# Nitrogen deficiency-induced molybdenum accumulation in wheat

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## Abstract

In the present study, we conducted experiments using wheat to elucidate whether the increased accumulation of molybdenum in leaves under nitrogen deficiency is due to the plant's own metabolic response, and further to estimate the role of molybdenum in the nitrogen deficiency response. Even under different growth conditions such as soil culture, hydroponic culture, and aseptic culture, the nitrogen deficiency always increased the molybdenum accumulation in leaves of wheat. Because molybdenum supply to the soil enhanced the growth of wheat under nitrogen deficiency but did not increase plant nitrogen concentration, the increased molybdenum uptake might be involved in the adaptive mechanisms to nitrogen deficiency by increasing nitrogen use efficiency. Wheat under nitrogen deficiency accumulated more molybdenum in lower leaves. Moreover, the nitrogen concentration of wheat grown under nitrogen deficiency increased in the lower leaves and decreased in the upper leaves with the application of molybdenum. These results suggest that molybdenum might affect nitrogen translocation from older to younger leaves.



Keywords: nitrogen deficiency; molybdenum; soybean; wheat


## Introduction

Currently, 17 essential inorganic elements are known to be required by plants (Marschner 2012). Molybdenum (Mo) is the essential element with the highest atomic weight and also with the lowest content in plants (Watanabe and Azuma, 2021). Mo is bound to pterin, and composes Molybdenum cofactor (Moco) in the active center of plant enzymes catalyzing key steps of nitrogen (N), carbon, and sulfur metabolisms (Zhang and Gladyshev, 2008). Well-known Mo-enzymes are nitrate reductase, sulfite

oxidase, xanthine dehydrogenase, aldehyde oxidase, and the mitochondrial amidoxime reductase (Mendel, 2011). In leguminous plants, Mo is also required for symbiotic $N_2$ fixation as the metal component of bacterial nitrogenase (Shah et al., 1984). Although more than 80 years have passed since Mo was noticed as an essential element (Arnon & Stout, 1939), however, its function might not be fully elucidated because Mo is contained in only a very small amount in plants.

Ionomics is the study of all metal, metalloid, and nonmetal accumulation in living organisms, regardless of whether the accumulated minerals are essential or nonessential (Huang and Salt, 2016; Salt et al. 2008). We previously examined the ionomic responses to nutrient deficiency in wheat, maize, sunflower, and soybean cultivated with four fertilizer treatments; complete fertilization (control), without N, without phosphorus (P), and without potassium (K) (Watanabe et al., 2015; Watanabe et al., currently under review in J. Plant Nutr.). In the results, we found that Mo accumulation in leaves increased in common for non-leguminous species cultivated under N-deficient conditions whereas it decreased and increased in leaves and roots of soybean, respectively (Watanabe et al., 2015; Watanabe et al., currently under review in J. Plant Nutr.). Changes in Mo distribution due to N deficiency in soybean, a leguminous plant, can be considered to be due to an increased demand for Mo in biological $N_2$ fixation of Rhizobium in the nodules (Ishizuka, 1981; Chu et al., 2016). However, the increase in Mo accumulation induced by N deficiency in the leaves of non-leguminous species is difficult to explain by known Mo-related metabolisms in plants. Moreover, external factors such as changes in Mo availability in soils, $N_2$-fixing rhizosphere microorganisms, and endophytes could affect Mo accumulation in plants. In this study, therefore, we conducted experiments using wheat to elucidate whether the increased accumulation of Mo in leaves under N deficiency is due to the plant's own metabolic

response to N deficiency, and further to estimate the role of Mo in the N deficiency response.

## Materials and method

### *Experiment 1: Effects of nitrogen deficiency on accumulation and distribution of molybdenum in wheat under field conditions*

In 2017, wheat (*Triticum aestivum* L. cv. Haruyokoi) plants were cultivated in complete fertilization (+NPK) and without N fertilization (−N) treatments of the long-term fertilizer application in the experimental field. This field was established in 1914, and the fertilizer treatments have been continuously applied for 103 years. The cultivation history of the field has been described in Table S1. 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 before sowing. Each plot was 5.25 × 18.5 m in size, and the soil type was classified as a brown lowland soil (Haplic Fluvisols). The general properties of the field soils were shown elsewhere (Watanabe et al. 2015). Seeds of wheat plant were sown on the 1$^{st}$ day of June and cultivated just before flowering stage. Then, each plant were separated into leaves (lower, middle, upper, and flag), stems and roots, and washed with deionized water. After determining the fresh weight of each plant sample, a part of each sample was then reweighed, and lyophilized. Dry weight of each lyophilized sample was determined, and each sample was stored at -20$^{o}$C before mineral analysis. Plant samples were ground and digested in 2 ml of 61 % (w/v) $HNO_3$ (EL grade; Kanto Chemical, Tokyo, Japan) at a temperature of 110$^{o}$C in a DigiPREP apparatus (SCP Science, Canada) for approximately 2 h until the solution had almost disappeared. When the samples had cooled, 0.5 ml of $H_2O_2$ (semiconductor grade; Santoku Chemical, Tokyo, Japan) was added and the samples were heated at a

temperature of 110ºC for another 20 min. As soon as the process of digestion was complete, the tubes were cooled and filled to 10 ml with 2 % (w/v) $HNO_3$ in Milli-Q water. The concentration of Mo in each organ was analyzed using an inductively coupled plasma-mass spectrometry (ICP-MS; ELAN DRC-e, Perkin Elmer, Waltham, MA, USA).

***Experiment 2: Effects of nitrogen deficiency on accumulation of molybdenum in wheat and soybean under hydroponic culture***

The experiment was conducted in a greenhouse in Hokkaido University. Seeds of wheat and soybean plants were surface-sterilized using 10% (v/v) sodium hypochlorite for 10 min and 1min, respectively, and then washed with distilled water. The surface-sterilized seeds were sown in trays filled with perlite moistened with distilled water. After germination, the seedlings were grown for 2 weeks. Then, the seedlings were transferred to 36-L containers (56 cm × 32 cm × 21 cm) containing standard nutrient solution, and grown for 7 days for preculture in hydroponics. 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. After that, they were transferred to 4-L containers containing standard nutrient solution with N treatments (High N: 2.14 mM N, Low N: 0.214 mM N). The pH of the solution was adjusted to 5.3 ± 0.1 with 0.1 M NaOH or 0.1 M HCl daily to avoid the precipitation of iron ion, and renewed every week. The seedlings were treated for 2 weeks with three replicates. After the treatment, seedlings were harvested and separated into leaves, stems and roots. The samples were flash-frozen under liquid nitrogen, lyophilized and then weighed. Mo concentrations of the plant samples were

then determined as described in Experiment 1. Nitrogen concentration contained in the plant samples was determined by the Kjeldahl method after wet digestion with $H_2SO_4$ and $H_2O_2$ (Fujiishi et al., 2019).

***Experiment 3: Effects of molybdenum application on growth responses of wheat to nitrogen deficiency under soil culture***

Pot experiment was conducted in a greenhouse of Hokkaido University. The soil used for the pot experiment was collected from the plough layer (0−10 cm) of −N plot in the long-term fertilizer experimental field of Hokkaido University. The soils were mixed with vermiculite in the ratio of 1:1 (v/v) to improve the physical properties of the soil, and then 2.6 L of the mixed soil was put in a plastic pot (3 L). To each pot, 0.25 g $P_2O_5$ and 0.25 g $K_2O$ were added as superphosphate and potassium sulfate, respectively. Four treatments were applied: +N+Mo (0.25 g N and 0.5 mg Mo $pot^{-1}$), +N−Mo (0.25 g N and 0 mg Mo $pot^{-1}$), −N+Mo (0 g N and 0.5 mg Mo $pot^{-1}$) and −N−Mo (0 g N and 0 mg Mo $pot^{-1}$) with 4 replicates. Nitrogen and Mo were applied in the form of $(NH_4)_2SO_4$ and $(NH_4)_6Mo_7O_{24}•4H_2O$, respectively. Seeds of wheat plant were surface-sterilized as described above. Then, six seeds were sown on each pot. After germination, seedlings were thinned to three. The soils were irrigated with deionized water daily to maintain the water content at approximately 60% of the maximum water-holding capacity. Seedlings were grown for 28 days. After cultivation, plants were sampled, and the dry weight and concentration of N and Mo were determined as described in Experiments 1 and 2.

***Experiment 4: Effects of nitrogen deficiency on molybdenum uptake of wheat under aseptic culture***

Seeds of wheat were surface-sterilized in 70% ethanol for 1 min, followed by 10% NaClO for 10 min under reduced pressure condition, and rinsed 10 times with sterile

125 Milli-Q water. Surface-sterilized seeds were placed on sterilized agar-medium
126 containing 0.7% agar and 2 mM $CaCl_2$, and germinated for 2 d at 25°C under dark
127 conditions. After germination, the seedlings were transferred to SCD (Soybean Casein
128 Digest) agar medium for another 3 days to check for their sterility. Two hundred ml of
129 vermiculite was placed in a plant box (Biomedical Science, Tokyo, Japan), then 150 ml
130 of a standard nutrient solution with N treatments (High N: 45 mg N $pot^{-1}$, Low N: 0.45
131 mg N $pot^{-1}$) was added to the vermiculite. The pH of the solution was adjusted to 5.5
132 with 0.1 M NaOH just before the application. After that, vermiculite in the plant box
133 was covered with 16 g of river sand to fix the seedlings, and autoclaved at 121 °C for 2
134 h. The autoclaved boxes were stood at room temperature for 24 h, then autoclaved for
135 another 2 h. After cooling to room temperature, an aseptic seedling was transplanted to
136 each plant box aseptically, and then 5 ml of the water extract of soil (1:2.5 soil-to-water
137 ratio, filtered through the filter paper) from flesh soil in −N plot of the long-term
138 fertilizer experimental field (non-sterilized treatment) or the membrane-filter-sterilized
139 (pore size = 0.22 μm) soil extract (sterilized treatment) was added. The plant boxes
140 were covered by a plastic bag, which was attached to filter paper for ventilation to
141 maintain aseptic conditions. Wheat seedlings were cultivated in a growth chamber (25
142 °C with day/night = 16/8) for 11 days with 5 replicates. After cultivation, chlorophyll
143 concentrations of leaves were measured with a chlorophyll meter (SPAD-502, Minolta
144 Camera Co. Ltd., Japan). Then, plants were sampled, and the concentration of Mo was
145 determined as described in Experiment 1. The fresh medium (vermiculite) was extracted
146 with water (vermiculite : water = 1 : 2.5, w/v) for the determination of water-extractable
147 Mo concentration in the medium. Mo concentration in the extracts was analyzed by
148 ICP-MS. Sterility was checked using the SCD agar medium before and after the

experiment. The cultivation were regarded as aseptic if microbial growth did not occur after 7 days of incubation at 30°C (Tokuhisa et al., 2010).

### *Experiment 5: Effects of molybdenum application on the distribution of nitrogen in wheat under nitrogen deficiency*

Wheat seedlings were cultivated hydroponically to examine the effects of Mo supply on N distribution in different plant parts. The seedlings were prepared as described above. After preculture in standard nutrient solution, the seedlings were transferred to 8-L containers containing low-N standard nutrient solution (0.214 mM N) with or without 0.05 μM Mo supply. Treatments were conducted with 3 replicates (7 seedlings per replicate). The pH of the solution was adjusted to 5.3 ± 0.1 with 0.1 M NaOH or 0.1 M HCl daily, and renewed weekly. After the treatment, seedlings were harvested and washed with deionized water. Then, seedlings were separated into leaves (1st, 2nd, 3rd, 4th, 5th, 6th leaves, separately), stems and roots, and then lyophilized. After determining the dry weight, each sample was ground and digested. Nitrogen and mineral concentrations in each organ were determined as described above.

### *Statistical analyses*

The mineral concentration data was analyzed on a dry weight basis. All statistical analyses were performed using Sigmaplot 11.0 (Systat Software, Inc., San Jose, CA, USA) and Excel 2013 (Microsoft, Redmond, WA, USA).

## Results

### *Experiment 1: Effects of nitrogen deficiency on accumulation and distribution of molybdenum in wheat under field conditions*

Wheat was cultivated in +NPK and −N treatments of the long-term fertilizer experimental field, and Mo concentration in leaves (lower, middle, upper, and flag) and

roots at just before flowering stage was analyzed. As a result, there was no significant difference in Mo concentration between different organs under N sufficient conditions (Figure 1). By contrast, N deficiency significantly increased Mo concentration in leaves and the increase was greater in the lower (old) leaves whereas it did not change the Mo concentration in roots (Figure 2).

### *Experiment 2: Effects of nitrogen deficiency on accumulation of molybdenum in wheat and soybean under hydroponic culture*

The dry weight and N concentration decreased under N deficiency in both wheat and soybean (Figure 2), and no nodule was found in soybean roots under hydroponics (data not shown). Increase in leaf Mo concentration of wheat plants due to N deficiency was also observed in hydroponic conditions as well as in field conditions (Figures 1 and 3A). By contrast, N deficiency did not increase Mo concentration in leaves of soybean under hydroponic conditions. In soybean, however, root Mo concentration was not increased due to the N deficiency (Figure 3B), while it was increased under field conditions (Watanabe et al., currently under review in J. Plant Nutr.).

### *Experiment 3: Effects of molybdenum application on growth responses of wheat to nitrogen deficiency under soil culture*

In order to elucidate whether the increase of Mo concentration in the leaves under N deficiency enhances N deficiency tolerance in wheat, a soil pot experiment was conducted with Mo application treatment. The growth of wheat was significantly enhanced with Mo application under N deficient conditions but no significant Mo effect was observed under N sufficient conditions (Figure 4A). The Mo application did not affect N concentration in roots and leaves regardless of its N nutritional status (Figures 4B and 4C). Leaf Mo concentration increased due to N deficiency while root Mo concentration was not affected regardless of the Mo application treatment (Figure S1).

### *Experiment 4: Effects of nitrogen deficiency on molybdenum uptake of wheat under aseptic culture*

In order to examine whether microorganisms are involved in the N-deficiency-induced increase in the concentration of Mo levels in the leaf, an aseptic culture experiment was conducted. Even under aseptic conditions, the N-deficiency-induced increase of Mo accumulation in wheat shoot was observed (Figure 5). The aseptic condition did not decrease growth and chlorophyll concentration of wheat (Figure S2). The N application treatment did not affect water-soluble Mo concentration in the medium (vermiculite) (Figure S3).

### *Experiment 5: Effects of molybdenum application on the distribution of nitrogen in wheat under nitrogen deficiency*

We cultivated wheat seedlings under N deficient hydroponic conditions to examine the effect of Mo application on N distribution in plants. Higher accumulation of Mo in lower leaves when subjected to N deficient conditions were observed in hydroponics irrespective of Mo application treatment (Figure 6B), as observed in the field experiment (Figure 1). The application of Mo significantly increased the concentration of N in lower leaves (2nd leaf) but decreased in upper leaves (5th leaf) (Figure 6A). Molybdenum application also affected the accumulation of other minerals both in leaves and roots (Table 1).

## Discussion

In order to understand the phenomenon that leaf Mo accumulation increases in non-leguminous species under N deficiency in the field (Watanabe et al., currently under review in J. Plant Nutr.), several cultivation experiments were conducted using wheat. As a result, increased wheat leaf Mo accumulation due to N deficiency was also observed in pot soil culture, hydroponics, and aseptic culture, as well as in field

223 conditions (Figures 1, 3, 4, 5, S1).

224 It is known that Mo is an element essential for nitrogenase of endophytes and other $N_2$-
225 fixing microorganisms in soils as well as rhizobia (Rubio & Ludden 2008). In the
226 previous study, a significant increase in Mo accumulation in roots of soybean was
227 observed under N deficient conditions in the field (Watanabe et al., currently under
228 review in J. Plant Nutr.), presumably due to its higher accumulation in root nodules
229 (Chu et al. 2016). This is supported by the result that soybean under hydroponics did not
230 form nodules and its root Mo concentration did not increase under N deficiency (Figure
231 3). Unlike wheat, in soybean with hydroponic culture, leaf Mo concentration did not
232 increase by the N deficient treatment (Figure 3), although N concentration in leaves
233 significantly decreased (Figure 2). Therefore, N deficiency-induced increase in Mo
234 concentration might be specific physiological response in non-leguminous species.

235 Under N deficient conditions, other $N_2$-fixing microorganisms also need more Mo for
236 fixing $N_2$, possibly resulted in increase of Mo accumulation of endophytes in plants or
237 increase of available Mo in soils solubilized by these $N_2$-fixing microorganisms.
238 Moreover, it is also possible that the solubility of Mo in the soil without fertilization of
239 inorganic N such as ammonium sulfate may increase because the soil pH is normally
240 higher than that with inorganic N fertilization (Vlek & Lindsay, 1977; Watanabe et al.,
241 2015). However, the accumulation of Mo increased even in aseptic culture (Figure 5),
242 and there was no influence on N fertilization on the water-soluble Mo concentration in
243 the medium (Figure S3), strongly suggesting that Mo is involved in the plant responses
244 to N deficiency directly, not indirectly by external factors such as microorganisms and
245 soil chemical properties.

246 While Mo application increased the growth of wheat under N deficiency, N

concentration in plant did not increase (Figure 4). From this, it is expected that Mo contributes not to the acquisition of N but to the improvement of N utilization efficiency in plants. Under N sufficient conditions, Mo concentration in leaves was low and almost constant regardless of the leaf position (Figure 1). However, N deficiency increased the Mo concentration in leaves, and the increase was greater in the lower leaves (Figure 1). When Mo was applied to wheat under N deficiency, the N concentration in the lower leaf (2nd leaf) increased and that in the upper leaf (5th leaf) decreased when compared with those without Mo application (Figure 6). These results suggest that under N deficiency, Mo increases N distribution in the lower leaf, probably by suppressing N retranslocaiton from lower to upper leaves. In general, N deficiency promotes leaf senescence, particularly in the lower leaves (Marschner 2012). This response exists due to retransfer N to young developing leaves, but senescent old leaves decrease their photosynthetic capacity (Escudero and Mediavilla, 2003). Schulte auf'm Erley et al. (2007) suggested that maintaining N concentration in lower leaves under N deficiency leads them to maintain photosynthesis (delayed senescence), resulting in increased yield of maize. Osaki (1995) also reported that maintaining photosynthesis in the lower leaves increases the distribution of photoassimilate to the roots, thereby enhancing the root activity. Molybdenum-induced increase in the concentrations of several essential elements in leaves and/or roots under N deficient conditions (Table 1) might be due to the enhanced root activity. Taken together, one of the roles of Mo under N deficiency is expected to suppress old leaf senescence for maintaining photosynthesis and root activity.

## Conclusion

This study showed that the increase in Mo accumulation in wheat leaves under N deficiency was due to the metabolic response of plants rather than the involvement of

changes in Mo availability in soils and $N_2$-fixing microorganisms. Furthermore, it was suggested that Mo may contribute to the suppression of senescence in the lower leaves, but the specific metabolism in which Mo is involved is still unknown. In future, various omics such as the transcriptomics, metabolomics, and metalloproteomics (Shi and Chance, 2008) are expected to clarify the metabolic changes caused by Mo application under N deficient conditions.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Figure captions**

**Figure 1.** Concentration of molybdenum in leaves (lower, middle, upper) and roots of wheat cultivated with complete fertilization (+NPK) or without N (−N) in the long-term fertilizer experimental field. Data are means of three replicates (±standard error). Different letters indicate statistically significant differences among different leaf positions and root at $P < 0.05$ using Tukey's multiple-comparison test following one-way ANOVA. Asterisks indicate statistically significant differences between +NPK and −N treatments (Student's t-test, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant).

**Figure 2.** Dry weight and concentration of nitrogen in leaves and roots of wheat and soybean hydroponically cultivated with different N levels in the nutrient solution. Data are means of three replicates (± standard error of total dry weight or nitrogen concentration). High N: 2.14 mM N, Low N: 0.214 mM N. Asterisks indicate statistically significant differences between High N and Low N treatments (Student's t-test, ** and ***: $P < 0.01$ and 0.001, respectively, ns: not significant).

**Figure 3.** Concentration of molybdenum in leaves (A) and roots (B) of wheat and soybean hydroponically cultivated with different N levels in the nutrient solution. Data are means of three replicates (±standard error). High N: 2.14 mM N, Low N: 0.214 mM

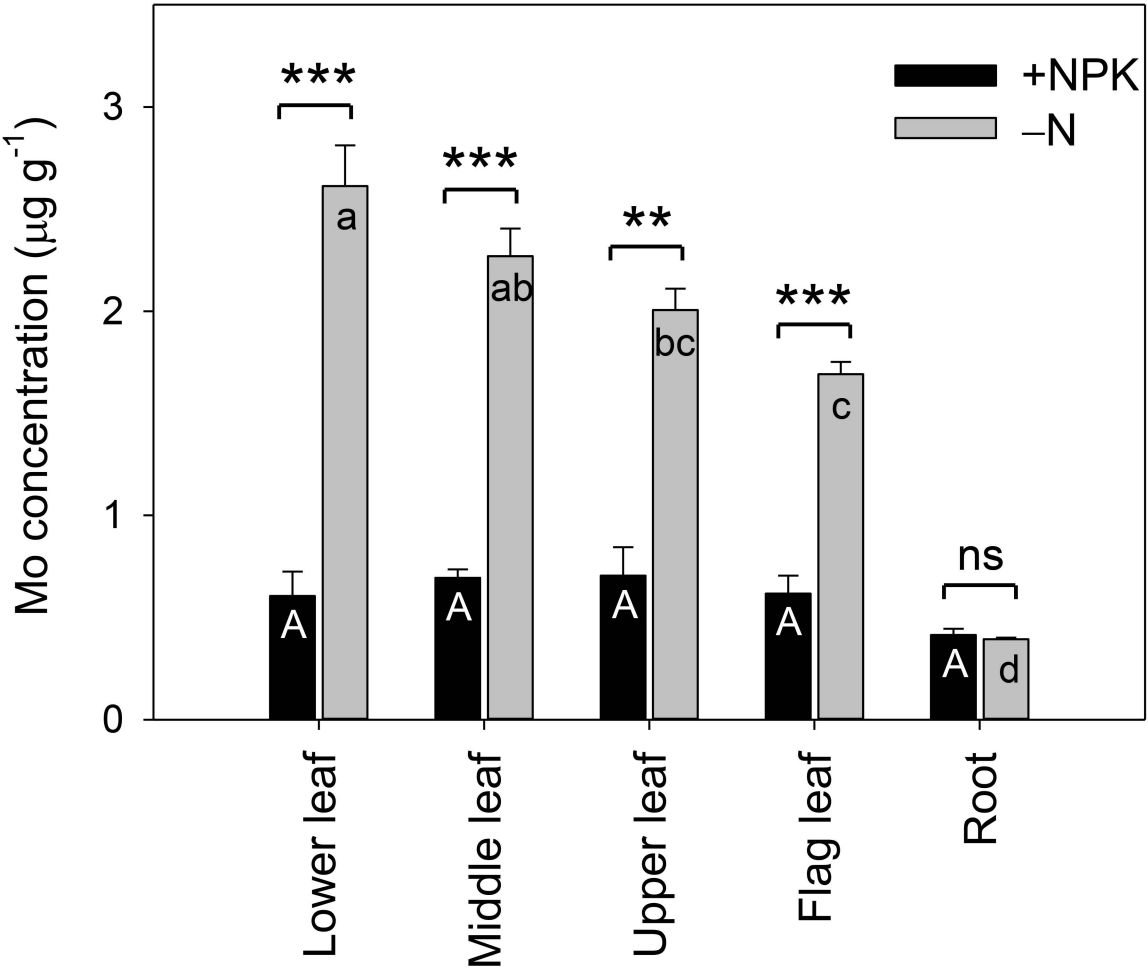
346 N. Asterisks indicate statistically significant differences between High N and Low N
347 treatments (Student's t-test, **: $P < 0.01$, ns: not significant).
348
349 **Figure 4.** Effects of nitrogen and molybdenum application on dry weight (A) and
350 nitrogen concentration in leaves (B) and roots (C) of wheat grown in soil pot culture.
351 Data are means of three replicates (± standard error of total dry weight or nitrogen
352 concentration). +N: 0.25 g N $pot^{-1}$, −N: 0 g N $pot^{-1}$, +Mo: 0.5 mg Mo $pot^{-1}$, −Mo: 0 mg
353 Mo $pot^{-1}$. Asterisks indicate statistically significant differences between −Mo and +Mo
354 treatments in each N treatment (Student's t-test, **: $P < 0.01$, ns: not significant).
355
356 **Figure 5.** Molybdenum concentration in shoots and roots of wheat grown aseptically or
357 nonaseptically under different nitrogen nutrient conditions. Data are means of five
358 replicates (±standard error). High N: 45 mg N $pot^{-1}$, Low N: 0.45 mg N $pot^{-1}$. Asterisks
359 indicate statistically significant differences between High N and Low N treatments
360 (Student's t-test, ***: $P < 0.001$, ns: not significant).
361
362 **Figure 6.** Effects of molybdenum application on nitrogen (A) and molybdenum (B)
363 concentrations in roots and leaves at different leaf positions in wheat grown under N
364 deficient conditions. Data are means of three replicates (±standard error). Asterisks in
365 graph above indicate statistically significant differences between −Mo and +Mo
366 treatments in each leaf position or root (Student's t-test, *: $P < 0.05$, ns: not significant).
367 Different letters in the graph below indicate statistically significant differences among
368 different leaf positions and root in each Mo treatment at $P < 0.05$ using Tukey's
369 multiple-comparison test following one-way ANOVA.

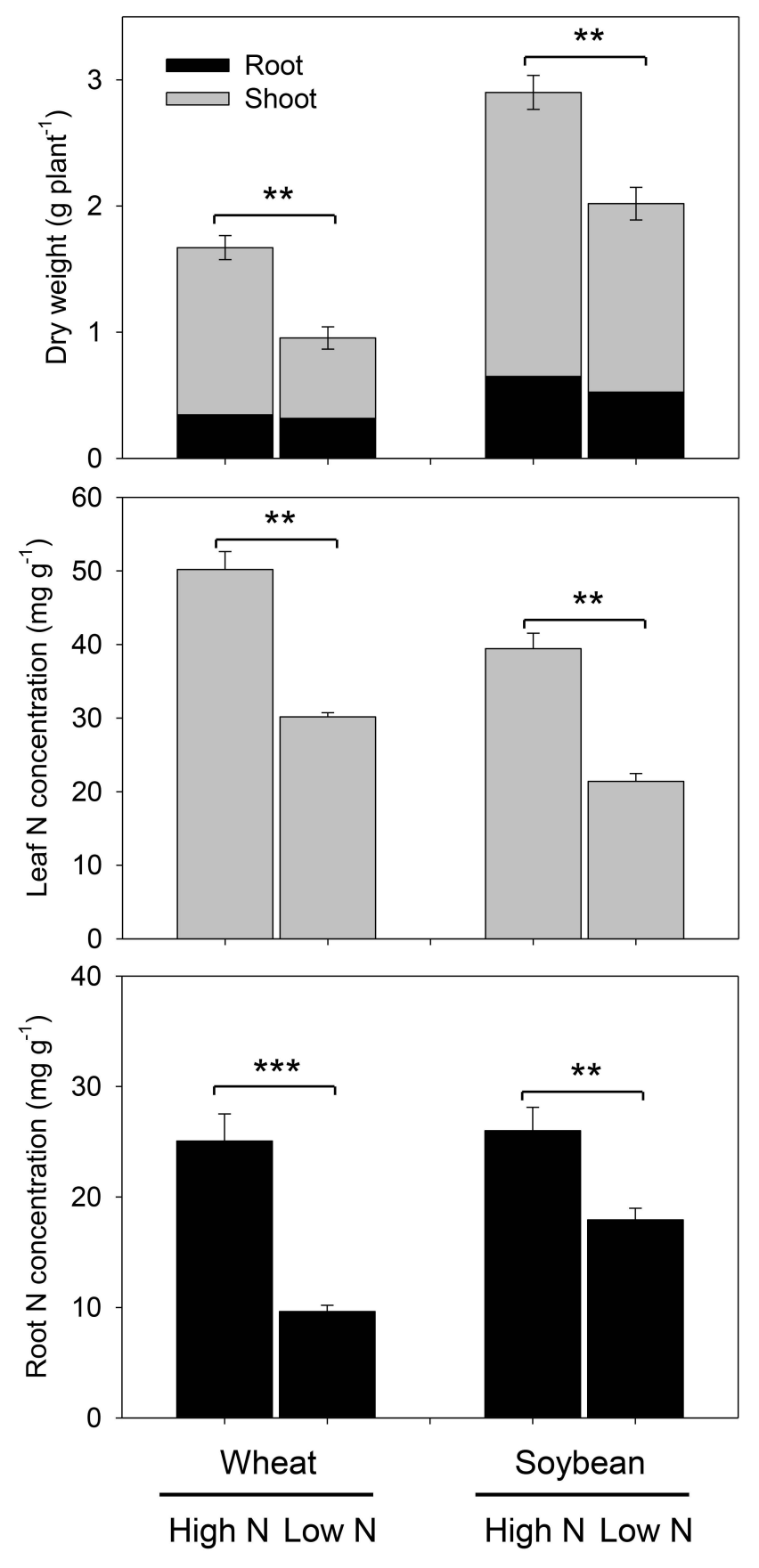
Table 1. Effect of molybdenum application on concentration (mg $g^{-1}$) of each element in different organs of wheat.

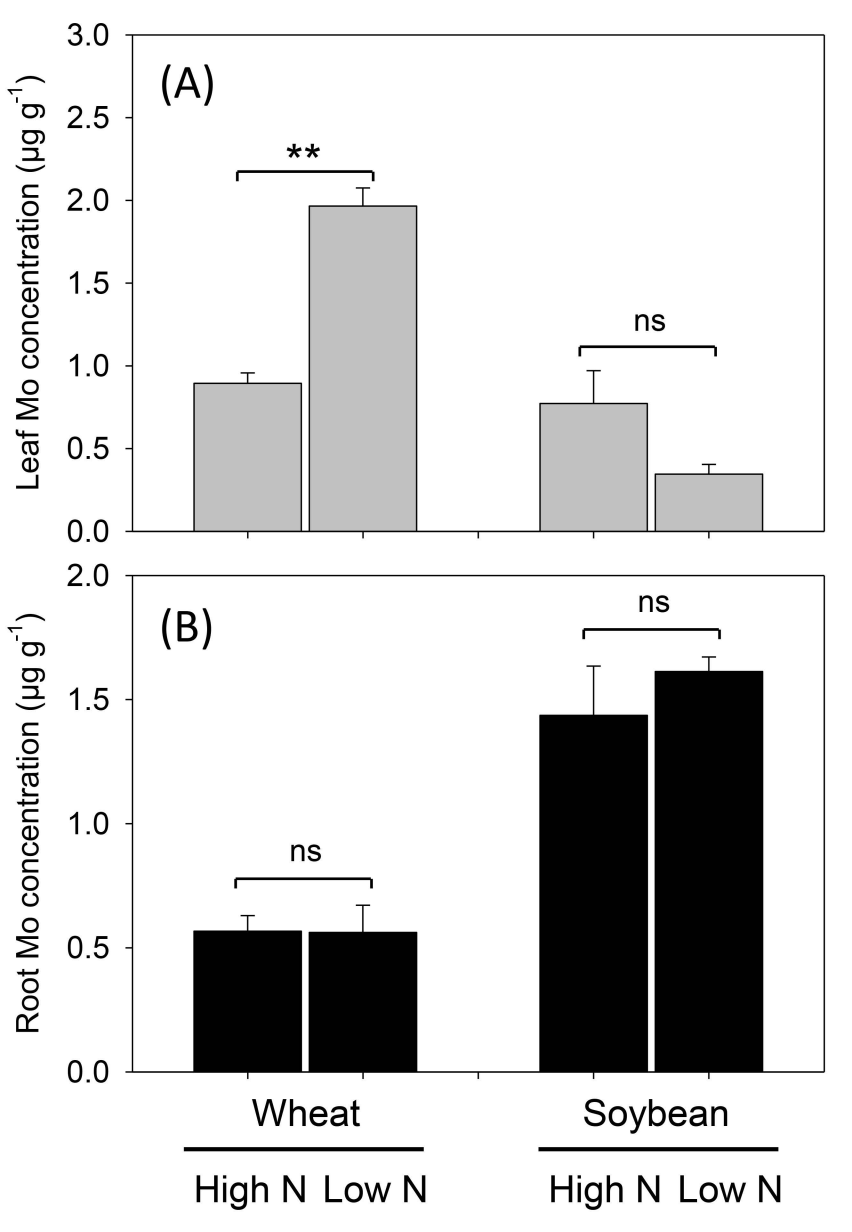
| | | K | | P | S | | Ca | | Mg | Fe | Mn | Zn | | B | Cu | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1st leaf | −Mo | 41.7 | | 32.4 | 17.6 | | 31.0 | | 12.5 | 0.241 | 0.656 | 0.129 | ** | 0.260 | 0.0427 | |
| | +Mo | 42.0 | | 29.1 | 15.8 | | 30.4 | | 12.2 | 0.253 | 0.613 | 0.095 | | 0.263 | 0.0404 | |
| 2nd leaf | −Mo | 45.8 | | 30.8 | 14.7 | | 21.8 | | 8.13 | 0.271 | 0.398 | 0.103 | | 0.223 | 0.0493 | |
| | +Mo | 48.4 | | 29.4 | 14.5 | | 22.2 | | 8.51 | 0.266 | 0.370 | 0.083 | | 0.215 | 0.0476 | |
| 3rd leaf | −Mo | 48.7 | | 20.7 | 7.78 | | 8.07 | | 3.62 | 0.168 | 0.233 | 0.057 | * | 0.111 | 0.0194 | |
| | +Mo | 46.7 | | 20.7 | 7.25 | | 8.01 | | 3.65 | 0.178 | 0.208 | 0.040 | | 0.122 | 0.0191 | |
| 4th leaf | −Mo | 44.5 | * | 13.3 | 6.51 | | 5.61 | | 2.63 | 0.138 | 0.186 | 0.054 | | 0.067 | 0.0206 | |
| | +Mo | 56.2 | | 13.7 | 6.58 | | 6.06 | | 2.81 | 0.160 | 0.189 | 0.044 | | 0.070 | 0.0206 | |
| 5th leaf | −Mo | 45.0 | | 11.0 | 5.63 | | 4.39 | | 2.06 | 0.120 | 0.139 | 0.054 | * | 0.048 | 0.0201 | |
| | +Mo | 44.8 | | 10.4 | 5.12 | | 5.23 | | 2.03 | 0.113 | 0.122 | 0.039 | | 0.046 | 0.0196 | |
| 6th leaf | −Mo | 46.7 | ** | 8.14 | 4.87 | | 2.44 | * | 1.70 | 0.105 | 0.096 | 0.056 | | 0.022 | 0.0207 | |
| | +Mo | 57.9 | | 8.04 | 4.68 | | 2.91 | | 1.78 | 0.113 | 0.087 | 0.051 | | 0.019 | 0.0206 | |
| Root | −Mo | 36.9 | | 13.3 | 3.02 | ** | 1.79 | ** | 1.26 | 18.42 | 0.112 | *** | 0.094 | ** | 0.005 | 0.067 | ** |
| | +Mo | 37.9 | | 12.8 | 2.42 | | 2.47 | | 1.21 | 18.05 | 0.390 | | 0.156 | | 0.005 | 0.101 | |

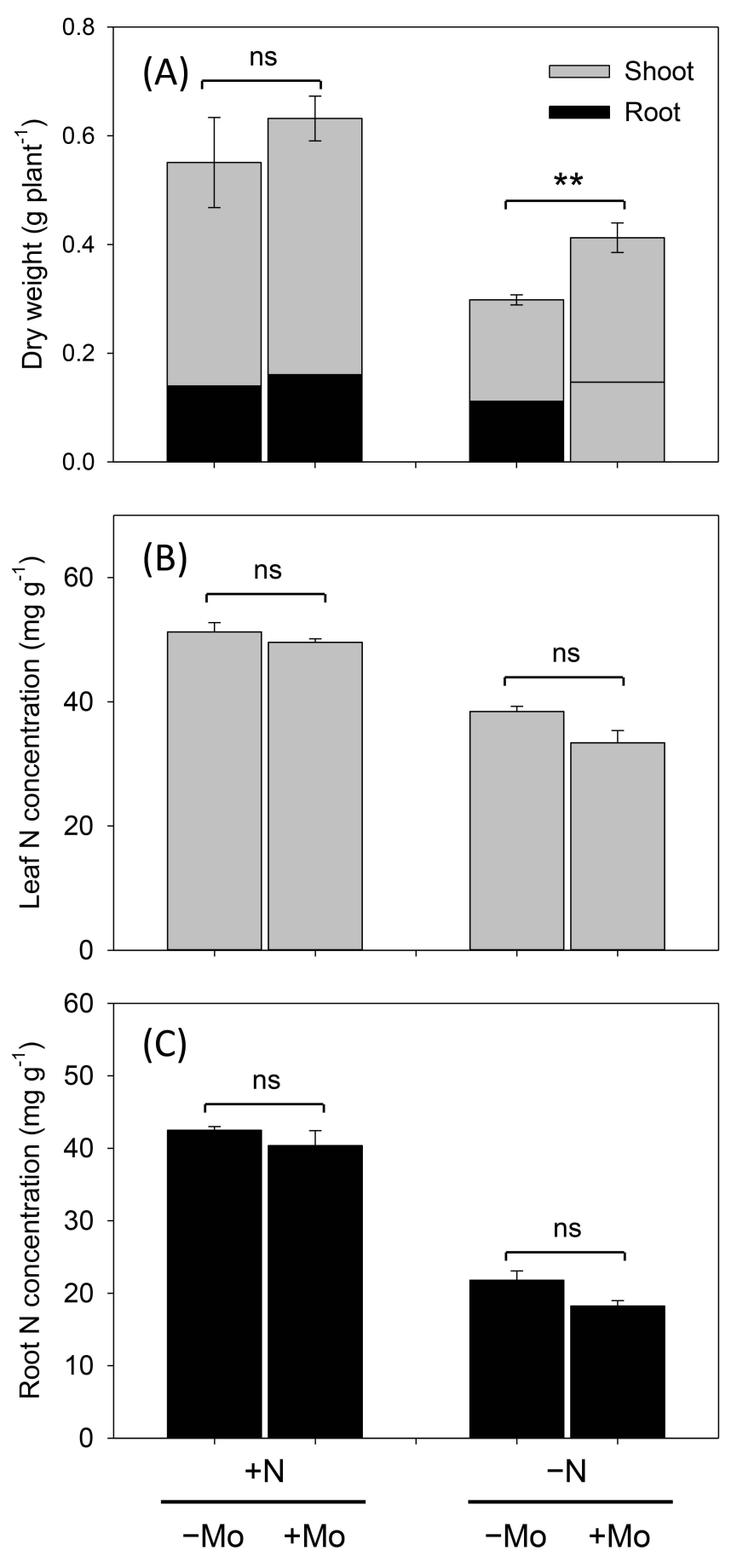
Values are means of three replicates.

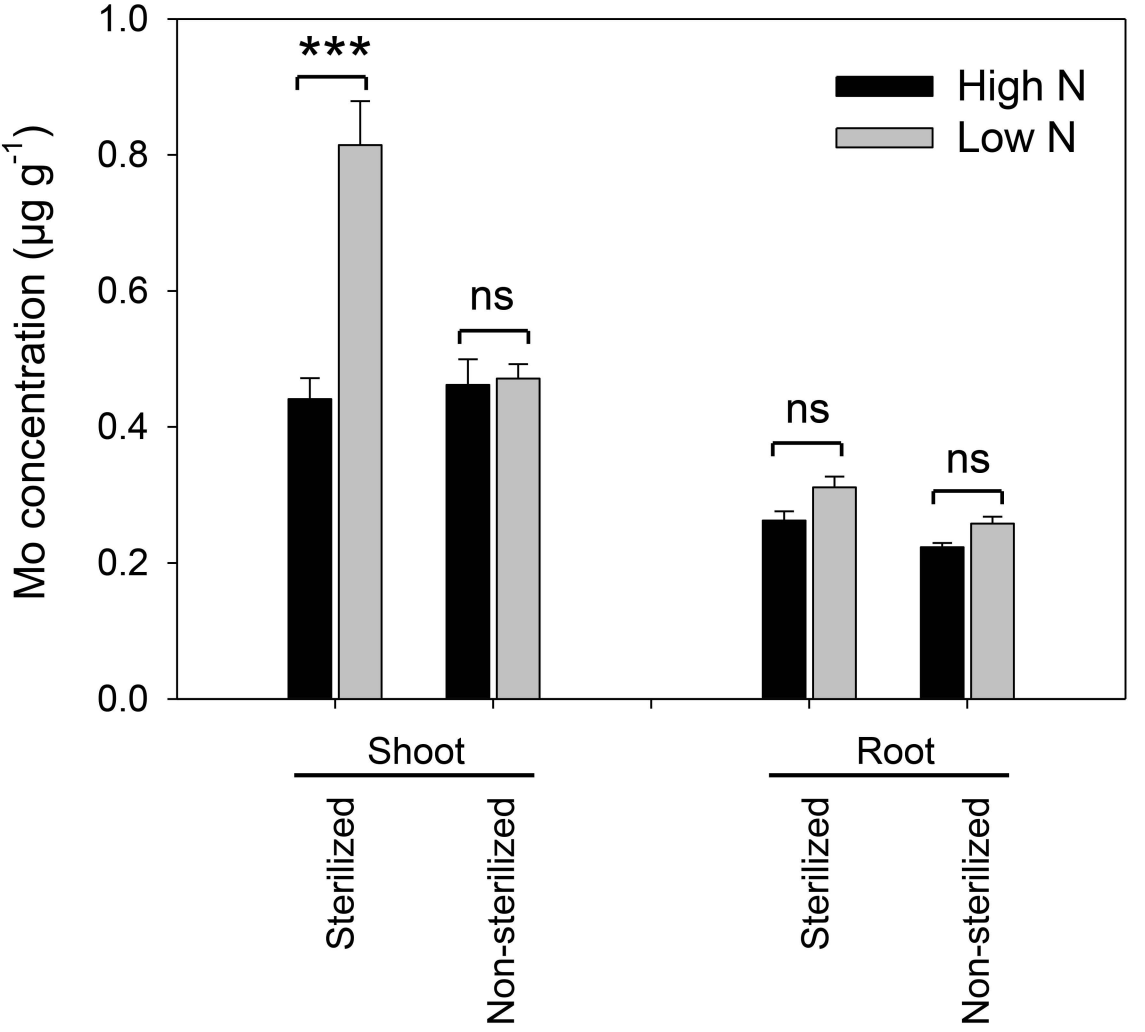
*, **, and *** indicate significant difference between −Mo and +Mo treatments at $P<0.05$, 0.01, and 0.001, respectively (Student's *t*-test).

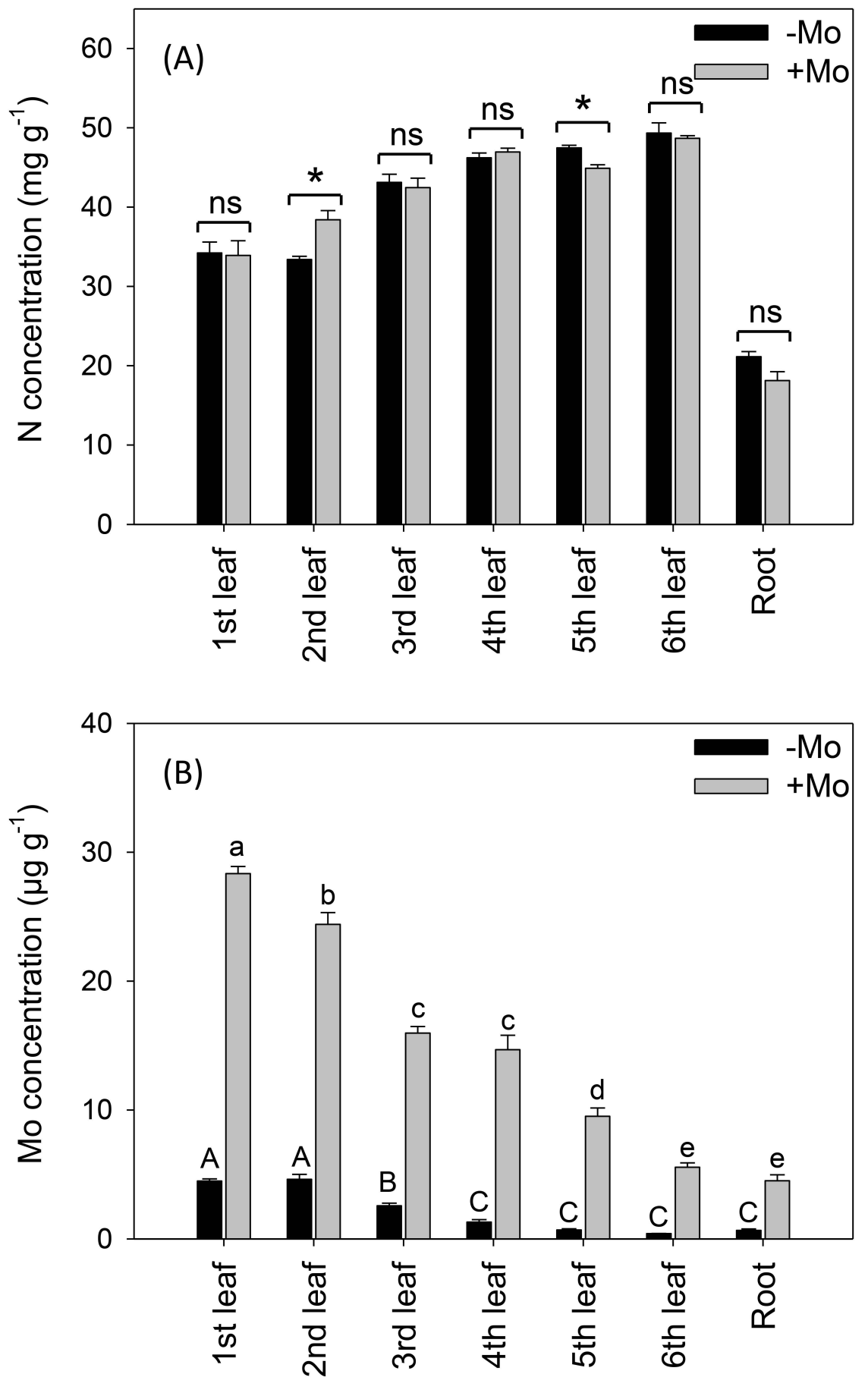

Mo concentration (μg g-1)
3
2
1
0
+NPK
–N
***
***
**
***
ns
a
ab
bc
c
A
A
A
A
A
d
Lower leaf
Middle leaf
Upper leaf
Flag leaf
Root


Root
Shoot
Dry weight (g plant-1)
0
1
2
3
**
**
Leaf N concentration (mg g-1)
0
10
20
30
40
50
60
**
**
Root N concentration (mg g-1)
0
10
20
30
40
***
**
Wheat
Soybean
High N
Low N
High N
Low N


(A)
Leaf Mo concentration (μg g-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
ns
(B)
Root Mo concentration (μg g-1)
2.0
1.5
1.0
0.5
0.0
ns
ns
Wheat
Soybean
High N
Low N
High N
Low N


(A)
ns
**
Shoot
Root
Dry weight (g plant-1)
0.0
0.2
0.4
0.6
0.8
(B)
ns
ns
Leaf N concentration (mg g-1)
0
20
40
60
(C)
ns
ns
Root N concentration (mg g-1)
0
10
20
30
40
50
60
+N
−N
−Mo
+Mo
−Mo
+Mo


1.0
0.8
0.6
0.4
0.2
0.0
Mo concentration (μg g-1)
***
ns
ns
ns
High N
Low N
Shoot
Root
Sterilized
Non-sterilized
Sterilized
Non-sterilized


(A)
-Mo
+Mo
N concentration (mg g-1)
60
50
40
30
20
10
0
ns
*
ns
ns
*
ns
ns
1st leaf
2nd leaf
3rd leaf
4th leaf
5th leaf
6th leaf
Root
(B)
-Mo
+Mo
Mo concentration (μg g-1)
40
30
20
10
0
a
b
c
c
d
e
e
A
A
B
C
C
C
C
1st leaf
2nd leaf
3rd leaf
4th leaf
5th leaf
6th leaf
Root