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Nitrate Accumulation, Assimilation, and Transport by Decapitated Corn Roots[1]

EFFECTS OF PRIOR NITRATE NUTRITION

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CHARLES T. MACKOWN[2], RICHARD J. VOLK, WILLIAM A. JACKSON
Department of Soil Science, North Carolina State University, Raleigh, North Carolina 27650

ABSTRACT

The effects of accumulated [^{14}N]nitrate and its utilization in decapitated, 5-day-old dark-grown corn roots on influx, accumulation, xylem deposition, and reduction of concurrently absorbed nitrate during an 18-hour exposure to 0.5 millimolar $K^{15}NO_3$ nutrient solution were examined. A 20-hour pretreatment in 15.0 millimolar $K^{14}NO_3$ high nitrate (HN) resulted in a 2-fold greater tissue nitrate level than pretreatment in 0.5 millimolar $K^{14}NO_3$ low nitrate (LN). Upon transfer to the 0.5 millimolar $K^{15}NO_3$ solution, the net nitrate uptake rate in HN roots after 2 hours was 52% of the LN rate, but increased to 93% at the end of the uptake period. Despite an enhanced [^{14}N]nitrate efflux from HN roots to the uptake solution, the efflux differences between the two pretreatments did not compensate for the decrease in net nitrate uptake. The [^{15}N]nitrate influx rate was initially restricted by 33% in the HN roots compared to LN roots, but it had decreased to 7% by the end of the 18-hour uptake period. At this time, the total tissue nitrate levels were similar for both pretreatments. The rate of accumulation of [^{15}N]nitrate in the tissue was relatively constant for both pretreatments, but was 25% less in HN roots. Of the previously accumulated [^{14}N]nitrate, 52 and 46% remained after 18 hours in the LN and HN roots, respectively. The [^{14}N]nitrate decline for HN roots was initially more rapid than in the LN roots which was linear over time. Xylem transport and efflux more than accounted for the decline in [^{14}N]nitrate of LN roots and all but 4% in the HN roots which was attributed to reduction. Compartmentation of the previously accumulated nitrate was evident from the higher atom per cent ^{15}N of xylem nitrate compared to that of the tissue nitrate of both LN and HN roots. During the first 2 hours, xylem transport of [^{14}N]nitrate by the HN roots was 49% greater than for LN roots, while [^{15}N]nitrate transport was 9% less in HN roots compared to LN roots. Even though the reduction of [^{15}N]nitrate in HN roots was 31% less than LN roots during the first 2 hours, [^{15}N]nitrate was reduced more rapidly than the previously accumulated [^{14}N]nitrate. After the first 4 hours, the relative partitioning of absorbed [^{15}N]nitrate between accumulation, reduction, and translocation was similar regardless of pretreatment.

Previously accumulated and concurrently absorbed nitrate are subject to several competing processes in roots of higher plants. These include reduction, xylem deposition, transport across the tonoplast, and efflux. The transport and reduction processes occurring in the root exert an initial control on the movement of nitrate and reduced nitrogen to the shoots, thus affecting the entire nitrate assimilation pathway of the plant. In addition, the nitrate status within the root tissue, resulting from prior nutritional conditions, may influence each of the pathway processes. Delineating the mode in which previously accumulated nitrate exerts these influences requires quantitative measurement of the exogenous and endogenous nitrate fluxes through each pathway.

Net nitrate uptake is restricted in roots of plants previously exposed to high nitrate concentrations for extended periods (5, 12, 16). The net rate of nitrate uptake is the difference between influx and efflux across the plasmalemma, which appear to be relatively independent processes subject to different controls (12, 17). Restriction in net uptake, resulting from high endogenous nitrate concentrations, may in part be accounted for by an enhanced efflux (12). Also, transinhibitory effects of accumulated nitrate (22) or nitrate plus chloride (4), on nitrate uptake may be exerted in a manner similar to the endogenous allosteric potassium influx regulation as proposed by Glass (9).

The extent to which previously accumulated nitrate may be utilized for efflux, translocation, or reduction is apparently dependent upon the supply of exogenous nitrate. In the absence of solution nitrate, a sizable depletion of the previously accumulated nitrate in intact plants may occur (8, 12), whereas in the presence of concurrently absorbed nitrate, the decline is restricted (8). The reduction of previously accumulated nitrate in the absence of exogenous nitrate accounted for a relatively large nitrate decline in bush bean leaves (14) and in intact wheat seedlings (12). However, when solution nitrate was present, reduction of previously accumulated nitrate was limited (1), indicating the probable comparmentation of nitrate within the root tissue.

The objectives of this investigation were to determine the effect of prior nitrate nutrition on the accumulation, assimilation, and transport of concurrently absorbed nitrate in the root system, and to measure the major pathways in the utilization of previously accumulated nitrate, thereby obtaining information on the extent of its compartmentation.

MATERIALS AND METHODS

Plant Growth. Corn seeds (*Zea mays* L. DeKalb XL-45) were placed in paper rolls moistened with 0.1 mM $CaSO_4$ and germinated in the dark for 3 days in an incubator at 30 C and 98% RH. Seedlings were removed from the germination paper and the secondary roots excised before threading the primary root through culture holders, consisting of hollow plastic stoppers (No. 7) punched with holes to accept five seedlings. After covering the endosperms with cotton moistened with 0.1 mM $CaSO_4$, the cultures were placed in tanks containing aerated, nitrogen-free nutri-

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[2] Present address: Department of Agronomy, United States Department of Agriculture, Science and Education Administration, Agricultural Research, University of Kentucky, Lexington, KY 40546.

ent solution and returned to the incubator for 28 h. The nitrogen-free, [^{14}N]nitrate pretreatment and subsequent [^{15}N]nitrate uptake solutions all contained the following basal nutrient solution: 0.05 mM K_2SO_4, 0.4 mM KH_2PO_4, 0.25 mM $MgSO_4$, 1.0 mM $CaSO_4$, 1.0 mg Fe/l as FeEDTA, and trace elements at two-fifths Hoagland strength (10). The basal nutrient solutions were adjusted to pH 6.1 with 1 M KOH. Approximately 4 h prior to nitrate pretreatment, the shoots were excised below the first node, cotton covering the endosperms was removed and replaced with black polypropylene beads, and a small funnel-shaped plastic exudate collector was attached to each mesocotyl and sealed with silicone grease. Cultures of five decapitated seedlings with attached exudate collectors were transferred to aerated tanks of LN[3] or HN pretreatment solution containing 0.5 or 15.0 mM $K^{14}NO_3$ for 20 h to assure induction (11) and establish different levels of tissue [^{14}N]nitrate.

Prior to placement of cultures in the ^{15}N-enriched uptake solution, the roots were rinsed with distilled H_2O and placed in 1.0 mM $CaSO_4$ at 30 C for 15 min to allow [^{14}N]nitrate to diffuse from the root free space. Individual cultures then were placed in culture tubes containing 250 ml aerated 0.5 mM $K^{15}NO_3$ nutrient solution with an enrichment of 98.25 A%. Uptake solutions in the culture tubes were maintained at a constant temperature of 30 C. Four LN cultures and four HN cultures were harvested at 0, 2, 4, 6, 12, and 18 h after exposure to $K^{15}NO_3$. Immediately after removal from the uptake solution, roots were rinsed with distilled H_2O and placed in cold (<5 C) 1.0 mM $CaSO_4$ to remove surface and free space nitrate. Analysis of this solution revealed no evidence for an inordinate loss of tissue [^{15}N]nitrate or [^{14}N]nitrate due to the cold treatment. The tissue was then blotted dry and divided into roots and seed pieces (see plus mesocotyl). Fresh weights were recorded and the tissues immediately frozen for lyophilizing prior to grinding. Cumulative exudate from the respective harvested cultures also was collected and kept chilled with ice. Uptake solutions were changed at 2, 4, 6, 9, and 15 h for the remaining cultures.

Nitrogen and Isotope Analysis. The uptake solution and exudate were analyzed for nitrate by a modification of the Lowe and Hamilton (13) procedure and for A% ^{15}N by MS procedures permitting direct determination of ^{15}N without prior reduction of nitrate to ammonium (25). Hot water extracts (1:50, w/v) from the combined ground plant material were analyzed for nitrate (13) and nitrate A% ^{15}N (25). To minimize foaming during the nitrate A% ^{15}N analysis, the extracts were treated with 0.5 ml 30% H_2O_2 to oxidize organic compounds and then evaporated to dryness at 97 C. Reduced nitrogen and A% ^{15}N in the exudate were determined on the same samples used to measure nitrate A% ^{15}N. The exudate samples were further treated to minimize reduction of any nitrate remaining during Kjeldahl digestion. Each sample was sonicated 1 additional h beyond the 15-min sonication used in the nitrate A% ^{15}N procedure (25), then treated with 0.5 ml 30% H_2O_2 and heated at 97 C to concentrate the samples and volatilize any nitrate remaining in the exudate prior to Kjeldahl digestion by the McKenzie and Wallace procedure (15). Ammonium nitrogen in the digest was determined colorimetrically (3) and A% ^{15}N by the MS freeze-layer method (24) after trapping the ammonia by diffusion. Total reduced nitrogen in tissue was determined also by Kjeldahl digestion after removal of tissue nitrate to minimize the possibility of its reduction during digestion (19). Ammonium nitrogen and A% ^{15}N were determined as for the exudate reduced N.

Calculations and Statistical Analysis. The solution analyses result in a determination of [^{15}N]nitrate influx and net [^{14}N]nitrate efflux, where

$$[^{15}\text{N}]\text{Nitrate influx} = (\mu\text{mol N} \times \text{A\% } ^{15}\text{N})_{\text{Initial}} - (\mu\text{mol N} \times \text{A\% } ^{15}\text{N})_{\text{Final}}$$

and

$$\text{Net } [^{14}\text{N}]\text{nitrate efflux} = (\mu\text{mol N} \times \text{A\% } ^{14}\text{N})_{\text{Final}} - (\mu\text{mol N} \times \text{A\% } ^{14}\text{N})_{\text{Initial}}$$

An estimation of the actual efflux of the previously accumulated [^{14}N]nitrate, a portion of which is available for absorption, thus can be made. Actual efflux represents the net [^{14}N]nitrate efflux plus the calculated mean hourly [^{14}N]nitrate influx, where

$$[^{14}\text{N}]\text{Nitrate influx} = ([^{15}\text{N}]\text{nitrate influx})\,([^{14}NO_3^-]/[^{15}NO_3^-])$$

assuming that influx of [^{15}N]nitrate and reabsorption of effluxed [^{14}N]nitrate occur proportionally to their mean ambient solution concentrations, and that [^{15}N]nitrate efflux is negligible.

Data presented are means of four replicates ± SE unless otherwise indicated. Reduction of previously accumulated nitrate for the HN pretreatment was calculated using values of tissue [^{14}N]-nitrate, net [^{14}N]nitrate efflux, and translocated [^{14}N]nitrate predicted by regression models. Regression lines passing through the origin were force fitted.

RESULTS

Nitrate pretreatment with 0.5 mM and 15.0 mM KNO_3 nutrient solution resulted in total tissue nitrate levels of 52.1 ± 1.0 and 105.6 ± 1.6 μmol/culture, respectively. The net nitrate uptake rate of HN roots was 48% less than that of LN roots during the first 2 h, whereas [^{15}N]nitrate influx rate during this period in HN roots was 33% less than in LN roots (Fig. 1). At the end of the 18-h uptake period, both net nitrate uptake and [^{15}N]nitrate influx rates of HN roots approached that of LN roots (Fig. 1). Mean root fresh weight difference between treatments within a time period was not significantly different at $P = 0.05$ level (data not shown). However, root fresh weight change over time was highly significant ($P < 0.01$) and increased from 1.24 to 2.07 g/culture.

The accumulation of [^{15}N]nitrate was 25% less in HN than in LN roots after 18 h, while the decline in the previously accumulated [^{14}N]nitrate initially was more rapid (Fig. 2). The previously accumulated [^{14}N]nitrate decreased approximately 50% by 18 and 12 h for the LN and HN roots, respectively. Total tissue nitrate (^{14}N + ^{15}N) concentration at the end of the 18-h uptake period

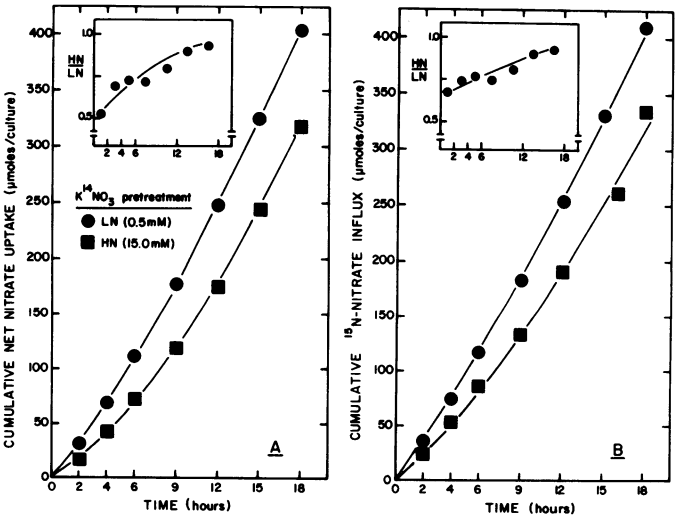

FIG. 1. Time course of net nitrate uptake (A) and [^{15}N]nitrate influx (B) in 5-day-old dark-grown decapitated corn roots (5/culture) exposed to 0.5 mM $K^{15}NO_3$ solution after a 20-h $K^{14}NO_3$ pretreatment. Insets: ratio of mean hourly rate (μmol culture^{-1} h^{-1}) of HN (15.0 mM $K^{14}NO_3$ pretreated) to LN (0.5 mM $K^{14}NO_3$ pretreated) roots. Data plotted represents means of 20, 16, 12, 8, 8, 4, and 4 observations for 2–18 h, respectively.

[3] Abbreviations: LN, low nitrate; HN, high nitrate; A%, atom per cent.

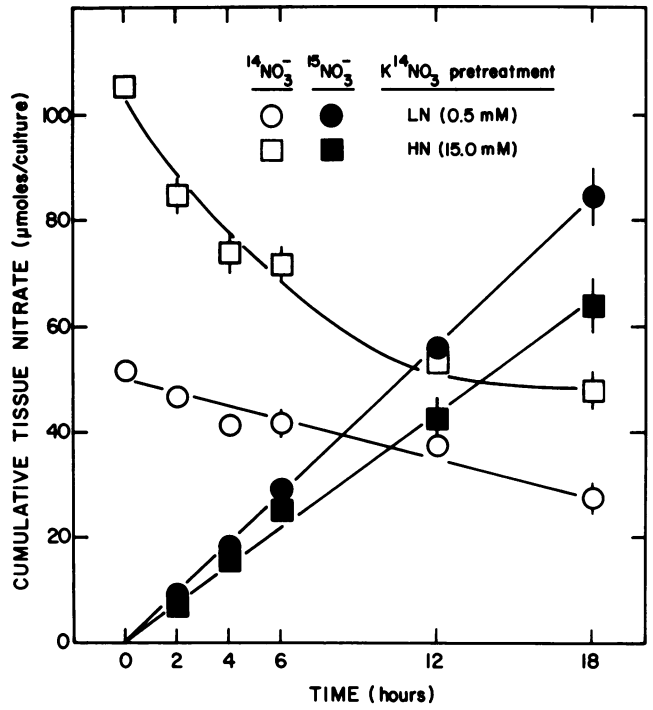

FIG. 2. Time course of tissue excess [^{15}N]nitrate accumulation and [^{14}N]nitrate decline in 5-day-old dark-grown decapitated corn roots (5/culture) exposed to 0.5 mM $K^{15}NO_3$ solution after a 20-h pretreatment in 5.0 mM (LN) or 15.0 mM (HN) $K^{14}NO_3$ solution. Vertical bars on symbols indicate the mean of four replicates ± SE. Regression analysis of cumulative [^{14}N]nitrate decline over 18 h for LN roots is $Y = 50.3 - 1.27$ h ($R^2 = 0.812$) and for HN roots is $Y = 103.1 - 7.18$ h + 0.233 h^2 ($R^2 = 0.958$).

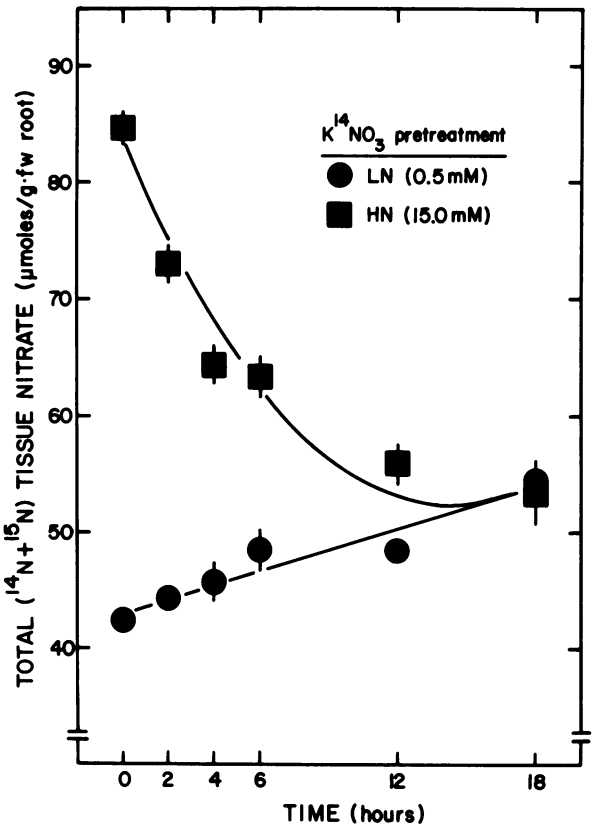

FIG. 3. Time course of tissue nitrate (^{14}N + ^{15}N) concentration in 5-day-old dark-grown decapitated corn roots (5/culture) pretreated in 0.5 mM (LN) and 15.0 mM (HN) $K^{14}NO_3$ for 20 h prior to transfer to 0.5 mM $K^{15}NO_3$. Vertical bars on symbols indicate SE of four replicates.

was 160.0 ± 7.6 and 158.9 ± 11.7 μmol/g fresh weight roots for the LN and HN roots, respectively, with the initial tissue nitrate decline of the HN roots being completely offset at the end of the experiment (Fig. 3).

The patterns of [^{14}N]nitrate decline (Fig. 2) were accounted for mostly by the processes of efflux and translocation. Net [^{14}N]-nitrate efflux from LN and HN roots occurred throughout the 18-h uptake period (Fig. 4), but was more rapid from HN roots. Both LN and HN root cumulative net efflux declined after 12 h. During the 18-h uptake period, nitrate A% ^{15}N uptake solutions was always significantly less than the original solution (data not shown). Estimated actual [^{14}N]nitrate efflux followed a similar pattern as net efflux, but did not decrease during the final hours (Fig. 4). During the first 2 h, actual efflux was 73% greater from HN roots than from LN roots. Translocated nitrate (^{14}N + ^{15}N) during the uptake period was similar for both pretreatments (data not shown). A significant proportion (30 and 35% for the LN and HN roots, respectively) of the concurrently absorbed [^{15}N]nitrate was translocated during the first 2 h (Fig. 5), accounting for 62 and 43% total nitrate translocated during this period. The lower deposition of [^{15}N]nitrate into the xylem of the HN roots throughout the experimental period was associated with an enhanced [^{14}N]nitrate translocation (Fig. 6). Translocation of [^{14}N]nitrate occurred throughout the 18-h uptake period for both pretreatments and at the end of the uptake period was 22.2 ± 1.3 and 36.0 ± 5.2 μmol/culture for the LN and HN roots, respectively. Compartmentation of the previously accumulated nitrate is evident from the greater ^{15}N enrichment in the nitrate translocated to the xylem compared to that in the tissue at a given time (Fig. 7). The effect was especially marked during the initial periods and was more evident in LN than in HN seedlings. As the initial tissue [^{14}N]nitrate declined and the tissue [^{15}N]nitrate increased (Fig. 2), the tissue nitrate A% ^{15}N approached the exudate nitrate A% ^{15}N

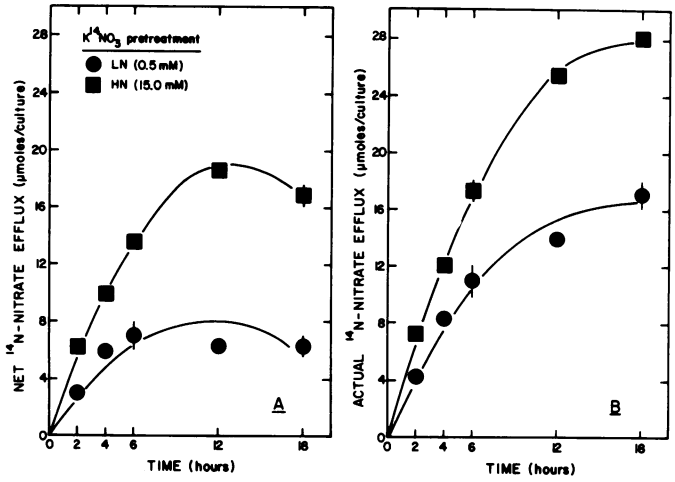

FIG. 4. Time course of (A) net [^{14}N]nitrate efflux and (B) estimated actual [^{14}N]nitrate efflux from 5-day-old dark-grown decapitated corn roots (5/culture) pretreated 20 h in $K^{14}NO_3$ prior to transfer to 0.5 mM $K^{15}NO_3$. Vertical bars on symbols indicate the mean of four replicates ± SE. Regression analysis of cumulative net [^{14}N]nitrate efflux over 18 h for LN (0.5 mM $K^{14}NO_3$ pretreated) roots is $Y = 1.41$ h − 0.061 h^2 ($R^2 = 0.935$) and for HN (15.0 mM $K^{14}NO_3$ pretreated) roots is $Y = 2.89$ h − 0.109 h^2 ($R^2 = 0.996$).

(Fig. 7).

Accumulation and transport of reduced nitrogen (^{14}N + ^{15}N) were similar between pretreatments (data not shown). However, accumulation of reduced ^{15}N in the tissue and translocation of reduced ^{15}N were less in the 15.0 mM pretreatment (Table I). Per

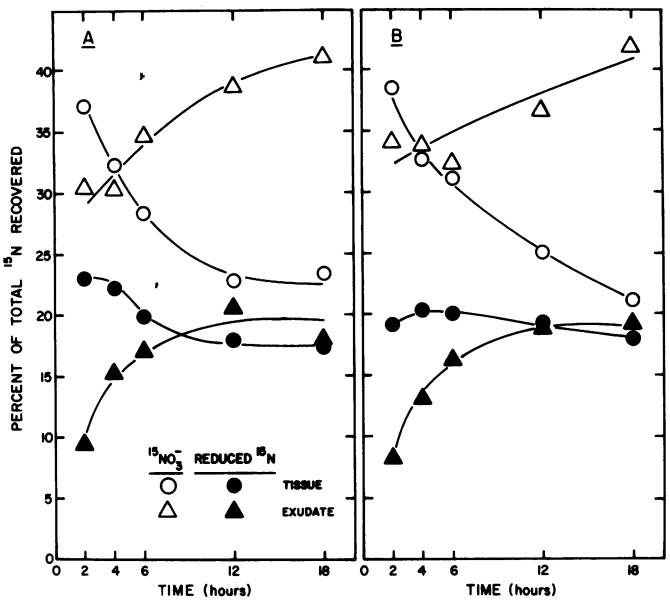

FIG. 5. Relative partitioning of ^{15}N with time in LN (A) and HN (B) roots of 5-day-old dark-grown corn roots pretreated 20 h with 0.5 mM and 15.0 mM $K^{14}NO_3$ solution, respectively, prior to transfer to 0.5 mM $K^{15}NO_3$ solution.

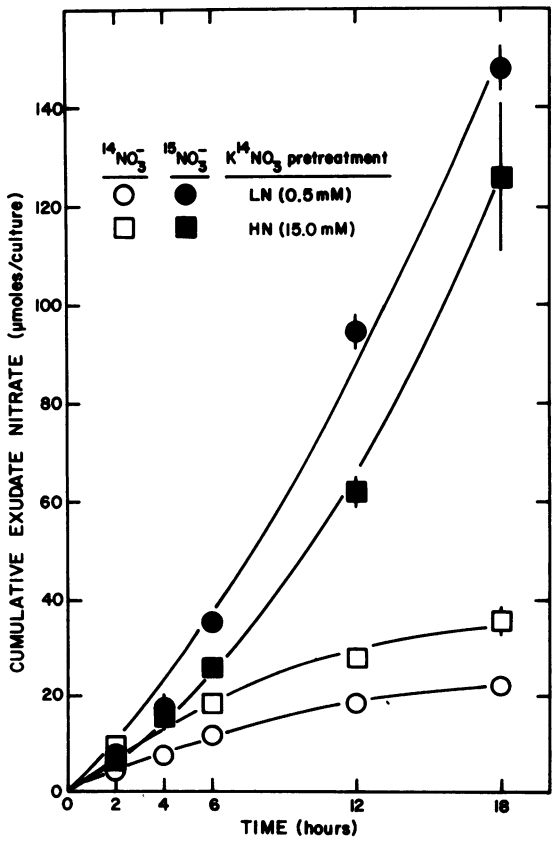

FIG. 6. Time course of [^{15}N]- and [^{14}N]nitrate translocated by 5-day-old dark-grown decapitated corn roots (5/culture) pretreated 20 h in $K^{14}NO_3$ prior to transfer to 0.5 mM $K^{15}NO_3$. Regression analysis of cumulative [^{14}N]nitrate translocated over 18 h for LN (0.5 mM $K^{14}NO_3$ pretreated) roots is $Y = 2.26\ h - 0.057\ h^2$ ($R = 0.998$) and for HN (15.0 mM $K^{14}NO_3$ pretreated) roots is $Y = 3.67\ h - 0.096\ h^2$ ($R^2 = 0.983$).

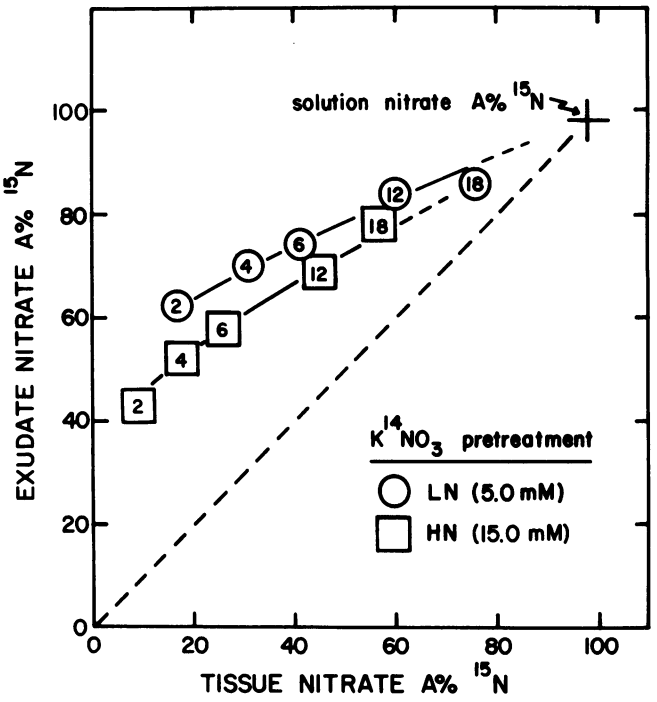

FIG. 7. Relationship of the nitrate A% ^{15}N of the tissue and exudate at the end of five uptake periods in LN (0.5 mM $K^{14}NO_3$ pretreated) and HN (15.0 mM $K^{14}NO_3$ pretreated) roots transferred to 0.5 mM $K^{15}NO_3$ after nitrate pretreatment. Numbers within the symbols indicate the hour at the end of each measurement period.

Table I. *Cumulative Accumulation and Translocation of Reduced ^{15}N in Excised Corn Roots Pretreated 20 h with 0.5 mM (LN) or 15.0 mM $K^{14}NO_3$ (HN) Nutrient Solution Prior to Transfer to 0.5 mM $K^{15}NO_3$ Uptake Solutions*

	KNO_3 Pretreatment			
	Tissue-Reduced Excess ^{15}N		Exudate-Reduced Excess ^{15}N	
Time	0.5 mM	15.0 mM	0.5 mM	15.0 mM
h	*μmol culture*$^{-1}$			
2	5.7 ± 0.2[a]	3.9 ± 0.7	2.4 ± 0.1	1.7 ± 0.1
4	12.5 ± 0.4	9.8 ± 0.5	8.9 ± 1.4	6.4 ± 0.4
6	20.4 ± 0.5	16.2 ± 0.3	17.6 ± 0.9	13.2 ± 0.7
12	43.9 ± 2.0	32.5 ± 3.1	50.4 ± 1.4	31.9 ± 1.0
18	62.9 ± 5.1	53.0 ± 4.7	65.4 ± 2.8	57.8 ± 3.2

[a] Value reported is the mean of four replicates ± SE.

cent reduction of cumulative ^{15}N during the 18-h uptake period ranged from 32.7 to 38.5% for LN roots and from 27.9 to 38.2% for HN roots (Fig. 5). Reduction of previously accumulated nitrate was estimated by the difference in tissue [^{14}N]nitrate decrease and [^{14}N]nitrate translocation plus net efflux. For LN roots, translocation, and net efflux of [^{14}N]nitrate more than accounted for the decline in tissue [^{14}N]nitrate, whereas for HN roots reduction ranged from 8 to 4% during the 18-h uptake period (data not shown). Since the calculated [^{14}N]nitrate reduction values depend upon analyses involving solution, exudate, and tissue nitrate, their ^{15}N enrichment, as well as the normal variation among the seedlings, we feel that they should be considered as tentative yet plausible. The implication that the currently absorbed nitrate is preferentially reduced by the root is currently being examined further.

DISCUSSION

Efflux and Influx. Aside from the decrease in KNO_3 concentration from 15.0 to 0.5 mM for the HN pretreatment, and the ^{15}N enrichment of the KNO_3, care was taken to maintain the composition of the uptake solution identical with that of the pretreatment solution. The 2-fold increase in tissue nitrate concentration of the HN roots compared to LN roots after nitrate pretreatment is indicative of the responsiveness of nitrate uptake to the ambient concentration (7, 20, 23). Upon transfer of the pretreated tissues to 0.5 mM $K^{15}NO_3$ nutrient solution, significant loss of [^{14}N]nitrate to the ambient solution occurred with both pretreatments, with an enhanced actual efflux being sustained by HN roots during the 18-h uptake period (Fig. 4B). Evidently the pool size of previously accumulated nitrate available for efflux was larger in the HN roots and/or the transfer of previously accumulated [^{14}N]nitrate to the efflux pool continued to exceed that from LN roots during the first 12 h. A similar continued net efflux occurred in intact wheat seedlings through a 6-h period (12), and the present data confirm the observation that efflux to the ambient solution is a major component of the decline in tissue [^{14}N]nitrate which occurs upon exposure of roots to [^{15}N]nitrate.

Cumulative net [^{14}N]nitrate efflux (Fig. 4A) from both LN and HN roots declined after 12 h, reflecting a decrease in the availability of previously accumulated [^{14}N]nitrate (Fig. 2) for efflux. During the later uptake intervals, the reabsorption of effluxed [^{14}N]nitrate and a portion of the originally present [^{14}N]nitrate (2.19 μmol) in the 98.25 A% [^{15}N]nitrate nutrient solution occurred. This resulted in a decrease in the cumulative net [^{14}N]nitrate efflux.

The restricted net nitrate uptake by the HN roots compared to LN roots could not be accounted for entirely by the difference in net [^{14}N]nitrate efflux between HN and LN roots (*cf.* Fig. 1A with Fig. 4A), indicating a specific restriction in [^{15}N]nitrate influx in the HN roots. The inhibition was associated with significantly higher total tissue nitrate concentrations during the first 12 h, corresponding to the period of restricted [^{15}N]nitrate influx (*cf.* Fig. 3 with Fig. 1B). Accumulation of [^{15}N]nitrate was relatively constant during the 18-h uptake period for both pretreatments, whereas the decline of previously accumulated [^{14}N]nitrate was constant for the LN roots and curvilinear for the HN roots (Fig. 2). The tissue nitrate (^{14}N + ^{15}N) concentration in LN roots increased with time, whereas that in HN roots rapidly declined (Fig. 3); eventually both were equal.

Three possibilities may be advanced for the restricted [^{15}N]-nitrate influx, on the assumption that the effect was solely a consequence of the altered nitrogen status of the tissue. One is transinhibition of the plasmalemma transport system due to initially greater tissue nitrate concentrations (4, 12, 22), perhaps via a process similar to the allosteric regulation model proposed for potassium influx (9). However, studies with a nitrate reductase deficient mutant of *Arabidopsis thaliana* have provided indirect evidence against the involvement of tissue nitrate in the regulation (5). A second is that efflux and recycling of previously accumulated [^{14}N]nitrate within the outer unstirred layer and apoplast region may compete with the [^{15}N]nitrate absorption, thereby restricting influx of the latter species. In this process, the extent of the competition would be dependent in part on the [^{14}N]nitrate concentration of the efflux pool. The third possibility is that a product of nitrate reduction may exert end product inhibition or repression of the transport system. Evidence in support of this mode of regulation in higher plants has been obtained from the inhibitory effects of exogenously supplied amino acids on net nitrate uptake by *A. thaliana* (6). At present, there is insufficient information to delineate the relative importance of these possible modes of regulation of nitrate influx by root tissue.

In addition to these possible direct effects of altered nitrogen status, indirect effects associated with the HN pretreatment could be involved. A limited energy supply seems to be precluded because the presence of the endosperm on the dark-grown decapitated seedlings used provides a source of continual rapid carbohydrate translocation into the root tissue. This is indicated by the marked increase in root fresh weight during the 18-h experimental period (relative growth rate = 0.68 day^{-1}) with no significant difference between the LN and HN pretreatments. Moreover, other experiments not reported here show that pretreatments with KNO_3 up to 50 mM for 20 h resulted in no consistent difference in the soluble carbohydrate (45 μmol/g fresh weight) or malate (4.3 μmol/g fresh weight) concentrations in the root tissue. On the other hand, elevated tissue potassium concentrations as a result of the HN pretreatment could have indirectly restricted [^{15}N]nitrate influx or enhanced [^{14}N]nitrate efflux. Net potassium uptake (data not presented) exhibited a pattern similar to net nitrate uptake (Fig. 1A) with both LN and HN pretreatments. Because other studies (2, 12) indicate that potassium uptake was dependently linked to nitrate uptake, the restricted [^{15}N]nitrate influx observed as a result of preloading (Fig. 1B) was more likely a result of the altered nitrogen status of the tissue than a consequence of restricted potassium influx. This interpretation has been extended to the effects of preloading on assimilation, partitioning, and compartmentation in the following paragraphs.

^{15}N Assimilation and Partitioning. Reduction of the entering [^{15}N]nitrate was restricted in the HN roots (Table I). After the initial 2 h (LN) or 4 h (HN), a relatively constant (36–38%) percentage reduction occurred during the remainng 18-h uptake period. The initial restriction in [^{15}N]nitrate reduction in HN roots may be attributed in part to the availability of the previously accumulated [^{14}N]nitrate for reduction. In both cases, a greater proportion of the reduced ^{15}N remained in the root tissue during the first 4 to 6 h. After this period, the relative proportions of reduced ^{15}N translocated and accumulated reduced ^{15}N were similar within and between both pretreatments (Fig. 5). The relative partitioning of [^{15}N]nitrate between accumulation and translocation was also unaffected by [^{14}N]nitrate pretreatment (Fig. 5). However, the proportion translocated increased with time.

^{14}N Assimilation and Partitioning. Only 52 and 46% of the previously accumulated nitrate remained at the end of the 18-h uptake period in the LN and HN roots, respectively, indicating the rather labile nature of the previously accumulated [^{14}N]nitrate (Fig. 2). Reduction of the previously accumulated [^{14}N]nitrate was not measurable in the LN roots, but accounted for 8% [^{14}N]-nitrate decline in HN roots during the first 2 h. The rate of [^{14}N]nitrate reduction declined during the 18-h uptake period and at the end accounted for 4% decline. The apparent absence of reduction in LN roots and decreasing reduction rates in HN roots may be attributed to differential fluxes of [^{15}N]- and [^{14}N]nitrate through the metabolic pool rather than to the total tissue level of nitrate (21). The LN root rate of tissue [^{14}N]nitrate decline predicted by regression was 1.3 μmol $culture^{-1}$ h^{-1} during the 18-h uptake period (Fig. 2), while [^{15}N]nitrate influx increased from 17.6 to 26.3 μmol $culture^{-1}$ h^{-1} (Fig. 1B). Similarly, the HN root predicted rate of decline in tissue [^{14}N]nitrate decreased from 6.7 to 0.2 μmol $culture^{-1}$ h^{-1}, whereas [^{15}N]nitrate influx increased from 11.8 to 24.4 μmol $culture^{-1}$ h^{-1} (Figs. 2 and 1B), resulting in a decreasing availability of previously accumulated [^{14}N]nitrate for reduction. If nitrate reduction is associated with the influx process, some of the measurable [^{14}N]nitrate reduction in HN roots may have been a consequence of the more rapid [^{14}N]nitrate efflux and subsequent reabsorption. Different regions of the root tissue may differ in nitrate reductase activity (18, 26). It is possible that reabsorption in regions of high nitrate reductase activity may have resulted in the greater percentage of the previously accumulated nitrate being reduced in the presence of concurrently absorbed [^{15}N]nitrate. Nevertheless, the magnitude of such a se-

quence, if it indeed occurred, was small.

In contrast to the limited reduction of [^{14}N]nitrate, a large proportion of the previously accumulated [^{14}N]nitrate was translocated (Fig. 5) and effluxed (Fig. 4). Translocated [^{14}N]nitrate was approximately 1.3 times greater than actual efflux (Fig. 4B). However, actual efflux most likely underestimates the total [^{14}N]nitrate movement outward across the plasmalemma as recycling within unstirred layers may occur (17).

[^{14}N]Nitrate Compartmentation. Enrichment of the tissue [^{15}N]nitrate was about 17 A% excess in the LN roots and 9 A% excess in the HN roots at 2 h after transfer to the 0.5 mM $K^{15}NO_3$ uptake solution (Fig. 2). In contrast, the A% ^{15}N in translocated nitrate was approximately 62% for the LN roots and 43% for the HN roots (Fig. 6). This differential in enrichment between exudate and tissue nitrate indicates marked compartmentation of the previously accumulated [^{14}N]nitrate and the concurrently absorbed [^{15}N]nitrate (Fig. 7). This compartmentation may be attributed to pool differences within the cell and/or on a larger scale, to zonal differences within the root.

SUMMARY

Overall the data indicate that differential effects were exerted by HN pretreatment on each of the nitrate assimilation pathway processes. During the first 2 h, for example, [^{15}N]nitrate influx was decreased 33%, accumulation by 16%, xylem deposition by 9%, and reduction by 31% in HN compared to LN roots. After 4 h, partitioning of the concurrently absorbed [^{15}N]nitrate was similar between the two pretreatments, while influx continued to be restricted. Concurrently absorbed nitrate was preferentially reduced compared to previously accumulated nitrate despite a significant decline of the latter. The rate of decline in tissue [^{14}N]nitrate due to efflux and translocation, as well as the distinct compartmentation of root tissue [^{14}N]nitrate perhaps from sites of major nitrate reduction, were probable factors related to the limited magnitude of [^{14}N]nitrate reduction compared to the reduction of concurrently absorbed [^{15}N]nitrate.

LITERATURE CITED

1. ASHLEY DA, WA JACKSON, RJ VOLK 1975 Nitrate uptake and assimilation by wheat seedlings during initial exposure to nitrate. Plant Physiol 55: 1102–1106
2. BLEVINS DG, AJ HIATT, RH LOWE 1974 The influence of nitrate and chloride uptake on expressed sap pH, organic acid synthesis, and potassium accumulation in higher plants. Plant Physiol 54: 82–87
3. CATALDO DA, LE SCHRADER, VL YOUNGS 1974 Analysis by digestion and colorimetric assay of total nitrogen in plant tissues high in nitrate. Crop Sci 14: 854–856
4. CRAM WJ 1973 Internal factors regulating nitrate and chloride influx in plant cells. J Exp Bot 24: 328–341
5. DODDEMA H, JJ HOFSTRA, WJ FEENSTRA 1978 Uptake of nitrate by mutants of *Arabidopsis thaliana*, disturbed in uptake or reduction of nitrate. I. Effect of nitrogen source during growth on uptake of nitrate and chlorate. Physiol Plant 43: 343–350
6. DODDEMA H, H OTTEN 1979 Uptake of nitrate by mutants of *Arabidopsis thaliana*, disturbed in uptake or reduction of nitrate. III. Regulation. Physiol Plant 45: 339–346
7. DODDEMA H, GP TELKAMP 1979 Uptake of nitrate by mutants of *Arabidopsis thaliana*, disturbed in uptake or reduction of nitrate. II. Kinetics. Physiol Plant 45: 332–338
8. FRIEDRICH JW, LE SCHRADER 1979 N deprivation in maize during grain-filling. II. Remobilization of ^{15}N and ^{35}S and the relationship between N and S accumulation. Agron J 71: 466–472
9. GLASS ADM 1976 Regulation of potassium absorption in barley roots: An allosteric model. Plant Physiol 58: 33–37
10. HOAGLAND DR, DI ARNON 1950 The water culture method for growing plants without soil. Calif Agric Exp Stn Circ 347
11. JACKSON WA, D FLESHER, RH HAGEMAN 1973 Nitrate uptake by dark-grown corn seedlings: Some characteristics of apparent induction. Plant Physiol 51: 120–127
12. JACKSON WA, KD KWIK, RJ VOLK, RG BUTZ 1976 Nitrate influx and efflux by intact wheat seedlings: Effects of prior nitrate nutrition. Planta 132: 149–156
13. LOWE RH, JL HAMILTON 1967 Rapid method for determination of nitrate in plant and soil extracts. J Agric Food Chem 15: 359–361
14. MARTIN P 1973 Nitrate nitrogen in bush bean leaves under the viewpoint of compartmentation of cells. Z Pflanzenphysiol 70: 158–165
15. MCKENZIE HA, HS WALLACE 1954 The Kjeldahl determination of nitrogen: A critical study of digestion conditions-temperature, catalyst and oxidizing agent. Aust J Chem 7: 55–70
16. MINOTTI PL, DC WILLIAMS, WA JACKSON 1969 Nitrate uptake by wheat as influenced by ammonium and other cations. Crop Sci 9: 9–14
17. MORGAN MA, RJ VOLK, WA JACKSON 1973 Simultaneous influx and efflux of nitrate during uptake by perennial ryegrass. Plant Physiol 51: 267–272
18. OAKS A, W WALLACE, D STEVENS 1972 Synthesis and turnover of nitrate reductase in corn roots. Plant Physiol 50: 649–654
19. PACE GM, CT MACKOWN, RJ VOLK 1980 An alternative method for removing nitrate interference in Kjeldahl digestion. Plant Physiol 65: S-55
20. RAO KP, DW RAINS 1976 Nitrate absorption by barley. I. Kinetics and energetics. Plant Physiol 57: 55–58
21. SHANER DL, JS BOYER 1976 Nitrate reductase activity in maize (*Zea mays* L.) leaves. I. Regulation by nitrate flux. Plant Physiol 58: 499–504
22. SMITH FA 1973 The internal control of nitrate uptake into excised barley roots with differing salt contents. New Phytol 72: 769–782
23. VAN DEN HORNERT TH, JJM HOOYMANS 1955 On the absorption of nitrate by maize in water culture. Acta Bot Néerl 4: 376–384
24. VOLK RJ, WA JACKSON 1979 Preparing nitrogen gas for nitrogen-15 analysis. Anal Chem 51: 463
25. VOLK RJ, CJ PEARSON, WA JACKSON 1979 Redution of plant tissue nitrate to nitric oxide for mass spectrometric ^{15}N analysis. Anal Biochem 97: 131–135
26. WALLACE W 1973 A nitrate reductase inactivating enzyme from the maize root. Plant Physiol 52: 197–201