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Ionome of Soybean Seed Affected by Previous Cropping with Mycorrhizal Plant and Manure Application

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ABSTRACT: Two field experiments were conducted to investigate the effects of previous cultivation of an arbuscular mycorrhizal (AM) host plant and manure application on the concentration of 19 mineral elements in soybean (*Glycine max* L. Merr. cv. Tsurumusume) seeds. Each experiment ran for two years (experiment 1 took place in 2007−2008, and experiment 2 took place in 2008−2009) with a split plot design. Soybeans were cultivated after growing either an AM host plant (maize, *Zea mays* L. cv. New dental) or a non-AM host plant (buckwheat, *Fagopyrum esculentum* Moench. cv. Kitawase-soba) 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 the two experiments, manure application significantly increased the available potassium (K) and decreased the available iron (Fe) and cesium (Cs) in the soil. However, higher concentrations of cadmium (Cd) and barium (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. The present study showed that the identity of the prior crop and manure application changed the mineral contents of the soybean seed and suggests a connection between environmental factors and food safety.

KEYWORDS: *ionome, soybean seed, manure, AM fungi*

INTRODUCTION

The soybean is one of the most important seed crops in the world and supplies protein, oil, and mineral nutrients for human consumption. The seed ionome represents its mineral nutrient and trace element content 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.[1] Any alterations in these processes that transport inorganic ions from the soil to the seed could affect the seed ionome[2] and could eventually influence human 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 nonbiological (e.g., climate, soil condition, and fertilizer application) and biological (e.g., symbiosis and parasitism).[3] Plants may accumulate not only essential elements but also nonessential metals such as cadmium (Cd) and lead (Pb), when they are present in the environment.[4] Increasing essential elements in the edible parts of crops is an important method of solving mineral malnutrition in the human diet,[5] whereas nonessential metals can cause serious health problems if they enter the human body.[6] 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[7] but few reports of the ionomic response of the seed to different environmental parameters.

Fertilization and crop rotation are fundamental agronomic measures used to improve yield and avoid disease induced by monoculture in soybean cultivation; as there are two important environmental factors, the ionome of the soybean seed should also respond to their stimuli. Manure is important as a source of plant nutrients, but the actual supply depends somewhat on the type of manure.[8,9] A number of studies have demonstrated that the application of manure can improve the concentrations of the essential elements zinc (Zn) and iron (Fe) in soybean plants.[10] Moreover, manure has been proposed to reduce the phytoavailability of radionuclides in soils and decrease the content in plants by binding these minerals to organic substances.[11] It is noteworthy that the colonization of arbuscular mycorrhizal (AM) fungi improves host plant uptake of the mineral elements nitrogen (N), Zn, sodium (Na), sulfur (S), Cd, selenium (Se), cesium (Cs), Fe, manganese (Mn), and, especially, phosphorus (P).[12,13] It is known that growth of AM fungi can be improved[14] and restricted[15] through the use of organic amendments, but the mechanism remains unclear. No studies have considered the entire network of elements influenced by the application of manure and AM fungi colonization in field conditions.

In this paper, 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 mineral concentrations.

MATERIALS AND METHODS

Two field experiments were conducted on a volcanic ash soil, which is classified as a Melanudands under the Classification of U.S. Soil Taxonomy, at the National Agricultural Research Center for the

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Table 1. Chemical Properties of the Soil in Two Experimental Sites

					exchangeable cations ($cmol_c$ kg^{-1})			
site	pH (H_2O)	available P_2O_5[a] (mg kg^{-1})	total N (g kg^{-1})	CEC[b] ($cmol_c$ kg^{-1})	Ca	Mg	K	BS[c] (%)
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 according to the Truog method; [b]CEC, cation exchange capacity. [c]BS, basic saturation.

Table 2. Total Mineral Elements in Manure

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

[a]DM, dry matter.

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 °C and precipitation of 1.8 mm 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 °C 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 Tables 1 and 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 120−154−103 and 30−120−70 kg/ha of N−P_2O_5−K_2O according to the Fertilization Guide in Hokkaido.[16]

For soybean cultivation, each first-year plot had subplots with or without manure application. Manure (20 t/ha) was applied to the field in April, which was 1 month before soybean planting. N and K were applied as ammonium sulfate at a rate of 20 kg N/ha, and potassium sulfate was applied 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 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 better examine the significant effect of AM fungi colonization on the change of ionome, P fertilizer was not applied during the soybean growth season because the increase of soil P after fertilizer application has a negative effect on 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 nonwoven fabric sheet (Paopao 90, MKV DREAM, Tokyo, Japan) for 3 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.[13] Soybean seeds were air-dried after 98 days of growth and were ground. Of the seed samples, 0.05 g was digested with 2 mL of 61% HNO_3 (EL grade; Kanto Chemical, Tokyo) in a tube at 110 °C in a DigiPREP apparatus (SCP Science, Quebec, Canada) for 3 h, and 0.5 mL of hydrogen peroxide (semiconductor grade; Santoku Chemical, Tokyo) was added and heated at 110 °C until the solution became clear.[17] The tube was cooled to room temperature and then diluted to 15 mL with 2% HNO_3 and analyzed for elements by inductively coupled plasma mass spectrometry (ICP-MS) (ELAN, DRC-e; Perkin-Elmer, Waltham, MA, USA).

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. Next, 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 19 mineral elements with the four treatments, principal component analysis (PCA) was employed using Minitab 15 (Minitab, 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.

RESULTS

Mineral Elements in the Soil after Prior Crop Cultivation and Manure Application. Except for available calcium (Ca), which was increased by previous cropping with maize in experiment 1, there was no effect of the previous crop on the total and available minerals in the soil in the present experiments, whereas the application of manure changed the profile of the mineral elements in the soil (Table 3). Compared with no manure treatment (Table 3a,c), total K and Ba increased to 1.6- and 1.15-fold in experiment 1, but only K was significantly increased to 1.17-fold 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, that is, 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 3b), the available K, Mn, cobalt (Co), and barium (Ba) increased to 2.83-, 1.14-, 1.33-, and 1.32-fold, respectively. In comparison, the available Ca, Fe, Cs, and boron (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). Thus, manure application significantly increased the K content in the soil, especially the available K, and decreased the available Fe and Cs contents 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

Table 3. Mineral Elements in Soil in Experiments 1 and 2[a]

(a) Total Mineral Elements in Soil in Experiment 1

		mg g^{-1}					µg g^{-1}												
treatment		K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co	Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
B	+M	1.02	1.51	4.50	1.04	24.69	19.98	58.57	3.40	0.61	8.78	384.75	45.43	293.02	175.24	0.27	13.20	0.56	2.24
	−M	0.62	1.51	5.01	1.01	24.64	20.81	54.21	5.14	0.62	8.79	336.97	45.32	246.13	173.09	0.28	12.94	0.67	2.12
M	+M	0.98	1.51	4.22	0.86	24.97	19.80	66.30	2.88	0.60	8.26	360.34	42.33	274.58	172.21	0.26	12.76	0.68	2.17
	−M	0.64	1.54	5.30	1.24	26.56	21.47	58.95	5.09	0.69	10.51	349.49	47 0.08	245.58	187.12	0.29	13.34	0.78	2.22
previous crop (A)[b]		NS	NS	NS	NS	NS	NS	NS	NS	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	NS	NS	<0.001	NS	NS	NS	NS	NS
A × B[b]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

(b) Available Mineral Elements in Soil in Experiment 1

		mg g^{-1}					µg g^{-1}												
treatment		K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co	Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
B	+M	0.44	0.17	0.78	51.82	2.00	0.21	0.23	0.29	0.02	0.04	19.05	17.40	81.56	0.043	0.023	nd	0.021	0.130
	−M	0.15	0.16	1.58	46.49	5.90	0.14	0.21	0.52	0.02	0.03	20.01	15.84	68.72	0.033	0.023	nd	0.017	0.169
M	+M	0.41	0.18	1.02	58.66	1.99	0.10	0.31	0.27	0.02	0.04	27.10	17.25	82.29	0.052	0.024	nd	0.021	0.125
	−M	0.15	0.16	1.71	50.62	9.22	0.19	0.16	0.46	0.01	0.03	20.57	15.40	65.20	0.032	0.024	nd	0.014	0.168
previous crop (A)		NS	NS	<0.001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS
manure (B)		<0.001	NS	<0.001	<0.001	<0.01	NS	<0.05	<0.01	NS	<0.001	NS	NS	0.001	<0.05	NS		NS	<0.001
A × B		NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS

(c) Total Mineral Elements in Soil in Experiment 2

		mg g^{-1}					µg g^{-1}												
treatment		K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co	Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
B	+M	1.25	1.53	4.89	0.90	17.14	17.17	51.99	2.26	2.26	8.05	357.65	56.65	300.84	173.50	0.26	12.76	0.49	2.39
	-M	1.09	1.68	5.23	0.92	18.67	17.35	60.10	2.45	2.45	9.06	377.44	58.58	313.11	192.42	0.25	13.99	0.5	2.55
M	+M	1.29	1.59	4.69	0.90	20.03	17.14	53.46	2.34	2.34	8.43	337.2	57.51	322.76	195.00	0.26	13.84	0.52	2.58
	−M	0.99	1.65	5.40	0.85	17.85	16.63	54.18	4.29	4.29	7.81	369.4	56.73	298.25	184.12	0.27	12.92	0.50	2.48
previous crop (A)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
manure (B)		<0.001	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A × B		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

(d) Available Mineral Elements in Soil in Experiment 2

		mg g^{-1}					µg g^{-1}												
treatment		K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co	Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
B	+M	0.35	0.28	2.45	75.48	3.42	0.09	0.58	0.07	0.036	0.042	28.13	23.43	72.57	0.051	0.035	nd	0.014	0.052
	−M	0.20	0.35	2.78	86.62	4.46	0.27	0.54	0.11	0.026	0.049	28.06	28.14	86.32	0.052	0.040	nd	0.022	0.078
M	+M	0.44	0.32	2.11	91.72	3.36	0.33	0.56	0.16	0.028	0.05	33.23	27.14	85.29	0.051	0.039	nd	0.020	0.056
	−M	0.15	0.28	2.27	77.84	3.61	0.20	0.61	0.15	0.068	0.043	23.32	25.24	76.85	0.047	0.038	nd	0.019	0.069
previous crop (A)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
manure (B)		<0.001	NS	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05

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Table 3. continued

(d) Available Mineral Elements in Soil in Experiment 2

treatment	K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co	Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
	mg g^{-1}					μg g^{-1}												
A × B	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

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

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 4 weeks after sowing ($P < 0.01$; Figure 1) the colonization increased nearly 4-fold in experiment 1 and 2-fold in experiment 2 compared with previous cultivation with buckwheat (non-AM host plant). However, there were no significant differences in colonization caused by manure application.

Profiling of the Mineral Elements in Soybean Seeds after Different Treatments. The results of 19 mineral elements in four different treatments were subjected to PCA (Figure 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, whereas 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.

Effect of Previous Crop and Manure Application on the Mineral Concentration in Soybean Seeds. An ANOVA (Table 4a,b) was conducted to clarify the effect of the previous crop 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 copper (Cu) (to 1.11-fold), but a significant reduction in Cd (to 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. With manure application (Table 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 decrease due to manure application. However, it is difficult to detect any combined effect of manure and previous cultivation. In experiment 2 (Table 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.

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.[18] 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.[19] Heavy metals have been reported to display low mobility in the soil profile due to adsorption on organic matter, for example, Cs and Cd.[20,21] However, the ability of organic substances to fix minerals is influenced by the types and properties of organic amendments.[22,23] In the present study,

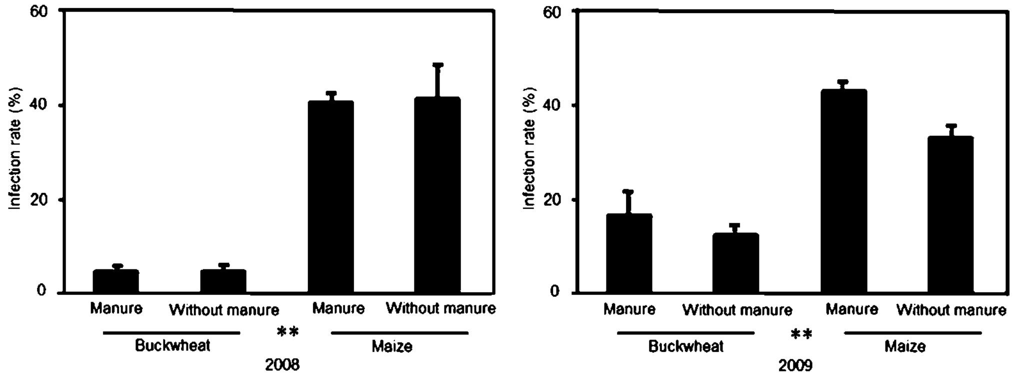

Figure 1. Effects of the previous crop and manure application on arbuscular mycorrhizal (AM) colonization. Bars indicate the standard errors (n = 4). ** indicates significant differences ($P < 0.001$) between treatments of different previous crops.

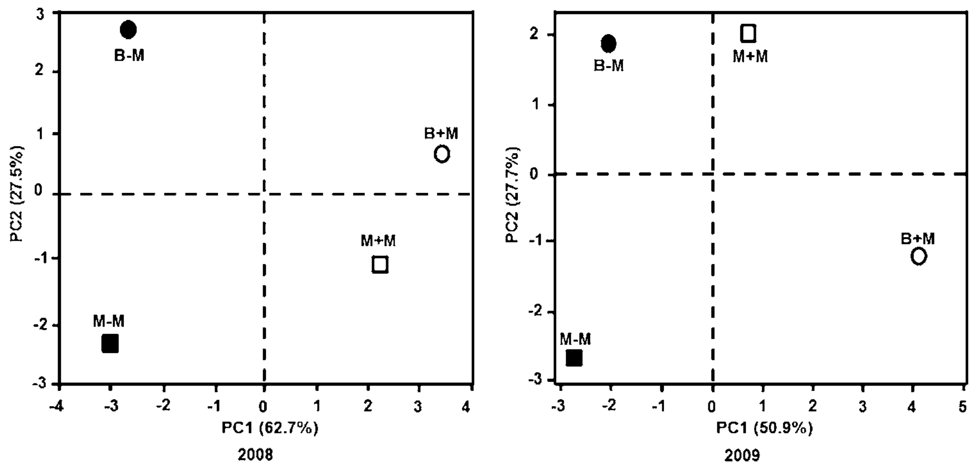

Figure 2. Sample scores for the first (PC1) and second (PC2) principal components drawn from the principal component analysis for 19 mineral elements. Labels next to the icons present the treatments: M, maize; B, buckwheat; +M, with manure application; −M, without manure application.

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.[24] In soil, available Cs is significantly decreased by manure application, which might be the main reason for lower Cs deposition in soybean seeds.[25] Conversely, it is known that K and Cs enter the root through 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), which may have inhibited root uptake of Cs.[26] 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,[27,28] whereas Ca^{2+} channels in the plasma membrane or root cells show significant discrimination between permeant cations, and Ba^{2+} is more permeable than either Sr^{2+} or Ca^{2+}.[29] 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. The P, K, magnesium (Mg), Ca, Mn, Cu, B, molybdenum (Mo), Na, strontium (Sr), and chromium (Cr) contents in soybean seeds were constant regardless of the manure treatments. It is highly likely that stable Mg, Cu, Na, Sr, and Mo contents in soybean seeds in the treatment with manure application were caused by the pool of available minerals in soil, whereas K, Ca, Mn, Cu, B, Ba, Cd, and Na in the seed were not affected by differences in 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 ion competition.[30,31]

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 nickel (Ni) (Table 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 experiment 1, but not in experiment 2, due to the higher average rainfall in 2009 of 1.4-fold that in 2008. Drought

Table 4. Mineral Concentrations in Soybean Seed in Experiments 1 and 2

(a) Mineral Concentrations in Soybean Seed in Experiment 1

	mg g^{-1}				μg g^{-1}														
treatment	P	K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co	Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
B +M	6.85	18.22	2.65	1.64	38.12	91.91	17.09	54.19	25.98	0.31	0.15	7.75	5.61	8.44	2.86	0.047	1.48	0.04	0.016
−M	6.66	17.63	2.57	1.71	36.71	86.40	18.12	49.22	28.68	0.19	0.12	6.84	5.76	5.61	2.93	0.031	1.18	0.08	0.036
M +M	6.75	18.16	2.56	1.75	37.22	95.39	19.49	52.36	26.85	0.31	0.15	7.86	6.04	8.77	2.51	0.036	1.44	0.03	0.014
−M	6.42	17.63	2.41	1.92	38.15	89.15	20.32	48.83	27.88	0.17	0.13	7.53	6.57	6.35	2.87	0.025	1.62	0.06	0.041
previous crop (A)[a]	NS	NS	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	NS	NS
manure (B)[b]	NS	NS	NS	NS	NS	<0.05	NS	<0.01	NS	NS	<0.05	NS	NS	<0.05	<0.05	<0.01	NS	<0.05	<0.001
A × B[c]	NS	NS	NS	NS	NS	NS	NS	0.548	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

(b) Mineral Concentrations in Soybean Seed in Experiment 2

	mg g^{-1}				μg g^{-1}														
treatment	P	K	Mg	Ca	Mn	Fe	Cu	Zn	B	Mo	Co	Na	Sr	Ba	Ni	Cd	Cr	Se	Cs
B +M	5.91	16.51	2.30	1.68	31.86	76.08	13.25	41.17	24.41	0.36	0.064	10.71	5.64	7.69	1.76	0.058	1.39	0.055	0.005
−M	5.63	17.09	2.35	1.73	32.28	69.36	13.26	40.52	24.23	0.44	0.056	6.90	6.05	6.30	1.60	0.047	0.97	0.052	0.007
M +M	5.81	16.72	2.31	1.60	32.10	66.53	13.65	41.12	24.00	0.47	0.060	7.47	5.24	6.79	1.67	0.049	1.24	0.04	0.004
−M	5.87	17.32	2.36	1.66	32.63	72.12	13.89	40.36	26.09	0.80	0.054	7.64	5.46	5.34	1.70	0.047	1.31	0.08	0.009
previous crop	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	<0.05	NS	NS	NS
manure (B)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	NS	<0.05	NS	NS	<0.05
A × B	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

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.[32] Increases in soil moisture 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. It was reported that drought increased arsenic (As) and Cd in *Erica multiflora* stems and decreased Cu in leaves, Ni in stems, and Pb in leaf litter of *Globularia alypum*.[33] Furthermore, a number of soil chemical, soil biological, and external factors contribute to the variance of the soil pool and distribution in plant.[34] The extractable concentrations of Zn, Cu, Cd, and Pb increased with increasing soil pH at the highest rate of addition of a cocomposted material, in contrast to the usual response to increasing soil pH, which generally reduces the availability of heavy metals in soil.[35] Cationic metals can form insoluble complexes with P and reduce the bioavailability of some heavy metals.[36] The differences of pH and available P in the two sites (Table 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.[37] Immobilization and mobilization of heavy metal cations such as Cd, Zn, and Cs by AM fungi have been studied for many years.[12,27,38] 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. It has been shown that legume crops are less tolerant to Cd toxicity compared to cereals and grasses.[39] In the present study, previous cropping with the AM host plant (maize) increased the AM fungi colonization rate in the soybean growth season (Figure 1), but there were lower Cd concentrations in soybean seed (Table 4) 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.[40−42] 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. 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 papers 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 Figure 3, as well as their transportability from soil to seed. Seed element concentrations varied by 8 orders of magnitude from the highest for K to the lowest for Cs, whereas in soil, the highest concentration was for Ca and the lowest was for Se (Figure 3). K, Mg, and Ca were present at relatively high concentrations in both soybean seed and soil solution, reflecting their natural abundance and their ease of transport within the plant.[43] On the basis of this study, Ba, Na, Cs, and Sr have lower transportability from soil to soybean seed, whereas Zn, B, K, Ni, and Cu have higher transportability.

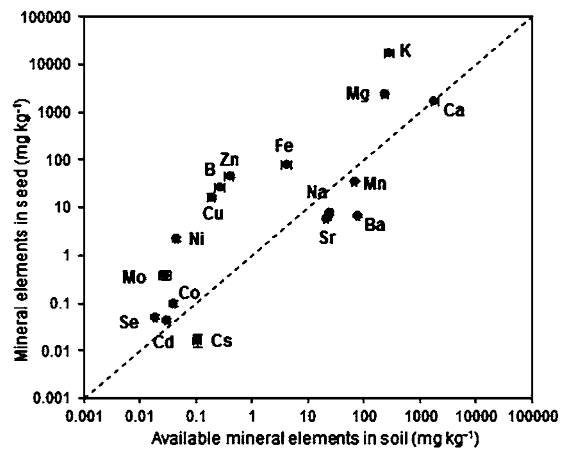

Figure 3. Transportability of element from soil to seed. Bars indicate the standard errors (n = 32).

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.[44,45] In the present study, correlations among elements in the two experiments were investigated (Table 5). There were significant positive correlations between K and Mg and between 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, and Na and Cr.

Many surveys have suggested correlations among minerals in the same group.[7,17,46] For example, Na, K, and Cs use a similar translocation mechanism in plants because of their chemical similarities, and increasing K could reduce Cs concentration in the plant because K is more effectively transported to the shoot than Na and Cs in Fabaceae,[47] similarly to the present study's results on the relationship between K and Cs (Table 5) in the soybean seed.

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 5), suggesting cotransport and cochelation in the soybean. Compared with other plants,[45] growth media (hydroponic or pot experiment), and different organs of plants,[48] 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, for example, Mg^{2+}, Cu^{2+}, Fe^{2+}, Cd^{2+}, and Ba^{2+}.[29,43] 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.[49]

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

Table 5. Correlation Coefficients for Seed Composition of Mineral Elements in Two Experiments[a]

		Mg	K	Ca	Mn	Fe	Ni	Cu	Zn	Sr	Cd	Ba	Cr	Na	Co	Se	Cs	P	Mo
K	2008	0.6[b]																	
	2009	0.7[c]																	
Ca	2008	−0.0	0.0																
	2009	0.4	0.4																
Mn	2008	0.5[b]	0.4	0.6[b]															
	2009	−0.6[b]	−0.5	−0.0															
Fe	2008	0.5[b]	0.5	0.4	0.4														
	2009	0.2	0.2	0.4	0.2														
Ni	2008	0.2	−0.2	0.2	0.4	−0.2													
	2009	0.0	−0.2	0.1	0.3	0.7[c]													
Cu	2008	−0.3	−0.4	0.4	0.2	0.2	0.1												
	2009	0.7[c]	0.8[c]	0.3	−0.6[b]	−0.0	−0.3												
Zn	2008	0.1	0.4	−0.4	−0.2	0.2	−0.4	−0.7[c]											
	2009	0.6[b]	0.5[b]	0.5[b]	−0.5	0.1	0.1	0.7[c]											
Sr	2008	−0.2	−0.0	0.8[c]	0.4	0.3	−0.0	0.5	−0.4										
	2009	−0.4	−0.2	0.5[b]	0.7[c]	0.5	0.3	−0.4	−0.2										
Cd	2008	0.3	0.3	−0.7[c]	−0.3	−0.0	−0.3	−0.7[c]	0.8[c]	−0.6[b]									
	2009	0.4	0.5	0.1	−0.1	0.3	0.0	0.3	0.2	0.1									
Ba	2008	0.2	0.5	0.2	0.1	0.6[b]	−0.5	−0.2	0.6[b]	0.4	0.3								
	2009	0.4	0.4	0.5	−0.1	0.5[b]	0.4	0.3	0.5	0.4	0.7[c]								
Cr	2008	−0.3	−0.2	0.1	−0.0	0.2	0.1	0.4	0.0	0.2	−0.0	0.2							
	2009	0.1	−0.4	−0.6	−0.1	0.1	0.5	−0.5	−0.5	−0.3	−0.0	−0.1							
Na	2008	0.1	0.3	0.0	0.1	0.3	−0.2	0.0	0.3	0.0	0.3	0.3	0.8[c]						
	2009	0.1	−0.3	−0.1	−0.0	0.6[b]	0.8[c]	−0.4	−0.1	0.0	−0.1	0.1	0.7[c]						
Co	2008	−0.1	0.3	−0.5[b]	−0.3	0.1	−0.5	−0.4	0.8[c]	−0.3	0.8[c]	0.5[b]	0.1	0.3					
	2009	0.6[b]	0.5	0.1	−0.3	0.4	0.0	0.5	0.3	−0.2	0.7[c]	0.5[b]	−0.0	−0.0					
Se	2008	−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				
	2009	−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	2008	−0.4	−0.5[b]	0.5	0.1	−0.5	0.5	0.3	−0.7[c]	0.4	−0.7[c]	−0.6[b]	0.0	−0.2	−0.7[c]	0.2			
	2009	0.2	−0.5[b]	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	2008	0.4	0.5[b]	−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		
	2009	0.8[c]	0.7[c]	0.2	−0.7[c]	0.2	−0.0	0.8[c]	0.6[b]	−0.6[b]	0.4	0.3	0.0	0.0	0.7[c]	−0.2	0.3		
Mo	2008	0.1	0.1	−0.1	−0.4	0.4	−0.2	−0.3	0.6[c]	−0.2	0.4	0.5[b]	0.1	0.1	0.6[b]	0.4	−0.5[b]	−0.2	
	2009	−0.1	−0.4	0.1	0.0	0.1	0.4	−0.1	0.3	−0.0	−0.7[c]	−0.2	0.2	0.9[c]	−0.2	0.8[c]	0.0	0.1	
B	2008	0.3	−0.1	0.4	0.4	0.2	0.5[b]	0.5	−0.8[c]	0.2	−0.7[c]	−0.6[b]	−0.1	−0.2	−0.7[c]	0.3	0.5	0.1	−0.4
	2009	−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

[a]Coefficients are derived from pairwise regression of mineral elements in two years. [b]Significant at 0.05 probability level. [c]Significant at 0.01 probability level.

coregulated in *Lotus japonicas*.[46] In the present study, there were positive correlations between Co and Ba and between Co and Cd, suggesting coregulation of these elements in the soybean.

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 phytoextraction of heavy metal elements.

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