Assimilation of $^{15}NO_3^-$ Taken Up by Plants in the Light and in the Dark[1]

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ABSTRACT

An experiment was conducted to determine the extent that NO_3^- taken up in the dark was assimilated and utilized differently by plants than NO_3^- taken up in the light. Vegetative, nonnodulated soybean plants (*Glycine max* L. Merrill, 'Ransom') were exposed to $^{15}NO_3^-$ throughout light (9 hours) or dark (15 hours) phases of the photoperiod and then returned to solutions containing $^{14}NO_3^-$, with plants sampled subsequently at each light/dark transition over 3 days. The rates of $^{15}NO_3^-$ absorption were nearly equal in the light and dark (8.42 and 7.93 micromoles per hour, respectively); however, the whole-plant rate of $^{15}NO_3^-$ reduction during the dark uptake period (2.58 micromoles per hour) was 46% of that in the light (5.63 micromoles per hour). The lower rate of reduction in the dark was associated with both substantial retention of absorbed $^{15}NO_3^-$ in roots and decreased efficiency of reduction of $^{15}NO_3^-$ in the shoot. The rate of incorporation of ^{15}N into the insoluble reduced-N fraction of roots in darkness (1.10 micromoles per hour) was somewhat greater than that in the light (0.92 micromoles per hour), despite the lower rate of whole-plant $^{15}NO_3^-$ reduction in darkness.

A large portion of the $^{15}NO_3^-$ retained in the root in darkness was translocated and incorporated into insoluble reduced-N in the shoot in the following light period, at a rate which was similar to the rate of whole-plant reduction of $^{15}NO_3^-$ acquired during the light period. Taking into account reduction of NO_3^- from all endogenous pools, it was apparent that plant reduction in a given light period (~13.21 micromoles per hour) exceeded considerably the rate of acquisition of exogenous NO_3^- (8.42 micromoles per hour) during that period. The primary source of substrate for NO_3^- reduction in the dark was exogenous NO_3^- being concurrently absorbed. In general, these data support the view that a relatively small portion (<20%) of the whole-plant reduction of NO_3^- in the light occurred in the root system.

Plants can assimilate NO_3^- during both the light and dark portions of the photoperiod, although rates of assimilation may differ. In experiments using $^{15}NO_3^-$ and excised leaves or leaf disks, it has been amply demonstrated that NO_3^- reduction can occur in darkness (8, 11, 24, 32), and the rate of reduction is associated with availability of carbohydrate (2, 15). Nevertheless, it was found consistently that reduction rates were lower in darkness. Furthermore, activities of nitrate reductase in leaves

sampled in darkness are lower than in leaves sampled in the light (for reviews, see 4, 29). Results from experiments with root tissues generally indicate that NO_3^- reduction in roots does not decrease when plants are in darkness (2, 25), although a substantial decline in root nitrate reductase activity was noted in one instance (7).

The extent to which the rate of NO_3^- assimilation in intact plants is diminished during the dark phase of a 'normal' photoperiod has not been examined. The present experiment was conducted with that intent. Nonnodulated vegetative soybean plants were exposed to $^{15}NO_3^-$ throughout light or dark periods to determine whole-plant NO_3^- assimilation during the period in which it was absorbed. In addition, following exposure to $^{15}NO_3^-$, sets of plants were returned to nutrient solutions containing $^{14}NO_3^-$. The subsequent partitioning of ^{15}N among plant parts and the reduction of endogenous $^{15}NO_3^-$ with incorporation of ^{15}N into soluble and insoluble reduced-N compounds were followed over 3 d to determine if acquisition of $^{15}NO_3^-$ in the light or dark was associated with differential utilization within the plant.

MATERIALS AND METHODS

Plant Culture. Seeds of soybean (*Glycine max* (L.) Merrill, 'Ransom') were germinated in moistened paper at 25°C and 98% RH in a dark germination chamber located in the North Carolina State University phytotron unit of the Southeastern Plant Environment Laboratory. The seeds were kept moist by capillary action from a 0.1 mM $CaSO_4$ solution. On day 4, 60 seedlings with radicle lengths between 8 and 12 cm were selected and placed into two 210-L continuous flow, hydroponic culture systems with temperature and pH control (26). The culture system was located within a controlled-environment growth room programmed for 26/22°C light/dark temperature, a 9-h light period, and a 15-h dark period. A photosynthetic photon flux density of 700 ± 50 μE m^{-2} s^{-1} between wavelengths of 400 and 700 nm, with a photomorphogenic radiation of 12 w m^{-2} between wavelengths of 700 and 850 nm, was provided during the 9-h light period from a combination of fluorescent and incandescent lamps at an input wattage ratio of 10:3. The ambient CO_2 concentration was maintained at 400 ± 25 μl/l. The environmental conditions used were sufficient to sustain net photosynthesis rates which exceeded those of soybean plants grown in the field or greenhouse.

The culture solution temperature was maintained at 24 ± 0.5°C and pH 5.6 ± 0.1 by additions of 0.01 N $Ca(OH)_2$ or 0.01 N H_2SO_4. Nutrient concentrations in solution were 1.0 mM NO_3^-, 0.25 mM $H_2PO_4^-$, 1.25 mM K^+, 1.0 mM Ca^{2+}, 1.0 mM Mg^{2+}, 2.0 mM SO_4^{2-}, 17.0 μM B, 3.0 μM Mn, 0.3 μM Zn, 0.1 μM Cu, 0.04 μM Mo, and 1 mg Fe/l as Fe-EDTA. The solutions were changed

every 2 d to avoid depletion effects.

Experimental Conditions. The experiment involved exposure of 2 groups of plants to $^{15}NO_3^-$ either during a light period or during the following dark period. The experiment began on day 24 after germination. At the beginning of the light period, 28 plants were removed from the culture system in which they were growing and placed into a separate culture system (in the same growth room) containing an identical nutrient solution except with $^{15}NO_3^-$ (~99 atom% ^{15}N) substituted for $^{14}NO_3^-$. The plants remained in the $^{15}NO_3^-$ solution throughout the 9-h light period. At the end of the light period, four plants were harvested with leaves, stems (including petioles), and roots separated and frozen promptly. Roots were rinsed in a cold 2.0 mM $CaSO_4$ solution prior to freezing. At the same time as the harvest, the remaining 24 plants were removed from the $^{15}NO_3^-$ solution, roots were dipped into 2.0 mM $CaSO_4$ (24°C), and the plants transferred back into the culture system containing nutrient solution with $^{14}NO_3^-$. During the following 3 d, four plants were harvested at the end of each subsequent dark and light period.

At the beginning of the dark period, also on day 24, a second group of 24 plants was removed from the culture system, in which the plants had been growing with $^{14}NO_3^-$, and placed into nutrient solution containing $^{15}NO_3^-$. The plants remained in the $^{15}NO_3^-$ solution during the 15-h dark period, after which four plants were harvested as before. Roots of the remaining 20 plants were rinsed with 2.0 mM $CaSO_4$ (24°C) and the plants then were transferred back into nutrient solution with $^{14}NO_3^-$, with four of the plants harvested at each light-dark transition over the following 3 d.

No nodules were found on plant roots during the initial growth or experimental periods. Therefore, the plants were acquiring nitrogen solely as NO_3^- from the nutrient solution. The concentrations of $^{14}NO_3^-$ and $^{15}NO_3^-$ in solution were depleted less than 5%. Thus, the experiment was conducted in steady-state conditions with respect to exogenous NO_3^- availability throughout.

Tissue Analysis. The tissue samples were analyzed for NO_3^- and soluble and insoluble reduced nitrogen. After being freeze-dried, weighed, and ground, the tissue was extracted with methanol:chloroform:water (13:4:3, v/v/v) (19). Following separation of the chloroform from the methanol:water fractions, the chloroform was added back to the tissue residue, with this constituting the insoluble N fraction. Total nitrogen in the insoluble N fraction was determined by Kjeldahl digestion (14) and colorimetric analysis of NH_4^+ (5). The NH_4^+ in the remaining digest was recovered by diffusion and the atom % ^{15}N determined mass spectrometrically using a freeze-layer procedure (30).

The methanol:water fraction was analyzed for NO_3^- and soluble reduced N. After the methanol was evaporated using aeration, an aliquot was taken and NO_3^- was determined using a manual modification of the method of Lowe and Hamilton (12). The atom % ^{15}N of the NO_3^- fraction was determined by mass spectrometry using a nitric oxide procedure (31). Nitrate remaining in the water fraction was removed (19), and the soluble reduced N and atom % ^{15}N determined as in analysis of the insoluble reduced N.

Data Presentation. Tissue data sometimes are presented (Table I) or discussed as reduced N, which represents the combined total of the insoluble and soluble reduced nitrogen fractions. The data in Figures 1, 2, and 3, and Table III, which depict distribution of ^{15}N among plant parts and partitioning of ^{15}N among N fractions, are presented as percentages of the total ^{15}N present in plants at each harvest. Overall rates of change during specific harvest intervals are in some instances presented in the figures and in the text. For clarity of presentation, the rates were calculated using the % data at relevant harvests multiplied by the mean of the total ^{15}N present in all plants, which was 83.5 ± 3.0 μmol plant^{-1} for the 28 plants exposed to ^{15}N in the 9-h light

period, and 137.3 ± 6.6 μmol plant^{-1} for the 24 plants exposed to ^{15}N in the 15-h dark period. The rates were similar to and led to the same interpretation as rates calculated using ^{15}N data from the plants sampled at each harvest. We emphasize that these overall rates reflect an average during a given light or dark period. They are presented to facilitate comparisons between periods which differed in length. Shorter-term rates could have deviated appreciably from the overall rates throughout the photoperiod.

The ^{15}N content of all plants exposed to $^{15}NO_3^-$ in the light or dark was similar throughout the 3-d $^{14}NO_3^-$ chase period. Thus, there was no indication of significant loss of ^{15}N due to volatilization.

RESULTS

Assimilation of $^{15}NO_3^-$ during the Uptake Periods. Total $^{15}NO_3^-$ uptake by vegetative soybean plants was greater in the dark phase of the photoperiod because the 15-h dark period was considerably longer than the 9-h light period (Table I). The rate of $^{15}NO_3^-$ uptake, however, was similar in the light and dark when expressed on an hourly basis, 8.42 and 7.93 μmol h^{-1}, respectively). Even though the rate of $^{15}NO_3^-$ uptake was similar in light and darkness, the rate of reduction of $^{15}NO_3^-$ was twice as great in the light, 5.63 compared to 2.58 μmol h^{-1}. As a result, 33% of the absorbed ^{15}N remained in the tissue as $^{15}NO_3^-$ at the end of the light period, while 67% of the ^{15}N absorbed in the dark period was still in the $^{15}NO_3^-$ fraction at the end of that time.

A much larger proportion of the ^{15}N acquired by plants in the light had accumulated in the shoot at the end of the uptake period (Table II, D). Of the 75.75 μmol of ^{15}N absorbed in the light (Table I), 45.48 μmol or 60% was recovered in leaves and stems. In the dark, however, of the 118.9 μmol absorbed, only 32.83 μmol or 28% was found in the shoot.

In addition, a greater proportion of the ^{15}N accumulated in the shoot during the light uptake period was in reduced fractions (Table II, B and C). In leaves, for example, of the 34.82 μmol of ^{15}N present, 2.80 μmol or only 8% was $^{15}NO_3^-$. In contrast, of the 17.42 μmol of ^{15}N in leaves at the end of the dark uptake period, 8.38 μmol or 48% was $^{15}NO_3^-$.

Increased retention of ^{15}N in roots in the dark was associated with substantial accumulation of $^{15}NO_3^-$ (Table II, A). The rate of $^{15}NO_3^-$ accumulation in roots in the dark (4.23 μmol h^{-1}) was more than twice the rate of accumulation in the light (1.99 μmol h^{-1}). The rate of ^{15}N accumulation in the insoluble nitrogen fraction of root tissues also was somewhat higher in darkness than in the light (Table II, C), despite the lower rate of whole-plant reduction of $^{15}NO_3^-$ (Table I) and the lower rate of accumulation of ^{15}N in reduced fractions in the shoot (Table II, B and C) in darkness.

Distribution of Endogenous ^{15}N within Plants with Time. Plants were returned to nutrient solutions containing $^{14}NO_3^-$ at the end of each $^{15}NO_3^-$ uptake period. In the days which followed, the distribution of ^{15}N within plants changed appreciably, and the patterns were different for ^{15}N absorbed in the light and dark (Fig. 1). After the light uptake period, there were adjustments in endogenous ^{15}N distribution until about hour 48, which primarily involved gradual transfer of ^{15}N to leaves (~0.48 μmol h^{-1}; Fig. 1A). In contrast, a large portion of the ^{15}N retained in the root at the end of the dark uptake period was transferred to the shoot in the subsequent light period (Fig. 1B). The rate of accumulation of ^{15}N in the shoot during this period (~5.49 μmol h^{-1}) was much greater than the rate of ^{15}N accumulation in the shoot during the previous dark uptake period (2.19 μmol h^{-1}; Table II, D), during which they were absorbing $^{15}NO_3^-$. Although most of the ^{15}N accumulated in leaves, ^{15}N in stems also increased. During the next dark period, however, stem ^{15}N declined whereas ^{15}N in leaves continued to increase.

Table I. *Uptake of $^{15}NO_3^-$ and Tissue Accumulation of $^{15}NO_3^-$ amd Reduced ^{15}N in Soybean Plants Exposed to $^{15}NO_3^-$ Solutions in the Light or Dark*

Data are presented as total μmol plant^{-1} at the end of each exposure period and as μmol plant^{-1} h^{-1} to allow rate comparisons. Reduced ^{15}N represents soluble and insoluble reduced ^{15}N fractions combined. Percentage of total ^{15}N in plants as $^{15}NO_3^-$ or reduced ^{15}N at the end of each exposure period is shown in parenthesis.

Exposure Period	Uptake		Plant $^{15}NO_3^-$		Plant Reduced ^{15}N	
	μmol	μmol h^{-1}	μmol	μmol h^{-1}	μmol	μmol h^{-1}
Light (9 h)	75.75 ± 3.67	8.42	25.11 ± 0.67 (33)	2.79	50.64 ± 3.26 (67)	5.63
Dark (15 h)	118.90 ± 7.44	7.93	80.14 ± 5.96 (67)	5.34	38.76 ± 5.43 (33)	2.58
Total	194.65		105.25		89.40	

Table II. *Accumulation of ^{15}N in Nitrogen Fractions of Different Tissues of Soybean Plants Exposed to $^{15}NO_3^-$ Solutions in the Light or Dark*

Data are presented as total μmol plant^{-1} at the end of each exposure period, and as μmol plant^{-1} h^{-1} to allow rate comparisons

Exposure Period	Material Analyzed	Tissue N Fractions							
		(A) NO_3^-		(B) Soluble reduced		(C) Insoluble reduced-N		(D) Total-^{15}N	
		μmol	μmol h^{-1}	μmol	μmol h^{-1}	μmol	μmol h^{-1}	μmol	μmol h^{-1}
Light (9 h)	Leaves	2.80 ± 1.19	0.31	9.31 ± 1.18	1.03	22.71 ± 1.54	2.52	34.82	3.87
	Stems	4.37 ± 1.24	0.49	3.88 ± 0.58	0.43	2.41 ± 0.23	0.27	10.66	1.18
	Shoot total	7.17	0.80	13.19	1.46	25.12	2.79	45.48	5.05
	Roots	17.94 ± 4.50	1.99	4.01 ± 0.65	0.45	8.32 ± 0.91	•0.92	30.27	3.36
Dark (15 h)	Leaves	8.38 ± 1.39	0.56	3.17 ± 0.59	0.21	5.87 ± 1.17	0.39	17.42	1.16
	Stems	8.30 ± 0.33	0.55	4.76 ± 0.76	0.32	2.34 ± 0.23	0.16	15.40	1.03
	Shoot total	16.68	1.11	7.93	0.53	8.21	0.55	32.82	2.19
	Roots	63.43 ± 5.56	4.23	6.12 ± 1.00	0.41	16.50 ± 2.49	1.10	86.08	5.74

The distribution of ^{15}N among plant parts had stabilized in all plants by hour 57, and the distribution was similar in plants exposed to ^{15}N in light and darkness. The only notable exception was that plants exposed to ^{15}N in the dark tended to retain a slightly larger proportion of ^{15}N in their roots. For the last three harvests, the mean proportion of ^{15}N in roots was 27.3% and 21.3% in plants exposed to ^{15}N in the dark and light, respectively.

Whole-Plant Assimilation of Endogenous $^{15}NO_3^-$ with Time. In the days following the ^{15}N uptake periods, the pattern of assimilation of the endogenous $^{15}NO_3^-$ remaining in the tissue into reduced-N fractions also was noticeably different for $^{15}NO_3^-$ which had been absorbed in the light and dark (Fig. 2). Of the $^{15}NO_3^-$ absorbed in the light, 33% was unreduced at the end of the uptake period (Table I; Fig. 2A). Reduction of this endogenous $^{15}NO_3^-$ was gradual but continuous until about hour 48, after which time the small amount of $^{15}NO_3^-$ present remained relatively constant. The soluble reduced-^{15}N was greatest at the end of the uptake period, declined during the subsequent dark period, and remained constant thereafter. Incorporation of ^{15}N into insoluble reduced-^{15}N proceeded steadily until hour 57, reciprocal to the decreases in endogenous $^{15}NO_3^-$ and soluble reduced-^{15}N.

A substantial amount of the $^{15}NO_3^-$ in the plant at the end of the dark uptake period was reduced during the following light period (Fig. 2B), coincident with the rapid transfer of ^{15}N to the shoot (Fig. 1B). The proportion of the plant ^{15}N in the $^{15}NO_3^-$ fraction decreased from 67 to 29%, and the rate of reduction (~5.80 μmol h^{-1}) was more than twice the rate of reduction during the dark uptake period (2.58 μmol h^{-1}; Table I). Thereafter, the $^{15}NO_3^-$ remaining in the tissue was reduced at a much slower rate. The relative amount of ^{15}N in the soluble reduced-^{15}N fraction changed only from 12% to 6% during the experiment. ^{15}N in the insoluble reduced-^{15}N fraction increased reciprocal to decreases in the other two nitrogen fractions; thus, the

largest increase occurred in the first light period following the dark uptake phase. Partitioning of ^{15}N among nitrogen fractions was similar at the final harvest (hour 81) in plants which had acquired ^{15}N both in the light and dark (compare Figs. 2A and 2B).

Changes in ^{15}N Fractions in Plant Parts with Time. At the end of the light uptake period, the majority of the endogenous $^{15}NO_3^-$ was in root tissues (Table II; Fig. 3). Almost all of the $^{15}NO_3^-$ in roots was depleted by hour 33 (Fig. 3C). Soluble reduced-^{15}N was elevated noticeably at the end of the light uptake period in each plant part, but declined in the subsequent dark period and then remained relatively stable in all parts at 1% to 4% of the plant ^{15}N content. Insoluble reduced-^{15}N had approached maximal levels in all parts by hour 48.

The large amount of $^{15}NO_3^-$ in roots at the end of the dark uptake period (Table II) was depleted in the subsequent light period (Fig. 3F), concurrent with the highest rates of net transfer of ^{15}N to the shoot (Fig. 1B) and whole-plant $^{15}NO_3^-$ reduction (Fig. 2B). Further depletion of root $^{15}NO_3^-$ (after hour 33) occurred at a slower rate. Stem $^{15}NO_3^-$ was maximal at the end of the phase of rapid depletion of $^{15}NO_3^-$ in roots, and then declined gradually during the remainder of the experiment (Fig. 3E). The largest increase in insoluble reduced-^{15}N in roots occurred during exposure to $^{15}NO_3^-$ (Fig. 3F); in leaves and stems, it occurred during the initial light period immediately following exposure to $^{15}NO_3^-$.

In general, by the end of the experiment, the ^{15}N content of all plants was partitioned among nitrogen fractions of each tissue similarly.

Total N(^{14}N + ^{15}N) Acquisition, Distribution, and Partitioning among N Fractions. Direct comparisons cannot be made between daily absorption and reduction rates of $^{14}NO_3^-$ prior to and after the exposure to $^{15}NO_3^-$ because of plant to plant variability in ^{14}N accumulation. However, longer-term experiments in the

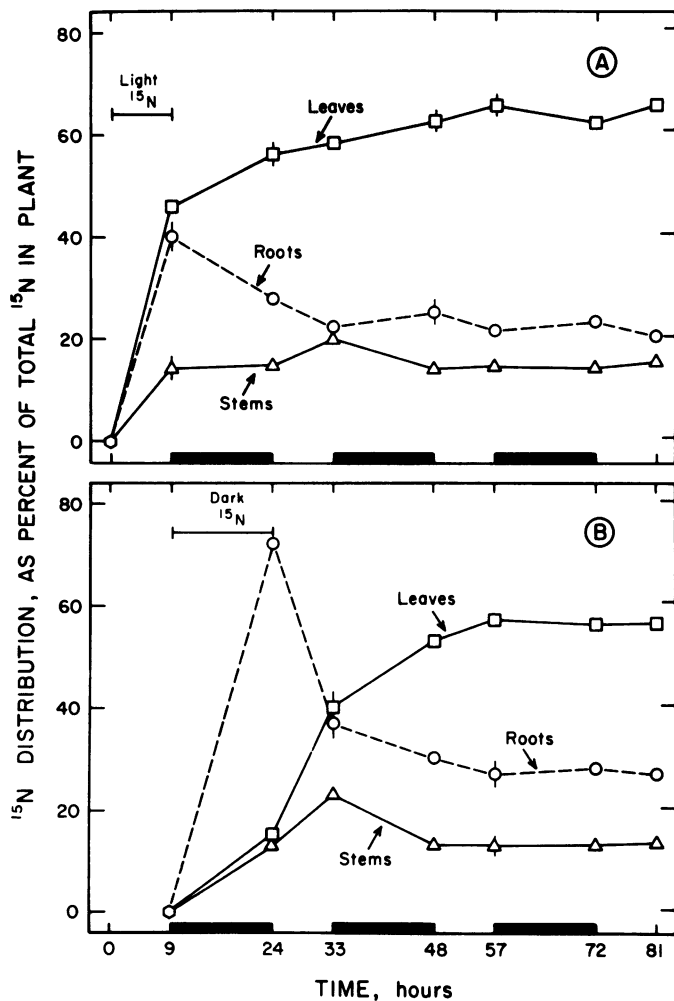FIG. 1. Alterations in distribution of ^{15}N among plant parts following exposure of plants to 1.0 mM ^{15}NO$_3^-$ in the light (hours 0–9, A) or in the dark (hours 9–24, B). After ^{15}NO$_3^-$ exposure, plants were in solutions containing 1.0 mM ^{14}NO$_3^-$. Each symbol is the mean of four replicates ± SE, as indicated by a vertical line when larger than the symbol.
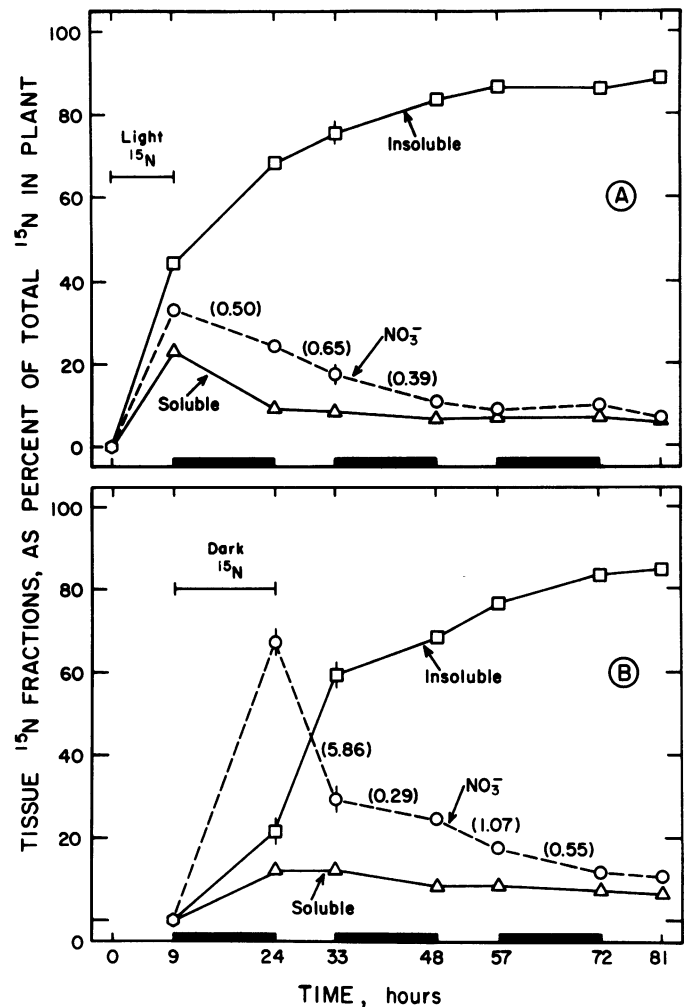
FIG. 2. Alterations in distribution of ^{15}N among nitrogen fractions in plants following exposure to 1.0 mM ^{15}NO$_3^-$ in the light (hours 0–9, A) or in the dark (hours 9–24, B). After ^{15}NO$_3^-$ exposure, plants were in solutions containing 1.0 mM ^{14}NO$_3^-$. Rates of endogenous ^{15}NO$_3^-$ assimilation are presented in parentheses (μmol h^{-1} plant^{-1}) for particular time intervals when significant assimilation occurred. Each symbol is the mean of four replicates ± SE, as indicated by a vertical line when larger than the symbol.

same growth environment have shown the accumulation of N to be exponential during this growth phase, the relative accumulation rate of total N being 0.115 to 0.125 μmol μmol^{-1} present in tissue d^{-1} (26). There were approximately 1800 μmol of N present in plants on the 1st d of the experiment. Thus, the predicted range of absorption (207–225 μmol NO$_3^-$ d^{-1}) was in good agreement with the mean ^{15}NO$_3^-$ absorption of 221 μmol for all plants used in the experiment (*cf.* "Materials and Methods").

Nitrogen (^{14}N + ^{15}N) distribution among plant parts and among nitrogen fractions was nearly identical in all plants harvested in the experiment. As shown in Table III, 24% of the plant N was in roots, 14% in stems, and 62% in leaves; values very similar to those observed with ^{15}N following stabilization (Fig. 1). A comparison of data in Table III with those in Figures 2 and 3 reveals that partitioning of N among N fractions in the whole plant and plant parts also was similar to that of ^{15}N toward the end of the experiment. Clearly, the acquired ^{15}N had approached an equilibrium with respect to plant distribution and partitioning among fractions within the experimental period. The data (Table III) further indicate that plants contained about 180 μmol of NO$_3^-$ in the tissue at the time of exposure to ^{15}NO$_3^-$ (10% of 1800). Thus, sustained reduction of NO$_3^-$ and accu-

mulation of reduced-N was strongly dependent upon continuous absorption of NO$_3^-$.

DISCUSSION

Decreased Whole-Plant Reduction of ^{15}NO$_3^-$ During the Dark Uptake Period. The ^{15}NO$_3^-$ absorbed in the dark phase of the photoperiod was reduced less efficiently by plants during the uptake period than was ^{15}NO$_3^-$ absorbed in the light. The whole-plant rate of reduction in the dark was less than half that in the light even though the ^{15}NO$_3^-$ absorption rates were similar (Table I). The lower rate of reduction in the dark predominantly reflected a decline in the rate of incorporation of ^{15}N into reduced-N fractions in leaves. This was associated with a substantial decrease in total ^{15}N accumulated in leaves as well as a decrease in the efficiency of reduction of ^{15}NO$_3^-$ present in leaves (Table II).

The large proportion of absorbed ^{15}NO$_3^-$ retained in roots during the dark uptake period (Table II) clearly implies that ^{15}NO$_3^-$ translocation out of the root system was restricted relative to that in the light. Results from other experiments in which

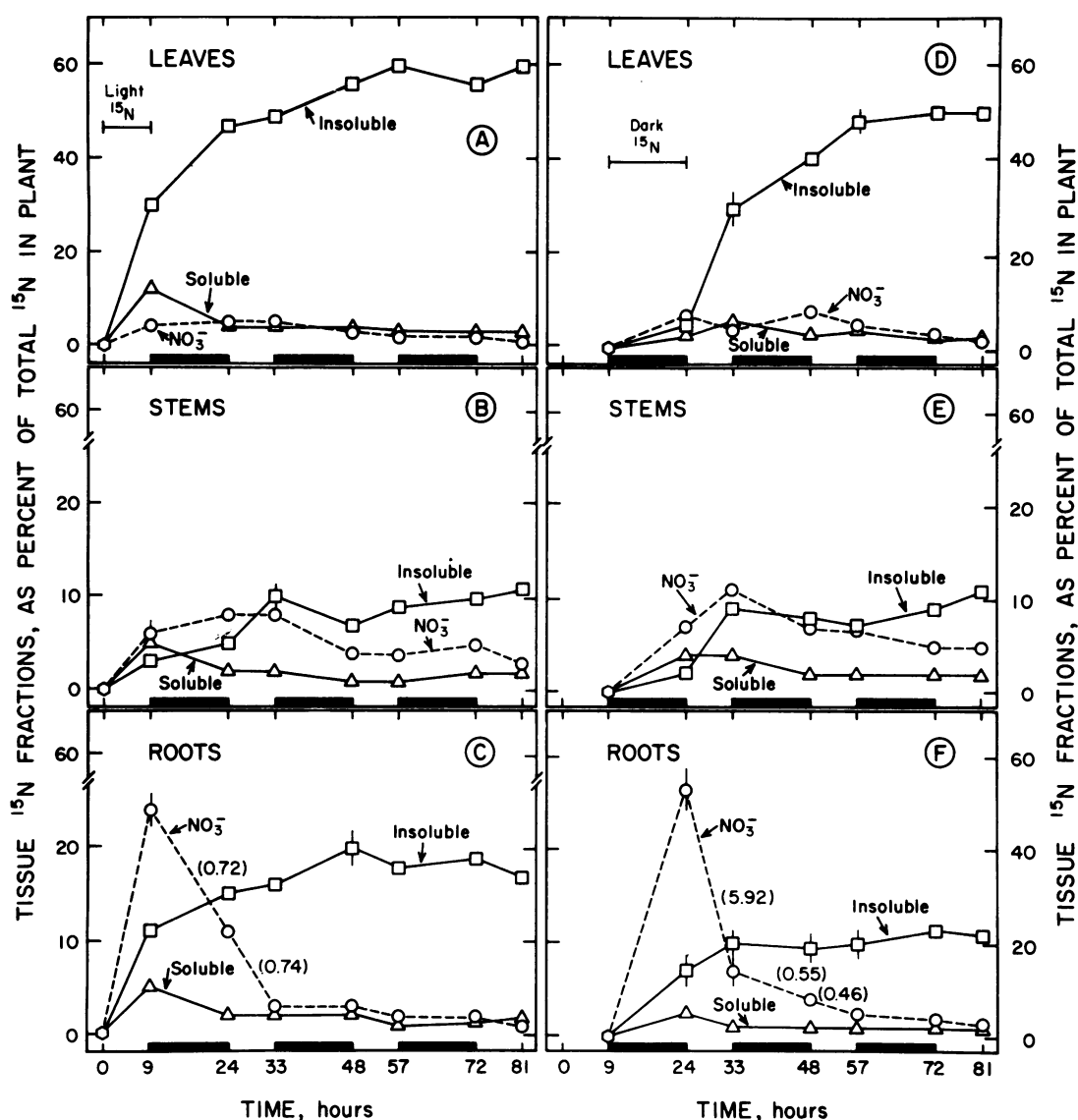FIG. 3. Alterations in distribution of ^{15}N among nitrogen fractions in each plant part following exposure to 1.0 mM $^{15}NO_3^-$ in the light (hours 0–9; A, B, and C) or in the dark (hours 9–24; D, E, and F). After $^{15}NO_3^-$ exposure, plants were in solutions containing 1.0 mM $^{14}NO_3^-$. Rates of endogenous $^{15}NO_3^-$ depletion from roots are presented in parentheses (μmol h^{-1} plant^{-1}) for particular time intervals when significant depletion occurred. Note that the ordinate scale of B, C, and E is double that of A, D, and F. Each symbol is the mean of four replicates ± SE, as indicated by a vertical line when larger than the symbol.

Table III. *Summary of Total Nitrogen ($^{14}N + ^{15}N$) Distribution among Plant Parts and Nitrogen Fractions in All Plants Harvested during the Experiment*

Data are presented as per cent of the total N (^{14}N + ^{15}N) in plants and are means from 52 plants.

Material Analyzed	Tissue N fractions			
	NO_3^-	Soluble reduced-N	Insoluble reduced-N	Total N
	%			
Leaves	2.54 ± 0.18	4.23 ± 0.23	55.54 ± 0.62	62.23 ± 0.64
Stems	3.08 ± 0.21	2.00 ± 0.11	8.54 ± 0.39	13.62 ± 0.37
Roots	4.38 ± 0.47	2.08 ± 0.08	17.46 ± 0.35	23.92 ± 0.55
Whole plant	10.08 ± 0.63	8.23 ± 0.20	81.54 ± 0.64	

plants were exposed to $^{15}NO_3^-$ at intervals (20, 21) or continuously (17) during the light/dark cycle also have shown that translocation of recently absorbed $^{15}NO_3^-$ from roots to leaves decreased in the dark. Precise reasons for the restriction of translocation in darkness cannot be ascertained from our data.

A logical explanation is that transpiration and ion fluxes in the xylem declined in response to stomatal closure in darkness. It also is conceivable that translocation was limited by energy availability (22), although other energy-dependent processes in the root were not similarly affected: uptake of $^{15}NO_3^-$ was not

decreased appreciably and incorporation of ^{15}N into the insoluble reduced-N fraction in roots increased (Table II).

Despite the restricted translocation in darkness, ^{15}NO$_3^-$ accumulation in leaves was 3-fold higher in darkness than in light (Table II, A). It thus seems logical to conclude that ^{15}NO$_3^-$ reduction in leaves was not limited by availability of ^{15}NO$_3^-$ substrate in darkness. However, it is possible that much of the ^{15}NO$_3^-$ measured in leaf tissue was actually in the leaf veins and hence sequestered from sites of reduction. Other regulatory effects which could have contributed to less efficient reduction of ^{15}NO$_3^-$ in darkness include a limitation in the availability of carbohydrate with the result that protein synthesis and/or generation of reductant were diminished (2, 15), the presence of nitrate reductase inhibitors (1, 10), and decreased transport of ^{15}NO$_3^-$ out of storage compartments (3, 9, 28).

In darkness the rate of ^{15}NO$_3^-$ accumulation in roots was more than double that in shoots. Reduction in roots could have been limited by total enzyme activity or by availability of carbohydrate; however, it also is possible a significant amount of the ^{15}NO$_3^-$ in the root tissue was compartmentalized and not available for reduction. If compartmentation was involved, it is evident that a large portion of the ^{15}NO$_3^-$ was sequestered in darkness in a pool from which it could be readily depleted during the subsequent light period (Fig. 3F).

Pools of Endogenous ^{15}N. The root was the primary site of endogenous ^{15}NO$_3$ storage at the end of light and dark uptake periods (Table II; Fig. 3). The marked depletion of dark-absorbed ^{15}NO$_3^-$ from roots in the immediately following, initial light period contrasted with slower rates of depletion which occurred subsequently. Significant retention of ^{15}NO$_3^-$ during dark absorption in the root symplasm or in cells with close proximity to vascular tissue is implied. Stems also served as storage sites for endogenous ^{15}NO$_3^-$, but levels characteristically were lower than in the root at the end of either of the uptake periods.

Nitrate is assimilated into soluble reduced-N, *e.g.* amino acids, prior to incorporation into insoluble reduced-N, *e.g.* proteins and nucleic acids. The transient nature of the soluble reduced-N pool was evident in this experiment as the plant content of soluble reduced-^{15}N was elevated only at times when ^{15}NO$_3^-$ reduction and incorporation into insoluble reduced-^{15}N were most rapid (Fig. 2). Nevertheless, a storage component was discernible, as 6% of the plant ^{15}N content remained in the soluble reduced-^{15}N fraction after assimilation of endogenous ^{15}NO$_3^-$ had virtually ceased.

Net increases in ^{15}N incorporation into the insoluble reduced-N fraction occurred during the experimental period, and in each organ the insoluble reduced-^{15}N was relatively stable once maximal levels were reached (*cf.* Fig. 3). This contrasts with results from an experiment with reproductive soybean plants, where a substantial release of ^{15}N from the insoluble reduced-N fraction of all plant parts occurred within 3 d after a ^{15}NO$_3^-$ exposure period (18). The implication is that types or stabilities of proteins or other insoluble components may be different in vegetative and reproductive plants.

Origins of Substrate. Plants in this experiment were growing rapidly, and because less than a 1-d supply of endogenous NO$_3^-$ was stored in the plants, sustained growth and accumulation of insoluble reduced-N were dependent upon continual acquisition of exogenous NO$_3^-$. Less than 10% of the ^{15}NO$_3^-$ absorbed in the light and dark remained in the plant as ^{15}NO$_3^-$ at the end of the experiment (Fig. 2). Since tissue NO$_3^-$ was maintained at about 10% of the total plant N during this growth phase (Table III), the temporal cycle of assimilation of absorbed ^{15}NO$_3^-$ was, for the most part, completed. Therefore, the pattern of ^{15}NO$_3^-$ assimilation over time can reasonably be integrated into a single light/dark time frame to evaluate the relative contributions of concurrently absorbed NO$_3^-$ and various endogenous NO$_3^-$

pools in supplying substrate for reduction during a typical day.

It is evident, for example, that substrate for NO$_3^-$ reduction in the light was supplied primarily from two sources, NO$_3^-$ absorbed during that light period and endogenous NO$_3^-$ which had been absorbed in the previous dark period, most of which was retained in the root system. Estimated rates of reduction from the two sources were similar, 5.63 μmol h^{-1} for concurrently absorbed ^{15}NO$_3^-$ (Table I) and 5.86 μmol h^{-1} for ^{15}NO$_3^-$ from the endogenous pool (Fig. 2B). Low rates of reduction of endogenous ^{15}NO$_3^-$ during other light periods ($\sim$1.72 μmol h^{-1}; *cf.* hours 24–33, Fig. 2A; hours 48–57, Fig. 2B) suggest relatively small contributions of substrate for reduction from older pools. When contributions of substrate during these light periods are considered collectively, it is apparent that the rate of whole-plant NO$_3^-$ reduction in the light ($\sim$13.21 μmol h^{-1}) exceeded considerably the concurrent rate of acquisition of exogenous substrate (*i.e.* NO$_3^-$ uptake; 8.42 μmol h^{-1}, Table I).

The primary source of substrate for NO$_3^-$ reduction in the dark apparently was the NO$_3^-$ being absorbed by the plant during that dark period. The rate of reduction of concurrently absorbed ^{15}NO$_3^-$ was 2.58 μmol h^{-1} (Table I). The total contribution of substrate from all endogenous sources was important ($\sim$1.73 μmol h^{-1}; *cf.* hours 9–24 and hours 33–48, Fig. 2A; hours 33–48 and hours 57–72, Fig. 2B). However, the rate of assimilation of ^{15}NO$_3^-$ originating from any one endogenous pool was not nearly as great as the rate from exogenously acquired ^{15}NO$_3^-$. Including reduction of endogenous ^{15}NO$_3^-$, it can be estimated that the whole-plant rate of reduction in the dark was about 33% of that in the light (4.31 and 13.21 μmol h^{-1}, respectively), which was somewhat lower than the 46% which would have been predicted from reduction during the ^{15}NO$_3^-$ exposure periods (Table I).

Site of NO$_3^-$ Reduction in the Light. In NO$_3^-$-fed soybean plants, considerable nitrate reductase activity is found both in roots and leaves (6, 16, 23). Also, in this experimental system concentrations of NO$_3^-$ and reduced-N in xylem exudate are nearly equal (27). However, available evidence would suggest only a small proportion of the whole-plant reduction of NO$_3^-$ in the light occurs in the root system *in situ*. In a previous experiment, it was found that during absorption of ^{15}NO$_3^-$ in the light, little ^{15}N enrichment occurred in reduced-N fractions in the root or in the soluble reduced-N fraction in xylem exudate (27). Most absorbed ^{15}NO$_3^-$ either was accumulated in the root or translocated to the shoot as ^{15}NO$_3^-$. It was concluded that less than 17% of the concurrently absorbed ^{15}NO$_3^-$ was reduced in the root. Furthermore, endogenous NO$_3^-$ in root tissue of corn is relatively unavailable for reduction in the root even though it can be readily translocated (13). If this applies also to soybean, then the marked depletion of dark absorbed ^{15}NO$_3^-$ from roots (Fig. 3F) coincident with transfer of ^{15}N to leaves (Fig. 1B) in the initial light period (hours 24–33) primarily would reflect translocation of ^{15}NO$_3^-$ and not reduction in the root with translocation of soluble reduced-^{15}NO$_3^-$. The increase in ^{15}NO$_3^-$ in stems at the end of the rapid transfer period, while soluble reduced-^{15}N in stems was unchanged, (Fig. 3E) indirectly supports that contention.

Additional consideration of data from this experiment also leads to the conclusion that reduction in roots in the light was not substantial. If it is assumed energy in the root in darkness was no less available than in the light, the rate of incorporation of ^{15}N into reduced-N fractions in the root during the dark uptake period might provide an estimate of the reductive capacity of the root. During that time, translocation of ^{15}N was restricted and NO$_3^-$ substrate levels were elevated (Fig. 3F), but the rate of ^{15}N incorporation in reduced fractions in the root (1.51 μmol h^{-1}, Table II) was only 11% of the estimated rate of whole-plant NO$_3^-$ reduction in the light ($\sim$13.21 μmol h^{-1}). Alternatively, the rate of whole-plant reduction during the dark uptake period

might reflect the reductive capacity of the root, and take into account translocation of reduced-^{15}N to leaves. Even so, the rate (2.58 μmol h^{-1}, Table I) was less than 20% of the estimated whole-plant reduction rate in the light ($\sim$13.21 μmol h^{-1}).

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