ni, Co, Mo, and Se were significantly different between the two periods, with the increasing of K, Mn, Cu and Co and decreasing of

Ni and Co. The effects of prior AM-host crop and P application on the changes of available elements at seedling and after harvest were revealed by ANOVA analysis. However, no significant difference was found by the treatments for all the elements we analyzed.

Table 2.1. Chemical properties of the soils used in the experiment.

pH (H_2O)	available P_2O_5 (mg kg^{-1})	total N (g kg^{-1})	CEC ($cmol_c$ kg^{-1})	exchangeable cations ($cmol_c$ kg^{-1})			Basic saturation (%)
				Ca	Mg	K_2O	
5.3	212	4.2	39.4	15.1	2.1	0.6	44.4

Table 2.2a. Concentration of elements in soybean at seedling stage.

Treatment			Mg	K	Ca	Mn	Fe	Cu	Zn	Ni	B	Co	Mo	Na	Sr	Ba	Cd	Cr	Se	Rb	Li	Cs
				mg g^{-1}										μg g^{-1}								
Sunflower	P 0	L	4.35	22.82	17.14	115.46	100.59	5.35	32.25	2.11	22.88	0.062	0.005	5.96	109.21	79.63	0.083	1.36	0.072	8.12	0.005	0.013
		S	5.33	38.81	15.96	25.58	63.77	7.75	27.96	1.33	19.34	0.042	0.291	16.28	138.29	91.26	0.090	0.86	0.065	10.28	0.009	0.011
		R	3.96	17.25	4.86	0.58	909.09	73.41	156.52	55.49	17.73	6.571	6.249	334.83	481.55	533.04	2.928	17.95	1.558	7.27	0.505	0.058
	P 1	L	4.48	22.89	17.37	113.93	114.31	5.22	32.02	2.62	24.19	0.063	-	7.21	115.07	80.69	0.085	1.17	0.057	8.36	0.003	0.012
		S	5.24	37.61	15.07	24.79	47.35	6.88	26.26	1.22	18.23	0.033	0.519	16.18	131.8	85.43	0.085	0.58	0.071	9.76	0.012	0.010
		R	2.66	8.53	5.50	1.52	1715.37	75.47	167.74	92.93	13.70	9.541	4.976	309.04	502.23	1385.07	2.138	18.51	1.936	3.96	0.879	0.089
	P 2	L	4.46	21.48	17.29	116.41	113.81	4.98	29.01	2.15	23.75	0.068	0.082	6.64	115.03	78.16	0.089	1.15	0.080	6.67	0.007	0.010
		S	5.27	39.09	15.17	25.63	65.96	7.08	26.87	1.24	18.16	0.037	0.356	16.51	133.69	83.96	0.091	0.56	0.050	8.63	0.012	0.009
		R	6.15	22.89	4.94	0.63	850.29	70.68	182.68	52.39	16.26	7.661	7.26	408.60	467.95	437.58	4.510	16.51	1.598	9.08	0.506	0.063
Mustard	P 0	L	3.91	18.47	15.6	109.26	104.69	3.90	31.75	2.61	22.52	0.057	0.094	4.61	102.93	70.69	0.078	1.05	0.050	7.28	0.011	0.010
		S	4.92	32.88	15.64	28.27	71.59	4.22	28.35	1.38	17.99	0.034	0.301	16.82	138.62	91.36	0.088	0.81	0.057	9.21	0.009	0.010
		R	5.51	18.43	4.36	0.56	779.80	58.56	161.34	50.25	15.14	6.158	6.936	280.87	446.67	455.62	3.747	14.52	1.246	9.53	0.512	0.058
	P 1	L	3.92	19.10	16.20	113.12	95.70	4.18	30.36	2.75	23.47	0.056	-	5.84	107.67	71.27	0.082	1.11	0.069	7.76	0.003	0.010
		S	4.81	33.12	15.88	28.92	60.66	3.97	27.55	1.37	18.06	0.033	0.254	22.54	139.71	87.70	0.086	0.73	0.038	9.80	0.009	0.010
		R	3.14	12.66	5.22	1.33	1582.12	66.99	158.64	92.48	14.62	9.593	5.634	215.51	471.52	539.65	4.475	20.29	1.85	6.43	0.900	0.097
	P 2	L	4.10	18.92	16.87	120.71	102.24	3.76	29.92	2.59	23.33	0.059	-	6.55	113.02	73.42	0.098	1.09	0.078	7.15	0.003	0.009
		S	4.89	33.61	15.80	28.67	55.76	4.30	28.76	1.36	18.47	0.033	0.237	17.61	138.11	85.42	0.101	0.75	0.048	9.40	0.012	0.009
		R	4.92	15.10	4.64	0.88	945.20	56.11	166.32	57.37	14.93	7.136	4.737	261.17	457.05	579.06	3.979	14.73	1.746	7.72	0.552	0.061
L		A[a]	<0.0001	<0.0001	<0.01	NS	NS	<0.0001	NS	NS	NS	<0.001	NS	NS	NS	<0.01	NS	NS	NS	NS	NS	<0.01
		B[b]	NS	NS	NS	NS	NS	NS	<0.05	<0.05	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	<0.001	NS	NS
		A*B[c]	NS	<0.05	NS	NS	<0.05	NS	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	<0.05	NS	NS
S		A[a]	<0.01	<0.01	<0.05	<0.001	NS	<0.001	NS	NS	NS	<0.05	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS
		B[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	NS	<0.01	NS	NS
		A*B[c]	NS	NS	<0.05	NS	NS	<0.0001	NS	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	<0.05	<0.01	NS	NS
R		A[a]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	NS	<0.05	NS	NS	NS	NS	NS
		B[b]	NS	NS	<0.05	NS	<0.05	NS	NS	<0.05	NS	<0.05	NS	NS	<0.05	<0.05	NS	NS	NS	NS	NS	<0.05
		A*B[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

P0, P1 and P2 indicate the P 0, P50 and P 150 treatments respectively; L, S, R,SE,P indicate the leaf, stem, root, seed and pod, respectively; *a*, *b* and *c* indicate the previous crop, P treatment and interaction of previous crop and P treatments respectively.

Table 2.2b. Concentration of elements in soybean at late flowering.

Treatment			Mg	K	Ca	Mn	Fe	Cu	Zn	Ni	B	Co	Mo	Na	Sr	Ba	Cd	Cr	Se	Rb	Li	Cs
		P	3.53	21.55	10.00	392.55	54.69	101.48	288.62	13.64	33.38	0.551	0.304	24.86	516.88	408.56	0.313	9.23	0.447	15.90	0.013	0.014
	P 0	L	4.05	17.02	22.71	155.96	74.16	4.88	48.49	1.44	40.28	0.084	0.089	10.75	131.61	79.10	0.072	1.51	0.090	9.35	0.0096	0.012
		S	4.04	19.42	11.38	203.98	35.90	50.16	74.84	5.76	21.27	0.419	0.149	48.64	1651.32	716.10	0.465	12.90	0.347	9.11	0.012	0.011
		R	2.74	10.70	2.17	219.73	324.37	68.41	81.12	22.67	13.98	2.14	2.48	497.20	351.44	306.73	1.52	22.97	0.910	6.10	0.150	0.027
		P	3.55	22.34	10.77	433.82	55.66	105.16	303.03	14.33	34.67	0.517	0.22	29.07	545.88	419.53	0.330	8.85	0.29	16.58	0.016	0.017
Sunflower	P 1	L	3.79	16.40	20.96	154.63	71.87	5.44	47.44	1.52	40.09	0.087	0.084	11.82	120.66	71.56	0.069	1.43	0.072	9.33	0.0097	0.013
		S	3.64	18.41	9.11	163.45	30.58	49.80	81.57	5.30	21.02	0.381	0.146	50.59	1350.07	543.28	0.48	11.65	0.329	9.91	0.012	0.013
		R	2.89	12.48	2.30	231.94	343.82	72.25	100.22	29.72	13.77	2.75	2.83	581.53	366.78	323.52	2.07	37.04	1.08	7.15	0.190	0.028
		P	3.54	21.76	10.28	411.84	53.88	95.98	293.47	12.55	34.91	0.604	0.249	24.06	531.78	393.39	0.344	8.33	0.187	14.24	0.018	0.014
	P 2	L	3.95	17.05	22.43	176.82	68.51	4.51	47.81	1.29	37.04	0.082	0.077	9.90	128.34	75.84	0.077	1.13	0.084	8.05	0.010	0.010
		S	4.06	20.31	11.37	186.47	37.45	45.77	83.46	4.63	21.49	0.417	0.141	47.62	1650.85	617.62	0.497	10.24	0.379	8.95	0.009	0.011
		R	2.92	12.12	2.58	242.67	373.05	71.93	96.6	31.98	13.94	2.33	3.54	526.76	397.29	307.57	1.62	34.61	1.21	6.21	0.200	0.026
		P	3.53	21.44	10.27	411.51	55.41	88.27	296.47	13.73	33.90	0.638	0.493	22.17	504.30	413.13	0.346	8.64	0.46	16.63	0.015	0.013
	P 0	L	4.24	17.80	24.19	174.12	72.98	4.25	49.88	1.33	37.85	0.078	0.113	10.27	145.08	84.04	0.086	1.41	0.095	10.42	0.0096	0.011
		S	4.20	19.39	11.08	210.28	40.91	41.57	82.51	4.99	21.91	0.32	0.182	50.16	1633.18	668.36	0.461	12.90	0.119	10.59	0.012	0.010
		R	3.10	13.04	3.29	263.13	362.62	63.92	104.61	29.25	14.43	2.16	4.54	540.13	388.22	328.08	1.82	35.78	0.96	7.70	0.180	0.028
		P	3.53	21.68	10.38	370.36	52.77	91.71	286.9	12.57	32.53	0.515	0.421	25.70	524.14	379.51	0.337	8.66	0.342	17.30	0.012	0.013
Mustard	P 1	L	4.04	17.39	23.06	164.36	67.26	4.43	46.63	1.28	37.76	0.073	0.112	9.84	134.12	71.45	0.083	1.54	0.070	10.38	0.0101	0.011
		S	4.34	20.25	10.94	204.37	36.97	44.27	91.22	5.25	22.12	0.358	0.175	50.13	1610.80	622.12	0.524	12.65	0.322	11.25	0.012	0.009
		R	3.10	12.01	2.54	235.49	342.71	59.47	88.43	28.13	13.27	2.00	4.07	494.61	360.98	292.61	1.93	34.93	0.88	7.49	0.170	0.025
		P	3.52	20.77	10.31	367.2	55.40	87.79	269.99	13.03	33.49	0.487	0.473	21.78	500.28	389.92	0.331	8.79	0.317	15.34	0.014	0.013
	P 2	L	4.05	16.58	23.19	181.53	66.80	4.51	46.12	1.28	36.99	0.078	0.086	9.11	135.54	79.48	0.089	1.34	0.103	9.26	0.0119	0.011
		S	4.43	16.47	11.46	197.69	36.12	45.65	87.99	7.05	21.53	0.419	0.23	41.42	1667.13	669.21	0.548	15.09	0.211	9.33	0.013	0.008
		R	3.14	12.61	3.18	296.63	489.16	62.13	100.99	41.82	12.49	3.04	4.13	528.8	356.59	335.34	2.09	50.52	0.820	7.26	0.250	0.032
		A[a]	NS	NS	NS	NS	NS	<0.0001	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	<0.01
P		B[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	NS	NS
		A*B[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A[a]	<0.05	NS	<0.01	NS	NS	NS	NS	NS	NS	<0.01	NS	<0.01	<0.05	NS	<0.001	NS	NS	<0.05	NS	NS
L		B[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A*B[c]	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A[a]	NS	<0.05	NS	<0.05	NS	NS	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	<0.05
S		B[b]	NS	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A*B[c]	<0.01	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A[a]	NS	NS	NS	NS	<0.001	<0.01	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	NS	NS	<0.05	NS	NS
R		B[b]	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A*B[c]	NS	NS	<0.01	NS	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 2.2c. Concentration of elements in soybean in mid-mature.

Treatment			Mg	K	Ca	Mn	Fe	Cu	Zn	Ni	B	Co	Mo	Na	Sr	Ba	Cd	Cr	Se	Rb	Li	Cs
Sunflower	P 0	SE	2.21	16.98	1.97	19.36	104.62	15.10	38.80	1.95	32.98	0.063	1.12	2.09	5.10	3.71	0.025	1.44	0.017	15.17	0.005	0.020
		P	5.34	17.93	8.32	37.56	19.48	6.69	11.14	0.31	28.65	0.038	-	7.64	75.89	20.44	0.029	1.22	0.022	9.85	-	0.015
		L	3.47	7.71	32.77	345.50	96.92	2.27	48.91	1.27	62.76	0.131	0.343	12.65	265.54	115.32	0.090	1.65	0.151	3.46	0.016	0.018
		S	3.32	8.38	8.41	16.69	28.01	5.50	7.26	0.92	15.34	0.065	0.094	39.66	129.5	39.98	0.081	1.67	0.043	4.48	0.013	0.012
		R	2.39	18.06	4.36	466.53	1031	88.63	143.30	71.23	13.57	6.84	2.00	768.71	458.34	515.42	3.94	64.66	1.577	9.42	0.542	0.077
	P 1	SE	2.36	16.39	2.21	21.03	89.03	15.75	36.82	1.73	33.39	0.067	0.519	2.77	5.74	4.43	0.021	1.65	0.045	13.09	0.008	0.015
		P	4.73	18.26	7.57	38.97	20.04	6.46	11.12	0.39	28.46	0.039	-	8.38	58.35	20.24	0.028	1.32	0.005	9.95	-	0.014
		L	3.81	8.26	34.35	342.33	89.72	2.23	54.45	1.32	63.36	0.126	0.279	11.27	252.63	121.27	0.083	1.64	0.123	3.63	0.016	0.017
		S	3.64	8.33	8.71	17.63	31.33	5.89	7.13	0.82	15.45	0.057	-	39.60	117.51	53.88	0.081	1.33	0.045	4.50	0.011	0.010
		R	2.26	18.59	4.56	508.40	1190	89.09	158.40	81.15	14.05	8.04	2.04	624.88	468.44	559.72	4.54	56.66	2.044	9.29	0.664	0.077
	P 2	SE	2.28	16.47	2.01	20.09	93.50	16.00	38.81	1.77	33.62	0.094	0.261	1.93	5.46	3.77	0.022	1.63	0.031	12.27	0.004	0.015
		P	5.03	18.78	7.55	36.67	18.85	6.65	11.03	0.30	29.18	0.042	-	7.74	44.51	17.59	0.035	1.06	0.017	9.43	-	0.015
		L	3.84	7.84	37.75	369.30	97.45	2.16	53.65	1.18	67.51	0.137	0.367	13.08	298.54	117.14	0.095	1.85	0.133	3.40	0.021	0.018
		S	3.68	8.37	8.67	15.72	25.75	5.09	6.79	0.77	15.64	0.061	-	42.47	118.20	40.28	0.072	1.78	0.022	4.28	0.013	0.010
		R	2.29	15.98	4.45	461.08	853	88.81	143.71	65.29	12.17	6.33	1.73	578.67	468.98	555.85	4.25	51.91	1.254	7.69	0.573	0.067
Mustard	P 0	SE	2.41	16.56	2.12	20.50	91.36	14.94	37.82	1.93	33.74	0.070	0.328	2.67	5.44	3.70	0.027	1.64	0.007	13.65	0.002	0.017
		P	5.23	17.01	8.01	34.63	18.70	4.93	9.51	0.36	28.47	0.033	-	7.55	50.56	18.61	0.027	1.22	0.022	10.08	-	0.014
		L	4.43	8.08	38.80	384.65	105.17	1.77	52.75	1.21	64.71	0.130	0.470	10.80	284.08	131.02	0.100	1.57	0.182	3.74	0.028	0.019
		S	3.69	8.36	9.23	16.00	24.74	4.70	7.56	0.92	15.63	0.056	-	41.83	122.35	45.23	0.078	2.42	0.037	4.95	0.013	0.011
		R	2.78	15.13	4.36	501.91	1179	83.35	151.55	73.36	13.84	6.78	1.98	515.23	456.03	506.94	4.66	63.51	1.561	9.17	0.656	0.079
	P 1	SE	2.36	16.40	1.89	20.12	85.17	14.53	36.42	1.76	33.61	0.053	0.298	2.24	5.62	4.14	0.022	1.55	0.033	14.27	0.003	0.014
		P	4.65	19.27	7.30	36.30	21.31	5.81	10.22	0.37	30.07	0.035	-	7.79	49.52	17.65	0.029	1.08	0.024	12.08	-	0.014
		L	4.09	8.40	40.91	389.83	99.61	1.78	50.55	1.34	64.65	0.124	0.299	10.16	277.15	126.64	0.098	1.93	0.183	4.38	0.020	0.018
		S	4.47	9.03	9.21	16.17	35.03	5.12	7.20	0.90	16.96	0.057	0.06	39.28	123.48	37.78	0.086	1.73	0.051	5.56	0.014	0.010
		R	2.57	14.97	4.17	405.86	765	77.80	142.27	62.60	13.40	5.69	1.48	567.63	445.73	463.78	4.00	55.11	1.619	9.65	0.495	0.061
	P 2	SE	2.38	16.30	2.14	20.65	98.14	15.13	38.64	1.87	34.28	0.054	0.343	2.13	5.49	3.89	0.029	1.84	0.021	14.82	0.003	0.015
		P	5.12	17.75	7.55	36.25	19.02	5.39	9.64	0.34	28.17	0.037	-	7.51	43.42	17.29	0.033	0.99	0.028	10.02	-	0.013
		L	4.11	7.66	38.76	421.53	105.98	1.79	49.37	1.30	66.64	0.137	0.337	11.36	270.14	119.86	0.111	1.73	0.201	3.41	0.017	0.018
		S	4.13	8.53	9.92	17.57	28.34	4.53	6.95	0.79	16.10	0.055	-	38.97	128.06	39.29	0.091	1.88	0.027	4.74	0.011	0.010
		R	2.57	17.55	4.39	515.60	1325	80.35	148.57	95.82	13.62	7.17	1.92	687.58	451.00	502.48	5.10	67.51	1.454	10.94	0.764	0.077
SE		A[a]	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS
		B[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A*B[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P		A[a]	NS	NS	NS	NS	NS	0.0001	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	NS	NS
		B[b]	<0.01	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	NS	NS
		A*B[c]	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	<0.05	NS	NS
L		A[a]	NS	NS	NS	<0.05	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	NS	<0.05	NS	NS	NS
		B[b]	NS	NS	NS	<0.05	NS	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A*B[c]	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S		A[a]	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS
		B[b]	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A*B[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R		A[a]	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		B[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A*B[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 2.2d. Concentration of elements in soybean at harvest.

Treatment			Mg	K	Ca	Mn	Fe	Cu	Zn	Ni	B	Co	Mo	Na	Sr	Ba	Cd	Cr	Se	Rb	Li	Cs
Sunflower	P 0	SE	2.69	17.53	2.11	25.20	109.03	16.9	44.36	2.11	41.70	0.102	0.296	6.03	6.09	4.61	0.031	3.42	0.068	13.14	0.013	0.018
		P	5.37	17.79	9.13	54.38	33.39	6.23	10.44	0.79	35.24	0.062	0.229	43.58	91.3	21.61	0.041	1.75	0.051	10.47	0.052	0.017
		S	2.30	5.60	4.69	17.34	39.38	5.40	4.62	0.81	12.00	0.045	0.035	92.92	40.05	31.21	0.083	1.96	0.002	2.94	0.026	0.008
		R	0.79	5.15	2.07	414.27	1077.13	70.53	90.66	65.78	7.92	3.44	1.31	317.86	330.47	363.56	1.33	44.10	0.900	2.68	0.550	0.050
	P 1	SE	2.72	17.6	2.21	20.68	98.11	17.38	42.46	1.75	41.11	0.092	0.264	5.48	6.00	4.09	0.027	1.95	0.063	14.15	0.040	0.023
		P	5.63	17.82	9.18	31.27	19.93	7.01	10.86	0.46	33.49	0.048	0.114	43.26	88.60	20.80	0.036	1.62	0.043	10.47	0.053	0.017
		S	2.27	4.94	4.10	14.62	25.39	4.33	3.39	0.54	9.77	0.028	0.053	56.48	42.43	31.29	0.068	1.33	0.016	2.64	0.015	0.007
		R	0.82	5.38	2.45	350.15	704.96	69.16	82.87	46.04	8.35	3.15	1.68	298.39	315.77	338.73	1.19	51.74	0.430	2.99	0.530	0.050
	P 2	SE	2.73	17.64	1.99	19.40	100.21	17.9	42.70	1.65	41.42	0.092	0.522	4.77	5.40	3.17	0.030	2.04	0.056	13.38	0.032	0.024
		P	5.83	17.02	9.10	31.74	22.04	6.62	10.86	0.47	33.11	0.053	0.129	43.94	86.02	19.34	0.038	1.27	0.037	9.53	0.047	0.017
		S	2.77	6.67	5.11	18.88	24.82	5.33	5.36	0.70	13.30	0.084	0.251	79.53	46.56	32.46	0.097	1.33	0.019	3.36	0.026	0.010
		R	0.71	3.90	2.06	290.63	497.25	68.14	77.86	41.83	7.99	2.5	1.23	219.16	299.41	304.29	1.06	34.95	0.790	2.54	0.370	0.040
Mustard	P 0	SE	2.71	17.09	2.00	21.36	93.89	15.33	40.54	1.6	43.11	0.08	0.435	5.50	5.71	3.85	0.028	1.78	0.071	14.19	0.034	0.020
		P	5.98	17.13	9.00	32.26	23.68	4.93	9.69	0.56	33.67	0.046	0.364	47.50	86.79	20.05	0.036	1.60	0.062	10.29	0.062	0.016
		S	2.14	6.41	4.92	17.88	40.18	4.56	4.88	1.01	12.11	0.055	0.028	109.06	43.58	30.18	0.081	2.62	0.010	3.55	0.028	0.009
		R	0.73	4.92	2.21	343.65	701.63	59.58	73.76	53.34	8.15	2.98	1.27	288.55	287.92	325.7	1.19	44.18	0.720	2.86	0.500	0.050
	P 1	SE	2.70	17.34	1.98	20.63	91.17	15.57	39.99	1.64	41.24	0.083	0.297	5.56	5.84	3.83	0.028	1.77	0.095	14.98	0.024	0.019
		P	5.83	16.81	8.75	31.71	19.64	5.05	8.59	0.45	31.74	0.045	0.187	43.76	85.52	19.43	0.034	0.89	0.058	10.45	0.043	0.014
		S	2.04	5.96	4.65	15.90	31.37	4.57	4.94	0.81	11.77	0.041	0.013	109.85	42.43	26.65	0.076	1.78	0.017	3.40	0.021	0.007
		R	0.85	4.57	2.36	271.06	595.86	64.36	82.98	38.15	8.09	2.44	1.64	330.97	311.50	317.69	1.25	44.64	0.770	3.16	0.440	0.040
	P 2	SE	2.79	17.53	2.11	22.87	100.66	16.35	42.38	1.89	42.48	0.100	0.353	5.12	5.72	3.81	0.031	2.00	0.051	14.76	0.014	0.021
		P	5.79	18.94	8.57	29.59	17.65	6.06	9.87	0.41	33.60	0.045	0.547	41.01	73.97	18.93	0.045	1.21	0.052	11.35	0.044	0.019
		S	2.47	6.10	5.37	18.45	52.06	5.06	8.04	1.44	13.28	0.052	0.051	92.34	56.49	31.20	0.102	2.96	-	3.47	0.027	0.008
		R	0.79	5.25	2.05	418.58	850.98	62.78	79.44	55.57	8.72	3.27	1.45	324.82	300.58	330.06	1.37	50.22	0.54	3.22	0.490	0.050
SE		A[a]	NS	NS	NS	NS	NS	<0.0001	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		B[b]	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	<0.05	NS	NS	<0.05	NS	NS	NS	NS	NS	NS
		A*B[c]	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	<0.05	NS	NS	<0.05	NS	NS	NS	NS	NS	NS
P		A[a]	NS	NS	NS	NS	<0.05	<0.01	<0.05	<0.001	NS	<0.01	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	NS
		B[b]	NS	NS	NS	NS	<0.01	NS	NS	<0.0001	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	NS
		A*B[c]	NS	NS	NS	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S		A[a]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	<0.05	NS	NS	NS	NS
		B[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A*B[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R		A[a]	NS	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		B[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		A*B[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	NS

Table 2.3 Available mineral elements in soil.

Treatment		Mg (g kg^{-1})		K (g kg^{-1})		Ca (g kg^{-1})		Mn (mg kg^{-1})		Fe (mg kg^{-1})		Cu (mg kg^{-1})		Zn (mg kg^{-1})		Ni (mg kg^{-1})		B (mg kg^{-1})	
		S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H
Sunflower	P 0	0.213	0.199	0.313	0.208	2.05	1.90	20.40	6.33	1.99	1.93	0.098	0.030	0.323	0.325	0.037	0.043	0.273	-0.012
	P 1	0.219	0.216	0.300	0.225	2.09	2.02	21.26	6.85	2.00	2.12	0.146	0.008	0.347	0.326	0.035	0.047	0.156	0.175
	P 2	0.216	0.217	0.276	0.232	2.03	2.00	18.32	7.08	1.86	1.93	0.091	-0.006	0.365	0.292	0.033	0.042	0.151	0.096
Mustard	P 0	0.210	0.205	0.283	0.210	2.09	1.97	19.41	6.60	2.03	2.03	0.119	0.003	0.414	0.290	0.039	0.043	0.468	0.103
	P 1	0.205	0.201	0.279	0.209	2.06	1.98	20.35	6.49	2.17	2.08	0.112	0.075	0.469	0.342	0.038	0.047	0.212	0.356
	P 2	0.209	0.239	0.291	0.264	2.16	2.47	19.66	8.52	2.10	2.71	0.105	0.007	0.352	0.425	0.037	0.060	0.554	-0.089
The difference in each element after sowing and after harvest																			
P value		0.907		<0.0001		0.790		<0.0001		0.347		<0.0001		0.282		0.001		0.110	
ANOVA																			
A		0.166	0.777	0.183	0.720	0.475	0.249	0.869	0.396	0.061	0.145	0.983	0.378	0.190	0.501	0.073	0.245	0.105	0.761
B		0.992	0.279	0.429	0.155	0.937	0.207	0.448	0.112	0.545	0.338	0.372	0.258	0.677	0.750	0.405	0.379	0.428	0.198
A*B		0.686	0.533	0.134	0.479	0.550	0.304	0.643	0.375	0.556	0.180	0.404	0.182	0.499	0.457	0.806	0.213	0.537	0.433

Treatment		Co (mg kg^{-1})		Mo (mg kg^{-1})		Na (mg kg^{-1})		Sr (mg kg^{-1})		Ba (mg kg^{-1})		Cd (mg kg^{-1})		Se (mg kg^{-1})		Rb (mg kg^{-1})		Cs (mg kg^{-1})	
		S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H
Sunflower	P 0	0.018	0.012	0.011	0.011	34.52	30.26	21.07	19.68	57.92	56.75	0.021	0.018	0.023	0.006	0.662	0.627	0.052	0.047
	P 1	0.019	0.014	0.005	0.013	30.25	31.76	21.26	20.90	58.28	60.56	0.021	0.019	0.025	0.012	0.678	0.658	0.053	0.049
	P 2	0.017	0.014	0.005	0.020	30.52	32.02	21.06	20.72	60.26	62.06	0.021	0.018	0.025	0.009	0.614	0.621	0.049	0.046
Mustard	P 0	0.018	0.012	0.014	0.013	31.31	28.92	20.79	20.17	55.60	55.54	0.021	0.017	0.026	0.008	0.676	0.661	0.054	0.049
	P 1	0.017	0.013	0.006	0.028	28.43	29.65	20.99	20.60	55.29	56.92	0.021	0.018	0.021	0.016	0.694	0.694	0.056	0.052
	P 2	0.019	0.017	0.013	0.015	31.73	37.36	21.57	25.78	56.46	72.61	0.022	0.024	0.029	0.010	0.684	0.832	0.055	0.063
The difference in each element after sowing and after harvest																			
P value		<0.0001		0.017		0.803		0.282		0.155		0.055		<0.0001		0.607		0.321	
ANOVA																			
A		0.793	0.406	0.375	0.352	0.412	0.864	0.974	0.287	0.101	0.694	0.953	0.386	0.700	0.132	0.152	0.102	0.040	0.074
B		0.927	0.127	0.372	0.250	0.193	0.503	0.762	0.238	0.689	0.169	0.919	0.259	0.586	0.021	0.412	0.463	0.592	0.387
A*B		0.080	0.326	0.740	0.129	0.489	0.663	0.697	0.355	0.938	0.445	0.365	0.164	0.586	0.772	0.502	0.330	0.578	0.267

S and H in the table indicate the mineral elements in seedling stage and after harvest, respectively.

2.4 Discussion

Dynamic changes of mineral elements in individual organs during successive growth stages

Except mineral nutrient supply, physiological age of a plant or plant part is the most important factor affecting plant nutrient concentration (Marschner, 1995). It was widely reported that across–season growth influenced the nutrient concentrations and ratios (Güsewell, 2004, Han et al., 2011), but these studies are limited to several elements or individual tissue. Therefore, it is important to investigate the distribution of multiple elements including essential and non-essential elements in plant tissues and changes during growth. In present study, the dynamic changes of 20 mineral elements in soybean organs during successive growth stages were shown in fig.2.1. In seedling stage, concentrations of Mg and K in plant tissues were at the highest values and in the order of Stem > Leaf > Root, after that, their concentrations decreased and the difference between leaf and stem became smaller but still higher than that in the root. Mg and K in pod and seed became increasing from late flowering and mid mature, respectively. In mature stage, Mg and K concentrations in plant tissues were in the order: Pod ≥ Seed > Stem > Root. K and Mg were considered mobile elements, and the older leaves acting as sources for the export of large amounts to growing organs. The present investigation provides evidence of internal redistribution of K and Mg in soybean, which showed the same results as the studies of Osaki (Osaki et al., 1991). It is likely legumes accumulate more Mg and K in their above-ground portions. Especially, angiosperms accumulate more Mg in their seeds than in their vegetative parts. Meristematic tissues and other rapidly metabolizing tissues also accumulate high Mg concentrations (Humphries and Devonald, 1977, Wilkinson et al., 1990). Ca and B have the similar trend in plant: with the extremely high concentrations in leaf and keep increasing during all the successive growth stages, especially Ca. The leaf vacuole is widely considered to be the major Ca store in plant tissue, which results in highly transpiring organs amassing large concentrations of Ca. Ca in tissue is often found to be tightly linked to transpiration, once Ca is deposited in vacuoles it is not redistributed because most intracellular Ca is relatively immobile (White and Broadley, 2003, Gilliham et al., 2011). It was reported in the majority of plant species, B has restricted mobility between plant organs like Ca, but it is present in phloem and is retranslocated in phloem, often in sufficient amounts to satisfy the demands of growing organs that do not readily transpire (Brown and Shelp, 1997, Miwa and Fujiwara, 2010), that might be the reason that B has the high accumulation in soybean leaf, pod and seed. Except the elements above mentioned and Rb, other elements in plant were accumulated in root, and change of their concentrations during successive growth stages like “M-shaped”: the higher in seedling stage, decreased in late flowering, increased in the mid-mature, and decreased in mature. However, except Mg, K, Ca, Fe and Mo, the change of other elements in stem were in the “bell-shaped”, with the highest concentration in late flowering stage. Whilst, most of the elements in pod process the highest concentrations at the late flowering stage. Mineral elements uptake by plant is closely related to the crop growth rate. The rate of crop growth and the element uptake change significantly during soybean development. It was widely known the maximum soybean growth rate occurs near late vegetative to early reproductive stages. It was reported that approximately 50% to 90% of elements in plant at flowering moves from the leaves and stem to the developing seed (Marschner, 1995). In the late flowering, plant turns into the reproductive growth, with the decreasing root activities and the ability of element absorption. At this stage, redistribution of elements in plant tissues became the major physiological activity: most of the elements transported to the reproductive organs, and finally accumulated in the seeds. These might be the reason why the special shaped curves in root, stem and pod. Many studies showed that micronutrients can be translocated to seeds from various tissues, including root, stem, leaf, and reproductive organs (Waters and Sankaran, 2011, Grusak, 1994).

At harvest, it was interesting that most of the essential elements in soybean seed have higher contents compared with the non-essential elements, indicating the more harmful the elements is, the less transfer to the next generation.

Transportability of elements in the soil-plant system

Plant internal metabolic processes changed during different growth stages, as well as the mineral elements uptake and translocation in different organs. The soil/root ration and the rations of different organs showed the translocations of mineral elements from soil to root and the transport in plant in different stages (Fig.2.2). The root/soil rations showed mineral elements composition of plant tissues does not reflect the availability of nutrients in the soil solution. This difference results from selective uptake and transport of element by the root system. The rate of nutrient uptake by roots depends on the concentration of the particular nutrient at the root surface, root properties or plant species, and requirements of the plant. At higher solution concentrations of nutrients, the rate of uptake is typically higher, but plants do exhibit selectivity in that, they preferentially take up some elements and exclude others. Plants can take up nutrients in excess of their needs, termed luxury consumption, and store these in the vacuole. However, there are feedback mechenisms that reduce ion uptake as internal concentrations increase maintaining a balance between demand and acquisition. In the present experiment, in the seedling stage and after harvest, except Mg, K, Ca, Mn, Sr, Ba, Na, Li, Cs and B, the soil/root ration of other elements are extremely higher than the ration of leaf/stem, stem/root and seed/pod, indicating their high accumulation in roots. During all the successive growth stages, most of the elements have higher leaf/stem ratio than stem/root, suggesting their active unload in the leaves. However, most of the ratios changed in the successive growth stages. These results suggested that plant growth can change the translocation pattern of a number of elements.

In conclusion, based on the experiment, it was found that large amounts of Ca accumulated in the shoots, especially in leaves, while Fe, Ni, Na, Co, Mo, Cd, Cr, Se, Li and Cs remained in roots during all the soybean growth stages. Moreover, Cu concentrations in soybean were significantly increased by prior cultivation with AM-host crop sunflower, regardless of the tissue and growth stage.

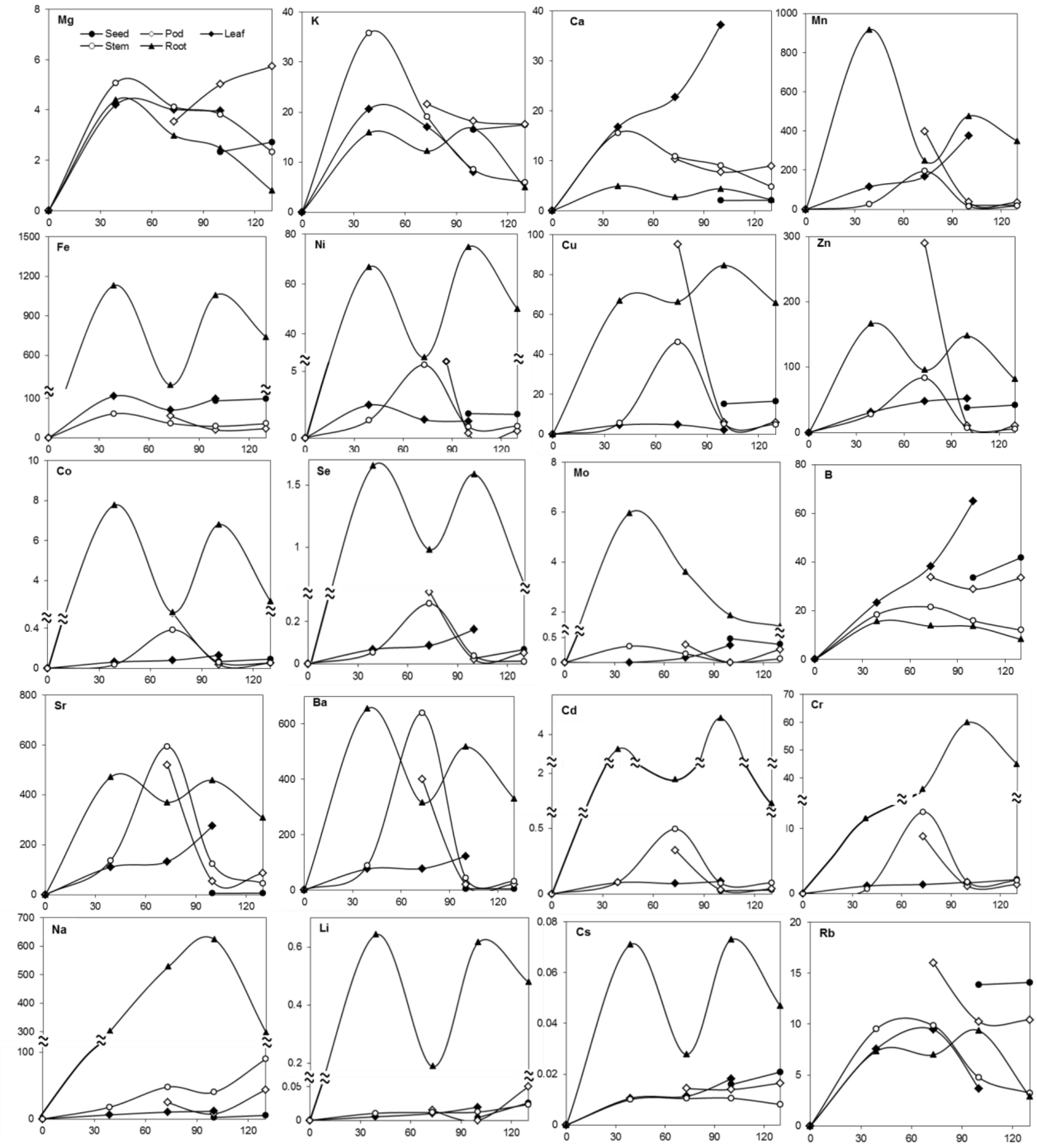


Fig.2.1. Dynamic changes of mineral elements in individual organs during successive growth stages

x and y-axis indicate the mineral element concentration and days after soybean sowing, respectively. Mg, K and Ca are shown as g kg^{-1} dry weight, other elements are shown as mg kg^{-1} dry weight.

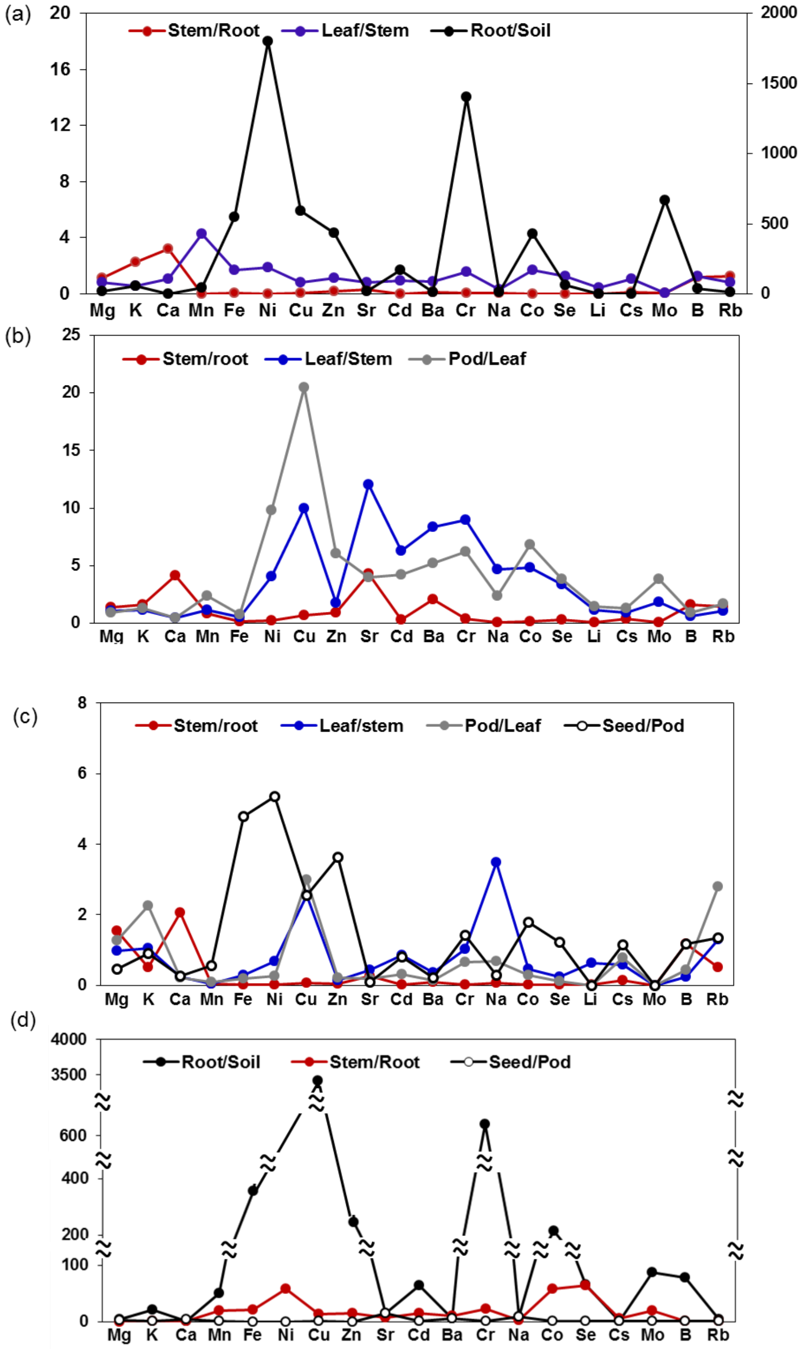


Fig.2.2. Transportability of elements among soil-soybean system during the successive growth stages: (a), (b), (c) and (d) indicate seedling stage, late flowering stage, mid-mature and mature, respectively. The second y-axis in (a) indicates the ratio of Root/ Soil.

CHAPTER 3

Ionome of soybean seed affected by previous cropping with mycorrhizal plant and manure application

3.1 Introduction

Elemental dietary imbalance affects approximately three billion people, where multiple deficiencies or excessive are possible in the same person or population (Welch and Graham, 2004). Mg, K, Fe, Zn and Ca dietary deficiencies are not uncommon even in the Global North (Broadley and White, 2010), whilst As, Cs and Cd excessive also lead to serious health problems around the world (Ishikawa et al., 2010). Biofortification and detoxification are the possible solutions for the malnutrition, but most of the studies just focus on one or two of the element, the complicated metabolism of elements and interactions between the elemental composition of different genotypes and environmental factors made it difficult to well understood.

Seed composition is an indicator of the plant's interaction with the environment (Baxter and Dilkes, 2012). The soybean seed ionome represents its mineral nutrient and trace element content regardless of essential or non-essential to plant, and is controlled by multiple processes including mobilization from the soil, uptake by the root, translocation and redistribution within the plant, and deposition in the seed (Grusak et al., 1999, Baxter, 2010). Any alterations in these processes that transport inorganic ions from the soil to the seed could affect the seed ionome and could eventually influence human and animal health through the food chain. Of these processes, the first step is the most polygenic and is affected by many environmental factors that are both non-biological (e.g., climate, soil condition and fertilizer application) and biological (e.g., symbiosis and parasitism) (Baxter, 2009). Plants may accumulate not only essential elements but also nonessential metals such as Cd and Pb, when they are present in the environment. Increasing essential elements in the edible parts of crops is an important method of solving mineral malnutrition in the human diet, while nonessential metals can cause serious health problems if they enter the human body (Vreugdenhil et al., 2004, White and Broadley, 2009). For this reason, revealing the relationship between the ionome and environmental parameters may help in developing strategies for better nutrient management in the future. To date, there have been many ionomic studies on leaves and shoots in various plants, but few reports on the ionomic response of the seed to different environmental parameters (Watanabe et al., 2007).

Fertilization and crop rotation are fundamental agronomic measures used to improve yield and avoid disease induced by monoculture in soybean cultivation. As two important environmental factors, the ionome of the soybean seed should also respond to the stimuli of fertilization and crop rotation. Manure is important as a source of plant nutrients, for example, K, but the actual supply depends somewhat on the type of manure (Citak and Sonmez, 2009, Citak and Sonmez, 2010). A number of studies have demonstrated that the application of manure can improve the concentrations of the essential elements Zn and Fe in soybean plants (Mekki and G.Ahmed, 2005). Moreover, manure has been proposed to reduce the phyto-availability of radionuclides in soils and decrease the content in plants by binding these minerals to organic substances (Ehlken and Kirchner, 2002). It is noteworthy that the colonization of arbuscular mycorrhizal (AM) fungi improves host plant uptake of the mineral elements N, Zn, Na, S, Cd, Se, Cs, Fe, Mn and, especially, P (Berreck and Haselwandter, 2001, Oka et al., 2010). It is known that growth of AM fungi can be improved or restricted through the use of organic amendments, but the mechanism remains unclear. No reports have considered the entire network of elements influenced by the application of manure and AM fungi colonization in field conditions (Gryndler et al., 2009).

In this chapter, the influence of previous cropping with mycorrhizal plants and manure application on the ionome of the soybean seed was investigated, which is important to improve the knowledge of the environmental control of plant seed mineral concentrations.

3.2 Materials and methods

Soybean cultivation

Two field experiments were conducted on a volcanic ash soil, which is classified as a Thapto-upland Wet Andosols under the Classification of Cultivated Soil in Japan and Melanudands under the Classification of US Soil Taxonomy, at the National Agricultural Research Center for the Hokkaido Region, Sapporo, Hokkaido. Each experiment ran for two years, with maize (*Zea mays* L. cv. New dental, AM-host crop) and buckwheat (*Fagopyrum esculentum* Moench. cv. Kitawase-soba, non-AM-host crop) being grown in the fields in the first year as the main effects, and soybean (*Glycine max* L. Merr. cv. Tsurumusume) being cultivated in the same fields in the following year and treated with manure (0 and 20 t/ha) as split plots. The first experiment was conducted in site 1 over the course of 2007–2008, with a mean temperature of 15.8 ℃/day and precipitation of 1.8 mm/day during the soybean growth period (May–October) in 2008. The second experiment was carried out in site 2 over the course of 2008–2009, with a mean temperature of 15.6 ℃ and precipitation of 2.5 mm during the soybean growth period in 2009. The chemical properties of the soils and manure from samples taken before the experiments began are shown in Table 3.1 and 3.2, respectively. Experiments were arranged in randomized complete blocks in a split-plot arrangement with four replications. Each replication contained previous crop plots and manure plots.

The first-year crop plots consisted of AM-host crops and non-AM-host crops fields. Maize and buckwheat were fertilized at planting with 120-240-120 and 30-120-70 kg/ha of N-P_2O_5-K_2O according to the Fertilization Guide in Hokkaido.

For soybean cultivation, each first-year plot had sub-plots with or without manure application. Manure (20 t/ha) was applied into the field in April, which was one month before soybean planting. N and K were applied as ammonium sulfate at a rate of 20 kg N/ha and potassium sulfate at the rate of 80 kg K_2O/ha in late May when the soybeans were sown. According to the available soil P contents of the fields (Table 3.1) and the Fertilization Guide in Hokkaido (Hokkaido Government Department of Agriculture 2002), the standard P application rate for soybean was 150 kg P_2O_5/ha. However, to examine the significant effect of AM fungi colonization on the change of ionome better, P fertilizer was not applied during the soybean growth season because the increase of soil P after fertilizer application has negative effect on the AM symbiosis. The subplot was 4.2 m × 4.2 m, interplant distance was 20 cm and row width was 60 cm. The fields were covered with a non-woven fabric sheet (Paopao 90, MKV DREAM, Tokyo, Japan) for three weeks to retain heat temperature and avoid damage by wildlife.

Plant and soil analysis

Four weeks after sowing, the roots of the soybean were collected, and AM colonization was determined according to the method of Oka *et al* (Oka et al., 2010). The elements in soybean seed, total and available elements in soil were analyzed according to the same method as chapter 1.

Statistics

To visualize the differences among the 19 mineral elements with the four treatments, principal component analysis (PCA) was employed using MiniTable 15 (MiniTable, State College, PA, USA). The results were subjected to analysis of variance (ANOVA) of a split-plot analysis using SAS 9.1 to reveal significant differences among treatments.

3.3 Results

Mineral elements in the soil after prior crop cultivation and manure application

Except for available Ca, which was increased by previous cropping with maize compared with previous cropping with buckwheat in experiment 1, there were no changes on the total and available minerals in the soil in the present experiments by the previous cropping, while the application of manure changed the profile of the mineral elements in the soil (Table 3.3a–d). Compared with no manure treatment (Table 3.3a, c), total K and Ba increased to 1.6- and 1.15-fold in soil in experiment 1, but only K was significantly increased to 1.17-fold in soil in experiment 2. In contrast, Ca decreased to 0.85- and 0.9-fold in experiments 1 and 2, respectively. However, only certain elements in the soil can be used by plants, i.e., the available elements. The availability of minerals depends on their solubility in the growth media and their binding strength to soil particles. With manure application (Table 3.3b), the available K, Mn, Co and Ba increased to 2.83-, 1.14-, 1.33- and 1.32-fold, respectively. In comparison, the available Ca, Fe, Cs and B decreased by 0.54-, 0.26-, 0.75- and 0.57-fold in experiment 1, respectively. The available K significantly increased by 2.26-fold, but Fe and Cs decreased by 0.84- and 0.73-fold in experiment 2, respectively (Table 3.3d). Thus, manure application significantly increased the K content in the soil, especially the available K, and decreased the available Fe and Cs content in the soil in both experiments.

AM fungal colonization of soybean roots

Soil microorganisms play a major role in the biogeochemical processes of soil–plant interactions. AM fungi can be intimately associated with plant roots and occupy an important position in the soil–root interaction. In this study, previous cropping with maize (i.e., an AM-host plant) significantly increased AM fungal colonization of soybean roots at four weeks after sowing ($P < 0.01$; Fig. 3.1), the colonization increased nearly fourfold in experiment 1 and twofold in experiment 2 compared with previous cultivation with buckwheat(non AM-host plant). However, there were no significant differences in colonization rate caused by manure application.

Profiling of the mineral elements in soybean seeds after different treatments

The results of 19 mineral elements concentrations in four different treatments were subjected to PCA (Fig. 3.2). Plots of the first and second principal component scores (i.e., PC1 and PC2, respectively) revealed differences in the mineral profiles. In experiment 1, with independent components that clearly corresponded to differences related to the manure application and previous crops, PC1 explains 62.7% and discriminated between the treatment with or without manure, while PC2 accounted for 27.5% of the total variance and discriminated between the previous crops: maize and buckwheat. Other factors contributed to the remaining 9.8% of variance. In experiment 2, PC1 accounted for 50.9% of the total variation and separated the manure treatments, but PC2 does not separate the previous crops.

Effect of previous crop and manure application on the mineral concentration in soybean seeds

An ANOVA (Table 3.4a, 3.4b) was conducted to clarify the effect of the previous cropping and manure application on the mineral concentration in the soybean seed. Regardless of the effect of manure, when the soybean was previously cropped with maize, there was a significant increase in the concentration of Cu (1.11-fold), but a significant reduction in Cd (0.78-fold) compared to previous cropping with buckwheat in experiment 1. In experiment 2, the concentrations of Cd and Ba decreased to 0.91- and 0.87-fold, respectively, when previously cultivated with maize compare with preciously cultivated with buckwheat. With manure application (Table 3.4a), the essential elements of Fe, Zn and Co in the soybean seed significantly increased (1.07-, 1.08- and 1.26-fold, respectively) in experiment 1. For the nonessential minerals, manure application significantly increased Cd (to 1.5-fold) and Ba (to 1.44-fold), but decreased Ni (to 0.76-fold) and Se (to 0.5-fold) concentrations in soybean seeds. For Cs, there was a nearly 0.39-fold due to manure application. However, it is difficult to detect any combined effect of manure and previous cultivation. In experiment 2 (Table 3.4b), the concentrations of three elements were altered by manure application, with Ba and Cd increasing to 1.24- and 1.15-fold, respectively, and Cs decreasing to 0.56-fold; these values were compatible with those of experiment 1

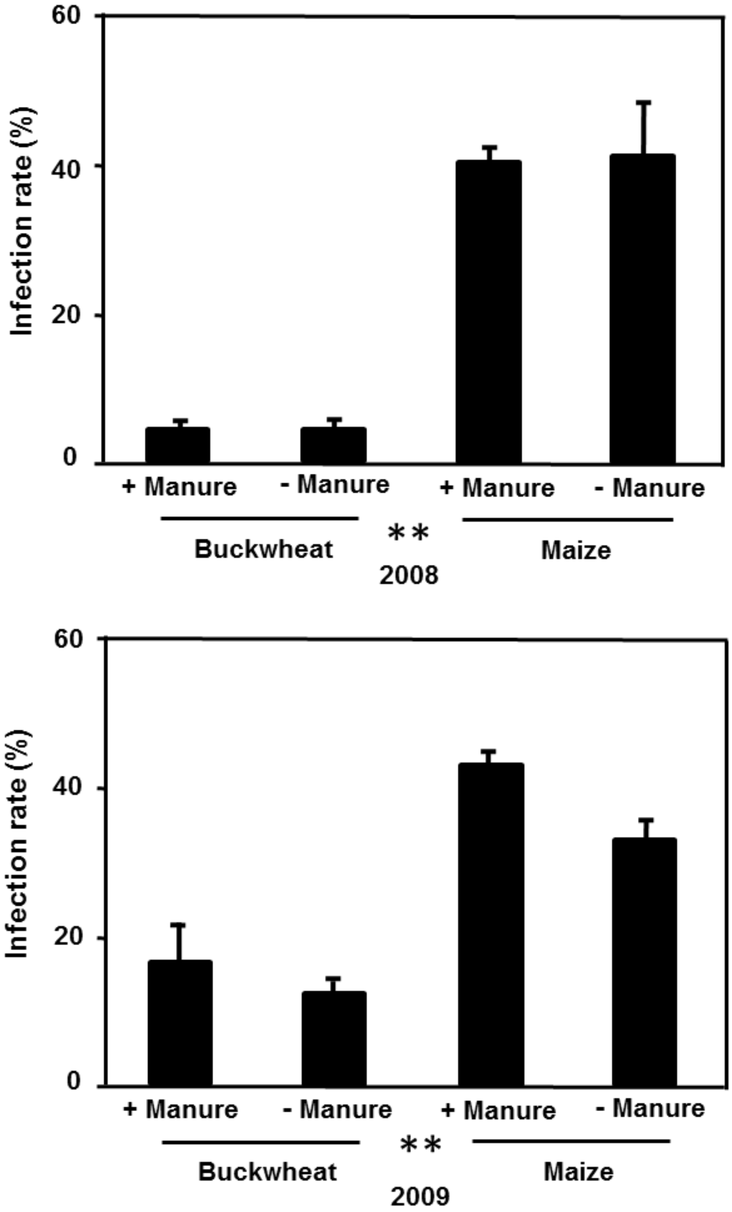


Fig. 3.1. Effects of the previous crop and manure application on the colonization of arbuscular mycorrhizal (AM).

Bars indicate the standard errors (n =4). ** indicates significant differences ($p < 0.001$) between treatments of different previous crops.

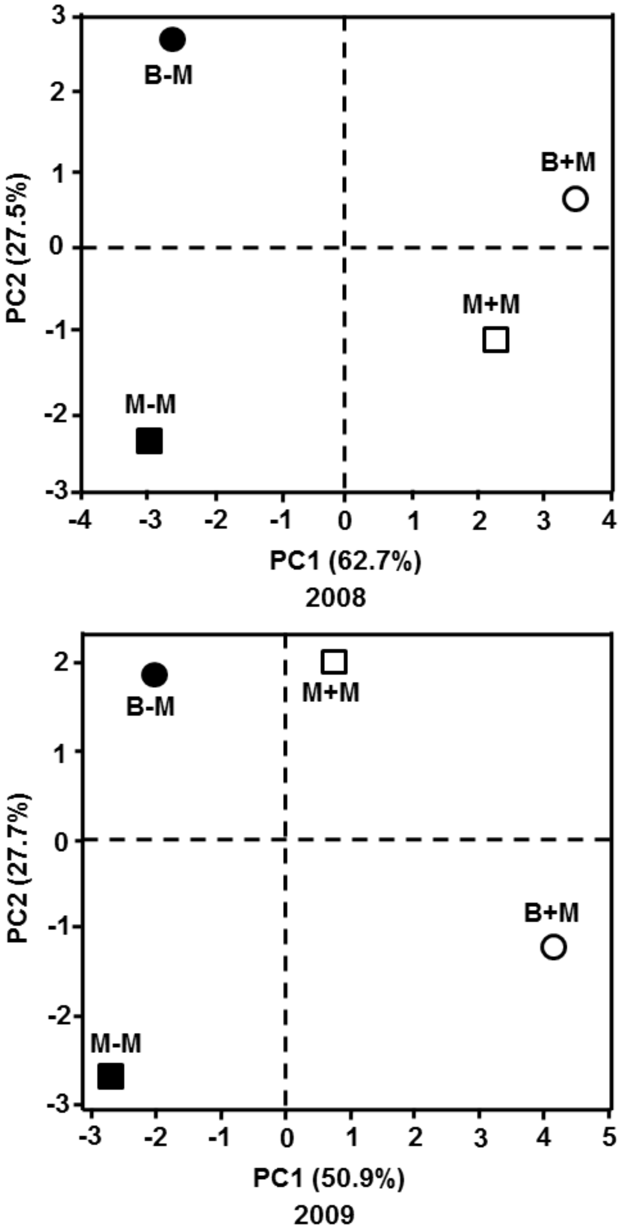


Fig. 3.2. Sample scores for the first (PC1) and second (PC2) principal components drawn from the principal component analysis for 19 mineral elements.

Labels next to the icons present the treatments: M, maize; B, buckwheat; +M, with manure application; −M, without manure application.

Table 3.1. Chemical properties of the soil in two experimental sites.

Site	pH	Available P_2O_5	Total N	CEC	Exchangeable cations ($cmol_c$ kg^{-1})			BS (%)
	(H_2O)	(mg kg^{-1}) [a]	(g kg^{-1})	($cmol_c$ kg^{-1})	Ca	Mg	K	
1	5.4	198	4.5	38.25	9.8	1.3	0.4	30.0
2	5.7	315	3.4	35.10	15.5	2.4	0.5	52.6

[a] P_2O_5 was determined by the Truog method; CEC, cation exchange capacity; BS, basic saturation.

Table 3.2 Total mineral elements in manure.

P	K	Mg	Ca	Mn	Fe	Cu	Zn	B
		g kg^{-1} DM					mg kg^{-1} DM	
7.72	25.87	10.85	17.55	0.40	3.7	65.6	200.04	13.84
Co	Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
				mg kg^{-1} DM				
3.76	5700	62.09	62.72	48.31	0.19	9.13	0.71	0.17

DM, dry matter; water contents, 32.5%.

Table 3.3a. Total mineral elements in soil (0-20cm) in experiment 1.

Treatment		K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co
		mg g^{-1}					μg g^{-1}				
B	+M	1.02	1.51	4.50	1.04	24.69	19.98	58.57	3.40	0.61	8.78
	–M	0.62	1.51	5.01	1.01	24.64	20.81	54.21	5.14	0.62	8.79
M	+M	0.98	1.51	4.22	0.86	24.97	19.80	66.30	2.88	0.60	8.26
	–M	0.64	1.54	5.30	1.24	26.56	21.47	58.95	5.09	0.69	10.51
Previous crop (A)[a]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Manure (B)[b]		**< 0.001**	NS	**< 0.01**	NS	NS	NS	NS	**0.05**	NS	NS
A*B[c]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Treatment		Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
		μg g^{-1}							
B	+M	384.75	45.43	293.02	175.24	0.27	13.20	0.56	2.24
	–M	336.97	45.32	246.13	173.09	0.28	12.94	0.67	2.12
M	+M	360.34	42.33	274.58	172.21	0.26	12.76	0.68	2.17
	–M	349.49	47 .08	245.58	187.12	0.29	13.34	0.78	2.22
Previous crop (A)[a]		NS	NS	NS	NS	NS	NS	NS	NS
Manure (B)[b]		NS	NS	**< 0.001**	NS	NS	NS	NS	NS
A*B[c]		NS	NS	NS	NS	NS	NS	NS	NS

Values are the means of 4 replications in each treatment. M indicates previous crop of maize, B is buckwheat; +M and –M indicates with and without manure application, respectively; Numbers and letters in rows [a], [b] and [c] indicate significances obtained by the ANOVA; nd, not detected by machine; NS, no significant difference.

Table 3.3b. Available mineral elements in soil in experiment 1.

Treatment		K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co
		mg g^{-1}					µg g^{-1}				
B	+M	0.44	0.17	0.78	51.82	2.00	0.21	0.23	0.29	0.02	0.04
	–M	0.15	0.16	1.58	46.49	5.90	0.14	0.21	0.52	0.02	0.03
M	+M	0.41	0.18	1.02	58.66	1.99	0.10	0.31	0.27	0.02	0.04
	–M	0.15	0.16	1.71	50.62	9.22	0.19	0.16	0.46	0.01	0.03
Previous crop (A)[a]		NS	NS	< 0.001	NS	NS	NS	NS	NS	NS	NS
Manure (B)[b]		< 0.001	NS	< 0.001	< 0.001	< 0.01	NS	< 0.05	< 0.01	NS	< 0.001
A*B[c]		NS	NS	< 0.05	NS	NS	NS	NS	NS	NS	NS

Treatment		Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
		µg g^{-1}							
B	+M	19.05	17.40	81.56	0.043	0.023	nd.	0.021	0.130
	–M	20.01	15.84	68.72	0.033	0.023	nd.	0.017	0.169
M	+M	27.10	17.25	82.29	0.052	0.024	nd.	0.021	0.125
	–M	20.57	15.40	65.20	0.032	0.024	nd.	0.014	0.168
Previous crop (A)[a]		NS	NS	NS	NS	NS	-	NS	NS
Manure (B)[b]		NS	NS	0.001	< 0.05	NS	-	NS	< 0.001
A*B[c]		NS	NS	NS	NS	NS	-	NS	NS

Table 3.3c. Total mineral elements in soil in experiment 2.

Treatment		K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co
		mg g^{-1}					μg g^{-1}				
B	+M	1.25	1.53	4.89	0.90	17.14	17.17	51.99	2.26	2.26	8.05
	–M	1.09	1.68	5.23	0.92	18.67	17.35	60.10	2.45	2.45	9.06
M	+M	1.29	1.59	4.69	0.90	20.03	17.14	53.46	2.34	2.34	8.43
	–M	0.99	1.65	5.40	0.85	17.85	16.63	54.18	4.29	4.29	7.81
Previous crop (A)[a]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Manure (B)[b]		< 0.001	NS	< 0.05	NS	NS	NS	NS	NS	NS	NS
A*B[c]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Treatment		Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
		μg g^{-1}							
B	+M	357.65	56.65	300.84	173.50	0.26	12.76	0.49	2.39
	–M	377.44	58.58	313.11	192.42	0.25	13.99	0.5	2.55
M	+M	337.2	57.51	322.76	195.00	0.26	13.84	0.52	2.58
	–M	369.4	56.73	298.25	184.12	0.27	12.92	0.50	2.48
Previous crop (A)[a]		NS	NS	NS	NS	NS	NS	NS	NS
Manure (B)[b]		NS	NS	NS	NS	NS	NS	NS	NS
A*B[c]		NS	NS	NS	NS	NS	NS	NS	NS

Table 3.3d. Available mineral elements in soil in experiment 2.

Treatment		K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co
		mg g^{-1}					µg g^{-1}				
B	+M	0.35	0.28	2.45	75.48	3.42	0.09	0.58	0.07	0.036	0.042
	–M	0.20	0.35	2.78	86.62	4.46	0.27	0.54	0.11	0.026	0.049
M	+M	0.44	0.32	2.11	91.72	3.36	0.33	0.56	0.16	0.028	0.05
	–M	0.15	0.28	2.27	77.84	3.61	0.20	0.61	0.15	0.068	0.043
Previous crop (A)[a]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Manure (B)[b]		< 0.001	NS	NS	NS	< 0.05	NS	NS	NS	NS	NS
A*B[c]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Treatment		Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
		µg g^{-1}							
B	+M	28.13	23.43	72.57	0.051	0.035	nd.	0.014	0.052
	–M	28.06	28.14	86.32	0.052	0.040	nd.	0.022	0.078
M	+M	33.23	27.14	85.29	0.051	0.039	nd.	0.020	0.056
	–M	23.32	25.24	76.85	0.047	0.038	nd.	0.019	0.069
Previous crop (A)[a]		NS	NS	NS	NS	NS	NS	NS	NS
Manure (B)[b]		NS	NS	NS	NS	NS	NS	NS	< 0.05
A*B[c]		NS	NS	NS	NS	NS	NS	NS	NS

Table 3.4a. Mineral concentrations in soybean seed in experiment 1.

Treatment		P	K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co
		mg g^{-1}				μg g^{-1}						
B	+M	6.85	18.22	2.65	1.64	38.12	91.91	17.09	54.19	25.98	0.31	0.15
	–M	6.66	17.63	2.57	1.71	36.71	86.40	18.12	49.22	28.68	0.19	0.12
M	+M	6.75	18.16	2.56	1.75	37.22	95.39	19.49	52.36	26.85	0.31	0.15
	–M	6.42	17.63	2.41	1.92	38.15	89.15	20.32	48.83	27.88	0.17	0.13
Previous crop (A)[a]		NS	NS	NS	NS	NS	NS	< 0.01	NS	NS	NS	NS
Manure (B)[b]		NS	NS	NS	NS	NS	< 0.05	NS	< 0.01	NS	NS	< 0.05
A*B[c]		NS	NS	NS	NS	NS	NS	NS	0.548	NS	NS	NS

		Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
		μg g^{-1}							
B	+M	7.75	5.61	8.44	2.86	0.047	1.48	0.04	0.016
	–M	6.84	5.76	5.61	2.93	0.031	1.18	0.08	0.036
M	+M	7.86	6.04	8.77	2.51	0.036	1.44	0.03	0.014
	–M	7.53	6.57	6.35	2.87	0.025	1.62	0.06	0.041
Previous crop (A)[a]		NS	NS	NS	NS	< 0.05	NS	NS	NS
Manure (B)[b]		NS	NS	< 0.05	< 0.05	< 0.01	NS	< 0.05	< 0.001
A*B[c]		NS	NS	NS	NS	NS	NS	NS	NS

Table 3.4b. Mineral concentrations in soybean seed in experiment 2.

Treatment		P	K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co
		mg g^{-1}				µg g^{-1}						
B	+M	5.91	16.51	2.30	1.68	31.86	76.08	13.25	41.17	24.41	0.36	0.064
	–M	5.63	17.09	2.35	1.73	32.28	69.36	13.26	40.52	24.23	0.44	0.056
M	+M	5.81	16.72	2.31	1.60	32.10	66.53	13.65	41.12	24.00	0.47	0.060
	–M	5.87	17.32	2.36	1.66	32.63	72.12	13.89	40.36	26.09	0.80	0.054
Previous crop (A)[a]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Manure (B)[b]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A*B[c]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Treatment		Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
		µg g^{-1}							
B	+M	10.71	5.64	7.69	1.76	0.058	1.39	0.055	0.005
	–M	6.90	6.05	6.30	1.60	0.047	0.97	0.052	0.007
M	+M	7.47	5.24	6.79	1.67	0.049	1.24	0.04	0.004
	–M	7.64	5.46	5.34	1.70	0.047	1.31	0.08	0.009
Previous crop (A)[a]		NS	NS	< 0.05	NS	< 0.05	NS	NS	NS
Manure (B)[b]		NS	NS	< 0.01	NS	< 0.05	NS	NS	< 0.05
A*B[c]		NS	NS	NS	NS	NS	NS	NS	NS

3.4 Discussion

Effect of manure on ionomic profile changes

Manure can supply soil with mineral elements directly and can improve mineral availability indirectly by changing the pH and biological activity in soil (Amiri and Fallahi, 2011). At the same time, manure provides more stable humic substances with large surface areas and components of long-chain fatty acids, aliphatic alcohols and linear hydrocarbons, which account for the fixation of minerals (Staunton and Roubaud, 1997). Heavy metals have been reported to display low mobility in the soil profile due to adsorption on organic matter, e.g., Cs and Cd (Ciecko et al., 2001, Rautaray et al., 2003). However, the ability of organic substances to fix minerals is influenced by the types and properties of organic amendments (Norvell et al., 2000, Singh and Agrawal, 2007). In the present study, the nonessential elements Cs, Ba and Cd in soybean seeds were the most strikingly divergent ions in both experiments, with manure treatments containing 0.39 and 0.56 times less Cs, 1.44 and 1.24 times more Ba, and 1.5 and 1.15 times more Cd than the treatment without manure in experiments 1 and 2, respectively. It has been suggested that organic matter may modify the capacity of clays to immobilize minerals, and the affinity of cations to the clays follows the order Cs > NH_4^+ > K > Na > Ca (Staunton and Roubaud, 1997). In soil, available Cs is significantly decreased by manure application, which might be the main reason for lower Cs deposition in soybean seeds (Kim et al., 2007). Conversely, it is known that K and Cs enter the root though the same channel.

In the present experiments, total K and available K in soil were significantly enriched by 2–3-fold with manure application, leading to a high accumulation of K around the root zone (Table 3.3a–d), which may have inhibited root uptake of Cs (Zhu and Smolders, 2000). The behavior of Cd and Ba in soybean seeds was less correlated with their availabilities in soil solution, which had a higher accumulation in the manure treatment, even when the available Cd in soil was stable in both experiments and only Ba was enhanced in experiment 1 by manure application. It has been extensively reported that Cd enters plants via transport processes that normally function in Fe uptake (Clemens, 2001, Siedlecka and Krupa, 1999), while Ca^{2+} channels in the plasma membrane or root cells show significant discrimination between divalent cations, and Ba^{2+} is more permeable than either Sr^{2+} or Ca^{2+} (White, 2001). The decrease of available Fe and Ca in soil might be possible reasons for the higher Cd and Ba concentrations in the soybean seed after manure treatments. In the present study, there are no adequate data to clarify the whole metabolic processing of elements, such as translocation, circulation, distribution and deposition within the plants, and therefore this subject requires further research. Phosphorus, K, Mg, Ca, Mn, Cu, B, Mo, Na, Sr and Cr contents in soybean seeds were constant regardless of the manure treatments. It is highly likely that constant Mg, Cu, Na, Sr and Mo content in soybean seeds in the treatment with manure application were caused by the constant pool of available minerals in soil. While K, Ca, Mn, Cu, B, Ba, Cd and Na in the seed showed little correlations with available minerals in the soil, which means the mineral concentration in seed was not only determined by the mineral's availability in the soil, but also affected by the dilution effect, plant active absorption, and antagonism (Berreck and Haselwandter, 2001, Baxter, 2010).

In addition to Ba, Cd and Cs, nearly half of the elements that were found in the soybean seed were significantly changed by manure application in Experiment 1, including increases for Fe, Zn and Co and a decrease for Ni (Table 3.4a). However, these differences did not occur in Experiment 2. It is likely that the alterations in the mineral content of the soybean seed correspond closely with the available minerals in the soil solution in the two experiments. There may be significant differences in soil available elements and in soybean seeds between the treatments in experiment 1, but not in experiment 2, due to the higher average rainfall in 2009 of 1.4-fold that in 2008. Drought can affect the release of soluble trace elements into the soil solution via the lysis of bacterial cells and the destruction of soil aggregates in drying–rewetting events. Increases in soil moisture also improve the soil diffusion capacity and increase the activities of some enzymes, which increase the plant capture capacity for certain elements. Climatic change can also affect plant metabolism and the internal distribution of elements, thereby changing mobilization and retranslocation of elements within plant organs (Quadir et al., 2011). It was reported that drought increased As and Cd in *Erica multiflora* stems, and decreased Cu in leaves, Ni in stems and lead (Pb) in leaf litter of *Globularia alypum* (Sardans et al., 2008). Furthermore, a number of soil chemical, soil biological and external factors contribute to the variance of the soil pool and distribution in plant (Baghour et al., 2002a, Baghour et al., 2002b). The extractable concentrations of Zn, Cu, Cd and Pb increased with increasing soil pH at the highest rate of addition of a co-composted material, in contrast to the usual response to increasing soil pH, which generally reduces the availability of heavy metals in soil (Smith, 2009). Cationic metals can form insoluble complexes with P, and reduce the bioavailability of some heavy metals (Maenpaa et al., 2002). The differences of pH and available P in the two sites (Table 3.1) may also explain the different results in two years.

Effect of AM-fungi colonization on ionomic profile changes

AM fungi regulate the host plant ionome by modulating membrane transport proteins that control the nutrition and ion homeostasis of the host, influencing its ions and water absorption; in turn, the host plant provides photoassimilates necessary for fungal energy supply, growth and reproduction (Sanchez et al., 2000). Immobilization and mobilization of heavy metal cations such as Cd, Zn and Cs, by AM fungi have been studied for many years (Berreck and Haselwandter, 2001, Clemens, 2001, Joner et al., 2000). However, both the increase and reduction in metal content in the host plant have been observed, depending on the growth conditions as well as on the fungi and plant species involved. In the present study, previous cropping with the AM-host plant (maize) increased the AM fungi colonization rate in the soybean growth season compared to precious cropping with the non-AM host plant (buckwheat) (Fig. 3.1), but there were lower Cd concentrations in soybean seed (Table 3.4a, 3.4b) when the previous crop was maize (AM-host crop) in both experiments. This finding may be a consequence of the dilution effects caused by the plant greater growth (the rate of increase was 10% in

2008 and 9% in 2009 compared with previous cropping with buckwheat) or exclusion by precipitation of polyphosphate granules, and compartmentalization into the seed (Garg and Aggarwal, 2011, Azcón et al., 2010, Kruglov et al., 2009). In addition, the Cu and Ba levels in the soybean seed increased in the two experiments, in accordance with the AM infection rate, suggesting that Cu and Ba might be transported by AM fungi to the host plant or the indirect effect of other nutrient uptake and resulted the elemental balance. Moreover, the same results were recorded for an experiment in which the soybean seed ionome was affected by the previous crop and conducted at the same site 1 during 2006–2007: Cu was increased and Cd decreased in the soybean seed by previous cropping with maize (data not shown). Although there are many reports related to the correlation of mineral translocation and AM fungi infection, direct evidence clarifying the mechanism between AM fungi infection and element uptake by plants is insufficient, and requires more attention.

Transportability of elements from soil to seed in different treatments

The performance of each element in soybean seeds and ammonium acetate (NH_4-AC) extracted soil solution is shown in Fig. 3.3, as well as their transportability from soil to seed. Seed element concentrations varied by eight orders of magnitude from the highest for K to the lowest for Cs, while in soil, the highest concentration was for Ca and the lowest was for Se (Fig. 3.3). K, Mg and Ca were present at relatively high concentrations both in soybean seed and soil solution, reflecting their natural abundance and their ease of transport into plants (Kanter et al., 2010). Based on this study, Ba, Na, Cs and Sr have lower transportability from soil to soybean seed, while Zn, B, K, Ni and Cu have higher transportability.

Correlation among elements

The homeostatic mechanisms that control the levels of different elements are reported to be interconnected, and certain interconnections are largely independent of the organ, population or environment (Eide et al., 2005, Ghandilyan et al., 2009). In the present study, correlations among elements in the two experiments were investigated (Table 3.5). There were significant positive correlations between K and Mg and; K and P; and negative correlations between K and Cs. Additional positive correlations were found between Ca and Sr; Fe and Ba; Co and Cd, Co and Ba; Na and Cr.

Many surveys have suggested correlations among minerals in the same group (Watanabe et al., 2007, Quadir et al., 2011, Chen et al., 2009a). For example, Na, K and Cs use a similar translocation mechanism in plants because of their chemical similarities, and increasing K availability in the soil could reduce Cs concentration in the plant because K is more effectively transported to the shoot than Na and Cs in Fabaceae – similarly to the present study's results on the relationship between K and Cs (Table 3.5) in the soybean seed (Isaure et al., 2006). Sr can compete with the transporter for Ca when Sr is at a notably high level. In this experiment, which is contrary to the normal results, the Ca and Sr in soybean seed showed a significant positive correlation (Table 3.5), suggesting co-transport and co-chelation in the soybean. Compared with other plants (Ghandilyan et al., 2009), growth media (hydroponic or pot experiment) and different organs of plants (Waters and Grusak, 2008), negative correlations have also been found, possibly caused by limited availability of transport proteins or chelator molecules causing competition between minerals. It is possible that Ca uptake channels might also be regulated by other divalent ions, e.g., Mg^{2+}, Cu^{2+}, Fe^{2+}, Cd^{2+} and Ba^{2+} (White, 2001, Karley and White, 2009). Therefore, an important source of the toxicity of heavy metal elements is their chemical similarity with essential elements, deregulating the homeostasis of the essential elements or causing their displacement from proteins (Verbruggen et al., 2009).

Moreover, as Co shares high chemical similarity with Ni, it was thought that the two elements entered cells by the same plasma membrane carriers; however, they were found to be co-regulated in *Lotus japonicas* (Chen et al., 2009a). In the present study, we did not find the significant correlations between Co and Ni, but there were positive correlations between Co and Ba and between Co and Cd, suggesting that the different plants and different media will caused the different results.

In summary, the present study provides evidence of the effects of previous crop and manure application on the ionome of the soybean seed and detailed information regarding element interactions. These findings suggested that manure application and previous crop should be given high attention in agricultural development and ionomic studies, which is relevant to food safety and the phyto-extraction of heavy metal elements from soil.

Table 3.5. Correlation coefficients for seed composition of mineral elements in two experiments.

		Mg	K	Ca	Mn	Fe	Ni	Cu	Zn	Sr	Cd	Ba	Cr	Na	Co	Se	Cs	P	Mo
K	08	0.6[a]																	
	09	0.7[b]																	
Ca	08	-0.0	0.0																
	09	0.4	0.4																
Mn	08	0.5[a]	0.4	0.6[a]															
	09	-0.6[a]	-0.5	-0.0															
Fe	08	0.5[a]	0.5	0.4	0.4														
	09	0.2	0.2	0.4	0.2														
Ni	08	0.2	-0.2	0.2	0.4	-0.2													
	09	0.0	-0.2	0.1	0.3	0.7[b]													
Cu	08	-0.3	-0.4	0.4	0.2	0.2	0.1												
	09	0.7[b]	0.8[b]	0.3	-0.6[a]	-0.0	-0.3												
Zn	08	0.1	0.4	-0.4	-0.2	0.2	-0.4	-0.7[b]											
	09	0.6[a]	0.5[a]	0.5[a]	-0.5	0.1	0.1	0.7[b]											
Sr	08	-0.2	-0.0	0.8[b]	0.4	0.3	-0.0	0.5	-0.4										
	09	-0.4	-0.2	0.5[a]	0.7[b]	0.5	0.3	-0.4	-0.2										
Cd	08	0.3	0.3	-0.7[b]	-0.3	-0.0	-0.3	-0.7[b]	0.8[b]	-0.6[a]									
	09	0.4	0.5	0.1	-0.1	0.3	0.0	0.3	0.2	0.1									
Ba	08	0.2	0.5	0.2	0.1	0.6[a]	-0.5	-0.2	0.6[a]	0.4	0.3								
	09	0.4	0.4	0.5	-0.1	0.5[a]	0.4	0.3	0.5	0.4	0.7[b]								
Cr	08	-0.3	-0.2	0.1	-0.0	0.2	0.1	0.4	0.0	0.2	-0.0	0.2							
	09	0.1	-0.4	-0.6	-0.1	0.1	0.5	-0.5	-0.5	-0.3	-0.0	-0.1							
Na	08	0.1	0.3	0.0	0.1	0.3	-0.2	0.0	0.3	0.0	0.3	0.3	0.8[b]						
	09	0.1	-0.3	-0.1	-0.0	0.6[a]	0.8[b]	-0.4	-0.1	0.0	-0.1	0.1	0.7[b]						
Co	08	-0.1	0.3	-0.5[a]	-0.3	0.1	-0.5	-0.4	0.8[b]	-0.3	0.8[b]	0.5[a]	0.1	0.3					
	09	0.6[a]	0.5	0.1	-0.3	0.4	0.0	0.5	0.3	-0.2	0.7[b]	0.5[a]	-0.0	-0.0					
Se	08	-0.0	0.1	0.2	0.0	0.0	0.4	-0.3	0.2	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1				
	09	-0.3	-0.4	0.2	0.3	0.4	0.4	-0.3	0.1	0.3	-0.5	-0.3	0.1	0.5	-0.5				
Cs	08	-0.4	-0.5[a]	0.5	0.1	-0.5	0.5	0.3	-0.7[b]	0.4	-0.7[b]	-0.6[a]	0.0	-0.2	-0.7[b]	0.2			
	09	0.2	-0.5[a]	0.4	-0.1	0.4	0.0	0.3	0.1	0.3	0.1	0.1	-0.1	0.1	0.2	0.5			
P	08	0.4	0.5[a]	-0.5	0.2	0.1	-0.1	0.0	0.1	-0.3	0.3	-0.1	-0.3	0.0	0.2	-0.1	-0.5		
	09	0.8[b]	0.7[b]	0.2	-0.7[b]	0.2	-0.0	0.8[b]	0.6[a]	-0.6[a]	0.4	0.3	0.0	0.0	0.7[b]	-0.2	0.3		
Mo	08	0.1	0.1	-0.1	-0.4	0.4	-0.2	-0.3	0.6[b]	-0.2	0.4	0.5[a]	0.1	0.1	0.6[a]	0.4	-0.5[a]	-0.2	
	09	-0.1	-0.4	0.1	0.0	0.1	0.4	-0.1	0.3	-0.0	-0.7[b]	-0.2	0.2	0.9[b]	-0.2	0.8[b]	0.0	0.1	
B	08	0.3	-0.1	0.4	0.4	0.2	0.5[a]	0.5	-0.8[b]	0.2	-0.7[b]	-0.6[a]	-0.1	-0.2	-0.7[b]	0.3	0.5	0.1	-0.4
	09	-0.2	-0.0	-0.1	0.4	0.4	-0.1	-0.1	-0.4	0.2	-0.0	-0.2	-0.1	-0.1	0.3	0.2	0.3	-0.1	-0.1

Coefficients are derived from pair-wise regression of mineral elements in two years.

[a],significant at 0.05 probability level. [b]significant at 0.01 probability level.

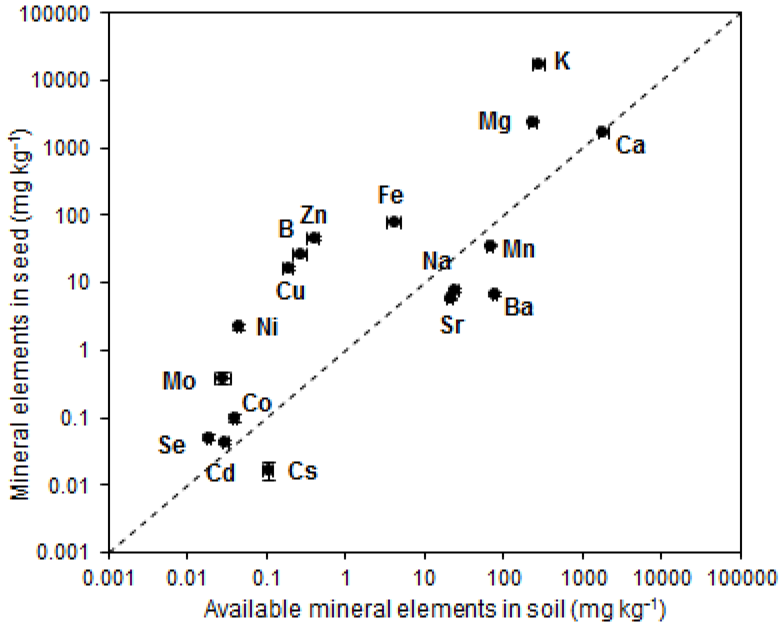


Fig. 3.3 Transportability of element from soil to seed

Bars indicated the standard errors (n = 32)

General conclusion

Soybean (*Glycine max* (L.) Merr.) is one of the most important seed crops in the world and supplies protein, oil, and mineral nutrients for human and animal consumption. The soybean ionome represents its mineral nutrients and trace elements of the plant, which is a dynamic network of elements that are controlled by the physiology and biochemistry of the plant, and ultimately controlled by the genome, in response to the various environmental factors. Any alterations in these processes that transport inorganic ions from the soil to the seed could affect the soybean ionome and could eventually influence human health through the food chain. Thus, it is important to investigate the linkages between the soybean ionome and agricultural practices to develop strategies for better nutrient management and food security of the sustainable agriculture. Fertilization and crop rotation are fundamental agronomic practices used to improve growth and yield in soybean cultivation, and as important environmental factors, they should have impacts on the ionome of soybean, also. Hence, three independent experiments were carried out to investigate the environmental control of ionome in soybean.

Firstly, field experiments were conducted to investigate the impacts of fertilizer applications on the concentration of 21 mineral elements of soybean shoots with 44 varieties. Soybeans were cultivated in the field with three different fertilizer treatments: manure (M, 20 t/ha), chemical fertilizer (20 kg N/ha, 80 kg P_2O_5/ha and 80 kg K_2O/ha) and only with potassium fertilizer (80 kg K_2O/ha). Sixty days after sowing, soybean shoots were sampled, and analyzed by using ICP-MS. On the basis of this experiments, the mean concentrations of the 21 elements in soybean shoots vary in the order N (nitrogen) > K (potassium) > Ca (calcium) > Mg (magnesium) > P (phosphorus) > Fe (iron) > Na (sodium) > Sr (strontium) > Mn (manganese) > B (boron) > Ba (barium) > Zn (zinc) > Cr (chromium) > Ni (nickel) > Cu (copper) > V (vanadium) > Li (lithium) > Co (cobalt) > Se (selenium) > Cs (cesium) > Cd (cadmium). The concentrations of P, K, Sr, Ba and B showed significant variation associated with fertilizer treatments, whilst Cd was sensitive to soybean genotypes. Genotype × fertilizer interactions were indicated for other elements (N, Ca, Mg, Fe, Na, Mn, Zn, Cr, Ni, Cu, V, Li, Co, Se and Cs). Principal component analysis (PCA) revealed the ionomic response of soybean shoots to fertilizer treatment, with the increase of P, K, Sr, Zn and Ba in manure treatment, and increase of Co, Fe, Cs, Ni, V and Cr in the chemical fertilizer treatment.

In the second experiment, the distribution of mineral elements in plant tissues during vegetative stages were detected by growing soybean (cv. Tsurumusume) in the field which previous cropped with arbuscular mycorrhizal (AM) host crop and P application. The experiment ran for two years with a split-plot design. AM host plant (sunflower) or a non-AM host plant (mustard) were cultivated in the field in the first year as the main plots, and 3 P levels (0, 50, 150 kg P_2O_5/ha) were set during the soybean growth season as split plots from both main plots. Soybeans were sampled at four important growth stages (seedling, late flowering, mid-mature, and mature) and separated into leaf, stem, root, pod and seed for the elemental analysis. Based on the experiment, it was found that large amounts of Ca accumulated in the shoots, especially in leaves, while Fe, Ni, Na, Co, Mo, Cd, Cr, Se, Li and Cs remained in roots during all the soybean vegetative stages. Moreover, Cu concentrations in soybean were significantly increased by prior cultivation with the AM host plant, regardless of the tissue and vegetative stage, suggesting that AM fungi has the ability of transporting Cu from soil to host plant.

As ionome of seed is a consequence of complex plant process and plant-environment interactions with serious nutritional implications to human health, the third experiment were conducted to investigate the effects of previous cultivation of an AM host plant and manure application on the concentration of mineral elements in soybean (cv. Tsurumusume) seeds. Soybeans were cultivated after growing either an AM host plant (maize) or a non-AM host plant (buckwheat) in the first year in the main plots, with manure application (0 and 20 t/ha) during the soybean season in split plots from both main plots. On the basis of this experiment, manure application significantly increased the available K and decreased the available Fe and Cs in the soil. However, higher concentrations of Cd and Ba and lower concentrations of Cs in the seed were induced by the application of manure. Cd levels in the seed were decreased by prior cultivation with the AM host plant.

In conclusion, this study offers detailed information of the elemental distribution and interactions in soybean, reveals genotypic and environmental control of the ionome of soybean, and develops the understanding of the correlations among the ionomic study, agricultural practice, and food safety.

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References

Ai C, Liang G, Sun J, Wang X, He P, Zhou W. 2013. Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. *Soil Biology and Biochemistry,* **57**: 30-42.

Amiri ME, Fallahi E. 2011. Impact of Animal Manure on Soil Chemistry, Mineral Nutrients, Yield and Fruit Quality in 'Golden Delicious' Apple.pdf. *Journal of Plant Nutrition,* **32**: 610-617.

Arao T, Ae N, Sugiyama M, Takahashi M. 2003. Genotypic differences in Cadmium uptake and distribution in soybeans. *Plant and Soil,* **251**: 247-253.

Azcón R, del Carmen Perálvarez M, Roldán A, Barea JM. 2010. Arbuscular mycorrhizal fungi, Bacillus cereus, and Candida parapsilosis from a multicontaminated soil alleviate metal toxicity in plants. *Microbial ecology,* **59**: 668-677.

Baghour M, Moreno DA, Villora G. 2002a. The infuluence of the root zone temperatures on the phytoextraction of boran and alumminium with potato plants growing in the field. *J. Environ. sci. Health,* **37**: 939-953.

Baghour M, Moreno DA, Villora G. 2002b. Root-zone Temperature influences the distribution of Cu and Zn in Potato-Plant Organs. *Journal of Agriculture and*

Food Chemistry, **50**: 140-146.

Baxter I. 2009. Ionomics: studying the social network of mineral nutrients. *Curr Opin Plant Biol,* **12**: 381-6.

Baxter I. 2010. Ionomics: The functional genomics of elements. *Brief Funct Genomics,* **9**: 149-56.

Baxter I, Dilkes BP. 2012. Elemental profiles reflect plant adaptations to the environment. *Science,* **336**: 1661-3.

Baxter I, Hermans C, Lahner B, Yakubova E, Tachibana M, Verbruggen N, Chao DY, Salt DE. 2012. Biodiversity of mineral nutrient and trace element accumulation in *Arabidopsis thaliana*. *PLoS one,* **7**: e35121.1-12.

Baxter I, Ouzzani M, Orcun S, Kennedy B, Jandhyala SS, Salt DE. 2007. Purdue ionomics information management system. An integrated functional genomics platform. *Plant Physiol,* **143**: 600-11.

Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot ML, Salt DE. 2008. The leaf ionome as a multivariable system to detect a plant's physiological status. *Proc Natl Acad Sci U S A,* **105**: 12081-6.

Benkeblia N, Shinano T, Osaki M. 2007. Metabolite profiling and assessment of metabolome compartmentation of soybean leaves using non-aqueous fractionation and GC-MS analysis. *Metabolomics,* **3**: 297-305.

Berreck M, Haselwandter M. 2001. Effect of the arbuscular mycorrhizal symbiosis upon uptake of cesium and other cations by plants.pdf. *Mycorrhiza,* **10**: 275-280.

Bilyeu KD, Ratnaparkhe MB, Kole C. 2010. *Genetics, genomics and breeding of soybean,* Enfield, NH

Boca Raton, FL, Science Publishers ;

Distributed by CRC Press.

Bogdanovic D, Ubavic M, Cuvardic M. 1999. Effect of phosphorus fertilization on Zn and Cd contents in soil and corn plants. *Nutrient Cycling in Agroecosystems,* **54**: 49-56.

Botwright Acuña TL, Wade LJ. 2012. Genotype × environment interactions for root depth of wheat. *Field Crops Research,* **137**: 117-125.

Broadley MR, Bowen HC, Cotterill HL, Hammond JP, Meacham MC, Mead A, White PJ. 2004. Phylogenetic variation in the shoot mineral concentration of angiosperms. *Journal of Experimental Botany,* **55**: 321-336.

Broadley MR, White PJ. 2010. Eats roots and leaves. Can edible horticultural crops address dietary calcium, magnesium and potassium deficiencies? *Proc Nutr Soc,* **69**: 601-12.

Broadley MR, White PJ, Hammond JP, Zelko I, Lux A. 2007. Zinc in plants. *New Phytol,* **173**: 677-702.

Brown PH, Shelp BJ. 1997. Boron mobility in plants. *Plant and Soil,* **193**.

Buescher E, Achberger T, Amusan I, Giannini A, Ochsenfeld C, Rus A, Hoekenga O, Yakubova E, Harper JF, Guerinot ML, Zhang M, Salt DE, Baxter I. 2012. Natural Genetic Variation in Selected Populations of *Arabidopsis thsliana* Is Associated with Ionomic Difference. *PLoS one,* **5**: e11081.1-10.

Bystrzejewska-Piotrowska G, Urban PL. 2004. Accumulation and translocation of cesium-137 in onion plants (Allium cepa). *Environmental and Experimental Botany,* **51**: 3-7.

Cacagnaro TR, Jackson LE, Six J, Ferris H, Goyal H, Asami D, Scow KM. 2006. Arbuscular mycorrhizas, microbial communities, nutrient availability and soil aggregates in organic tomato production. *Plant and Soil,* **282**: 209-225.

Chao DY, Gable K, Chen M, Baxter I, Dietrich CR, Cahoon EB, Guerinot ML, Lahner B, Lu S, Markham JE, Morrissey J, Han G, Gupta SD, Harmon JM, Jaworski JG, Dunn TM, Salt DE. 2011. Sphingolipids in the root play an important role in regulating the leaf ionome in Arabidopsis thaliana. *Plant Cell,* **23**: 1061-81.

Chen Z, Watanabe T, Shinano T, Ezawa T, Wasaki J, Kimura K, Osaki M, Zhu YG. 2009a. Element interconnections inLotus japonicus: A systematic study of the effects of element additions on different natural variants. *Soil Science and Plant Nutrition,* **55**: 91-101.

Chen Z, Watanabe T, Shinano T, Okazaki K, Mitsuru O. 2009b. Rapid characterization of plant mutants with an altered ion-profile: a case study using Lotus japonicus. *New Phytol*, **181**: 795-801.

Cheng WD, Zhang GP, Yao HG, Wu W, Xu M. 2006. Genotypic and environmental variation in cadmium, chromium, arsenic, nickel, and lead concentrations in rice grains. *J Zhejiang Univ Sci B,* **7**: 565-71.

Cheng Y, Ishimoto K, Kuriyama Y, Osaki M, Ezawa T. 2012. Ninety-year-, but not single, application of phosphorus fertilizer has a major impact on arbuscular mycorrhizal fungal communities. *Plant and Soil,* **365**: 397-407.

Ciecko Z, Wyszkowski M, Krajewski W, Zabielska J. 2001. Effect of organic matter and liming on the reduction of cadmium uptake from soil by triticale and spring oilseed rape. *The Science of the Total Environment,* **281**: 37-45.

Citak S, Sonmez S. 2009. Mineral contents of organically and conventionally grown spinach (Spinacea oleracea L.) during two successive seasons. *J Agric Food Chem,* **57**: 7892-8.

Citak S, Sonmez S. 2010. Influence of organic and conventional growing conditions on the nutrient contents of white head cabbage (Brassica oleracea var. capitata) during two successive seasons. *J Agric Food Chem,* **58**: 1788-93.

Clark RB. 1983. Plant genotype differences in the uptake, translocation, accumulation, and use of mineral elements required for plant growth. *Plant* *and Soil,* **72**: 175-196.

Clemens S. 2001. Molecular mechanisms of plant metal tolerance and homeostasis. *Planta,* **212**: 475-486.

Declerck S, Boulois HD, Bivort C, Delvaux B. 2003. Extraradical mycelium of the arbuscular mycorrhizal fungus Glomus lamellosum can take up, accumulate and translocate radiocaesium under root-organ culture conditions. *Environmental Microbioglogy,* **5**: 510-516.

Diez JA, Cartagena MC, Vallejo A. 1992. Controlling phosphorus fixation in calcareous soils by using caated diammonium phosphate. *Fertilizer Research,* **31**: 269-274.

Drinkwater LE, Wagoneer P, Sarrantonio M. 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. *Nature,* **396**: 262-264.

Ehlken S, Kirchner G. 2002. Environmental processes affecting plant root uptake of radioactive trace elements and variability of transfer factor data: a review. *Journal of environmental radioactivity,* **58**: 97-112.

Eide DJ, Clark S, Nair TM, Gehl M, Gribskov M, Guerinot ML, Harper JF. 2005. Characterization of the yeast ionome: a genome-wide analysis of nutrient mineral and trace element homeostasis in Saccharomyces cerevisiae. *Genome Biol,* **6**: R77.

Güsewell S. 2004. N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist,* **164**: 243-266.

Gadd GM. 2004. Microbial influence on metal mobility and application for bioremediation. *Geoderma,* **122**: 109-119.

Garg N, Aggarwal N. 2011. Effects of Interactions Between Cadmium and Lead on Growth, Nitrogen Fixation, Phytochelatin, and Glutathione Production in Mycorrhizal Cajanus cajan (L.) Millsp. *Journal of Plant Growth Regulation*: 1-15.

Ghandilyan A, Ilk N, Hanhart C, Mbengue M, Barboza L, Schat H, Koornneef M, El-Lithy M, Vreugdenhil D, Reymond M, Aarts MG. 2009. A strong effect of growth medium and organ type on the identification of QTLs for phytate and mineral concentrations in three Arabidopsis thaliana RIL populations. *J Exp Bot,* **60**: 1409-25.

Gilliham M, Dayod M, Hocking BJ, Xu B, Conn SJ, Kaiser BN, Leigh RA, Tyerman SD. 2011. Calcium delivery and storage in plant leaves: exploring the link with water flow. *J Exp Bot,* **62**: 2233-50.

Gong JM, Lee DA, Schroeder JI. 2003. Long-distance root-to-shoot transport of phytochelatins and cadmium in Arabidopsis. *Proc Natl Acad Sci U S A,* **100**: 10118-23.

Grusak MA. 1994. Iron transport to developing Ovules of Pisum sativum. *Plant physiol.,* **104**: 649-655.

Grusak MA, Dellapenna D, Welch RM. 1999. Physiologic processes affecting the content and distribution of phytonutrients in plants. *Natr. Rev.,* **57**: 26-33.

Gryndler M, Hrselova H, Cajthaml T, Havrankova M, Rezacova V, Gryndlerova H, Larsen J. 2009. Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. *Mycorrhiza,* **19**: 255-66.

Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX, Zhang WF, Christie P, Goulding KW, Vitousek PM, Zhang FS. 2010. Significant acidification in major Chinese croplands. *Science,* **327**: 1008-10.

Gyuricza V, Declerck S, Dupre de Boulois H. 2010. Arbuscular mycorrhizal fungi decrease radiocesium accumulation in Medicago truncatula. *J Environ Radioact,* **101**: 591-6.

Han WX, Fang JY, Reich PB, Ian Woodward F, Wang ZH. 2011. Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecol Lett,* **14**: 788-96.

Hermans C, Chen J, Coppens F, Inze D, Verbruggen N. 2011. Low magnesium status in plants enhances tolerance to cadmium exposure. *New Phytol,* **192**: 428-36.

Hsu JH, Lo SL. 2001. Effect of comosting on characterization and leaching of copper, manganese, and zinc from swine manure. *Environmental Pollution,*

114: 119-127.

Humphries PD, Devonald VG. 1977. The distribution of potassium, calcium and magnesium in young tomato plants grown in water culture. *Plant and Soil,* **48**: 435-455.

Isaure MP, Fraysse A, Deves G, Le Lay P, Fayard B, Susini J, Bourguignon J, Ortega R. 2006. Micro-chemical imaging of cesium distribution in Arabidopsis thaliana plant and its interaction with potassium and essential trace elements. *Biochimie,* **88**: 1583-90.

Ishikawa S, Abe T, Kuramata M, Yamaguchi M, Ando T, Yamamoto T, Yano M. 2010. A major quantitative trait locus for increasing cadmium-specific concentration in rice grain is located on the short arm of chromosome 7. *J Exp Bot,* **61**: 923-34.

Jeong ML, Jiang H, Chen HS, Tsai CJ, Harding SA. 2004. Metabolic profiling of the sink-to-source transition in developing leaves of quaking aspen. *Plant Physiol,* **136**: 3364-75.

Joner EJ, Briones R, Leyval C. 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant and Soil,* **226**: 227-234.

Kanter U, Hauser A, Michalke B, Draxl S, Schaffner AR. 2010. Caesium and strontium accumulation in shoots of Arabidopsis thaliana: genetic and physiological aspects. *J Exp Bot,* **61**: 3995-4009.

Karley AJ, White PJ. 2009. Moving cationic minerals to edible tissues: potassium, magnesium, calcium. *Curr Opin Plant Biol,* **12**: 291-8.

Kim Y, Kim K, Kang HD, Kim W, Doh SH, Kim DS, Kim BK. 2007. The accumulation of radiocesium in coarse marine sediment: effects of mineralogy and organic matter. *Mar Pollut Bull,* **54**: 1341-50.

Kovacevic V, Vragolovic A. 2011. Genotype and environmental effect on cadmium concentration in maize. *Journal of Life Science,* **5**: 926-932.

Kruglov SV, Anisimov VS, Lavrent'eva GV, Anisimova LN. 2009. Parameters of selective sorption of Co, Cu, Zn, and Cd by a soddy-podzolic soil and a chernozem. *Eurasian Soil Science,* **42**: 385-393.

Kurz，H., Schulz R, Romheld V. 1999. Selection of cultivars to reduce the concentration of cadmium and thallium in food and fodder plant. *J.Plant Nutr. Soil Sci.,* **162**: 323-328.

Lahner B, Gong J, Mahmoudian M, Smith EL, Abid KB, Rogers EE, Guerinot ML, Harper JF, Ward JM, McIntyre L, Schroeder JI, Salt DE. 2003. Genomic scale profiling of nutrient and trace elements in Arabidopsis thaliana. *Nat Biotechnol,* **21**: 1215-21.

Li G, Nunes L, Wang Y, Williams PN, Zheng M, Zhang Q, Zhu Y. 2013. Profiling the ionome of rice and its use in discriminating geographical origins at the regional scale, China. *Journal of Environmental Sciences,* **25**: 144-154.

Liu J, Li K, Xu J, Liang J, Lu X, Yang J, Zhu Q. 2003a. Interaction of Cd and five mineral nutrients for uptake and accumulation in different rice cultivars and genotypes. *Field Crops Research,* **83**: 271-281.

Liu JG, Liang JS, Li KQ, Zhang ZJ, Yu BY, Lu XL, Yang JC, Zhu QS. 2003b. Correlations between cadmium and mineral nutrients in absorption and accumulation in various genotypes of rice under cadmium stress. *Chemosphere*, **52**: 1467-1473.

Maenpaa KA, Kukkonen JV, Lydy MJ. 2002. Remediation of heavy metal-contaminated soils using phosphorus: evaluation of bioavailability using an earthworm bioassay. *Arch Environ Contam Toxicol,* **43**: 389-98.

Mandal KG, Hati KM, Misra AK. 2009. Biomass yield and energy analysis of soybean production in relation to fertilizer-NPK and organic manure. *Biomass and Bioenergy,* **33**: 1670-1679.

Marschner P. 1995. *Mineral Nutrition of Higher Plants,* UK, Academic press.

Mazen M. 1995. Assessment of heavy metal accumulation and performance Of some physiological parameters in zea mays l. And Vic1 a faba l. Grown on soil amended by sewage Sludge resulting from sewage water Treatment in the state of qatar.

Mekki BB, G.Ahmed A. 2005. Growth, Yield and Seed Quality of Soybean(*Glycine max* L.) As Affected by Organic, Biofertilizer and Yeast Application.

Research Journal of Agriculture and Biological Sciecnes, **1**: 320-324.

Melnitchouck A, Hodson M. 2004. Genotype and environment interaction in the uptake of Cs and Sr from soils by plants. *J.Plant Nutr. Soil Sci.,* **167**: 72-78.

Mendoza-Cozatl DG, Jobe TO, Hauser F, Schroeder JI. 2011. Long-distance transport, vacuolar sequestration, tolerance, and transcriptional responses induced by cadmium and arsenic. *Curr Opin Plant Biol,* **14**: 554-62.

Mendoza J, Garrido T, Castillo G, San Martin N. 2006. Metal availability and uptake by sorghum plants grown in soils amended with sludge from different treatments. *Chemosphere,* **65**: 2304-12.

Menkir A. 2008. Genetic variation for grain mineral content in tropical-adapted maize inbred lines. *Food Chemistry,* **110**: 454-464.

Messina MJ. 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. *The American Journal of Clinical Nutrition,* **70**: 439s-450s.

Michael A, Grausak. 2002. Tansprot minerals in plant review. *Journal of the American college of nutrition,* **21**: 178-183.

Miwa K, Fujiwara T. 2010. Boron transport in plants: co-ordinated regulation of transporters. *Ann Bot,* **105**: 1103-8.

Narwal R, Singh B. 1998. Effect of organic materials on partitioning, extractability and plant uptake of metals in an alum shale soil. *Water, Air, & Soil Pollution,*

103: 405-421.

Norton GJ, Deacon CM, Xiong L, Huang S, Meharg AA, Price AH. 2009. Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. *Plant and Soil,* **329**: 139-153.

Norvell W, Hopkins J, DG Welch R. 2000. Association of cadmium in durum wheat grain with soil chloride and chelate-extractable soil cadmium. *Soil Science Society of America Journal,* **64**: 2162.

Ohata M, Takaku Y, Inagaki K, Hioki A, Chiba K. 2009. Improvement of analytical sensitivity by Ar-N_2 Inductively Coupled Plasma in axially viewing optical emission spectrometry *Analytical Science,* **25**.

Oka N, Karasawa T, Okazaki K, Takebe M. 2010. Maintenance of soybean yield with reduced phosphorus application by previous cropping with mycorrhizal plants. *Soil Science and Plant Nutrition,* **56**: 824-830.

Okazaki K, Oka N, Shinano T, Osaki M, Takebe M. 2008. Differences in the metabolite profiles of spinach (Spinacia oleracea L.) leaf in different concentrations of nitrate in the culture solution. *Plant Cell Physiol,* **49**: 170-7.

Olayinka KO, Oyeyiola AO, Odujebe FO, Oboh B. 2011. Uptake of potentially toxic metals by vegetable plants grown on contaminated soil and their potential bioavailability using sequential extraction. *Journal of soil science* *and environmenal management,* **2**: 220-227.

Oldiges M, Lutz S, Pflug S, Schroer K, Stein N, Wiendahl C. 2007. Metabolomics: current state and evolving methodologies and tools. *Appl Microbiol Biotechnol,* **76**: 495-511.

Osaki M, Morikawa K, Shinano T, Urayama M, Tadano T. 1991. Productivity of high-yielding crops. *Soil Science and Plant Nutrition,* **37**: 445-454.

Outten CE, O'Halloran TV. 2001. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science,* **292**: 2488-92.

Puig S, Penarrubia L. 2009. Placing metal micronutrients in context: transport and distribution in plants. *Curr Opin Plant Biol,* **12**: 299-306.

Quadir QF, Watanabe T, Chen Z, Osaki M, Shinano T. 2011. Ionomic response ofLotus japonicusto different root-zone temperatures. *Soil Science and Plant Nutrition,* **57**: 221-232.

Rautaray S, Ghosh B, Mittra B. 2003. Effect of fly ash, organic wastes and chemical fertilizers on yield, nutrient uptake, heavy metal content and residual fertility in a rice–mustard cropping sequence under acid lateritic soils. *Bioresource Technology,* **90**: 275-283.

Sale PWG, Campbell LC. 1980. Patterns of mineral nutrient accumulation in soybean seed. *Field Crops Research,* **3**: 157-163.

Salt DE, Baxter I, Lahner B. 2008. Ionomics and the study of the plant ionome.

Annu Rev Plant Biol, **59**: 709-33.

Sanchez AL, Parekh NR, Dodd BA, Ineson P. 2000. Microbial component of radiocaesium retention in highly organic soils. *Soil Biology and Biochemistry,* **32**: 2091-2094.

Sardans J, Penuelas J, Estiarte M. 2008. Warming and drought change trace element bioaccumulation patterns in a Mediterranean shrubland. *Chemosphere,* **70**: 874-85.

Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J, Xu D, Hellsten U, May GD, Yu Y, Sakurai T, Umezawa T, Bhattacharyya MK, Sandhu D, Valliyodan B, Lindquist E, Peto M, Grant D, Shu S, Goodstein D, Barry K, Futrell-Griggs M, Abernathy B, Du J, Tian Z, Zhu L, Gill N, Joshi T, Libault M, Sethuraman A, Zhang XC, Shinozaki K, Nguyen HT, Wing RA, Cregan P, Specht J, Grimwood J, Rokhsar D, Stacey G, Shoemaker RC, Jackson SA. 2010. Genome sequence of the palaeopolyploid soybean. *Nature,* **463**: 178-83.

Sha Z, Oka N, Watanabe T, Tampubolon BD, Okazaki K, Osaki M, Shinano T. 2012. Ionome of soybean seed affected by previous cropping with mycorrhizal plant and manure application. *J Agric Food Chem,* **60**: 9543-52.

Sherene T. 2010. Mobility and transport of heavy metals in polluted soil environment. *Biological Forum*, **2**: 112-121.

Siedlecka A, Krupa Z. 1999. Cd/Fe interaction in higher plants - its consequences for the photosynthetic apparatus. *Photosynthetica*, **36**: 321-331.

Singh R, Agrawal M. 2007. Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of Beta vulgaris plants. *Chemosphere,* **67**: 2229-2240.

Smith SR. 2009. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ Int,* **35**: 142-56.

Staunton S, Hinsinger P, Guivarch A, Brechignac F. 2003. Root uptake and translocation or radiocaesium from agricultural soils by various plant species. *Plant and soil,* **254**: 443-455.

Staunton S, Roubaud M. 1997. Adsorption of ^{137}Cs on montmorillonite and illite: effect of charge compensating cation, ionic strength, concentration of Cs, K and fulvic acid. *Clays and clay minerals,* **45**: 251-260.

Sucharova J, Suchara I. 2006. Determination of 36 elements in plant reference materials with different Si contents by inductively coupled plasma mass spectrometry: comparison of microwave digestions assisted by three types of digestion mixtures. *Anal Chim Acta,* **576**: 163-76.

Sugiyama M, Ae N, Arao T. 2007. Role of roots in differences in seed cadmium concentration among soybean cultivars—proof by grafting experiment. *Plant and Soil,* **295**: 1-11.

Sugiyama M, Ae N, Hajika M. 2010. Developing of a simple method for screening soybean seedling cadmium accumulation to select soybean genotypes with low seed cadmium. *Plant and Soil,* **341**: 413-422.

Szpunar J. 2004. Metallomics: a new frontier in analytical chemistry. *Anal Bioanal Chem,* **378**: 54-6.

Szpunar J. 2005. Advances in analytical methodology for bioinorganic speciation analysis: metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics. *Analyst,* **130**: 442-65.

Tarighi H. 2011. Zinc availability of two wheat cultivars in soil amended with organic and inorganic Zn sources. *African Journal of Biotechnology,* **11**.

Tejada-Jimenez M, Galvan A, Fernandez E, Llamas A. 2009. Homeostasis of the micronutrients Ni, Mo and Cl with specific biochemical functions. *Curr Opin Plant Biol,* **12**: 358-63.

Ueno D, Kono I, Yokosho K, Ando T, Yano M, Ma JF. 2009a. A major quantitative trait locus controlling cadmium translocation in rice (Oryza sativa). *New Phytol,* **182**: 644-53.

Ueno D, Koyama E, Kono I, Ando T, Yano M, Ma JF. 2009b. Identification of a novel major quantitative trait locus controlling distribution of Cd between roots and shoots in rice. *Plant Cell Physiol,* **50**: 2223-33.

Uraguchi S, Mori S, Kuramata M, Kawasaki A, Arao T, Ishikawa S. 2009. Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *J Exp Bot,* **60**: 2677-88.

Verbruggen N, Hermans C, Schat H. 2009. Mechanisms to cope with arsenic or cadmium excess in plants. *Curr Opin Plant Biol,* **12**: 364-72.

Vreugdenhil D, Aarts MGM, Koornneef M, Nelissen H, Ernst WHO. 2004. Natural variation and QTL analysis for cationic mineral content in seeds of *Arabidopsis thaliana*. *Plant, Cell and Environment,* **27**: 828-839.

Watanabe T, Broadley MR, Jansen S, White PJ, Takada J, Satake K, Takamatsu T, Tuah SJ, Osaki M. 2007. Evolutionary control of leaf element composition in plants. *New Phytol,* **174**: 516-23.

Waters BM, Grusak MA. 2008. Quantitative trait locus mapping for seed mineral concentrations in two Arabidopsis thaliana recombinant inbred populations. *New Phytol,* **179**: 1033-47.

Waters BM, Sankaran RP. 2011. Moving micronutrients from the soil to the seeds: genes and physiological processes from a biofortification perspective. *Plant Sci,* **180**: 562-74.

Welch RM, Graham RD. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany,* **55**: 353-364.

White PJ. 2001. The pathways of calcium movement to the xylem. *Journal of Experimental Botany,* **52**: 891-899.

White PJ, Broadley MR. 2003. Calcium in plants. *Ann Bot,* **92**: 487-511.

White PJ, Broadley MR. 2009. Biofortification of crops with seven mineral elements often lacking in human diets--iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol,* **182**: 49-84.

White PJ, Broadley MR, Thompson JA, McNicol JW, Crawley MJ, Poulton PR, Johnston AE. 2012. Testing the distinctness of shoot ionomes of angiosperm families using the Rothamsted Park Grass Continuous Hay Experiment. *New Phytol*.

Wilkinson SR, Welch RM, Mayland HF, Grunes DL. 1990. Compendium on Magnesium and its role in Biology, Nutrition, and Physiology. In: Sigel H, Sigel A eds. *Metal ions in biological systems.* Switzerland, Institute of Inorganic Chemistry University of Basel.

Yafa C, Farmer JG. 2006. A comparative study of acid-extractable and total digestion methods for the determination of inorganic elements in peat material by inductively coupled plasma-optical emission spectrometry. *Analytica Chimica Acta,* **557**: 296-303.

Zeng F, Mao Y, Cheng W, Wu F, Zhang G. 2008. Genotypic and environmental variation in chromium, cadmium and lead concentrations in rice. *Environ Pollut*, **153**: 309-14.

Zhang GP, Fukami M, Sekimoto H. 2002. Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Filed Crops Research*, **77**: 93-98.

Zhao FJ, McGrath SP. 2009. Biofortification and phytoremediation. *Curr Opin Plant Biol*, **12**: 373-80.

Zhong W, Rosen K, Martensson A. 2007. Effects of ageing and microbial component on chemical availability of (137)Cs in a long-term experimental site. *Environ Monit Assess*, **129**: 309-14.

Zhu YG, Smolders E. 2000. Plannt uptake of radiocaesium: a review of mechanisms, regulation and application. *Journal of Experimental Botany*, **51**: 1635-1645.