Accumulation and Distribution of Fertilizer Nitrogen and Nodule-Fixed Nitrogen in Soybeans with Dual Root Systems

Rui Zhang, Cong Wang, Wenzhi Teng, Jing Wang, Xiaochen Lyu, Shoukun Dong, Shuang Kang, Zhenping Gong, Chunmei Ma

1 College of Agriculture, Northeast Agricultural University, Harbin 150030, China; zhang134rui@163.com (R.Z.); w15774517432@163.com (C.W.); twz1024@163.com (W.T; crystal900313@163.com (J.W.); xiaochenlyu@163.com (X.L.); shoukundong@163.com (S.D.)
2 Heilongjiang Academy of Land Reclamation Sciences, Harbin 150038, China; ks92511@163.com

* Correspondence: gzpyx2004@163.com (Z.G.); chunmm518@163.com (C.M.)

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Abstract: The soybean (Glycine max L. Merr.) is a crop with a high demand for nitrogen (N). The root nodules that form in soybeans can fix atmospheric N effectively, yet the goal of achieving high yields cannot be met by relying solely on nodule-fixed N. Nonetheless, the application of N fertilizer may inhibit nodule formation and biological N fixation (BNF), but the underpinning mechanisms are still unclear. In this study, we grafted the roots of non-nodulated soybeans onto nodulated soybeans to generate plants with dual root system. The experiment included three treatments conducted under sand culture conditions with NO$_3^-$ and NH$_4^+$ as N sources. Treatment I: The non-nodulated roots on one side received 50 mg·L$^{-1}$ $^{15}$NO$_3^-$ or $^{15}$NH$_4^+$, and the nodulated roots on the other side were not treated. Treatment II: The non-nodulated roots received 50 mg·L$^{-1}$ $^{15}$NO$_3^-$ or $^{15}$NH$_4^+$, and the nodulated roots received 50 mg·L$^{-1}$ $^{14}$NO$_3^-$ or $^{14}$NH$_4^+$. Treatment III: Both non-nodulated and nodulated roots received 50 mg·L$^{-1}$ $^{15}$NO$_3^-$ or $^{15}$NH$_4^+$. The results showed the following: (1) Up to 81.5%–87.1% of the N absorbed by the soybean roots and fixed by the root nodules was allocated to shoot growth, leaving 12.9%–18.5% for root and nodule growth. Soybeans preferentially used fertilizer N in the presence of a NO$_3^-$ or NH$_4^+$ supply. After the absorbed fertilizer N and nodule-fixed N was transported to the shoots, a portion of it was redistributed to the roots and nodules. The N required for root growth was primarily derived from the NO$_3^-$ or NH$_4^+$ assimilated by the roots and the N fixed by the nodules, with a small portion translocated from the shoots. The N required for nodule growth was primarily contributed by nodule-fixed N with a small portion translocated from the shoots, whereas the NO$_3^-$ or NH$_4^+$ that was assimilated by the roots was not directly supplied to the nodules. (2) Based on observations of the shoots and one side of the roots and nodules in the dual root system as an N translocation system, we proposed a method for calculating the N translocation from soybean shoots to roots and nodules during the R$_1$–R$_5$ stages based on the difference in the $^{15}$N abundance. Our calculations showed that when adding N at a concentration of 50 mg·L$^{-1}$, the N translocated from the shoots during the R$_1$–R$_5$ stages accounts for 29.6%–52.3% of the N accumulation in nodulated roots (Root$_n$) and 9.4%–16.6% of the N accumulation in Nodule$_n$ of soybeans. Through the study of this experiment, the absorption, distribution and redistribution characteristics of fertilizer N and root nodule N fixation in soybean can be clarified, providing a theoretical reference for analyzing the mechanisms of the interaction between fertilizer N and nodule-fixed N.

Keywords: soybean dual root system; N accumulation; distribution; remobilization; NO$_3^-$; NH$_4^+$
1. Introduction

Leguminous crops and rhizobia form nodules in soybean roots through complex interactions to efficiently fix atmospheric N for the N nutrient supply. However, achieving the goal of high yields in leguminous crops is not possible by relying solely on nodule-fixed N. The application of N fertilizer can considerably increase the yields of leguminous crops [1–4]. In peanut growing fields, the application of urea-N is a better way to increase the supply of N from root nodules and improves the N use efficiency [5]. Nonetheless, N application may inhibit nodule formation and N fixation [6–15]. Many researchers have reported that the application of NO$_3^-$-N reduces the weight of the root nodules because high levels of NO$_3^-$-N lead to a sharp decrease in the proportion of photosynthetic products transported to the root nodules and a corresponding increase to the stem and root [16–19]. In addition, Minchin et al. [18] and Carroll et al. [20] indicated that NO$_3^-$ inhibits the nitrogenase activity in root nodules by increasing their O$_2$ diffusion barrier. Moreover, Munns [21] and Wahab et al. [22] considered that NO$_3^-$ affects the number of root nodules by inhibiting root hair formation and rhizobial infections in leguminous crops. Gan et al. [23] found that applying a higher concentration of N fertilizer markedly reduced the nodule number and nodule-fixed N in soybeans, whereas a lower concentration of N fertilizer did the opposite.

Many experiments have used the split-root system [24]. Xia et al. [25] conducted a study using dual root system, in which a high concentration of N was added to one side of the roots and no N was added to the other side. They found that the number of root nodules decreased in the side receiving a high concentration of N, whereas the number increased in the side without added N. This finding shows that high N concentrations have a local contact effect in inhibiting the formation and growth of root nodules. Fujikake et al. [26] reported that following the addition of NO$_3^-$ to soybeans, the diameter growth of the root nodules completely stopped; however, after NO$_3^-$ withdrawal from the nutrient solution, the growth of the root nodules rapidly recovered to the original normal rate. This observation suggests that the NO$_3^-$-induced inhibition of root nodule growth is a reversible process. Using a split-root system, Kosslak et al. [27] inoculated rhizobia into one side of soybean roots, and 10 days later, they inoculated the other side of the roots. They showed that earlier rhizobia inoculation on one side of the roots inhibited nodulation on the other side. Using peas, van Brussel et al. [28] came to a similar conclusion in that the presence of nodules on one side of the roots inhibits nodule formation on the other side, which shows autoregulation.

The N utilization rate of plants determined by the $^{15}$N isotope tracer method can truly reflect the status of fertilizer utilization by plants. Oghoghorie and Pate [29] divided the pea roots into the upper and lower parts and separated the roots into different treatments, with $^{15}$N-labeled N$_2$ being added to the upper roots in one treatment and $^{15}$N-labeled NO$_3^-$ being added to the upper roots in the other treatment. Following treatment, $^{15}$N was detected not only in the shoots but also in the lower roots and nodules. Silva et al. [30] applied $^{15}$N urea to soybean leaves and stems, and found that in 71 days after marking, the content of $^{15}$N aboveground decreased with time, whereas it increased in the roots. Moreover, Oghoghorie and Pate [29] added $^{15}$N-labeled NO$_3^-$ onto the 3rd, 7th, and 12th leaves and detected $^{15}$N in both the aboveground and belowground parts of the peas. Akria et al. [9] used the soybean double root system to supply $^{15}$NO$_3^-$ at different concentrations on one side and no N on the other side. This study found that $^{15}$N markers were also detected in the roots and root nodules on the supplied side and increased with the increase in N concentration. Reynolds et al. [31] used the soybean root-dividing system, where $^{13}$NH$_4^+$ was applied on one side and no N was supplied on the other side, and detected an abundance of $^{13}$N amino acids in the N-supplied side of the root, and $^{15}$N markers were detected on both the nodes and the supplied side, indicating that N in the leaf stem was also transported to the root and root nodules.
Previous studies have shown that the N absorbed externally by legumes is transferred to other organs in the plant, but most studies do not show the ratio of N accumulation and distribution in the plant. In this study, $^{15}$N-labeled NO$_3^−$ and NH$_4^+$ were added to the root system planted under sand culture conditions. The N accumulation and $^{15}$N abundance in soybean plants at the R$_1$ and R$_5$ stages were measured and analyzed to understand how N is absorbed, distributed, and remobilized in soybeans. The results provide reference data to understand the characteristics of N translocation and unravel the systematic regulation of nodule formation in soybeans.

2. Materials and Methods

This study was conducted at an experimental area on the campus of Northeast Agricultural University in 2018 and 2019. The experimental area (N: 45°74′ and E: 126°73′) is located in the Xiangfang District of Harbin, Heilongjiang Province, China. The annual precipitation is 500–550 mm, and the ≥10 °C accumulated temperature is 2700 °C.

2.1. Experimental Design and Treatments

2.1.1. Preparation of Plant Materials with a Dual Root System

The soybean plants with dual root system was prepared based on the method in Xia et al. [25]. The treatments were conducted using plastic pots with a diameter of 0.3 m and a height of 0.3 m. Each pot was divided into two equal, independent spaces by vertically inserting a custom-made polycarbonate plate that was fitted for the inner shape of the pot and sealed with glue in the middle of the pot. The top of the partition plate was 2 cm below the rim of the pot. For each partitioned space, a drainage hole with a 1 cm diameter was drilled in the bottom of each pot. The hole was capped with a piece of gauze to prevent clogging by river sand. Each pot was filled with 20 kg of washed sand for cultivating the soybean plants with dual root.

Seeds of nodulated soybeans (*Glycine max* L. cv. Kenfeng 16) and non-nodulated soybeans (*Glycine max* L. cv. WDD01795, L8-4858, provided by the Crop Research Institute, Chinese Academy of Agricultural Sciences) were drilled into a fine sand medium at a depth of 2 cm and incubated in a growth chamber at 30 °C for 3 days. When the distance between the growing point of the cotyledon and the tip of the root was 7 to 10 cm, the roots of the soybean seedlings were rinsed with water and then used for grafting. Two seedlings of nodulated and non-nodulated soybeans were chosen and an incision of 0.5–1.0 cm (without cutting off) was made with a sterilized blade, which extended upward or downward slightly above the middle point of the hypocotyl. The non-nodulated seedling was cut from the cotyledon toward the root (Figure 1A), whereas the nodulated seedling was cut from the root toward the cotyledon (Figure 1B). The two seedlings were cross-inserted into their cuts (Figure 1C) and clipped with a grafting clip. The root system of the two seedlings were planted separately into the fine sand medium on both sides of the partition plate in the pot, with the grafting site exactly on the top of the partition plate. The grafted seedlings were allowed to grow inside a weather-tight enclosure for a week. The grafting clip was then removed and the upper part of each non-nodulated seedling was cut from the grafting site, leaving its combined site and lower parts. This procedure generated seedlings with dual root system (nodulated and non-nodulated) and the shoots of a nodulated cultivar. The plants were grown under farmland conditions and treated experimentally. (Figure 1D) shows the roots of a soybean plant with a dual root system at the time of sampling, with non-nodulated roots (Root$_{non}$) on the left and nodulated roots (Root$_{n}$) on the right side.
When the opposite true leaves were fully expanded, all the roots were inoculated with rhizobia as were washed, ground, and then added to the nutrient solutions at 5 g Bradyrhizobium japonicum. The prepared by adding 360.7 mg A. The method was feasible for soybean inoculation, and there would be spontaneous nodulation of this method was used to inoculate soybean with soil rhizobia continuously for 5 days. Previous experiments showed that this method was feasible for soybean inoculation, and there would be spontaneous nodulation of rhizobia in the soil community. The main N-fixing rhizobia in soil belonged to Bradyrhizobium, Bradyrhizobium japonicum, and B. liaoningense were the dominant bacteria.

![Figure 1. Soybean plant with dual root system. (A) Non-nodulated seedling, (B) nodulated seedling, (C) two seedlings cross-inserted into their cuts and clipped with a grafting clip, and (D) roots of a soybean plant with a dual root system at the time of sampling, with non-nodulated roots (Root_non) on the left and nodulated roots (Root_n) on the right side.](image-url)

### Table 1. Experimental treatments.

|   | NO$_3^-$ | NH$_4^+$ |
|---|----------|----------|
| I | $^{15}$NO$_3^-$ | N free | $^{15}$NH$_4^+$ | N free |
| II | $^{15}$NO$_3^-$ | $^{14}$NO$_3^-$ | $^{15}$NH$_4^+$ | $^{14}$NH$_4^+$ |
| III | $^{15}$NO$_3^-$ | $^{15}$NO$_3^-$ | $^{15}$NH$_4^+$ | $^{15}$NH$_4^+$ |

Before the full expansion of the opposite true leaves, the soybean seedlings were irrigated once a day with 250 mL of distilled water on each side of the root system. Following the full expansion of the opposite true leaves, the seedlings were irrigated once a day with 250 mL of the corresponding nutrient solution on each side of the root system until the R$_1$ stage. From the R$_1$ stage on, the seedlings were irrigated twice a day, once in the morning and once in the evening, with 250 mL of the corresponding nutrient solution for each side of the root system until the end of the experiment. When the opposite true leaves were fully expanded, all the roots were inoculated with rhizobia as follows: Soybean root nodules harvested from the field during the previous year and stored in a freezer were washed, ground, and then added to the nutrient solutions at 5 g L$^{-1}$. This method was used to inoculate soybean with soil rhizobia continuously for 5 days. Previous experiments showed that this method was feasible for soybean inoculation, and there would be spontaneous nodulation of rhizobia in the soil community. The main N-fixing rhizobia in soil belonged to Bradyrhizobium, Bradyrhizobium japonicum, and B. liaoningense were the dominant bacteria.
2.2. Sampling and Parameter Analysis

Samples were taken at the R1 and R5 stages [34]. The plants were separated into different parts, deactivated at 105 °C for 30 min, and then dried at 85 °C. Dry samples were used to analyze the 15N abundance, dry weight, and N content of each part.

Plant N content analysis: The plant N content was determined using a B324 automatic Kjeldahl analyzer after digestion with concentrated H2SO4 (K2SO4 and CuSO4 as catalysts).

15N abundance analysis: After plant N content analysis by the Kjeldahl method, the titrated samples were concentrated and allowed to react with lithium hypobromite to produce N2 under freezing-vacuum conditions. The 15N abundance was determined using a mass spectrometer (Thermo-Fisher Delta V Advantage IRMS) equipped with a dual-inlet system.

2.3. Data Calculations

The percent of 15N-labeled N derived from fertilizer (15Ndff%) in plants was calculated as:

\[ 15\text{Ndff} \% = \frac{f_{\text{treatment}} - f_{\text{nature}}}{f_{\text{fertilizer}} - f_{\text{nature}}} \times 100\% \]  

where \( f_{\text{nature}} \) is the natural 15N abundance, \( f_{\text{fertilizer}} \) is the 15N abundance of the fertilizer, and \( f_{\text{treatment}} \) is the 15N abundance of the treatment.

The percent of N derived from atmosphere (Ndfa%) in plants was calculated as:

\[ \text{Ndfa}\% = 1 - 15\text{Ndff}\% \]  

The percent of 14N derived from fertilizer (14Ndff%) plus the percent of N derived from atmosphere (Ndfa%) was calculated as:

\[ 14\text{Ndff}\% + \text{Ndfa}\% = 1 - 15\text{Ndff}\% \]  

Based on the 15N abundance of each organ, the ratio of N sources from 15N, 14N, and N-fixing root nodules in each organ can be calculated; if these values are then multiplied by the N accumulation, the N accumulation from 15N, 14N, and root nodules in each organ can be obtained.

2.4. Statistical Analyses

Descriptive statistics, one-way ANOVA, and correlation tests were performed on the data by IBM SPSS Software version 17.0. The results were mean ± standard deviation (SD) of three replicates. Duncan test was used for comparison between treatments (\( \alpha = 0.05 \)). All data were tested for normality and homogeneity of variance.

3. Results

3.1. Ratio of Fertilizer N and Root Nodule N Fixation in Soybean Plants

3.1.1. 15N Abundance (%) Difference in Soybean Plants with Dual Root

Table 2 shows the 15N abundance in the various parts of soybean plants with dual root system for Treatments I, II, and III. The 15N abundance in the vegetative organs of soybean plants differed significantly among the three treatments, indicating that the different treatments resulted in considerable differences in the 15N abundance in various organs.
Table 2. $^{15}$N abundance (%) in plant organs of soybean with dual root

| Stages | Organs | NO$_3^-$ | NH$_4^+$ |
|--------|--------|----------|----------|
|        | I      | II       | III      | I      | II       | III      |
| $R_1$  | Root$_{non}$ | 2.73 ± 0.11ab | 2.58 ± 0.06b | 2.91 ± 0.04a | 2.79 ± 0.05b | 2.59 ± 0.06c | 2.99 ± 0.02a |
|        | Root$_n$  | 0.96 ± 0.01b | 0.77 ± 0.04c | 2.45 ± 0.02a | 1.01 ± 0.01b | 0.71 ± 0.02c | 2.70 ± 0.03a |
|        | Nodule$_n$ | 0.63 ± 0.02b | 0.58 ± 0.03b | 0.88 ± 0.01a | 0.62 ± 0.01b | 0.56 ± 0.01c | 0.96 ± 0.01a |
|        | Shoot    | 1.45 ± 0.04b | 1.34 ± 0.06b | 2.16 ± 0.03a | 1.51 ± 0.02b | 1.34 ± 0.01c | 2.78 ± 0.02a |
| $R_5$  | Root$_{non}$ | 2.46 ± 0.09b | 2.27 ± 0.08b | 2.74 ± 0.01a | 2.55 ± 0.17a | 2.47 ± 0.11a | 2.76 ± 0.09a |
|        | Root$_n$  | 0.73 ± 0.02b | 0.76 ± 0.03b | 1.87 ± 0.08a | 0.80 ± 0.04b | 0.70 ± 0.03b | 1.98 ± 0.05a |
|        | Nodule$_n$ | 0.51 ± 0.00b | 0.52 ± 0.02b | 0.76 ± 0.00a | 0.52 ± 0.01b | 0.51 ± 0.01b | 0.75 ± 0.01a |
|        | Shoot    | 0.94 ± 0.01b | 0.94 ± 0.03b | 1.44 ± 0.03a | 0.94 ± 0.02b | 1.01 ± 0.02b | 1.61 ± 0.04a |

In the dual root system, Root$_{non}$ represents non-nodulated roots, Root$_n$ represents nodulated roots, and Nodule$_n$ represents nodules on the same side of nodulated roots. The values are the means ± standard error ($n = 3$). Different lowercase letters indicate significant differences between treatments at the 5% level. Treatments I, II, and III are compared horizontally.

In Treatment I, under NO$_3^-$ and NH$_4^+$ N sources, the $^{15}$N abundance in Root$_{non}$ at the $R_1$ and $R_5$ stages was lower than that of the N fertilizer (3.63%). However, the $^{15}$N abundance in Root$_n$ and Nodule$_n$ at the $R_1$ and $R_5$ stages remained higher than the natural $^{15}$N abundance (0.365%). The $^{15}$N abundance in the soybean Shoot at the $R_1$ stage was higher than the natural $^{15}$N abundance (0.365%) and lower than the $^{15}$N abundance of fertilizer N (3.63%). In Treatments II and III, under NO$_3^-$ and NH$_4^+$ N sources, the $^{15}$N abundance at the $R_1$ and $R_5$ stages in Root$_{non}$ was lower than that of fertilizer N (3.63%) and higher than the natural $^{15}$N abundance (0.365%). There were no significant differences in the $^{15}$N abundance between the NO$_3^-$ and NH$_4^+$ N sources for Treatments I, II, and III. However, during three treatments, the $^{15}$N abundance in all the soybean organs under different treatments at $R_5$ was lower than it was at the $R_1$ stage.

3.1.2. Ratio of N Absorbed from Different Sources in Dual Root Soybeans

Table 3 shows that for Treatment I under NO$_3^-$ and NH$_4^+$ sources at the $R_1$ and $R_5$ stages, most of the N in Root$_{non}$ came from the fertilizer N that was self-absorbed by the roots. However, these results illustrate that at the $R_1$ and $R_5$ stages, most of the N in Root$_n$ and Nodule$_n$ came from the nodule-fixed N in this root system. At the $R_1$ and $R_5$ stages, nodule-fixed N contributed a larger proportion to the supply for the Shoot.
In the dual root system, Root\textsubscript{non} represents non-nodulated roots, Root\textsubscript{n} represents nodulated roots, and Nodule\textsubscript{n} represents nodules on the same side as the nodulated roots. $^{15}$Ndff\% represents the proportion of $^{15}$N-labeled fertilizer N, Ndfa\% represents the proportion of nodule-fixed N, and $^{14}$Ndff\%+Ndfa\% represents the proportion of nodule-fixed N plus unlabeled fertilizer N. The values are the means ± standard error (n = 3). Different lowercase letters indicate significant differences between treatments at the 5% level. The two N sources are compared horizontally.

In Treatment II under NO$_3^-$ and NH$_4^+$ N sources, the results indicate that when N was added to both sides of the roots, the N in Root\textsubscript{non} primarily came from fertilizer N that was self-absorbed by the roots with small proportions from absorbed fertilizer N and nodule-fixed Root\textsubscript{n} N (translocated from the Shoot). A comparison with Treatment I revealed that there was little difference in the nutrient proportions of different N sources in Root\textsubscript{non} as contributed by the two roots of the dual root system with an addition to both sides versus one side. Furthermore, the proportions of different N sources in various parts of the soybean plants in Treatments I and II were compared. Similarly, the nutrient proportions contributed by the two roots of the dual root system did not change markedly in the Root\textsubscript{n}, Nodule\textsubscript{n}, or Shoot. These results indicate that the soybean plants contributed similarly to the plant N with or without N addition, showing the integrity of fertilizer N absorption and nodule N fixation.

No significant differences were detected in the proportions of various N sources between the NO$_3^-$ and NH$_4^+$ N sources among Treatments I, II, and III, indicating that the nutritional effect of NO$_3^-$ vs. NH$_4^+$ addition was not markedly different in soybean plants. However, compared with the R$_1$ stage, the R$_5$ stage was associated with a higher proportion of nodule-fixed N in various organs under different conditions for Treatments I, II, and III, indicating a larger contribution of nodule-fixed N to soybean plants at the R$_5$ stage than at the R$_1$ stage.
In both Treatments II and III, the same N concentration was added to both sides of the root system, and the only difference was related to the $^{15}$N abundance. To distinguish among the three N sources in Treatment II, we calculated the proportion of absorbed $^{14}$N-labeled fertilizer N in various parts of the soybean plants by summing up the proportion of absorbed $^{14}$N-labeled fertilizer N and the proportion of N from nodule fixation in each part of the soybeans from Treatment II and subtracting the proportion of N from nodule fixation in the same parts of the soybean plants from Treatment III (Table 4).

**Table 4. Proportions of N from different sources in various organs of soybean plants in Treatment II (%).**

| Stages | Organs     | NO$_3^-$ | $^{15}$Ndf% | $^{14}$Ndf% | Nd% | $^{15}$Nd% | $^{14}$Nd% | Ndfa% |
|--------|------------|----------|-------------|-------------|-----|-------------|-------------|-------|
| R$_1$  | Root$_{non}$ | 68.0 ± 1.92a | 10.0 ± 2.83c | 22.0 ± 1.15b | 68.1 ± 1.81a | 12.4 ± 1.84c | 19.5 ± 0.68b |
|        | Root$_n$    | 12.4 ± 1.24c | 51.3 ± 0.81a | 36.3 ± 0.66b | 10.7 ± 0.63c | 60.7 ± 0.54a | 28.6 ± 0.96b |
|        | Nodule$_n$ | 6.6 ± 0.80c | 9.2 ± 1.00b  | 84.2 ± 0.25a | 6.0 ± 0.30c  | 12.3 ± 0.60b | 81.7 ± 0.30a |
|        | Shoot      | 30.0 ± 0.49b | 25.0 ± 1.68c | 45.0 ± 1.23a | 30.0 ± 0.35b | 31.6 ± 1.18b | 38.4 ± 1.48a |
| R$_5$  | Root$_{non}$ | 58.5 ± 2.58a | 14.2 ± 2.63c | 27.3 ± 0.18b | 64.6 ± 3.42a | 8.8 ± 0.72c  | 26.6 ± 2.74b |
|        | Root$_n$    | 12.2 ± 0.86c | 33.8 ± 2.69b | 54.0 ± 2.52a | 10.2 ± 0.97c | 39.4 ± 0.32c | 50.4 ± 1.41a |
|        | Nodule$_n$ | 4.7 ± 0.42c | 7.2 ± 0.46b  | 88.1 ± 0.04a | 4.6 ± 0.28c  | 7.2 ± 0.03b  | 88.2 ± 0.31a |
|        | Shoot      | 17.6 ± 0.66b | 15.3 ± 1.20b | 67.1 ± 0.88a | 19.8 ± 0.37b | 18.3 ± 0.37b | 61.9 ± 0.78a |

In the dual root system, Root$_{non}$ represents non-nodulated roots, Root$_n$ represents nodulated roots, and Nodule$_n$ represents nodules on the same side as the nodulated roots. $^{15}$Ndf% represents the proportion of $^{15}$N-labeled fertilizer N, $^{14}$Ndf% represents the proportion of $^{14}$N-labeled fertilizer N, and Nd% represents the proportion of nodule-fixed N. The values are the means ± standard error (n = 3). Different lowercase letters indicate significant differences between treatments at the 5% level. Three N sources are compared horizontally.

In Treatment II, there were three N sources for soybean plants, i.e., absorbed fertilizer N from Root$_{non}$, absorbed fertilizer N from Root$_n$, and nodule-fixed N from Root$_n$. Under the NO$_3^-$ and NH$_4^+$ N sources in the R$_1$ stage, Root$_{non}$ preferentially absorbed the self-assimilated N. At the R$_1$ stage, 51.3% and 60.7%, respectively, were contributed by absorbed Root$_n$, fertilizer N, and 36.3% and 28.6%, respectively, were contributed by nodule-fixed Root$_n$. N. At the R$_5$ stage, the corresponding proportions were 33.8% and 39.4%, 54.0% and 50.4% for absorbed fertilizer N from Root$_{non}$, absorbed fertilizer N from Root$_n$, and nodule-fixed N from Root$_n$, respectively. These results show that when N was added to both sides of the root system, Root$_n$ also preferentially absorbed the N that was assimilated by this root system; the proportion of nodule-fixed N in Root$_n$ increased with the increasing N fixation capacity of the nodules. For the N in Nodule$_n$ at the R$_1$ and R$_5$ stages, nodule-fixed N was preferentially absorbed by Nodule$_n$. Moreover, the proportion of N in the root nodules contributed by the absorbed fertilizer N of the two roots of the dual root system was significantly different; that is, the N from Root$_{non}$ was less than that from Root$_n$. These results show that when N was added to both sides of the root system, the Shoot primarily absorbed fertilizer N at the R$_1$ stage, whereas nodule-fixed N was primarily absorbed during the R$_5$ stage. The supplies of fertilizer N from the two roots of the dual root system to the Shoot were almost identical, suggesting the same supply of absorbed fertilizer N to Shoot by each side in the dual root system.

A comparison of Treatments I and II revealed no major change in the proportions of N supplied to the Shoot by each root of the dual root system, irrespective of whether N was added to one or both sides. However, after the addition of N to both sides of the root system, the proportion of nodule-fixed N decreased, whereas the proportions of absorbed NO$_3^-$ and NH$_4^+$ supplied to the Shoot by Root$_{non}$ and Root$_n$ were similar.

### 3.2. N Accumulation and Source in Dual Root Soybeans

#### 3.2.1. N Accumulation in Dual Root Soybeans

Table 5 shows the N accumulation of organs in the dual root system of single-nodule soybeans. In Treatments I, II, and III under the NO$_3^-$ N source for soybean plants, the N accumulation of Root$_{non}$...
at the R₁ stage was significantly higher in Treatment III than in Treatments I and II. During the R₃ stage, there was no significant difference among Treatments I, II, or III. At the R₁ stage, the N accumulation of Rootₙ was significantly lower in Treatment I than in Treatments II and III. During the R₃ stage, there was no significant difference among Treatments I, II, and III. The N accumulation of Noduleₙ at the R₁ and R₃ stages was significantly higher in Treatment I than in Treatments II or III. In Treatment I, the N accumulation in the Shoot at R₁ was significantly lower than in Treatment II or III, and that at R₃ it was significantly lower than in Treatment II. The total N accumulation of Treatment I at the R₁ stage was significantly lower than in Treatment II or III, and the difference at the R₃ stage was not significant. In Treatments I, II, and III under the NH₄⁺ N source for soybean plants, the N accumulation of Rootₙ in Treatment I at the R₁ and R₃ stages was significantly lower than in Treatments II and III. At the R₁ stage, the N accumulation of Rootₙ in Treatment I was significantly lower than in Treatments II and III, whereas the difference was not significant at the R₃ stage. The N accumulation of Noduleₙ in Treatment I at the R₁ and R₃ stages was significantly higher than in Treatments II and III. At the R₁ stage, the N accumulation in the Shoot of Treatment I was significantly lower than in Treatments II and III. The R₁ and R₃ stages showed the opposite pattern. In Treatment I, the N accumulation of the Shoot in the R₁ stage was significantly lower than that in Treatments II and III, and the difference at the R₃ stage was not significant.

### Table 5. N accumulation in each organ of soybean in the dual root system (mg/plant).

| Stages | Organs          | NO₃⁻ | NH₄⁺ |
|--------|----------------|------|------|
|        | I              | II   | III  |
|        | I              | II   | III  |
| R₁     | Rootₙ         | 18.08 ± 1.19c | 23.38 ± 1.40b | 25.20 ± 0.77a |
|        | Rootₙ         | 22.54 ± 1.15b | 31.19 ± 2.71a | 34.53 ± 1.30a |
|        | Noduleₙ       | 35.79 ± 2.34a | 23.28 ± 2.63b | 24.70 ± 2.20b |
|        | Shoot         | 355.50 ± 5.98b | 418.10 ± 14.75a | 432.48 ± 7.85a |
|        | Total         | 431.91 ± 10.66b | 495.95 ± 21.49a | 516.91 ± 12.12a |
| R₃     | Rootₙ         | 46.89 ± 4.92a | 41.80 ± 4.86a | 37.05 ± 4.91a |
|        | Rootₙ         | 69.22 ± 3.15a | 76.83 ± 7.06a | 82.07 ± 7.89a |
|        | Noduleₙ       | 109.14 ± 9.68a | 76.94 ± 8.53b | 88.15 ± 6.63ab |
|        | Shoot         | 1012.07 ± 28.50b | 1105.38 ± 48.17a | 1045.11 ± 32.50ab |
|        | Total         | 1237.32 ± 46.33a | 1300.95 ± 68.62a | 1252.38 ± 51.93a |

The values are the means ± standard error (n = 3). Different lowercase letters indicate significant differences between treatments at the 5% level. Treatments I, II, and III are compared horizontally.

A comparison of Treatments I, II, and III showed that the N accumulation of Rootₙ and Rootₙ at the R₁ stage in Treatment I was lower than in Treatments II and III, whereas the N accumulation at the R₃ stage was not significantly different among the treatments. This finding indicates that local N application in the early stage of soybean growth affects the accumulation of N in the root, but local N application in the late stage of soybean growth has a weak effect on the accumulation of N in the root, possibly because the ratio of N fixation in the root nodules was increasing during the late stage of soybean growth. N accumulation of Noduleₙ in Treatment I was significantly higher than in Treatments II and III. This result indicates that the local application of N can inhibit the growth of root nodules on the N-treated side and promotes the growth of root nodules on the N-treated side, thus improving the N-fixing ability of root nodules and leading to an increase in N accumulation. There was no significant difference in N accumulation in each part of the treatment with a NO₃⁻ vs. NH₄⁺ N source, indicating that under an N concentration of 50 mg·L⁻¹, there was no difference in the nutritional effect of NO₃⁻ vs. NH₄⁺ on each part.

#### 3.2.2. Accumulation of Fertilizer N and N-Fixing Root Nodules in Dual Root Soybeans

As seen from Table 6, when N was applied on one side, the majority of Rootₙ N came from the fertilizer N absorbed by Rootₙ, whereas a small part came from the root nodules of Rootₙ. When N was not applied to Rootₙ, most of the N came from root nodule N fixation, and a small part came from the fertilizer N absorbed by Rootₙ. Under unilateral N application to soybean roots, most of the N in...
Nodule, came from the N-fixing root nodules of Root, and a small part came from fertilizer N absorbed from Root, which also indicates that not all N needed for root nodule growth came from internal N fixation and some N needed to be absorbed from the roots. In comparing the two N sources in the R, and R stages, N fixation from the root nodules was significantly higher than N from fertilizer N absorbed by Root. The total N accumulation from N-fixing root nodules was much higher than that from fertilizer.

Table 6. The accumulation of N fixation from fertilizer and nodule-fixed N in each parts of the dual root soybean (mg/plant).

| Stages | Organs | NO$_3^-$ | Nodule | NH$_3^+$ | Nodule |
|--------|--------|----------|---------|----------|---------|
| I      | Root$_{non}$ | 13.10 ± 0.63a | 4.99 ± 0.62b | 15.32 ± 0.33a | 5.34 ± 0.33b |
|        | Root$_{f}$ | 4.13 ± 0.08b | 18.41 ± 0.08a | 5.23 ± 0.09b | 21.29 ± 0.09a |
|        | Nodule$_{f}$ | 2.87 ± 0.18b | 32.92 ± 0.18a | 2.64 ± 0.11b | 30.88 ± 0.11a |
|        | Shoot | 11.67 ± 3.45b | 238.8 ± 3.45a | 109.99 ± 1.25b | 202.18 ± 1.25a |
|        | Total | 136.8 ± 4.33b | 295.12 ± 4.33a | 133.27 ± 1.78b | 259.69 ± 1.78a |
| II     | Root$_{non}$ | 30.04 ± 1.28a | 16.85 ± 1.28b | 24.97 ± 1.94a | 12.30 ± 1.94b |
|        | Root$_{f}$ | 7.74 ± 0.37b | 61.49 ± 0.37a | 9.11 ± 0.78b | 59.34 ± 0.79a |
|        | Nodule$_{f}$ | 4.68 ± 0.11b | 104.46 ± 0.11a | 4.87 ± 0.18b | 97.63 ± 0.18a |
|        | Shoot | 178.12 ± 3.79b | 833.95 ± 3.78a | 222.20 ± 4.69b | 947.67 ± 4.69a |
|        | Total | 220.38 ± 5.54b | 1016.75 ± 5.54a | 261.15 ± 7.59b | 1116.94 ± 7.59a |

In the dual root system, Root$_{non}$ represents non-nodulated roots, Root$_{f}$ represents nodulated roots, and Nodule$_{f}$ represents nodules on the same side as the nodulated roots. $^{15}$N represents the N accumulation of $^{15}$N-labeled fertilizer N, $^{14}$N represents the N accumulation of unlabeled fertilizer N, and Nodule represents the N accumulation of nodule-fixed N. The values are the means ± standard error (n = 3). Different lowercase letters indicate significant differences between treatments at the 5% level. The three N sources are compared horizontally.

Under the condition of bilateral N application, most of the N in Root$_{non}$ came from the fertilizer N absorbed by Root$_{non}$, and a small part came from the N absorbed and fixed by Root$_{f}$. However, most of the N in Root$_{f}$ came from the N absorbed and fixed by Root$_{f}$, whereas a small part came from the fertilizer N absorbed by Root$_{non}$ and transferred to Root$_{f}$. Most of the N in Nodule$_{f}$ came from N absorbed and fixed by Root$_{f}$, and a small part came from fertilizer N absorbed by Root$_{non}$ and transferred to Root$_{f}$ nodules. At the same time, it can be observed that not all the N required for root nodule growth comes from self-fixing N, and it is also necessary to absorb N via the roots. In comparing the three N sources in the Shoot at the R, and R stages, there was no significant difference between N accumulation in Root$_{non}$ and Root$_{f}$, but N accumulation was significantly lower than in the N-fixing root nodules of Root$_{f}$. This suggests that the supply of absorbed fertilizer N to Shoot was the same from the two parts of the dual root systems. As a whole, the total accumulation of N absorbed and fixed by Root$_{f}$ was much higher than the fertilizer N absorbed by Root$_{non}$, mainly because Root$_{non}$ had no root nodules for N fixation.
3.2.3. N Translocation from Shoot to Roots and Nodules of Soybeans

The fertilizer N absorbed by the two roots of the dual root system and the N fixed by the root nodules were transported to the Shoot, and after assimilation, they were transported to the roots and nodules in certain forms. Due to the different $^{15}$N abundance in various parts of the soybean plants in the treatments (Table 2), we regarded the Root$_n$ and the Shoot as one system, in which the Shoot served as a source of $^{15}$N for the Root$_n$. From the R$_1$ to the R$_5$ stage, the amount of $^{15}$N increasing in Root$_n$ can be obtained from the $^{15}$N accumulation of the R$_5$ stage minus the $^{15}$N accumulation of the R$_1$ stage, and this should be equal to the amount of $^{15}$N translocated from the Shoot plus the amount of naturally occurring $^{15}$N that was self-absorbed by the roots of Root$_n$ (including the supply by nodules) from the R$_1$ to the R$_5$ stage. If we let the amount of N translocated from the Shoot to Root$_n$ be $x$, then the $^{15}$N abundance of the N translocated downwards from the Shoot can be calculated using the mean $^{15}$N abundance in the Shoot at the R$_1$ and R$_5$ stages, where $x \times (f_{\text{shoot}R1} + f_{\text{shoot}R5})/2$ represents the amount of $^{15}$N translocated from the Shoot to Root$_n$ during R$_1$–R$_5$, $N_{R5}$–$N_{R1}$ represents the N accumulation in Root$_n$ during R$_1$–R$_5$, $N_{R1}$–$N_{R5}$–$x$ represents the N accumulation from Root$_n$ absorbed during R$_1$–R$_5$ (including the N supplied by nodules), $(N_{R5}$–$N_{R1}$–$x$) $\times f_{\text{nature}}$ represents the amount of $^{15}$N in Root$_n$ that is self-absorbed by the roots and supplied by the nodules during R$_1$–R$_5$, $N_{R5}$ $\times f_{\text{RS}}$ represents the total $^{15}$N in Root$_n$ at the R$_5$ stage, and $N_{R1}$ $\times f_{R1}$ represents the total $^{15}$N in Root$_n$ at the R$_1$ stage. This method can also be used to calculate the amount of N translocated from the Shoot to Nodule$_n$. For Treatments I and II, the calculation is as follows:

$$x = \frac{N_{R5} \times f_{R5} - N_{R1} \times f_{R1} - (N_{R5} - N_{R1}) \times f_{\text{nature}}}{(f_{\text{shoot}R1} + f_{\text{shoot}R5})/2 - f_{\text{nature}}}$$  \hspace{1cm} (4)

where $x$ is the amount of N translocated from the Shoot to Root$_n$ or Nodule$_n$, $N_{R1}$ is the N accumulation in Root$_n$ or Nodule$_n$ at the R$_1$ stage, $N_{R5}$ is the N accumulation in Root$_n$ or Nodule$_n$ at the R$_5$ stage, $f_{\text{nature}}$ is the natural $^{15}$N abundance, $f_{R1}$ is the $^{15}$N abundance in Root$_n$ or Nodule$_n$ at the R$_1$ stage, $f_{R5}$ is the $^{15}$N abundance in Root$_n$ or Nodule$_n$ at the R$_5$ stage, $f_{\text{shoot}R1}$ is the $^{15}$N abundance in the Shoot at the R$_1$ stage, and $f_{\text{shoot}R5}$ is the $^{15}$N abundance in the Shoot at the R$_5$ stage.

Similarly, when calculating the amount of N translocated from the Shoot to Root$_{non}$, we regarded the Root$_{non}$ and the Shoot as one system. Because $^{15}$N-labeled fertilizer N was added to Root$_{non}$, the calculation is as follows:

$$x = \frac{(N_{R5} - N_{R1}) \times f_{\text{fertilizer}} - N_{R5} \times f_{R5} + N_{R1} \times f_{R1}}{f_{\text{fertilizer}} - (f_{\text{shoot}R1} + f_{\text{shoot}R5})/2}$$ \hspace{1cm} (5)

where $x$ is the amount of N translocated from the Shoot to Root$_{non}$, $N_{R1}$ is the N accumulation in Root$_{non}$ at the R$_1$ stage, $N_{R5}$ is the N accumulation in Root$_{non}$ at the R$_5$ stage, $f_{\text{fertilizer}}$ is the $^{15}$N abundance in the fertilizer, $f_{R1}$ is the $^{15}$N abundance in Root$_{non}$ at the R$_1$ stage, $f_{R5}$ is the $^{15}$N abundance in Root$_{non}$ at the R$_5$ stage, $f_{\text{shoot}R1}$ is the $^{15}$N abundance in the Shoot at the R$_1$ stage, and $f_{\text{shoot}R5}$ is the $^{15}$N abundance in the Shoot at the R$_5$ stage.

The amount of N translocated from the Shoot to Root$_{non}$, Root$_n$, and Nodule$_n$ can be calculated using Equations (4) and (5), as shown in Table 7.
Table 7. N translocation from the Shoot to roots and nodules of soybean plants from the R<sub>1</sub>–R<sub>5</sub> stage.

| Organs       | NIA (mg/plant) | NTFS (mg/plant) | NTFS/NIA (%) | NIA (mg/plant) | NTFS (mg/plant) | NTFS/NIA (%) |
|--------------|----------------|-----------------|--------------|----------------|-----------------|--------------|
| Root<sub>n</sub> | 28.8           | 16.1            | 55.9%        | 16.6           | 9.5             | 57.0%        |
| Root<sub>n</sub> | 46.8           | 13.9            | 29.6%        | 41.9           | 14.6            | 34.9%        |
| Nodule<sub>n</sub> | 73.4           | 6.9             | 9.4%         | 68.9           | 8.4             | 12.2%        |
| Total        | 148.9          | 36.9            | 24.8%        | 127.4          | 32.5            | 25.5%        |
| Root<sub>n</sub> | 18.4           | 13.0            | 70.5%        | 26.6           | 13.8            | 51.8%        |
| Root<sub>n</sub> | 45.7           | 22.9            | 50.0%        | 43.6           | 17.6            | 40.3%        |
| Nodule<sub>n</sub> | 53.7           | 8.9             | 16.6%        | 47.5           | 7.3             | 15.3%        |
| Total        | 117.7          | 44.8            | 38.1%        | 117.6          | 38.7            | 32.9%        |

In the dual root system, Root<sub>n</sub> represents nodulated roots, Root<sub>non</sub> represents non-nodulated roots, and Nodule<sub>n</sub> represents nodules on the same side as the nodulated roots. ANT (mg) represents the accumulation of N translocated from the Shoot to roots and nodules during R<sub>1</sub>–R<sub>5</sub>. AIN (mg) represents the accumulation of increased N in various parts during R<sub>1</sub>–R<sub>5</sub>, and AIN/NIA (%) represents the proportion of total N from Shoot (NTFS) under AIN in various plant parts.

Table 7 shows that for Treatment I, in the NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> N sources between the R<sub>1</sub> and R<sub>5</sub> stages, the accumulation of increased N in Root<sub>n</sub> was 28.8 and 16.6 mg, respectively, and the accumulation of N translocated from the Shoot to Root<sub>n</sub> was 16.1 and 9.5 mg, respectively. The N translocated from the Shoot to Root<sub>non</sub> accounted for 55.9% and 57.0%, respectively, of the increased accumulation of N in Root<sub>n</sub>. In Root<sub>n</sub>, the increased accumulation of N was 46.8 and 41.9 mg, respectively, and the accumulation of N translocated from the Shoot was 13.9 and 14.6 mg, accounting for 29.6% and 34.9%, respectively, of the increased accumulation of N in Root<sub>n</sub>. In Nodule<sub>n</sub>, the increased accumulation of N was 73.4 and 68.9 mg, respectively, and the accumulation of N translocated from the Shoot was 6.9 and 8.3 mg, contributing to 9.4% and 12.2%, respectively, of the increased accumulation of N in Nodule<sub>n</sub>. In Treatment II with NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> N sources between the R<sub>1</sub> and R<sub>5</sub> stages, the increased accumulation of N in Root<sub>n</sub> was 18.4 and 26.6 mg, respectively, and the accumulation of N translocated from the Shoot to Root<sub>n</sub> was 13.0 and 13.8 mg, making up 70.5% and 51.8%, respectively, of the increased accumulation of N in Root<sub>n</sub>. In Root<sub>n</sub>, the increased accumulation of N was 45.7 and 43.6 mg, respectively, and the accumulation of N translocated from the Shoot was 22.9 and 17.6 mg, accounting for 50.0% and 40.3%, respectively, of the increased accumulation of N in Root<sub>n</sub>. In Nodule<sub>n</sub>, the increased accumulation of N was 53.7 and 47.5 mg, respectively, and the accumulation of N translocated from the Shoot was 8.9 and 7.3 mg, contributing to only 16.6% and 15.3%, respectively, of the increased accumulation of N in Nodule<sub>n</sub>.

4. Discussion

4.1. Sources of N in the Roots and Nodules of Soybeans

After adding <sup>15</sup>NO<sub>3</sub><sup>-</sup> to soybean (Glycine max L. Merr.) seedlings, Crafts-Brandner and Harper [35] detected <sup>15</sup>N in the reduced N from xylem sap and found that the <sup>15</sup>N abundance tended to increase with time, leading to a conclusion that soybean roots can reduce <sup>15</sup>NO<sub>3</sub><sup>-</sup>. Sprent and Thomas [36] indicated that Phaseolus vulgaris and Glycine max directly absorb and transport fertilizer NO<sub>3</sub><sup>-</sup> into the shoots for assimilation, whereas Pisum sativum and Vicia faba transport NO<sub>3</sub><sup>-</sup> into the shoots after assimilation in the roots. Following the application of different concentrations of NO<sub>3</sub><sup>-</sup> to six leguminous crops, Andrews [37] found that the assimilation of absorbed NO<sub>3</sub><sup>-</sup> occurred primarily in the roots of Cajanus cajan, Lupinus albus, Trifolium repens, and Pisum sativum, whereas Glycine max, and Phaseolus vulgaris primarily assimilated the absorbed NO<sub>3</sub><sup>-</sup> in the shoots. Kiyomiya et al. [38] treated the roots of rice with <sup>13</sup>NH<sub>4</sub><sup>+</sup> and then observed the <sup>13</sup>N at the bottoms of the shoots within 2 min. However, the rapid upward transport of <sup>13</sup>N was inhibited after the addition of glutamine synthetase inhibitor, indicating that most of the NH<sub>4</sub><sup>+</sup> was assimilated in the roots. In the present study, under <sup>15</sup>NO<sub>3</sub><sup>-</sup> and
15NH4+ treatments, the 15N abundance in the roots on the N-receiving side was always higher than in the shoots, indicating that part of the 15NO3− and 15NH4+ absorbed by the roots was used for root growth after assimilation in the roots (otherwise, if all the absorbed fertilizer N was transported to the shoots for assimilation, the 15N abundance would have been the same in both the shoots and roots). Tanaka et al. [9] and Reynolds et al. [31] established split-root system in soybeans and added 15NO3− and 13NH4+ to one side of the roots, and they then detected the 15N and 13N in the roots and nodules on the N-free side. In the present study, we added 15N-labeled fertilizer N to non-nodulated roots on one side of the dual root system, and yet a 15N concentration higher than the natural abundance was detected in both the nodulated roots and root nodules on the other side. This finding indicates that the N absorbed by the roots on one side was translocated to the roots and nodules on the other side via the Shoot.

Sato et al. [39] added 13NO3− to the culture nutrient solution of nodulated soybeans and then recorded data at 1-min intervals for 1 h. They found that 13N-labeled NO3− first appeared in soybean petioles and then in the leaves, with little detected in the nodules. This observation suggests that the NO3− absorbed by the roots was not translocated into the nodules within a short period of time. In Treatments I and II, we added 15N-labeled fertilizer N to non-nodulated roots in the dual root system, and yet a higher 15N was detected in the nodules on the other side than would be naturally available (0.365%). By combining these findings with the results of Sato et al. [39], we believe that the absorbed fertilizer N present in the nodules was translocated from the Shoot, rather than directly absorbed and supplied by the roots. In Treatment III, we added the same concentration of 15N-labeled fertilizer N to both sides of the dual root system. The 15N abundance in Rootnon was higher than that in Rootn, at both the R1 and R5 stages, reflecting that node-fixed N was directly supplied to Rootn and nodule growth.

Wery et al. [40] conducted an experiment using alfalfa with and without NH4+ addition, and no significant difference was found in N accumulation between the two treatments. However, with an N supply, the rate of N fixation in the nodules decreased, whereas the absorbed N increased. This phenomenon indicates that alfalfa preferentially selected combined N in the presence of both combined N and N2. In Treatment I, 50 mg L−1 N was supplied to Rootnon, and in Treatment II, there was a bilateral supply of 50 mg L−1 N to Rootnon and Rootn. It was found that the ratio of Rootn N supplied to aboveground N supply at the R1 and R5 stages was similar, but the proportion of nodule-fixed N supplied to aboveground N supply in root nodules was smaller; this further indicates that the fertilizer N was preferentially selected by soybeans in the presence of a fertilizer N supply.

Many researchers believe that N application can considerably increase soybean yields [1–4], yet there are divergent opinions regarding the differences in the effects of NO3− and NH4+ on plants. In a study using the soybean split-root system, Chaillou et al. [41] found that the dry weight of the roots on one side that received NO3− was higher than on the other side that received NH4+. However, Gan et al. [23] treated soybeans with NO3− and NH4+ and found that NH4+ application alone resulted in a higher biomass accumulation, nodule dry weight, total N accumulation, and N fixation in this crop. Saravitz et al. [42] found that when NH4+ and NO3− were applied to the dual roots of soybean, the cumulative absorption of NH4+ was about half that of NO3−. Abdellaaoui et al. [43] believed that both NH4+ and NO3− were easily absorbed by the root system in wheat seedlings, but only NO3− could accumulate in plants. The phloem in the root of the castor oil plant only absorbs NO3−, not ammonium salt, and NO3− is rapidly transported in xylem [44]. Although plants can effectively use NH4+, it is generally considered that NO3− is the main absorption form of plant N, mainly because NO3− is more soluble [45]. Savvas et al. [46] found that NH4+ could be nitrated in both soil and a hydroponic nutrient solution and that NO3− could be formed by nitrification even when NH4+ was supplied. Kumar [47] suggested that NH4+ was more easily converted into NO3− in soils with good ventilation. In this experiment, no significant differences were observed in the 15N abundance or N accumulation in various parts of the soybean plants after NO3− and NH4+ treatments, indicating that there were no major differences in the effects of NO3− and NH4+ on N nutrition in soybeans under the experimental conditions (50 mg L−1 N addition). This may be because NH4+ was nitrated to NO3−.
4.2. N Distribution in Shoot, Roots, and Nodules of Soybean Plants

In this study, a significantly higher N accumulation was observed in the Shoot than in the roots and nodules of soybeans (Table 6), suggesting that the N absorbed by the roots and fixed by the nodules was primarily transported to the aboveground part for Shoot growth, with only a small fraction supplied to the roots and nodules growth. After adding $^{15}\text{NO}_3^-$ or $^{15}\text{NH}_4^+$ to the leaf surface of sunflower, Ito, O. et al. [48] determined the $^{15}$N abundance in N-treated leaves and their upper and lower internodes. $^{15}$N was detected in both the upper and lower internodes, with a lower value for the upper than for the lower internode, revealing that the N added to the leaf surface can be transported not only upwards but also downwards and that the downward transport exceeds the upward transport. Tanaka et al. [9], Reynolds et al. [31], and our present study have all demonstrated that the N absorbed by roots on one side was transferred to the roots and nodules on the other side through the shoots, suggesting that the N assimilated by shoots can be translocated and redistributed to the roots and nodules.

In this study, using the Shoot and one side of the roots and nodules in the dual root system as an N translocation system, we established a method for calculating the translocation of N from the Shoot to the roots and nodules during the $R_1$–$R_5$ stages based on the difference in $^{15}$N abundance. The calculation showed that when N was added at a concentration of 50 mg L$^{-1}$, the N translocated from the Shoot to Root$_n$ accounted for 29.6%–52.3% of the N accumulation in Root$_n$, whereas the N translocated from the Shoot to Nodule$_n$ made up 9.4%–16.6% of the N accumulation in Nodule$_n$ during the $R_1$–$R_5$ stage. In the dual root system, the N absorbed and fixed by Root$_n$ and Nodule$_n$ was absorbed and utilized by the aboveground part whereas the fertilizer N was absorbed by Root$_{non}$. After the assimilation by the aboveground part, the N can be transported to the root and root nodules in a certain form.

5. Conclusions

1. Up to 81.5%–87.1% of the N absorbed by the soybean roots and fixed by the root nodules was supplied for shoot growth, leaving 12.9%–18.5% for root and nodule growth. Soybeans preferentially used fertilizer N in the presence of the NO$_3^-$ or NH$_4^+$ supply. After the absorbed fertilizer N and nodule-fixed N was transported to the shoot, a portion of it was redistributed to the roots and nodules. The nitrogen required for root growth was primarily derived from the NO$_3^-$ or NH$_4^+$ assimilated by the roots and the N fixed by the nodules, with a small portion translocated from the shoot. The N required for nodule growth was primarily contributed by nodule-fixed N with a small portion translocated from the shoot, whereas the NO$_3^-$ or NH$_4^+$ assimilated by the roots was not directly supplied to the nodules.

2. Using the shoot and one side of the roots and nodules in the dual root system as an N translocation system, we established a method for calculating the translocation of N from the shoots to the roots and nodules during the $R_1$–$R_5$ stages based on the difference in $^{15}$N abundance. The calculation showed that when N was added at a concentration of 50 mg L$^{-1}$, the N translocated from the Shoot to Root$_n$ accounted for 29.6%–52.3% of the N accumulation in Root$_n$, whereas the N translocated from the Shoot to Nodule$_n$ made up 9.4%–16.6% of the N accumulation in Nodule$_n$ during the $R_1$–$R_5$ stage.

3. This experiment systematically studied the transport characteristics of soybean N and the interaction mechanism of fertilizer N and root nodule N fixation, providing a theoretical basis and guidance for the rational application of N fertilizer.

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