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

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Short running title: Lupin roots solubilize minerals

1

2 **Abstract**

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

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

24

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

26

27 **1. Introduction**

28 The rhizosphere is the interface between plant roots and soil, where roots, soil microorganisms, and soils interact
29 with each other (Hinsinger et al. 2005). In the rhizosphere, roots influence soil chemical, physical, and biological
30 properties (Gregory and Hinsinger 1999; Wenhao et al. 2013), and these are referred to as “rhizosphere effects”. In
31 problem soils, roots are the plant organs that are directly exposed to soil stress. Plant roots adapt to soil stress by
32 altering the rhizosphere environment to prevent root damage caused by toxic elements (Kochian, Pineros, and
33 Hoekenga 2005) and by increasing the availability of nutrient elements (Hinsinger et al. 2011). *Lupinus albus* L.
34 (lupin) is an annual legume grown in temperate regions, and is highly tolerant of phosphorus (P) deficient soils as
35 it can alter the rhizospheric environment to increase the available P (Gardner and Parbery 1982). Bottlebrush-like
36 clusters of rootlets along its lateral roots, called cluster roots, are formed under conditions of nutrient deficiency,

37 particularly P deficiency (Watt and Evans 2003). These cluster roots efficiently exude phosphatase and organic
38 acids to decompose organic P and solubilize insoluble P, respectively, in the rhizosphere (Neumann et al. 2000;
39 Wasaki et al. 2003). These abilities of lupin roots are powerful, and in pot experiment where maize was mixed-
40 cropped with lupin, it was reported that the P nutrient status of maize in P-deficient soil was significantly improved
41 due to the mixed cropping with lupin (Dissanayaka et al. 2015; Dissanayaka and Wasaki 2021). Furthermore, it has
42 been found that in the lupin rhizosphere, organic nitrogen (N) may be decomposed by lupin roots, rather than
43 rhizosphere microorganisms (Fujiishi, Maejima, and Watanabe 2020). It has also been suggested that lupin roots
44 may be able to solubilize other elements in the rhizosphere soil (Dessureault-Rompré et al. 2008), but this requires
45 further investigation.

46 Currently, 17 essential elements are known to be required by plants (Marschner 2012). Nonessential elements also
47 occur in soils and are absorbed and translocated by plants alongside essential elements. Ionics is the study of all
48 metal, metalloid, and nonmetal accumulations in living organisms, regardless of whether the accumulated minerals
49 are essential or nonessential, and this helps us to better understand biological and physiological problems (Salt,
50 Baxter, and Lahner 2008). Ionics has been applied not only to plants but also to cultivated soils to analyze the
51 mineral dynamics between plants and soils (Watanabe et al. 2015). It is important to examine the rhizosphere soil
52 to elucidate the impact of roots on soil mineral dynamics (Chu et al. 2017). However, it is also difficult to obtain
53 accurate information to understand the mineral dynamics between plants and soils just from the analysis of
54 rhizosphere soils, as even though the roots can solubilize elements in the rhizosphere, they then absorb the

55 solubilized elements during growth (Chu et al. 2017). Therefore, we devised experiments using another control
56 plant to estimate the effect of the lupin roots on soil mineral dynamics in the rhizosphere.

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

69

70 **2. Materials and methods**

71 ***2.1. Effect of rhizosphere sharing with lupin on the leaf ionome of soybean under nitrogen, phosphorus, or***
72 ***potassium deficient conditions in the field***

73 *2.1.1. Cultivation*

74 In 2015, lupin (cv. Energy) and soybean (*Glycine max* (L.) Merr. cv. Wasemidori) were cultivated in the long-term
75 fertilizer experimental field of Hokkaido University. This field was established in 1914, and 4 fertilizer treatment
76 plots: complete fertilization (+NPK), without N (-N), without P (-P), and without K (-K), have been continuously
77 run here for 101 years. The N, P, and K fertilizers were applied as ammonium sulfate, superphosphate, and potassium
78 sulfate, respectively (100 kg N, P₂O₅, K₂O ha⁻¹), once in a year before sowing. Each plot was 5.25 × 18.5 m in size,
79 and the soil type was classified as a brown lowland soil (Haplic Fluvisols). The general properties of the field bulk
80 soils are shown in Table S1 (pH, concentration of total carbon, and total N) and Watanabe et al. (2015) (concentration
81 of available minerals). Seeds of each plant species (two seeds per hole) were sown on June 9 in each plot. Lupin
82 and soybean were cultivated alone (mono-cultured) or co-cultured with shared rhizospheres, as shown in Fig. 1. The
83 row and intra-row spacing was 50 cm × 30 cm.

84

85 *2.1.2. Sampling*

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

90

91 *2.1.3. Mineral analysis*

92 Each 70 mg leaf sample was digested in 2 mL of 61% (w/v) HNO₃ (EL grade; Kanto Chemical, Tokyo, Japan) at
93 110°C in a DigiPREP apparatus (SCP Science, Montreal, Canada) for approximately 2 h until the solution had
94 almost disappeared. After the samples had cooled, 0.5 mL H₂O₂ (semiconductor grade; Santoku Chemical, Tokyo,
95 Japan) was added and the samples were heated at 110°C for an additional 20 min. Once digestion was complete, the
96 tubes were cooled, and the samples were reconstituted to a volume of 10 mL by adding 2% (w/v) HNO₃ in ultrapure
97 water. The concentrations of 24 elements [potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), sulfur
98 (S), iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu), nickel (Ni), molybdenum (Mo), sodium (Na),
99 aluminum (Al), vanadium (V), chromium (Cr), cobalt (Co), arsenic (As), selenium (Se), rubidium (Rb), strontium
100 (Sr), cadmium (Cd), cesium (Cs), and barium (Ba)] were determined by using an inductively coupled plasma-mass
101 spectrometer (ELAN DRC-e; Perkin Elmer, Waltham, MA, USA). External calibration standards containing these
102 elements were measured after every 10 samples. To determine the plant N concentrations, each 70 mg sample was
103 digested with 1.25 mL of concentrated H₂SO₄ in a test tube at 200°C. At 30-min intervals, 0.3 mL of H₂O₂ was
104 added to each tube (eight times), following which the N concentration in the digests was determined using the micro-
105 Kjeldahl method (K-350 Distillation Unit, Büchi, Flawil, Switzerland).

106

107 ***2.2. Mineral accumulation of lupin and soybean under hydroponic conditions***

108 *2.2.1. Cultivation*

109 The hydroponic experiment was conducted in a greenhouse at Hokkaido University under natural light conditions.
110 Seeds of lupin and soybean were surface-sterilized with 70% (v/v) ethanol for 2 min, and then washed with
111 deionized water. The seeds were germinated in running water for 24 h, and the germinated seeds were sown in
112 vermiculite and precultured for 7 d in a greenhouse. Then, three seedlings per replicate in each plant species were
113 transferred to 8 L container (30 cm × 26 cm × 15 cm) containing 8 L of aerated standard nutrient solution
114 supplemented with 5 μM CsCl (mono-culture). The standard nutrient solution contained 2.14 mM N (NH₄NO₃), 32
115 μM P (NaH₂PO₄·2H₂O), 0.77 mM K (K₂SO₄:KCl = 1:1), 1.25 mM Ca (CaCl₂·2H₂O), 0.82 mM Mg (MgSO₄·7H₂O),
116 35.8 μM Fe (FeSO₄·7H₂O), 9.1 μM Mn (MnSO₄·4H₂O), 46.3 μM B (H₃BO₃), 3.1 μM Zn (ZnSO₄·7H₂O), 0.16 μM
117 Cu (CuSO₄·5H₂O), and 0.05 μM Mo ((NH₄)₆Mo₇O₂₄·4H₂O); total SO₄ = 1.06 mM. We set the P concentration in
118 the culture medium to 32 μM, as this concentration has already been reported to be sufficient for many crops,
119 including soybean (Tadano and Tanaka 1980). The pH of the solution was adjusted to 6.0 ± 0.1 with 0.1 M NaOH
120 or 0.1 M HCl daily, and the solution was renewed every week.

121

122 2.2.2. *Sampling and mineral analysis*

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

127

128 **2.3. Statistical analyses**

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

131

132 **3. Results**

133 **3.1. Effect of rhizosphere sharing with lupin on the leaf ionome of soybean under nitrogen, phosphorus, or**
134 **potassium deficient conditions in the field**

135 **3.1.1. Growth**

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

143

144 **3.1.2. Effect of rhizosphere sharing on the accumulation of mineral elements in leaves**

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

158

159 ***3.2. Differences in the mineral concentrations of leaves between lupin and soybean when cultivated in the field***
160 ***or hydroponic cultures***

161 Soybean and lupin were grown hydroponically in a nutrient solution containing essential elements and trace
162 concentrations of Cs, and the mineral concentrations in the leaves were determined (Table S4). The ratio of lupin to

163 soybean based on the concentrations in the leaves was calculated for each element in the hydroponically and field
164 (+NPK treatment of the above field experiment, monoculture) grown plants (Fig. 4). When grown hydroponically,
165 the Na and Mn concentrations were much higher in the lupin, while the concentrations of the other elements showed
166 little difference between lupin and soybean or tended to be lower in lupin (Fig. 4A). By contrast, in the field grown
167 plants, the Na and Mn concentrations were considerably higher in lupin as in hydroponics, but many other elements
168 were also higher in the lupin, especially the Cs (Fig. 4B).

169

170 **4. Discussion**

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

181 while the growth of lupin was reduced (Fig. 2). This suggests that lupin-induced changes in the rhizosphere
182 environment had beneficial effects on soybean growth.

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

198 In the -P treatment, as expected, a significant increase in P concentrations in the soybean leaves was observed when

199 co-cultured with lupin, suggesting that improved P nutrition was responsible for the improved growth of soybean.
200 Similar results have been observed in previous pot experiments cultivating maize with lupin (Dissanayaka et al.
201 2015; Dissanayaka and Wasaki 2021). Meanwhile, enhanced uptake of alkaline metal elements such as K and Rb
202 and alkaline earth metal elements such as Mg, Ca, Sr, and Ba was also observed in soybean co-cultured with lupin
203 in the -P treatment. The increase in the concentration of alkaline metal elements was also observed in the -K
204 treatment. Thus, it is suggested that the rhizosphere effect of the lupin enhanced the availability of various elements
205 in the soils and affected mineral element uptake in soybean that shared the rhizosphere. In addition, since the effect
206 of mixed cropping with lupin was also observed in the mineral concentrations of soybean leaves in the +NPK
207 treatment, it seems that lupin roots considerably change the rhizosphere environment even in soils with relatively
208 good nutrient conditions.

209 Under hydroponic conditions, the mineral absorption characteristics of plant roots directly affected the plant mineral
210 concentration as the rhizosphere effect is negligible under the conditions where the culture solution is well circulated,
211 and all mineral elements are solubilized. Therefore, to directly compare the ability to solubilize mineral elements in
212 the rhizosphere soil between lupin and soybean, the concentration ratios for the lupin and soybean leaves were
213 calculated for each essential element and Cs for each of the hydroponically and field (+NPK treatment of the above
214 field experiment, monoculture) grown cases (Fig. 4). The reason for selecting the +NPK treatment in the field
215 cultivation for comparison is that the complete nutrient solution was used in the hydroponic cultivation. It is known
216 that even under nutrient-rich conditions, lupin has significant rhizosphere effects such as the secretion of organic

217 acids from the roots (Egle, Römer, and Keller 2003). Since the results of the field trials suggested the solubilization
218 of alkaline metal elements in the lupin rhizosphere, Cs was also added at subtoxic concentrations. The results
219 showed that the lupin/soybean ratios of leaf mineral concentrations were larger in field cultivations than in
220 hydroponics for many elements, and the ratios of Na, K, Cs, P, Fe, Cu, and Mo in the field cultivation were more
221 than twice those in the hydroponics (Fig. 4). It is likely that lupin roots have a greater ability to solubilize these
222 elements in the rhizosphere soil than soybean roots.

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

235 alteration of biotite. Yang et al. (2019) also showed that organic acids increase the available K concentrations in the
236 soil, and there was a positive correlation between the amount of organic acids secreted by the roots of *Nicotiana*
237 *tabacum* and the available K concentration in the rhizosphere soil. These results strongly suggest that organic acid
238 secretion from the roots is involved in the solubilization of various elements, including alkaline metal elements, in
239 the lupin rhizosphere.

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

249 The element with the largest increase in its lupin/soybean ratio for leaf concentrations in field cultivation when
250 compared to hydroponics was Cs, an alkaline metal element. The leaf Cs concentration in hydroponics was not
251 significantly different between lupin and soybean (Fig. 4), and the large change in this ratio was due to the extremely
252 low Cs uptake by soybean in the field cultivation (Table S4). Most plant species, including soybean, have difficulty

253 absorbing Cs from the soil (Shinano et al. 2014; Watanabe and Azuma 2021). The primary reason for this is that Cs
254 fixed in soil is extremely difficult to leach into the soil solution. In fact, when the concentrations of water-soluble
255 elements relative to the total concentration contained in the soil of the +NPK treatment were calculated based on
256 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
257 feldspars, fixed in the interlayer of 2:1 layered clay minerals, or specifically adsorbed to the edge sites of the
258 interlayer, which are called frayed edge sites (Park, Alessi, and Baek 2019). These Cs are extremely stable and rarely
259 leach into the soil solution (Wauters et al. 1996). However, if organic acids can enhance the surface dissolution and
260 the structure alteration of the 2:1 layered clay minerals as described by Li et al. (2016), more Cs ions will be
261 dissolved into the soil solution. This may be one of the main reasons why lupin roots are able to solubilize more Cs
262 in the rhizosphere soil.

263 In the present study, the concentrations of many elements in the leaves of soybean were increased by mixed cropping
264 with lupin. However, B is the only element whose concentration increased significantly in the leaves of lupin due
265 to mixed cropping with soybean in all treatments except the +NPK treatment (Fig. 3). In Fig. 4, the lupin/soybean
266 ratio of the concentration of B was lower in the field cultivation than in the hydroponic cultivation. These results
267 indicate that soybean roots may solubilize B in the soil. It has been reported that readily soluble B is only 1%–2%
268 of the total B present in the soil (Padbhushan and Kumar 2017; Tsadilas et al. 1994). However, it has been suggested
269 that plants also absorb less soluble B as well as the readily soluble B, and the available forms of soil B seem to vary
270 with plant species (Jin, Martens, and Zelazny 1988; Tsadilas et al. 1994). Although little research has been conducted

271 on the solubilization of soil B by plant roots, soybean roots may have an unknown mechanism to solubilize B in
272 rhizosphere soils that lupin roots do not have.

273

274 **5. Conclusion**

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

289 studies on root secretion and root morphology, and their impact on mineral element dynamics in the rhizosphere
290 soil will be essential to elucidate the multi-nutrient acquisition mechanisms of lupin.

291

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295

296 **Disclosure statement**

297 No conflicts of interest declared.

298

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302

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403

404 **Figure captions**

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

408

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

415 respectively).

416

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

422

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

427



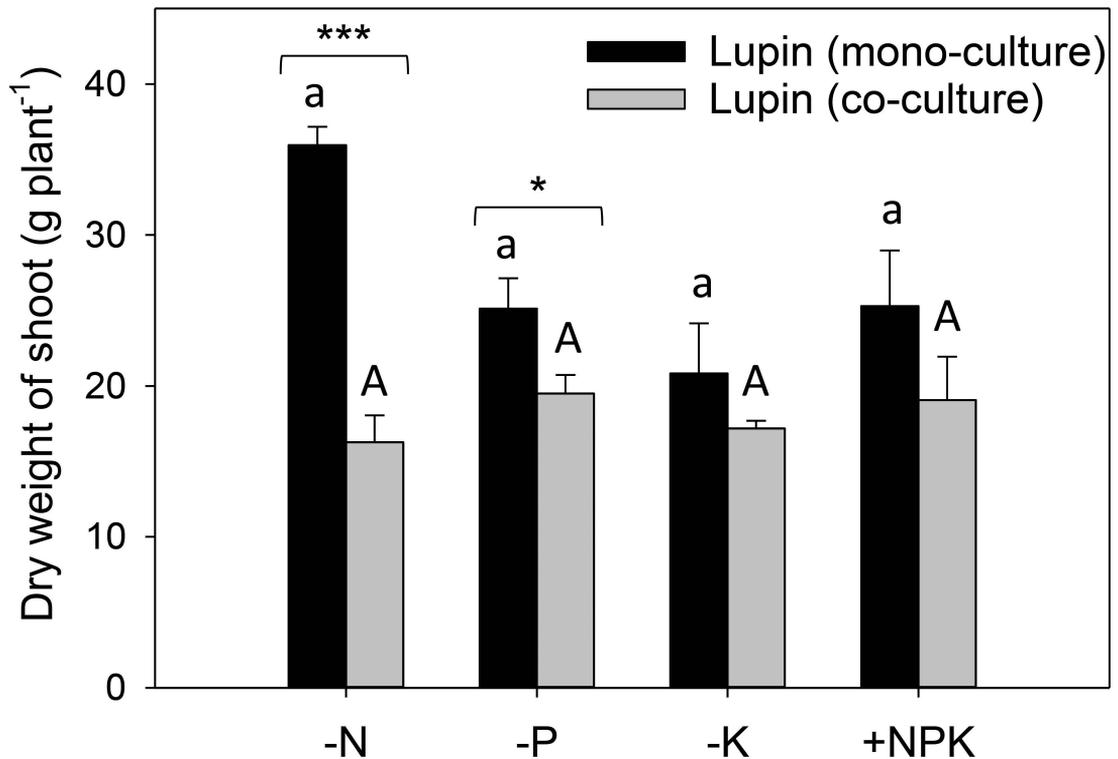
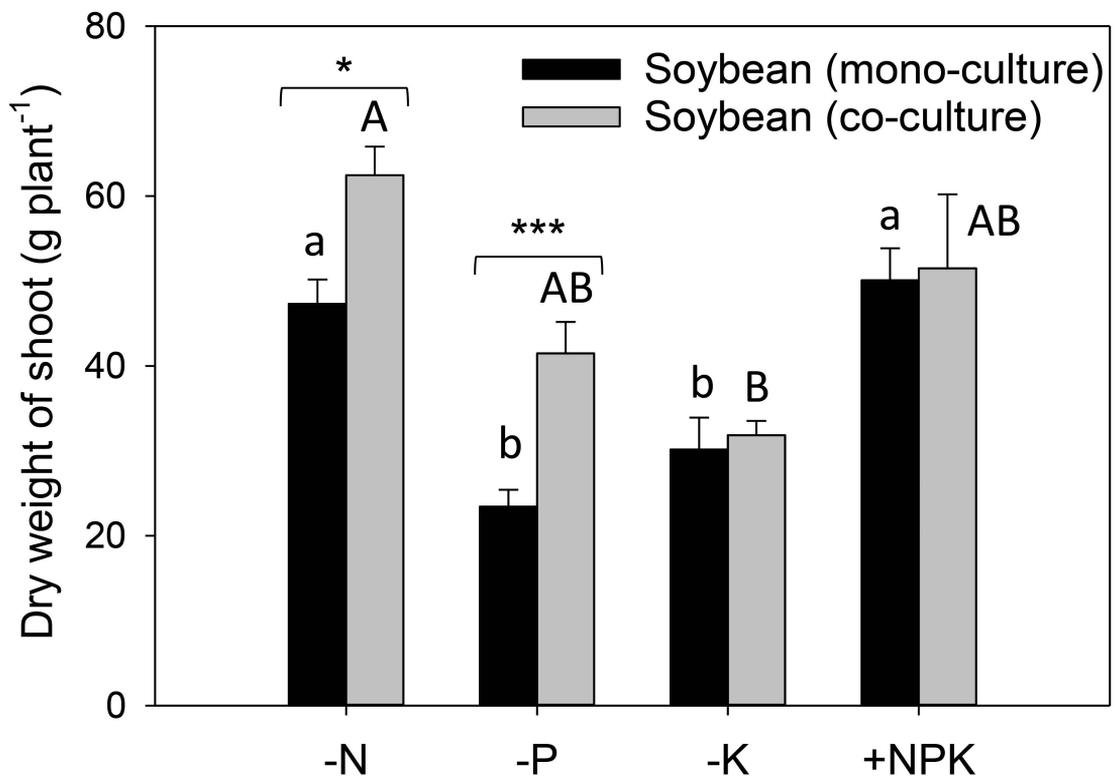
Soybean
Mono-cultured



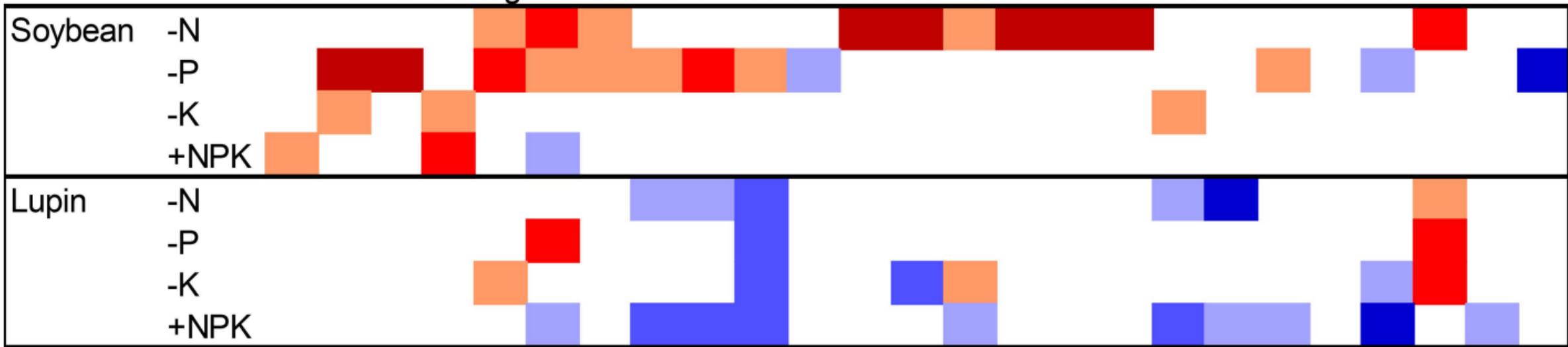
Lupin-soybean
Co-cultured



Lupin
Mono-cultured



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



Effect of mixed cropping on element concentration



