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Nitrate Reduction by Roots of Soybean (*Glycine max* [L.] Merr.) Seedlings

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STEVEN J. CRAFTS-BRANDNER AND JAMES E. HARPER
Department of Agronomy and United States Department of Agriculture, Agricultural Research, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Studies were conducted with 9 to 12 day-old soybean (*Glycine max* [L.] Merr. cv. Williams) seedlings to determine the contribution of roots to whole plant NO_3^- reduction. Using an *in vivo* $-NO_3^-$ nitrate reductase (NR) assay (no exogenous NO_3^- added to incubation medium) developed for roots, the roots accounted for approximately 30% of whole plant nitrate reductase activity (NRA) of plants grown on 15 mM NO_3^-.

Nitrogen analyses of xylem exudate showed that 53 to 66% of the total-N was as reduced-N, depending on the time of day of exudate collection. These observations supported enzyme data that suggested roots were contributing significantly to whole plant NO_3^- reduction. In short-term feeding studies using ^{15}N-NO_3^- significant and increasing atom percent ^{15}N excess was found in the reduced-N fraction of xylem exudate at 1.5 and 3 hours after feeding, respectively, which verified that roots were capable of reducing NO_3^-.

Estimated reduced-N accumulation by plants based on *in vivo* $-NO_3^-$ NR assays of all plant parts substantially over-estimated actual reduced-N accumulation by the plants. Thus, the *in vivo* NR assay cannot be used to accurately estimate reduced-N accumulation but still serves as a useful assay for relative differences in treatment conditions.

Soybean plants grown under midwest field conditions have been estimated to obtain from 25 to 60% of their total-N from symbiotic N_2 fixation (22). Therefore, much of the N in soybean plants must be soil derived, primarily as NO_3^-.

It is well established that many plants have the ability to reduce NO_3^- in both roots and leaves. Based on xylem exudate analyses, some plants, such as *Xanthium*, rely almost totally on leaves for NO_3^- reduction while others, such as *Pisum*, reduce nearly all of their NO_3^- in the roots (16). McClure and Israel (13) have shown that xylem exudate of non-nodulated soybean plants grown on high NO_3^- levels contained high levels (≃40%) of reduced-N throughout the growing season.

The reported amounts of NRA[1] in roots of soybean have been quite variable. This may be due in part to the fact that many different assays have been used and that the assays used have been developed for other plant parts, such as leaves. Also, much of the variability in results of root NR assays may be attributed to the basis of expressing the data. Different results may be expected when expressing the data on a fresh weight *versus* a mg protein basis. Randall *et al.* (19) found no detectable *in vivo* or *in vitro* NRA in soybean roots. Magalhães (12) showed *in vitro* NRA in soybean roots to be only 5% of *in vitro* leaflet NRA. Weissman (23) found comparable *in vitro* NRA of soybean roots and leaflets on a mg protein basis. However, considering the greater protein content of soybean leaflets than roots (J. E. Harper, unpublished data), the NRA reported by Weissman would be much greater in leaflets than roots on a fresh weight basis. Radin (18) assayed intact soybean parts by the *in vivo* $-NO_3^-$ NR assay (activity dependent on endogenous NO_3^-) and showed that soybean roots accounted for approximately 32% of the NRA of leaflets on a fresh weight basis. When NO_3^- was included with the *in vivo* assay medium, the NRA of the roots was 7% that of the leaflets. Hatam and Hume (9) reported considerable fresh weight NRA in soybean root tips relative to leaf discs. They also showed that cumulative NRA of soybean leaf discs, stems plus cotyledons, and root tips calculated on a whole plant basis agreed with actual reduced-N accumulation. In a more recent study, Hatam (8) showed soybean leaf disc and root tip NRA to be comparable on a fresh weight basis. However, leaflet NRA was much lower than is normally reported (7, 14, 18). The *in vivo* incubation medium used by Hatam and Hume (9) contained NADH which is known to interfere with the colorimetric assay of nitrite (20) and may have contributed to the low NRA values for leaves. In the more recent study, Hatam (8) used the *in vivo* NR assay method described by Jaworski (10) and yet the values reported for leaves were still quite low.

A knowledge of root contribution to NO_3^- reduction is needed to understand NO_3^- metabolism of the whole plant. Thus, the objectives of this study were to (a) investigate the contribution of roots to whole plant NO_3^- reduction based on *in vivo* $-NO_3^-$ NR assays and xylem exudate analyses, (b) verify with ^{15}N-NO_3^- the reduction of NO_3^- in roots, and (c) compare actual reduced-N accumulation of plants with reduced-N accumulation as estimated from integrated *in vivo* $-NO_3^-$ NR assays of intact plant parts.

MATERIALS AND METHODS

Plant Culture. Soybeans (*Glycine max* [L.] Merr. cv. Williams) were germinated in sand which was moistened with deionized H_2O. After 6 to 7 d, seedlings were transplanted into 2-L pots containing a modified Hoagland solution consisting of the following: 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, and 1 mM K-phosphate (pH 6.5). Micronutrients were supplied as follows: 50 μM KCl, 25 μM H_3BO_3, 36 μM Fe as sodium ferric diethylenetriamine pentaacetate, 5 μM $CuSO_4$, 6 μM $MnSO_4$, 2 μM $ZnSO_4$, and 0.015 μM $(NH_4)_6$ Mo_7O_{24}. There were six plants per pot. Plants were grown in growth chambers under a 14 h, 29°C light cycle and a 10 h, 19°C dark cycle. The light intensity was 600 μE m^{-2} s^{-1} supplied by incandescent and cool-white fluorescent lamps. Plants were harvested 9 to 12 DAP at various times during the light or dark cycle, depending on the experiment.

***In Vivo* NR Assay.** Soybean roots were assayed with a modification of the *in vivo* NR assay for soybean leaves reported by Nicholas *et al.* (14). The assay was optimized for solvent, phos-

[1] Abbreviations: NRA, nitrate reductase activity; NR, nitrate reductase; DAP, days after planting.

phate concentration, pH, and gaseous phase (4). The root assay medium in this study contained 100 mM K-phosphate (pH 7.0) and 2.0% (v/v) 1-propanol. In a typical assay, four intact root systems, previously rinsed with deionized H_2O, were placed into 50-ml Erlenmeyer flasks immersed in ice and containing 40 ml of incubation medium. The roots and incubation medium were vacuum infiltrated twice for 2 min each time and then incubated for 30 min in the dark in a shaking water bath at 30°C. N_2 was bubbled continuously (50 cc min^{-1}) through the root incubation medium. After incubation, flasks were placed into a boiling water bath for 10 min. The concentration of nitrite released to the medium was determined as described (7). Checks which included tetracycline (50 μg ml^{-1}) in the root incubation medium were carried out in order to determine the extent of bacterial dissimilatory NRA.

Intact unifoliolate leaflets, first trifoliolate leaflets, and stems plus petioles and cotyledons were assayed by the *in vivo* $-NO_3^-$ NR assay as described (14) except that assays were terminated by boiling for 10 min.

NRA of plant parts was calculated as described (7) except for the 4-d time course experiment where NRA was calculated from the linear portion of the assay period and NRA was expressed as μmol NO_2^- accumulated plant $part^{-1}$ h^{-1}. NRA $plant^{-1}$ was estimated by multiplying NRA $plant^{-1} \times 24 \times 0.8$ (diurnal factor calculated from Fig. 1 by dividing total plant average NRA for the day by the NRA at 4 h after the beginning of the light cycle). For Figure 1, NRA was determined at 2-h intervals over a 48-h period. Measurements for NRA were made at 30 and 19°C during the light and dark cycles, respectively, to approximate *in situ* reduction temperatures.

Preparation of Plant Material for N Analyses. One hundred mg subsamples of plant parts previously dried and ground were used for N determinations. Undiluted 0.2 to 0.5 ml subsamples of xylem exudate were analyzed immediately after collection.

Collection of Xylem Exudate. Soybean seedlings were detopped just below the cotyledonary node. For each plant, the first drop of exudate was blotted off and exudate was collected with microcapillary tubes for 15 min. These procedures were used in an attempt to overcome various problems associated with collection of xylem exudate (13, 15, 21). Xylem exudate was collected from 60 plants at 0, 2, 4, 6, and 8 h after the beginning of the light cycle for one experiment. For the ^{15}N experiment, exudate was collected from 72 plants at 1.5 and 3.0 h after placing plants into ^{15}N nutrient solution after 3.5 and 5 h of illumination, respectively.

N Determinations. Both total-N and NO_3^--N of tissue and xylem exudate were determined as described (3). Reduced-N was calculated by subtraction of NO_3^--N from total-N.

^{15}N Analysis of Xylem Exudate. Plant culture was as previously described except that the nutrient solution contained the following: 3 mM $MgSO_4$, 3 mM $CaCl_2$, 2 mM K_2SO_4, 0.25 mM K-phosphate (pH 6.5), 10 mM KNO_3, and 34.5 μM Fe as sodium ferric diethylenetriamine pentaacetate. Other micronutrients were as described previously. At the appropriate time, plants were transferred into pots containing the same nutrient solution except that the KNO_3 was replaced with 10 mM $K^{15}NO_3$ (15.7 atom % ^{15}N excess). The ^{15}N $-NO_3^-$ nutrient solution was the same temperature as the nutrient solution prior to transfer of the seedlings. The ^{15}N content of the total-N fraction of the exudate was determined following complete digestion to the NH_4^+ form as described (3). The ^{15}N content of the reduced-N fraction of the exudate was determined after it had been separated from the NO_3^--N fraction using anion exchange resin columns. One-ml subsamples of exudate were placed on 10 ml of Dowex AG 1 × 8 anion exchange resin (200–400 mesh, Cl^- form; columns were 10 ml blowout pipettes). Reduced-N was recovered by rinsing with 2 N HCl while NO_3^- remained adsorbed to the resin.

Digested total-N and reduced-N samples were neutralized with KOH to pH 5.0, and samples were dried to a volume of approximately 2 ml. One-half-ml aliquots of the samples (containing 40 to 80 μg NH_4^+-N) were transferred to Rittenberg tubes. Atom % ^{15}N excess was determined with a JASCO NIA ^{15}N analyzer[2] following oxidation of NH_4^+ to N_2 by alkaline sodium hypobromite (5). Atom % ^{15}N excess of NO_3^--N was calculated from the difference between total-N and reduced-N fractions.

Table I. *Effect of Various Assay Conditions on in Vivo $-NO_3^-$ NRA of Soybean Roots*

The basic incubation medium was 100 mM K-phosphate against which other assay parameters were tested. All samples were vacuum infiltrated with the exception noted.

Assay Conditions	NR Activity
	μmol NO_2^- $plant^{-1}$ h^{-1}
pH 7.0, 2.0% 1-propanol, N_2 bubbling	3.14
pH 7.5, 2.0% 1-propanol, N_2 bubbling	3.10
pH 7.0, 2.0% 1-propanol	3.02
pH 7.0, 2.0% 1-propanol, N_2 bubbling[a]	2.80
pH 7.5, 2.0% 1-propanol	2.78
pH 7.5, 1.0% 1-propanol, N_2 bubbling	2.12
pH 7.0, 1.0% 1-propanol, N_2 bubbling	2.02
pH 7.0, 1.0% 1-propanol	1.77
pH 7.5, 1.0% 1-propanol[b]	1.72
FLSD (0.05)	0.35

[a] No vacuum infiltration.

[b] The assay procedure developed for soybean leaves (14).

RESULTS

***In Vivo* NR Assay for Roots.** The assay procedure developed specifically for roots (100 mM K-phosphate, pH 7.0, 2.0% [v/v] 1-propanol, N_2 bubbling) resulted in an increase in NRA of 82% over that obtained using the *in vivo* assay developed by Nicholas *et al.* (14) for soybean leaves (Table I). The greatest improvement in the assay resulted from increasing the concentration of 1-propanol from 1.0 to 2.0% (v/v) and purging the incubation medium with N_2. The inclusion of tetracycline (50 μg ml^{-1}) in the incubation medium had no effect on root NRA (data not shown).

NRA of Plant Parts. Soybean seedlings (11 and 12 DAP) were sectioned into various parts and these intact plant parts assayed for *in vivo* $-NO_3^-$ NRA on 2 consecutive d (Table II). Dry weight of all fractions increased from 1 d to the next. Root NRA increased slightly while NRA of unifoliolate leaflets, and stems (including petioles and cotyledons), remained constant over the 2 d. First trifoliolate leaflets had no measurable NRA at 11 DAP but significant NRA was observed at 12 DAP. When expressed on a percentage of total plant basis, the roots accounted for over 30% of the total plant NRA while comprising only about 15% of the dry weight of the plant (Table II). Unifoliolate leaflets accounted for the majority of total plant NRA but activity of this fraction decreased 10% from 11 DAP to 12 DAP while NRA of first trifoliolate leaflets accounted for 10% of total plant NRA by the 2nd d. Stems with petioles and cotyledons comprised 10% of whole plant NRA on both days.

Analysis of Xylem Exudate for Total-N, NO_3^--N, and Reduced N. Xylem exudate was collected from seedlings (11 DAP) at 2-h intervals beginning with the light cycle and was analyzed for total-N, NO_3^--N and reduced-N (Table III). The amount of exudate

[2] Mention of a trademark, vendor, or proprietory product does not constitute a guarantee or warranty of the vendor or product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other vendors or products that may also be suitable.

Table II. *In Vivo* $-NO_3^-$ *NRA and Dry Weight of Whole Plant Parts of Soybean Seedlings.*

Plants were harvested 11 and 12 DAP and 5 h after the beginning of the light cycle. Values are means ± SE or as percent of total. There were 15 replicates for root and unifoliolate leaflet assays and 10 replicates for first trifoliolate leaflet and stem with petioles and cotyledons assays.

Plant Part	Dry Wt		NR Activity		Dry Wt		NR Activity	
	11 DAP	12 DAP	11 DAP	12 DAP	11 DAP	12 DAP	11 DAP	12 DAP
	mg plant part^{-1}		*μmol* NO_2^- *plant part*$^{-1}$ h^{-1}		*% of total*		*% of total*	
Roots	32.8 ± 0.9	42.7 ± 1.6	2.1 ± 0.1	2.6 ± 0.1	14.8	14.6	30.9	31.7
Unifoliolate leaflets	84.9 ± 1.8	105.8 ± 1.2	4.0 ± 0.1	4.0 ± 0.2	37.2	36.9	58.8	48.8
First trifoliolate leaflets	13.2 ± 0.3	33.6 ± 1.2	0.0 ± 0.0	0.8 ± 0.0	6.0	11.5	0.8	9.8
Stems with petioles and cotyledons	95.8 ± 3.2	108.8 ± 2.8	0.7 ± 0.1	0.8 ± 0.0	42.0	37.1	10.3	9.8
Total plant	227.0	293.4	6.8	8.2	100	100	100	100

collected per plant increased up to 4 h after the beginning of the light cycle and decreased thereafter. These data were consistent with a previous report that exudation follows a diurnal pattern (15). The total-N, NO_3^--N and reduced-N contents of the xylem exudate also peaked at 4 h into the light cycle and decreased with later samplings. In all cases, the amount of reduced-N exceeded the amount of NO_3^--N in the xylem exudate.

The concentration of total-N in the exudate increased with time (Table IV). The increase in total-N concentration was due to an increase in reduced-N concentration since NO_3^--N concentration remained constant.

Short-Term ^{15}N-NO_3^- Feeding Experiment. The total-N, NO_3^--N, and reduced-N concentrations in xylem exudate of seedlings (11 DAP) which had been in ^{15}N-NO_3^- nutrient solution for 1.5 and 3 h are shown in Table V. The reduced-N fraction accounted for approximately 50% of the total-N of the exudate at both samplings. There was a significant atom % ^{15}N excess in all N fractions at both sampling times (Table V). The ^{15}N concentration of the reduced-N fraction was 5 and 11% of the ^{15}N concentration of the NO_3^--N fraction at 1.5 and 3 h after transfer into ^{15}N nutrient solution, respectively.

Estimated *versus* Actual Reduced-N Accumulation. Dry weight increased over a 4-d period in all plant parts except the stems which remained constant until the 3rd and 4th sampling periods when dry weight increases were observed (Table VI). Total-N increased in all plant parts except for the stems which remained constant. The percent total-N in all plant parts was the same as the percent dry weight (relative to the whole plant) of the corresponding parts.

Nitrate-N increased in all plant parts over time (Table VI). However, only very low levels of NO_3^--N were found in first trifoliolate leaflets at the end of the time course. Nitrate-N contents of roots and stems were higher than in either unifoliolate or trifoliolate leaflets. Roots and stems contained 36.8 and 40.7%, respectively, of total plant NO_3^--N at 13 DAP.

Reduced-N increased in all plant parts except stems where reduced-N declined slightly after 10 DAP (Table VI). Reduced-N accumulation was most pronounced in the leaf tissue. At 13 DAP, the unifoliolate leaflets accounted for 39.2% of the total plant reduced-N, and the first trifoliolate leaflets accounted for 15.5% of the reduced-N. First trifoliolate leaflets comprised only 2.6% of the total plant NO_3^--N.

NRA of the fractions over the time course is shown in Table VII. Root NRA was quite low relative to unifoliolate NRA at 9 DAP, but by 13 DAP root NRA had nearly tripled and accounted for a larger percentage of total plant NRA than at 9 DAP. Unifoliolate NRA increased from day to day, but not nearly to the extent of root NRA. NRA of stems was relatively constant over the time course while NRA of first trifoliolate leaflets was insignificant at 12 DAP.

Daily NRA for the fractions was summed and converted to a 24 h basis by using a diurnal factor obtained from Figure 1 (also see "Materials and Methods"). These values were used to estimate reduced-N change per d (Table VIII). The total estimated reduced-N accumulation (based on *in vivo* $-NO_3^-$ NRA) was 1.6 times the actual reduced-N accumulation over the time course. It should be noted that the entire diurnal curve was used to obtain the diurnal factor from Figure 1.

Table III. *Total-N,* NO_3^-*-N, and Reduced-N Content of Xylem Exudate of Soybean Seedlings*

Plants were detopped at 11 DAP and at indicated time after the beginning of the light cycle. Values are means ± SE. There were three replicates for the 0, 2, 4, and 6 h sampling times and two replicates for the 8 h sampling time.

Time	Exudate Volume	Exudate Analyses		
		Total-N	NO_3^--N	Reduced-N
h	*μl plant*$^{-1}$	*μg N volume exudate collected*$^{-1}$		
0	ND[a]	ND	ND	ND
2	21.3 ± 1.2	15.5 ± 0.2	7.3 ± 0.2	8.2 ± 0.4
4	22.8 ± 3.0	17.0 ± 0.6	7.8 ± 0.1	9.2 ± 0.6
6	13.1 ± 3.3	11.5 ± 0.5	4.7 ± 0.2	6.8 ± 0.4
8	7.6 ± 1.9	8.0 ± 0.0	2.7 ± 0.1	5.3 ± 0.2

[a] Not detectable.

Table IV. *Total-N,* NO_3^-*-N, and Reduced-N Concentrations of Xylem Exudate of Soybean Seedlings*

Plants were from experiment described in Table II. Values are means ± SE. There were three replicates for the 2, 4, and 6 h sampling times and two replicates for the 8 h sampling time.

Time	Total-N	Nitrate-N	Reduced-N
h	*μg N ml exudate*$^{-1}$		
0	ND[a]	ND	ND
2	724 ± 10	341 ± 13	383 ± 19
4	747 ± 29	345 ± 4	402 ± 28
6	873 ± 40	359 ± 18	514 ± 29
8	1053 ± 0	354 ± 12	700 ± 25

[a] Not detectable.

DISCUSSION

The *in vivo* $-NO_3^-$ NR assay developed specifically for roots nearly doubled the root NRA over activity obtained with a soybean leaf NRA assay (Table I). We feel that using an assay developed for roots which relies on endogenous NO_3^- and energy, and expressing the data on a whole plant basis may result in a better indication of the NO_3^- reducing ability of roots.

Root NRA accounted for approximately 30% of the total plant NRA when measured at 11 and 12 DAP (Tables II and VII). At 9 and 10 DAP (Table VII), the root NRA was much lower relative to unifoliolate NRA than at 11 and 12 DAP. This result could be

Table V. *Concentration and Atom Percent ^{15}N Excess of Specific Nitrogen Fractions of Xylem Exudate of Soybeam Seedlings*

Plants (11 DAP) were transferred into ^{15}N-NO_3^- nutrient solution 2 h after the beginning of the light cycle. Xylem exudate was collected at 1.5 and 3 h after transferring plants into ^{15}N-NO_3^- solution. Values are means ± SE of two replications.

Time	Total-N		NO_3^--N		Reduced-N	
h	*μg N ml⁻¹*	*atom % ^{15}N excess*	*μg N ml⁻¹*	*atom % ^{15}N excess*	*μg N ml⁻¹*	*atom % ^{15}N excess*
1.5	659 ± 27	2.86 ± 0.02	345 ± 9	5.19 ± 0.01	313 ± 18	0.30 ± 0.00
3.0	799 ± 8	3.61 ± 0.06	382 ± 16	6.82 ± 0.03	418 ± 25	0.67 ± 0.10

Table VI. *Dry Weight and Nitrogen Contents of Whole Plant Parts of Soybean Seedlings*

All values are the composite of two separate experiments. For each experiment, dry weights were taken as the mean of 50 plants at 9 DAP and 30 plants at each subsequent d. Values for dry weight and N contents are means ± SE of two replications.

Plant Part	DAP	Dry Wt	Total-N	NO_3^-	Reduced-N
		mg plant part⁻¹			
Roots	9	33.5 ± 3.5	1.96 ± 0.02	0.46 ± 0.02	1.51 ± 0.01
	10	37.8 ± 4.3	2.22 ± 0.04	0.57 ± 0.04	1.65 ± 0.00
	11	49.4 ± 3.8	2.86 ± 0.03	0.75 ± 0.04	2.11 ± 0.02
	12	61.1 ± 7.0	3.35 ± 0.03	0.89 ± 0.05	2.46 ± 0.04
	13	72.3 ± 7.5	3.95 ± 0.02	1.11 ± 0.03	2.84 ± 0.02
Unifoliolate leaflets	9	43.5 ± 0.0	3.10 ± 0.02	0.23 ± 0.02	2.87 ± 0.04
	10	64.1 ± 1.0	4.23 ± 0.02	0.34 ± 0.02	3.89 ± 0.04
	11	87.2 ± 1.8	5.50 ± 0.06	0.44 ± 0.00	5.06 ± 0.06
	12	111.4 ± 6.1	6.68 ± 0.04	0.60 ± 0.00	6.07 ± 0.04
	13	130.2 ± 7.6	7.63 ± 0.10	0.60 ± 0.01	7.03 ± 0.09
Stems with petioles and cotyledons	9	89.4 ± 3.8	6.50 ± 0.13	0.65 ± 0.01	5.85 ± 0.12
	10	93.6 ± 2.2	6.87 ± 0.05	0.79 ± 0.00	6.08 ± 0.05
	11	90.0 ± 2.1	6.29 ± 0.08	0.90 ± 0.01	5.39 ± 0.07
	12	100.0 ± 0.3	6.51 ± 0.10	1.06 ± 0.02	5.45 ± 0.10
	13	107.7 ± 4.9	6.50 ± 0.05	1.23 ± 0.00	5.27 ± 0.05
First trifoliolate leaflets	11	10.7 ± 0.7	0.75 ± 0.01	nd[a]	0.75 ± 0.01
	12	25.0 ± 2.9	1.76 ± 0.07	0.03 ± 0.00	1.72 ± 0.06
	13	42.4 ± 5.9	2.85 ± 0.10	0.08 ± 0.00	2.77 ± 0.11

[a] Not detectable

Table VII. *In Vivo $-NO_3^-$ NRA of Whole Plant Parts of Soybean Seedlings*

Plants were harvested 4 h after the beginning of the light cycle on each of 4 successive d. The data are a composite of two separate experiments. Values are means ± SE of 12 replications.

Plant Part	NR Activity at Following DAP			
	9	10	11	12
	μmol NO_2^- plant part⁻¹ h⁻¹			
Roots	1.69 ± 0.14	2.54 ± 0.13	4.26 ± 0.21	4.90 ± 0.15
Unifoliolate leaflets	5.53 ± 0.22	6.30 ± 0.20	7.27 ± 0.22	8.22 ± 0.19
Stems with petioles and cotyledons	1.26 ± 0.03	1.36 ± 0.04	1.47 ± 0.06	1.42 ± 0.04
First trifoliolate leaflets	—[a]	—[a]	0.02 ± 0.00	0.32 ± 0.04

[a] Insufficient plant material for harvest.

linked to a constitutive (non-NO_3^- induced) NRA which is found in soybean leaves (6, 11) but not in the roots (4). Patterns of NRA of unifoliolate and first trifoliolate leaflets were similar to patterns obtained previously (7). NRA peaks in leaflets at approximately the time of full expansion (when leaflet flattens out) and decreases slowly thereafter. NRA of stems-petioles-cotyledons composites accounted for about 10% of total plant NRA. The cotyledons were probably responsible for most of this activity as Radin (17) has shown that cotyledons have significant NRA.

The ratio of reduced-N to NO_3^--N in xylem exudate is another procedure to approximate the amount of NO_3^- reduction by roots. However, there are inherent problems with this procedure. For example, reduced-N may be recycled from tops to roots and back to the tops. Reduced-N from protein turnover in the root may increase the amounts of reduced-N appearing in the xylem exudate. Contamination of xylem exudate may result from the excision wounds. Components of xylem exudate and rate of exudation may vary as a function of the time after decapitation. Procedures used in this work were selected to minimize as many of these problems as possible.

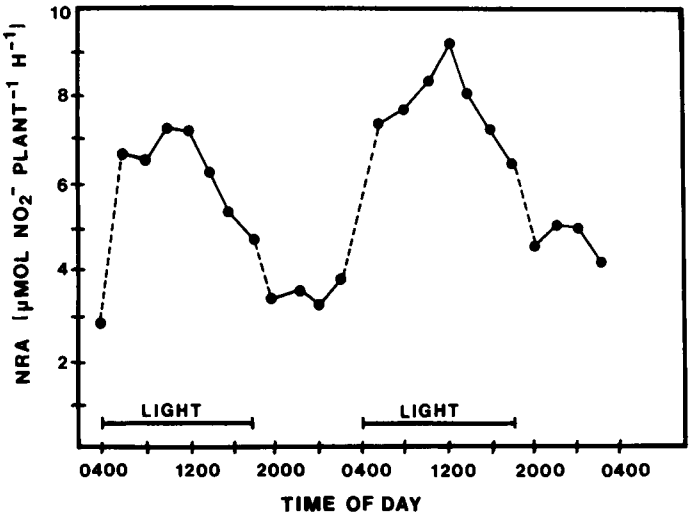

FIG. 1. Diurnal variation of *in vivo* $-NO_3^-$ NRA of soybean seedlings. Roots, unifoliolate leaflets, first trifoliolate leaflets, and stems with petioles plus cotyledons were assayed at 11 through 12 DAP. Assay procedure was as described in "Materials and Methods," except that dark cycle assays were run at 19°C. Each point is the sum of the means of four replicates of each tissue assayed.

Table VIII. *Comparison of Actual and Estimated Reduced-N Accumulation of Soybean Seedlings*

DAP	Actual Reduced-N Accumulation[a]	Estimated Reduced-N Accumulation[b]
	mg N increase plant^{-1} sample period^{-1}	
9–10	1.4	2.3
10–11	1.7	2.8
11–12	2.4	3.5
12–13	2.2	4.0
9–13	7.7	2.6

[a] Calculated from Table VI.

[b] Calculated from Table VII and Figure 1.

Our results show that the reduced-N fraction of xylem exudate comprised at least 50% of the total-N in the exudate (Table III). Furthermore, while exudate flow and the amount of all N fractions followed a diurnal pattern, the proportion of reduced-N in the exudate increased with time. Thus, it appeared that most NO_3^- flux to the tops occurred early in the light cycle. Since NO_3^- flux to the tops seems to control leaf NR (21), then perhaps most leaf NO_3^- reduction occurs early in the day while root NO_3^- reduction, as indicated from the amount of reduced-N in xylem exudate, occurs more consistently throughout the light cycle.

Collectively, root NRA and the level of reduced-N in xylem exudate indicate that roots play an important role in NO_3^- reduction of soybean seedlings.

Short-term ^{15}N-NO_3^- feeding studies should overcome the problem of recycled N in xylem exudate. This assumes that an exposure time of 1.5 and 3 h would preclude recycling of N from tops to roots and back to tops. The finding of reduced-^{15}N in the exudate after 1.5 and 3 h shows that NO_3^- was reduced in the roots (Table V). The low amount of reduced-^{15}N relative to ^{15}N-NO_3^- may be due to the equilibration rates of the two forms of N for deposit into the xylem stream. Preliminary work showed that if plants were switched into ^{15}N-NO_3^- nutrient solution and immediately detopped, significant atom % ^{15}N excess was found in the NO_3^--N fraction of exudate while only natural abundance ^{15}N was found in the reduced-N fraction (data not shown). These data were not used because of the observation that exudate collected immediately after transferring plants from one pot to another had a much higher NO_3^--N:reduced-N ratio relative to nontransferred controls. The NO_3^--N:reduced-N ratio of exudate collected 1.5 h after transfer from one pot to another was the same as nontransferred controls (data not shown). Therefore, the short feeding time and the rapidity with which ^{15}N-NO_3^- can move into the xylem stream could explain the low amount of reduced-^{15}N relative to ^{15}N-NO_3^- in xylem exudate. Furthermore, when optimizing the root NR assay only a slight increase in NRA was observed when exogenous NO_3^- was included in the incubation medium (4). This effect was also reported by Radin (18). The large amount of NO_3^- found in roots (Table VI) perhaps explains the small effect of exogenous NO_3^- on root NRA and suggests that root NR is nearly saturated with NO_3^-. This could also be a factor in the low amount of reduced-^{15}N relative to ^{15}N-NO_3^- in xylem exudate.

The 4-d time course experiment illustrated the limits of using *in vivo* NR assays. While Harper (6) found that leaf NRA over a growing season underestimated (by one-third) actual reduced-N accumulation by plants, the present study showed that total plant NRA (including roots) overestimated (by 1.6) actual reduced-N accumulation. One problem is the controversy over NO_3^- reduction in the dark (1, 2). The results of the time course experiment would have changed considerably if only light cycle NRA had been used in the calculations. *In vivo* assays may not be useful in estimating actual reduced-N accumulation by plants; however, such assays could be used to investigate NR under different treatment conditions. We have used the root and leaf assay to show a wide genotypic variation in both root and leaf NR over a diverse range of soybean genotypes (S. A. Ryan and S. J. Crafts-Brandner, manuscript in preparation).

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