Possible solubilization of various mineral elements in the rhizosphere of *Lupinus albus* L.

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Abstract

Lupinus albus L. (lupin) has a high tolerance for phosphorus deficient conditions as its roots can solubilize the unavailable phosphorus in the rhizosphere soil. The roots may also be able to solubilize other elements, but this requires further investigation. In this study, therefore, we conducted two experiments to comprehensively investigate the effects of lupin roots on the mineral dynamics of the rhizosphere soil. First, a mixed cropping experiment was conducted, in which lupin shared a rhizosphere with soybean (*Glycine max* (L.) Merr.) in a long-term experimental field with four fertilizer treatments: complete fertilization (+NPK), without nitrogen (−N), without phosphorus (−P), and without potassium (−K). The results of shoot dry weight of plants cultivated alone indicated that lupin is highly tolerant to all N, P, and K deficiencies, while soybean can adapt to N deficiency with the help of rhizobia but is less tolerant to P and K deficiencies than lupin. When mixed-cropped with lupin, the concentrations of many elements in the soybean leaf increased, particularly with the −N and −P treatments. Furthermore, soybean growth was significantly improved when cropped with lupin in the −N and −P treatments. Second, a comparison of the elemental profiles of hydroponically and field-soil-grown plants was conducted. Under hydroponic conditions, the rhizosphere effect is negligible when the culture medium is well circulated. The lupin/soybean ratios for leaf mineral concentrations were considerably larger in the field cultivated plants when compared with the hydroponic cultivations for elements such as sodium, potassium, cesium, phosphorus, iron, copper, and molybdenum, as there were lower concentrations of these elements in the soybean leaves in the field. These results indicate that lupin roots

can solubilize a variety of insoluble elements in the soil, which may be the reason why lupin can adapt to various nutrient deficient soils. In the lupin rhizosphere, the solubilization of cesium, which is generally strongly fixed by soil minerals and not easily leached, was particularly pronounced. This implies that the surface structure of clay minerals might be altered in the lupin rhizosphere, resulting in the fixed forms of various elements becoming available.

Key words: ionomics, *Lupinus albus* L., nutrient deficiency, rhizosphere, *Glycine max* (L.) Merr.

1. Introduction

The rhizosphere is the interface between plant roots and soil, where roots, soil microorganisms, and soils interact with each other (Hinsinger et al. 2005). In the rhizosphere, roots influence soil chemical, physical, and biological properties (Gregory and Hinsinger 1999; Wenhao et al. 2013), and these are referred to as “rhizosphere effects”. In problem soils, roots are the plant organs that are directly exposed to soil stress. Plant roots adapt to soil stress by altering the rhizosphere environment to prevent root damage caused by toxic elements (Kochian, Pineros, and Hoekenga 2005) and by increasing the availability of nutrient elements (Hinsinger et al. 2011). *Lupinus albus* L. (lupin) is an annual legume grown in temperate regions, and is highly tolerant of phosphorus (P) deficient soils as it can alter the rhizospheric environment to increase the available P (Gardner and Parbery 1982). Bottlebrush-like clusters of rootlets along its lateral roots, called cluster roots, are formed under conditions of nutrient deficiency, particularly P deficiency (Watt and Evans 2003). These cluster roots efficiently exude phosphatase and organic acids to decompose organic P and solubilize insoluble P, respectively, in the rhizosphere (Neumann et al. 2000; Wasaki et al. 2003). These abilities of lupin roots are powerful, and in pot experiment where maize was mixed-cropped with lupin, it was reported that the P nutrient status of maize in P-deficient soil was significantly improved due to the mixed cropping with lupin (Dissanayaka et al. 2015; Dissanayaka and Wasaki 2021). Furthermore, it has been found that in the lupin rhizosphere, organic nitrogen (N) may be decomposed by lupin roots, rather than rhizosphere microorganisms (Fujiishi, Maejima, and Watanabe 2020). It has also been suggested that lupin roots may be able to solubilize other elements in the rhizosphere soil (Dessureault-Rompré et al. 2008), but this requires further investigation.

Currently, 17 essential elements are known to be required by plants (Marschner 2012). Nonessential elements also occur in soils and are absorbed and translocated by plants alongside essential elements. Ionomics is the study of all metal, metalloid, and nonmetal accumulations in living organisms, regardless of whether the accumulated minerals are essential or nonessential, and this helps us to better understand biological and physiological problems (Salt, Baxter, and Lahner 2008). Ionomics has been applied not only to plants but also to cultivated soils to analyze the mineral dynamics between plants and soils (Watanabe et al. 2015). It is important to examine the rhizosphere soil to elucidate the impact of roots on soil mineral dynamics (Chu et al. 2017). However, it is also difficult to obtain accurate information to understand the mineral dynamics between plants and soils just from the analysis of rhizosphere soils, as even though the roots can solubilize elements in the rhizosphere, they then absorb the solubilized elements during growth (Chu et al. 2017). Therefore, we devised experiments using another control plant to estimate the effect of the lupin roots on soil mineral dynamics in the rhizosphere.

In this study, we conducted two experiments to comprehensively investigate the effects of lupin roots on the mineral dynamics of the rhizosphere soil without analyzing the rhizosphere soil. First, a mixed cropping experiment was conducted, in which lupin shared a rhizosphere with soybean (*Glycine max* (L.) Merr.). In this situation the rhizosphere effects of the lupin could alter the growth and elemental absorption of the soybean. As for example, mixed cropping with maize has been shown to improve the iron (Fe) nutrient status of peanuts, presumably due to the secretion of phytosiderophores from the roots of maize (Zuo et al. 2000). In this study, the alterations in the mineral element profiles of the soybean leaves due to the mixed cropping were investigated. We also compared the elemental profiles between hydroponic and soil-grown plants. The rhizosphere effect is negligible under hydroponic conditions where the culture medium is well circulated. Therefore, soybean was used as a control plant and the leaf mineral concentration ratios of lupin to soybean were compared between the hydroponic (without rhizosphere effects) and field cultivations (with rhizosphere effects). Then, the rhizosphere effects of lupin on the availability of various elements in the field soils were evaluated relative to the soybean.

2. Materials and methods

2.1. Effect of rhizosphere sharing with lupin on the leaf ionome of soybean under nitrogen, phosphorus, or potassium deficient conditions in the field

2.1.1. Cultivation

In 2015, lupin (cv. Energy) and soybean (*Glycine max* (L.) Merr. cv. Wasemidori) were cultivated in the long-term fertilizer experimental field of Hokkaido University. This field was established in 1914, and 4 fertilizer treatment plots: complete fertilization (+NPK), without N (−N), without P (−P), and without K (−K), have been continuously run here for 101 years. The N, P, and K fertilizers were applied as ammonium sulfate, superphosphate, and potassium sulfate, respectively (100 kg N, P_2O_5, K_2O ha^{-1}), once in a year before sowing. Each plot was 5.25 × 18.5 m in size, and the soil type was classified as a brown lowland soil (Haplic Fluvisols). The general properties of the field bulk soils are shown in Table S1 (pH, concentration of total carbon, and total N) and Watanabe et al. (2015) (concentration of available minerals). Seeds of each plant species (two seeds per hole) were sown on June 9 in each plot. Lupin and soybean were cultivated alone (mono-cultured) or co-cultured with shared rhizospheres, as shown in Fig. 1. The row and intra-row spacing was 50 cm × 30 cm.

2.1.2. Sampling

Shoots of lupin and soybean were sampled randomly with 4 replicates (two plants per replicate, per species) from September 14 to 16, when the soybean was in the R6 growth stage, and were separated into leaves, stems, and pods. Plant samples were washed with deionized water, dried in an oven at 70°C for 7 days, weighed, and ground for mineral analysis.

2.1.3. Mineral analysis

Each 70 mg leaf sample was digested in 2 mL of 61% (w/v) HNO_3 (EL grade; Kanto Chemical, Tokyo, Japan) at 110°C in a DigiPREP apparatus (SCP Science, Montreal, Canada) for approximately 2 h until the solution had almost disappeared. After the samples had cooled, 0.5 mL H_2O_2 (semiconductor grade; Santoku Chemical, Tokyo, Japan) was added and the samples were heated at 110°C for an additional 20 min. Once digestion was complete, the tubes were cooled, and the samples were reconstituted to a volume of 10 mL by adding 2% (w/v) HNO_3 in ultrapure water. The concentrations of 24 elements [potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), sulfur (S), iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu), nickel (Ni), molybdenum (Mo), sodium (Na), aluminum (Al), vanadium (V), chromium (Cr), cobalt (Co), arsenic (As), selenium (Se), rubidium (Rb), strontium (Sr), cadmium (Cd), cesium (Cs), and barium (Ba)] were determined by using an inductively coupled plasma-mass spectrometer (ELAN DRC-e; Perkin Elmer, Waltham, MA, USA). External calibration standards containing these elements were measured after every 10 samples. To determine the plant N concentrations, each 70 mg sample was digested with 1.25 mL of concentrated H_2SO_4 in a test tube at 200°C. At 30-min intervals, 0.3 mL of H_2O_2 was added to each tube (eight times), following which the N concentration in the digests was determined using the micro-Kjeldahl method (K-350 Distillation Unit, Büchi, Flawil, Switzerland).

2.2. Mineral accumulation of lupin and soybean under hydroponic conditions

2.2.1. Cultivation

The hydroponic experiment was conducted in a greenhouse at Hokkaido University under natural light conditions. Seeds of lupin and soybean were surface-sterilized with 70% (v/v) ethanol for 2 min, and then washed with deionized water. The seeds were germinated in running water for 24 h, and the germinated seeds were sown in vermiculite and precultured for 7 d in a greenhouse. Then, three seedlings per replicate in each plant species were transferred to 8 L container (30 cm × 26 cm × 15 cm) containing 8 L of aerated standard nutrient solution supplemented with 5 µM CsCl (mono-culture). The standard nutrient solution contained 2.14 mM N (NH_4NO_3), 32 µM P ($NaH_2PO_4 \cdot 2H_2O$), 0.77 mM K (K_2SO_4:KCl = 1:1), 1.25 mM Ca ($CaCl_2 \cdot 2H_2O$), 0.82 mM Mg ($MgSO_4 \cdot 7H_2O$), 35.8 µM Fe ($FeSO_4 \cdot 7H_2O$), 9.1 µM Mn ($MnSO_4 \cdot 4H_2O$), 46.3 µM B (H_3BO_3), 3.1 µM Zn ($ZnSO_4 \cdot 7H_2O$), 0.16 µM Cu ($CuSO_4 \cdot 5H_2O$), and 0.05 µM Mo ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$); total SO_4 = 1.06 mM. We set the P concentration in the culture medium to 32 µM, as this concentration has already been reported to be sufficient for many crops, including soybean (Tadano and Tanaka 1980). The pH of the solution was adjusted to 6.0 ± 0.1 with 0.1 M NaOH or 0.1 M HCl daily, and the solution was renewed every week.

2.2.2. Sampling and mineral analysis

The seedlings were treated for 2 weeks, with 3 replicates. After the treatment, seedlings were harvested and separated into leaves, stems, and roots. Plant samples were washed with deionized water, dried in an oven at 70°C for 7 days, weighed, and ground for mineral analysis. The concentrations of Na, K, Cs, Mg, Ca, P, N, Fe, Mn, Zn, Cu, Mo, B, and S in each sample were determined as described above.

2.3. Statistical analyses

The mineral concentration data was analyzed on a dry weight basis. All statistical analyses were performed using Sigmaplot 14.5 (Systat Software, Inc., San Jose, CA, USA) and Excel 2013 (Microsoft, Redmond, WA, USA).

3. Results

3.1. Effect of rhizosphere sharing with lupin on the leaf ionome of soybean under nitrogen, phosphorus, or potassium deficient conditions in the field

3.1.1. Growth

Shoot dry weight after cultivation is shown in Fig. 2. The dry weight of each organ of the shoot (leaf, pod, and stem) is shown in Table S2. Comparing the tolerance to nutrient deficient soils based on the results of the shoot dry weight for the mono-cultures, the growth of soybean was found to be inferior in P deficient and K deficient soils, while the growth of lupin was not reduced in any of the nutrient deficient soils (Fig. 2). Soybean grown with lupin (with shared rhizospheres) showed enhanced growth in the −N and −P treatments in comparison with the mono-cultured soybean. Conversely, in lupin, growth in −N and −P treatments was reduced when grown with soybean. Nodulation was observed in roots of both species, particularly in −N treatment (no data).

3.1.2. Effect of rhizosphere sharing on the accumulation of mineral elements in leaves

The concentrations for each element in the leaves of the soybean and lupin grown in the field are shown in Table S3. To evaluate the effect of the rhizosphere sharing on the absorption of mineral elements, the concentration of each element in the leaves was compared when each plant species was grown alone and when mixed, and the results were presented in a heatmap (Fig. 3). In soybean, a significant increase in the concentration of the elements was often observed when cultivated with lupin in all treatments, particularly the −N and −P (Fig. 3). In the −N treatment, alkaline earth metal elements such as Mg, Ca, and Sr, and metal elements such as Fe, Al, Mn, Cr, Co, and Ni had increased concentrations in the leaves of the soybean cultivated with lupin. In the leaves of the soybean in the −P treatment, there was an increase in the concentration of alkaline and alkaline earth metal elements such as K, Rb, Mg, Ca, Sr, and Ba as well as P and N due to the mixed cropping with lupin. Potassium and Cs concentrations in the −K treatment and Na and Cs concentrations in the +NPK treatment (control) also increased in the soybean leaves due to the mixed cropping with lupin. By contrast, in lupin, the increase in leaf mineral concentrations due to the mixed cropping with soybean was rare, and in some cases it even decreased. In particular, the N concentration decreased due to the mixed cropping with soybean in all treatments.

3.2. Differences in the mineral concentrations of leaves between lupin and soybean when cultivated in the field or hydroponic cultures

Soybean and lupin were grown hydroponically in a nutrient solution containing essential elements and trace concentrations of Cs, and the mineral concentrations in the leaves were determined (Table S4). The ratio of lupin to soybean based on the concentrations in the leaves was calculated for each element in the hydroponically and field (+NPK treatment of the above field experiment, monoculture) grown plants (Fig. 4). When grown hydroponically, the Na and Mn concentrations were much higher in the lupin, while the concentrations of the other elements showed little difference between lupin and soybean or tended to be lower in lupin (Fig. 4A). By contrast, in the field grown plants, the Na and Mn concentrations were considerably higher in lupin as in hydroponics, but many other elements were also higher in the lupin, especially the Cs (Fig. 4B).

4. Discussion

Plants adapt to nutrient deficient soils by altering the rhizosphere environment, but the characteristics of these adaptations depends on the plant species and the type of nutrient deficiency (Marschner et al. 1986). Lupin is a plant adapted to nutrient-poor soils and in particular, it is known to have a high tolerance for P deficient soils (Gardner and Parbery 1982). To evaluate the rhizosphere effects of lupin, the first experiment examined the ionome variation in soybean when its rhizosphere was shared by lupin in the field with different soil nutrient conditions. In the long-term fertilizer experimental field, the growth of mono-cultured soybean decreased with the −P and −K treatments, while there was no significant difference in the growth of mono-cultured soybean among the treatments (Fig. 2). This indicates that lupin is highly tolerant to all N, P, and K deficiencies, while soybean can adapt to N deficiency with the help of rhizobia but is less tolerant to P and K deficiencies than lupin. When soybean was co-cultured with lupin so that their rhizospheres were shared, the growth of soybean was enhanced in the −N and −P treatments, while the growth of lupin was reduced (Fig. 2). This suggests that lupin-induced changes in the rhizosphere environment had beneficial effects on soybean growth.

To estimate the variation of available mineral elements in the rhizosphere soil due to mixed cropping, the mineral concentrations of the leaves of co-cultured plants were compared with that of mono-cultured plants (Fig. 3). The results showed that the concentration of many elements in soybean leaf increased in the −N and −P treatments with mixed cropping, and the same trend was seen for the growth enhancement of soybean (Figs. 2 and 3). In the −N treatment, the N concentration of mono-cultured soybean was not lower than that in the other treatments (Table S3), and no increase in leaf N concentration due to mixed cropping with lupin was observed (Fig. 3, Table S3). These results indicates that the growth enhancement of soybean in the −N treatment by mixed cropping with lupin cannot be explained by the improvement of N nutrition. The long-term fertilizer experimental field used in this study have been continuously fertilized with the same fertilizer treatment more than 100 years, and as a result, there are differences in soil chemical properties among the treatments. In particular, the soil pH of the −N treatment, where no N fertilizer was applied for a long time, was higher than that of the other treatments (Table S1), resulting in the decline of availability of many trace metal elements in the soil of the −N treatment (Watanabe et al. 2015). Therefore, it is strongly suggested that the low availability of trace elements in the soil is a limiting factor for soybean growth in the −N treatment, and that the enhanced uptake of trace elements in soybean due to the mixed cropping with lupin (Fig. 2) caused its growth enhancement in the −N treatment.

In the −P treatment, as expected, a significant increase in P concentrations in the soybean leaves was observed when co-cultured with lupin, suggesting that improved P nutrition was responsible for the improved growth of soybean. Similar results have been observed in previous pot experiments cultivating maize with lupin (Dissanayaka et al. 2015; Dissanayaka and Wasaki 2021). Meanwhile, enhanced uptake of alkaline metal elements such as K and Rb and alkaline earth metal elements such as Mg, Ca, Sr, and Ba was also observed in soybean co-cultured with lupin in the −P treatment. The increase in the concentration of alkaline metal elements was also observed in the −K treatment. Thus, it is suggested that the rhizosphere effect of the lupin enhanced the availability of various elements in the soils and affected mineral element uptake in soybean that shared the rhizosphere. In addition, since the effect of mixed cropping with lupin was also observed in the mineral concentrations of soybean leaves in the +NPK treatment, it seems that lupin roots considerably change the rhizosphere environment even in soils with relatively good nutrient conditions.

Under hydroponic conditions, the mineral absorption characteristics of plant roots directly affected the plant mineral concentration as the rhizosphere effect is negligible under the conditions where the culture solution is well circulated, and all mineral elements are solubilized. Therefore, to directly compare the ability to solubilize mineral elements in the rhizosphere soil between lupin and soybean, the concentration ratios for the lupin and soybean leaves were calculated for each essential element and Cs for each of the hydroponically and field (+NPK treatment of the above field experiment, monoculture) grown cases (Fig. 4). The reason for selecting the +NPK treatment in the field cultivation for comparison is that the complete nutrient solution was used in the hydroponic cultivation. It is known that even under nutrient-rich conditions, lupin has significant rhizosphere effects such as the secretion of organic acids from the roots (Egle, Römer, and Keller 2003). Since the results of the field trials suggested the solubilization of alkaline metal elements in the lupin rhizosphere, Cs was also added at subtoxic concentrations. The results showed that the lupin/soybean ratios of leaf mineral concentrations were larger in field cultivations than in hydroponics for many elements, and the ratios of Na, K, Cs, P, Fe, Cu, and Mo in the field cultivation were more than twice those in the hydroponics (Fig. 4). It is likely that lupin roots have a greater ability to solubilize these elements in the rhizosphere soil than soybean roots.

These results lead us to question what is responsible for the solubilizing ability of lupin root for various mineral elements in the rhizosphere soil. The metabolites that lupin secretes from its roots have been studied in detail (Tomasi et al. 2008; Valentinuzzi et al. 2015), and among them, citric and malic acids, which are secreted in large amounts from lupin roots, are known to be able to solubilize not only P but also various metal elements (Gerke, Römer, and Jungk 1994). Dessureault-Rompré et al. (2008) showed that lupin increased the concentrations of Ca, Mg, Fe, Mn, and Al in the rhizosphere soil solution in a rhizobox experiment and suggested that these increases were due to the binding effect of citrate secreted by lupin roots. They did not mention alkaline metal elements, but the solubilization of these elements in the rhizosphere soils by secreted organic acids is possible. In the mixed cropping experiments in this investigation, the increase in the concentration of many elements in soybean leaves due to co-culture with lupin was observed in the −P treatment, where organic acid secretion from lupin roots is expected to be high (Fig. 3). Li et al. (2016) showed that citrate increased K release from biotite at pH 4, and suggested that the reason for this phenomenon is that citrate enhanced the surface dissolution and the structure alteration of biotite. Yang et al. (2019) also showed that organic acids increase the available K concentrations in the soil, and there was a positive correlation between the amount of organic acids secreted by the roots of *Nicotiana tabacum* and the available K concentration in the rhizosphere soil. These results strongly suggest that organic acid secretion from the roots is involved in the solubilization of various elements, including alkaline metal elements, in the lupin rhizosphere.

The results of this study did not support the solubilization of Mn in the rhizosphere soils under P deficient conditions (Fig. 3), while it has previously been found to be solubilized in the rhizosphere soil of lupin (Dessureault-Rompré et al. 2008). In their review, Lambers et al. (2015) suggest that plants that secrete organic acids from their roots as a response to P deficiency, such as lupin, tend to have higher Mn concentrations in their leaves because Mn is solubilized simultaneously with P solubilization. In the hydroponic cultivations in this study, the Mn concentration of the lupin leaves was found to be more than 10 times higher than that of soybean, indicating that the Mn uptake capacity of lupin roots is much higher than that of soybean (Fig. 4). The Mn uptake in soybean may not have been significantly affected by mixed cropping with lupin in −P treatment (Fig. 3) because lupin actively absorbed Mn that lupin itself solubilized in the rhizosphere.

The element with the largest increase in its lupin/soybean ratio for leaf concentrations in field cultivation when compared to hydroponics was Cs, an alkaline metal element. The leaf Cs concentration in hydroponics was not significantly different between lupin and soybean (Fig. 4), and the large change in this ratio was due to the extremely low Cs uptake by soybean in the field cultivation (Table S4). Most plant species, including soybean, have difficulty absorbing Cs from the soil (Shinano et al. 2014; Watanabe and Azuma 2021). The primary reason for this is that Cs fixed in soil is extremely difficult to leach into the soil solution. In fact, when the concentrations of water-soluble elements relative to the total concentration contained in the soil of the +NPK treatment were calculated based on the data of Watanabe et al. (2015), K was 0.017 while Cs was 0.0004. Most of the Cs in the soil is contained in feldspars, fixed in the interlayer of 2:1 layered clay minerals, or specifically adsorbed to the edge sites of the interlayer, which are called frayed edge sites (Park, Alessi, and Baek 2019). These Cs are extremely stable and rarely leach into the soil solution (Wauters et al. 1996). However, if organic acids can enhance the surface dissolution and the structure alteration of the 2:1 layered clay minerals as described by Li et al. (2016), more Cs ions will be dissolved into the soil solution. This may be one of the main reasons why lupin roots are able to solubilize more Cs in the rhizosphere soil.

In the present study, the concentrations of many elements in the leaves of soybean were increased by mixed cropping with lupin. However, B is the only element whose concentration increased significantly in the leaves of lupin due to mixed cropping with soybean in all treatments except the +NPK treatment (Fig. 3). In Fig. 4, the lupin/soybean ratio of the concentration of B was lower in the field cultivation than in the hydroponic cultivation. These results indicate that soybean roots may solubilize B in the soil. It has been reported that readily soluble B is only 1%–2% of the total B present in the soil (Padbhushan and Kumar 2017; Tsadilas et al. 1994). However, it has been suggested that plants also absorb less soluble B as well as the readily soluble B, and the available forms of soil B seem to vary with plant species (Jin, Martens, and Zelazny 1988; Tsadilas et al. 1994). Although little research has been conducted on the solubilization of soil B by plant roots, soybean roots may have an unknown mechanism to solubilize B in rhizosphere soils that lupin roots do not have.

5. Conclusion

In this study, lupin was shown to be more tolerant to K and P deficiencies than soybean. The decrease in K concentration in the lupin leaves in the −K treatment was much smaller than that of the soybean leaves (Table S3), and this high tolerance to K deficiency is due to the high K-acquisition capacity of the roots rather than the high efficiency of K utilization in the plant. Based on the results of this study, it is likely that this high K-acquisition capacity is not due to K-absorption capacity, but to the organic acids secreted from the lupin roots, as they alter the surface structures of clay minerals, including the fixed forms of some mineral elements such as available K. Furthermore, the secreted organic acids seem to solubilize many other elements in the rhizosphere. While organic acid secretion from lupin roots has been a primary concern for P and Fe solubilization in the rhizosphere soil, it may also be an effective strategy for the solubilization of various nutrients for this species and may be an essential mechanism for survival in nutrient-poor soils. However, there are limitations in analyzing root function based solely on elemental concentration in plant shoots, as in this study. To verify these predictions, the dynamics of inorganic and organic compounds in the rhizosphere soil should be directly investigated. Furthermore, it has been reported that mixed cropping with other plant species can change the root morphology of plants, which indirectly affects the elemental absorption (Zhang et al. 2016; Zuo et al. 2003). In addition to this study, therefore, future integrated studies on root secretion and root morphology, and their impact on mineral element dynamics in the rhizosphere soil will be essential to elucidate the multi-nutrient acquisition mechanisms of lupin.

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Disclosure statement

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Figure captions

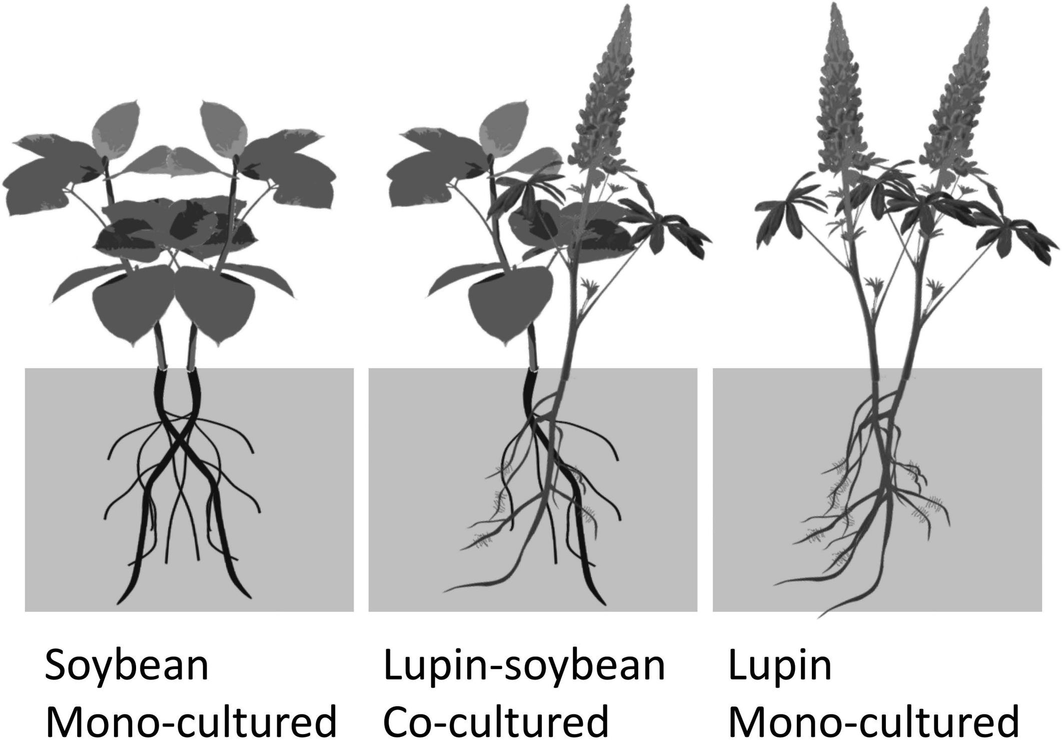
Figure 1. Planting method of lupin and soybean. The two plants were planted in the field in close proximity to each other to share the rhizosphere. Note: The plant picture is a conceptual drawing showing the characteristics of the species and should not be taken to indicate the size of the plant or specific growth stage.

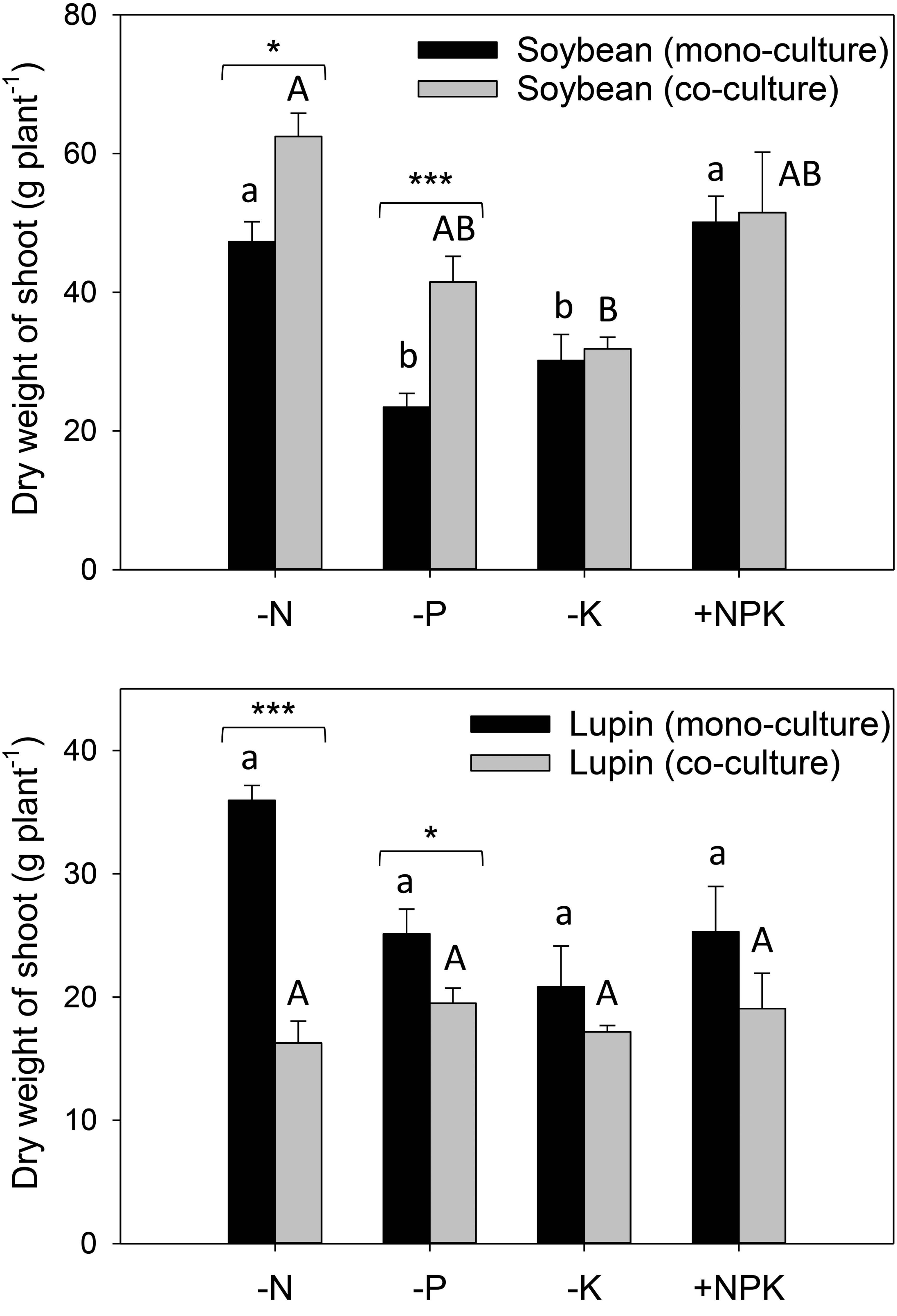
Figure 2. Effect of rhizosphere sharing on the growth of lupin and soybean under nitrogen, phosphorus, and potassium deficient conditions. −N, fertilization without N; −P, fertilization without P; −K, fertilization without K; +NPK, complete fertilization. Values are means of four replicates, and bars indicate ± standard errors for the total dry weight. Different letters indicate statistically significant differences in total dry weight ($P < 0.05$) using Tukey's multiple comparison test following a one-way ANOVA. Asterisks indicate statistically significant differences between mono-cultures and co-cultures in each treatment (Student's t-test, * and ***: $P < 0.05$ and 0.001,

respectively).

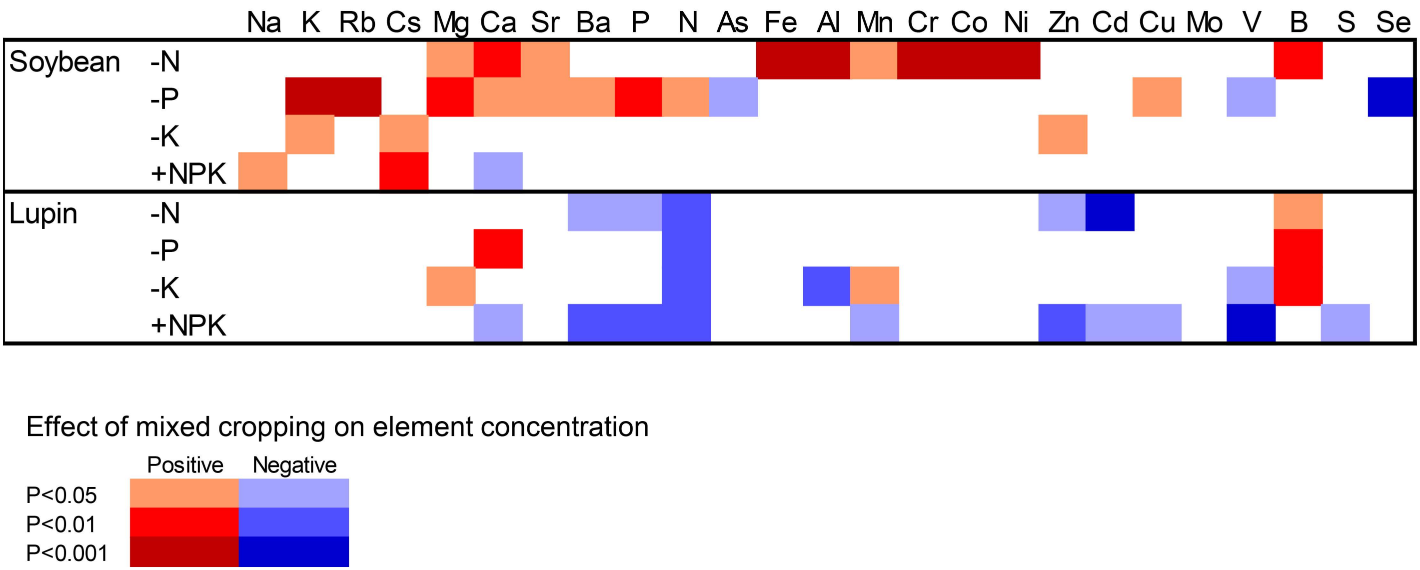
Figure 3. Heatmap analysis showing the effects of rhizosphere sharing on the accumulation of different elements in the leaves of soybean and lupin under different nutrient treatments. Leaf concentrations of each element were statistically compared between mono-cultures and co-cultures using the Student's t-test to evaluate the effect of mixed cropping on leaf element accumulation. −N, fertilization without N; −P, fertilization without P; −K, fertilization without K; +NPK, complete fertilization.

Figure 4. Lupin/soybean ratios for the concentrations of each element in the leaves under different growth conditions. a: hydroponics, b: field (+NPK treatment). Values are means of three and four replicates in hydroponics and field cultivation, respectively, and bars indicate ± standard errors. Values in the bar are the lupin/soybean ratios for each element concentration in the leaves.

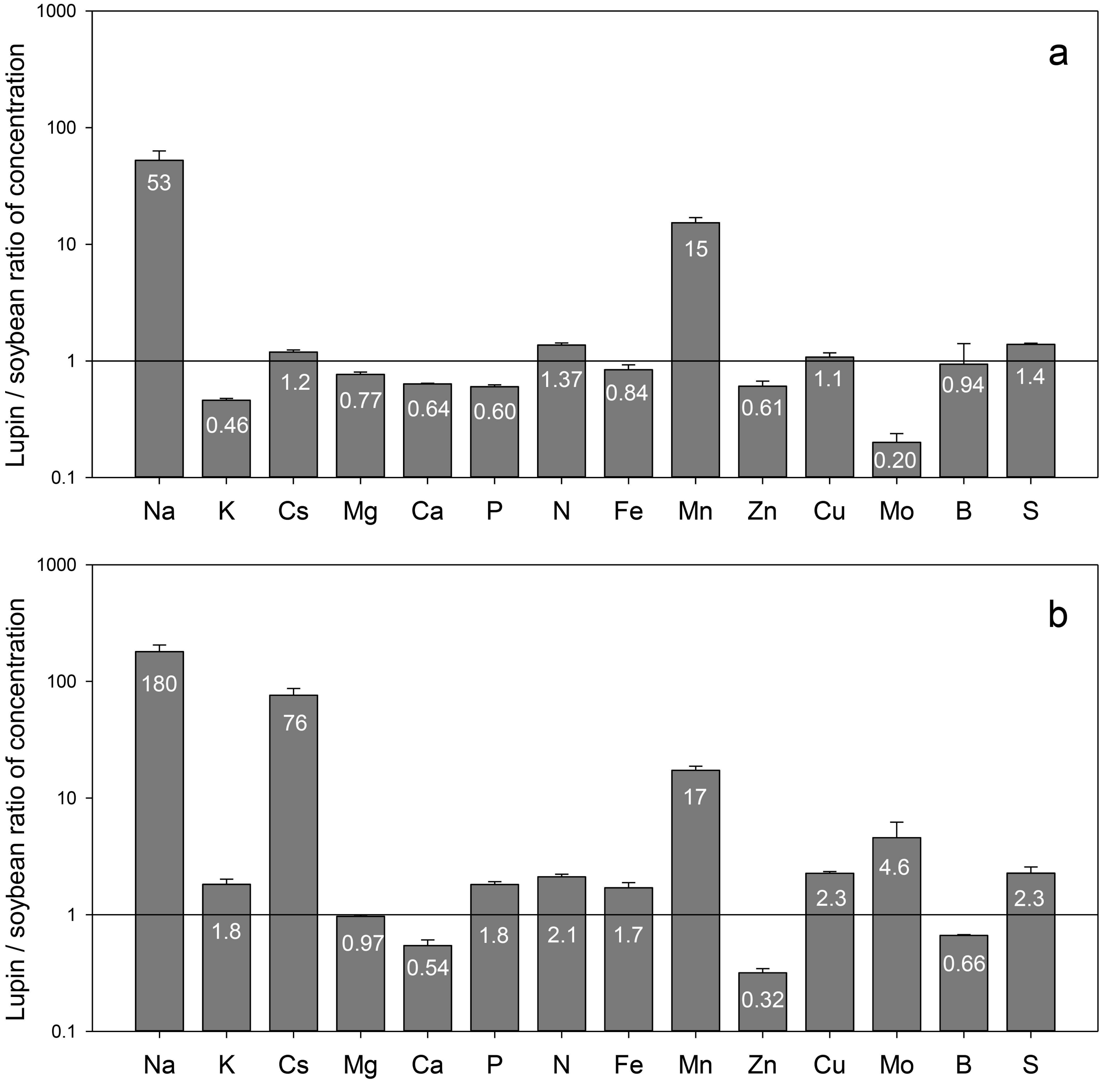

Soybean
Mono-cultured
Lupin-soybean
Co-cultured
Lupin
Mono-cultured



Dry weight of shoot (g plant-1)
80
60
40
20
0
Soybean (mono-culture)
Soybean (co-culture)
*
A
a
***
AB
b
b
B
a
AB
-N
-P
-K
+NPK
Dry weight of shoot (g plant-1)
40
30
20
10
0
Lupin (mono-culture)
Lupin (co-culture)
***
a
A
*
a
A
a
A
a
A
-N
-P
-K
+NPK



Na K Rb Cs Mg Ca Sr Ba P N As Fe Al Mn Cr Co Ni Zn Cd Cu Mo V B S Se
Soybean -N
-P
-K
+NPK
Lupin -N
-P
-K
+NPK
Effect of mixed cropping on element concentration
Positive Negative
P<0.05
P<0.01
P<0.001



a
Lupin / soybean ratio of concentration
1000
100
10
1
0.1
53
0.46
1.2
0.77
0.64
0.60
1.37
0.84
15
0.61
1.1
0.20
0.94
1.4
Na
K
Cs
Mg
Ca
P
N
Fe
Mn
Zn
Cu
Mo
B
S
b
Lupin / soybean ratio of concentration
1000
100
10
1
0.1
180
1.8
76
0.97
0.54
1.8
2.1
1.7
17
0.32
2.3
4.6
0.66
2.3
Na
K
Cs
Mg
Ca
P
N
Fe
Mn
Zn
Cu
Mo
B
S