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Simultaneous Influx and Efflux of Nitrate during Uptake by Perennial Ryegrass[1]

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ABSTRACT

Experiments with intact plants of *Lolium perenne* previously grown with $^{14}NO_3^-$ revealed significant efflux of this isotopic species when the plants were transferred to solutions of highly enriched $^{15}NO_3^-$. The exuded $^{14}NO_3^-$ was subsequently reabsorbed when the ambient solutions were not replaced. When they were frequently replaced, continual efflux of the $^{14}NO_3^-$ was observed. Influx of $^{15}NO_3^-$ was significantly greater than influx of $^{14}NO_3^-$ from solutions of identical NO_3^- concentration. Transferring plants to $^{14}NO_3^-$ solutions after a six-hour period in $^{15}NO_3^-$ resulted in efflux of the latter. Presence of Mg^{2+}, rather than Ca^{2+}, in the ambient $^{15}NO_3^-$ solution resulted in a decidedly increased rate of $^{14}NO_3^-$ efflux and a slight but significant increase in $^{15}NO_3^-$ influx. Accordingly, net NO_3^- influx was slightly depressed. A model in accordance with these observations is presented; its essential features include a passive bidirectional pathway, an active uptake mechanism, and a pathway for recycling of endogenous NO_3^- within unstirred layers from the passive pathway to the active uptake site.

Under appropriate experimental conditions, efflux of a range of plant tissue components has been noted even in the absence of metabolic stress (2, 4–6, 8, 13, 14, 21, 25), but evidence for NO_3^- efflux is quite limited. When wheat seedlings were exposed sequentially to $^{15}NO_3^-$ and $^{14}NO_3^-$, Ashley (1) failed to recover in the tissue all of the $^{15}NO_3^-$ previously removed from the solution. His data indicate that about one-fourth of the absorbed $^{15}NO_3^-$ may have been lost from the roots during exposure to $^{14}NO_3^-$. Net loss of NO_3^- to ambient solution has been observed when plants previously cultured on NO_3^- were placed in NO_3^--free solutions (15, 16). These results suggest that continuous NO_3^- efflux may be a common feature of net NO_3^- uptake by roots of higher plants.

In general, ion efflux studies have focused on the process itself rather than on the possible relationship between concurrent efflux and ion accumulation by plants. The present paper reports results from experiments conducted (*a*) to establish the NO_3^- efflux properties of intact perennial ryegrass seedlings and (*b*) to examine the relationship between NO_3^- adsorption and NO_3^- efflux in those seedlings. Use of NO_3^- highly enriched with ^{15}N permitted direct measurement of the simultaneously occurring fluxes. The results obtained are in agreement with a proposed NO_3^- uptake mechanism whose essential features include: (*a*) an active influx mechanism, (*b*) a passive, bidirectional pathway dependent only on diffusion and concentration gradients, and (*c*) an internal pathway for NO_3^- recycling.

MATERIALS AND METHODS

Experiment 1. Perennial ryegrass (*Lolium perenne*) seeds were surface sterilized in 5% H_2O_2 and germinated in opaque polyethylene cups with bottoms of stainless steel screen. After thinning, each cup (one culture) contained about 45 seedlings. Plants were grown in a chamber for 21 days at 22 ± 1 C during the light period (16 hr, 238 hlx) and 18 ± 1 C during darkness. Four cultures were placed in containers of 820 ml solution, containing 0.1 mM KH_2PO_4, 0.25 mM $Ca(^{14}NO_3)_2$, 0.25 mM $MgSO_4$, 1 mg of Fe as FeEDTA, and other trace elements supplied at one-fifth those in Hoagland's solution (10). Solutions were replaced every other day, and on the 19th day the $Ca(NO_3)_2$ was doubled. On the 21st day, the nutrient solutions were removed, the roots were rinsed with a strong jet of distilled water, and then in 10 changes of distilled water. After draining thoroughly, the plants were exposed to solutions containing 0.75 mM $Ca(^{15}NO_3)_2$ or $Mg(^{15}NO_3)_2$ with a ^{15}N enrichment of 97.5 atom %. Duplicate containers were harvested after 3, 6, 12, and 18 hr of continuous illumination (238 hlx) at 24 ± 1 C. The solutions were analyzed for total NO_3^- by ultraviolet absorption at 207 nm (3). The quantities of NO_2^- in the solutions were not sufficiently high to contribute significantly to absorbance, and absence of other ultraviolet-absorbing components was confirmed by spectral scanning. The atom per cent ^{15}N of all solutions was determined mass spectrometrically (23) following reduction of NO_3^- to NH_3 using Devarda's alloy. Solution NO_2^- was measured using equal volumes of 1% sulfanilamide in 3 N HCl and 0.01% N-(1-naphthyl)-ethylenediamine dihydrochloride. Ninhydrin-reactive amino nitrogen (before and after refluxing in 6 N HCl for 16 hr) was determined by the method of Yemm and Cocking (26).

Experiment 2. Perennial ryegrass plants were grown essentially as in experiment 1. On the day of the experiment, each culture was mounted in an opaque, 60-ml leaching funnel. A stopcock permitted rapid removal of the continuously aerated solutions (55 ml) as desired. Each of the four treatments consisted of six replications of these single culture assemblies. The experiment was conducted at 70.2 hlx and 23 ± 1 C. Prior to imposition of the treatments, all cultures received a pretreatment (initiated 1 hr after onset of the dark period) of 0.75 mM $Ca(^{14}NO_3)_2$, pH 5.0, for 3 hr. These solutions were then replaced with the appropriate treatment solutions after

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thoroughly rinsing the roots. The four treatments consisted of $Ca(^{14}NO_3)_2$, $Mg(^{14}NO_3)_2$, $Ca(^{15}NO_3)_2$, and $Mg(^{15}NO_3)_2$, each 0.75 mM, pH 5.0. The ^{15}N enrichment of the latter two salts was 97.5 atom %. At 1-hr intervals the solutions were withdrawn and stored under refrigeration. The roots were again rinsed and fresh volumes of the same treatment solutions were introduced into the funnels. The sequence of root exposure, solution withdrawal, washing, and solution replenishment was repeated six times.

Solution analyses were conducted as described for experiment 1. The various NO_3^- fluxes ($^{14}NO_3^-$ influx, $^{15}NO_3^-$ influx, $^{14}NO_3^-$ efflux, and $^{15}NO_3^-$ efflux) were calculated as the difference in the amount of each nitrogen species present in solution at the start and end of each hourly period.

Experiment 3. Perennial ryegrass was grown similarly to the first two experiments except that temperature was 27 ± 1 C during the light period and the light intensity was slightly less (205 hlx). In addition, the $Ca(^{14}NO_3)_2$ in the nutrient solution was increased to 0.5 mM, 24 hr before initiation of the experiment. The experimental techniques were similar to those of experiment 2 and consisted of two phases (6 hr each) after all cultures had been pretreated for 3 hr in 0.7 mM $Ca(^{14}NO_3)_2$. The sequence of events for the three treatments is shown in Table I. Light intensity was 157 hlx, and temperature was 25 ± 1 C throughout. Solution analyses were as described for experiment 1.

Table I. *Isotopic NO_3^- Treatment Designations Employed in Experiment 3*

Numerical values refer to concentrations in meq l^{-1}. Initial pH was 5.0, and all solutions were changed hourly during the treatment period.

Treatment No.	Pretreatment Period	Treatment Period	
		Phase A[1]	Phase B[2]
	0–3 hr	*3–9 hr*	*9–15 hr*
1	1.4 $Ca(^{14}NO_3)_2$	1.4 $Ca(^{14}NO_3)_2$	1.4 $Ca(^{15}NO_3)_2$[3]
2	1.4 $Ca(^{14}NO_3)_2$	1.4 $Ca(^{15}NO_3)_2$[3]	1.4 $Ca(^{14}NO_3)_2$
3	1.4 $Ca(^{14}NO_3)_2$	2.8 $Ca(^{15}NO_3)_2$[3]	2.8 $Ca(^{14}NO_3)_2$

[1] Replications were eight, nine, and nine for treatments 1, 2, and 3, respectively.

[2] All treatments had six replications.

[3] 97.5 atom % ^{15}N enrichment.

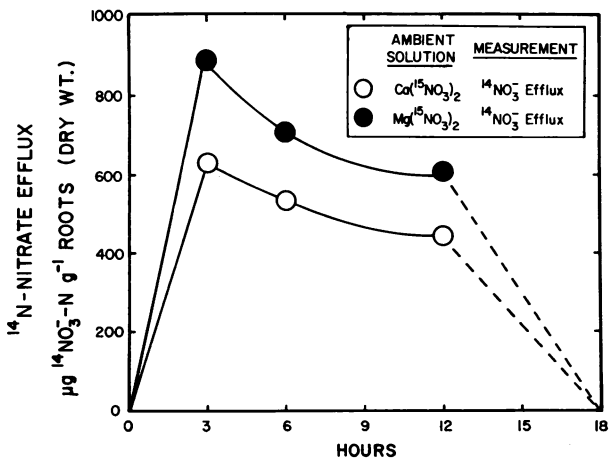

FIG. 1. Time course of $^{14}NO_3^-$ efflux to $^{15}NO_3^-$ solutions by 21-day-old seedlings in experiment 1.

Table II. *Efflux of NO_2^- by Roots of Ryegrass Seedlings*

Experiment 1 shows results during exposure to 1.5 meq l^{-1} Ca $(^{15}NO_3)_2$ or $Mg(^{15}NO_3)_2$.

Treatment	Nitrite Efflux			
	3 hr	6 hr	12 hr	18 hr
	μg NO_2^--N g^{-1} roots, dry wt			
$Ca(^{15}NO_3)_2$	40	46	28	19
$Mg(^{15}NO_3)_2$	35	47	30	16

Table III. *Efflux of Amino-N by Roots of Ryegrass Seedlings*

Experiment 1 shows results during exposure to 1.5 meq l^{-1} Ca $(^{15}NO_3)_2$ or $Mg(^{15}NO_3)_2$.

Solution	Treatment	Amino-N Efflux			
		3 hr	6 hr	12 hr	18 hr
		μg NH_2-N g^{-1} roots, dry wt			
Before hydrolysis	$Ca(^{15}NO_3)_2$	8	11	13	10
	$Mg(^{15}NO_3)_2$	16	16	15	16
Following hydrolysis	$Ca(^{15}NO_3)_2$	17	16	15	64
	$Mg(^{15}NO_3)_2$	23	33	49	133

The data of all three experiments are expressed per g dry weight (70 C) of the root tissue.

RESULTS

The time course of $^{14}NO_3^-$ efflux during $^{15}NO_3^-$ uptake from solutions of $Ca(^{15}NO_3)_2$ or $Mg(^{15}NO_3)_2$ in experiment 1 is shown in Figure 1. Sizeable amounts of $^{15}NO_3^-$ were absorbed during the periods when $^{14}NO_3^-$ appeared in the external solution. Cumulative values for $^{15}NO_3^-$-N absorbed (μg ^{15}N g^{-1} roots, dry weight) after 3-, 6-, 12-, and 18-hr were 2410, 5520, 10,360, and 13,000 for the $Ca(^{15}NO_3)_2$ treatment and 2080, 5490, 8840, and 12,700 for the Mg $(^{15}NO_3)_2$ treatment. Both solutions were competely depleted of NO_3^- between 12 and 18 hr. Maximal $^{14}NO_3^-$ efflux was observed at 3 hr and was 40% greater to $Mg(^{15}NO_3)_2$ than to $Ca(^{15}NO_3)_2$ (Fig. 1). After 3 hr, however, the amount of $^{14}NO_3^-$ in each solution declined, indicating reabsorption of exuded $^{14}NO_3^-$. Total reabsorption had occurred by 18 hr. Exudation of NO_2^- (Table II) and of ninhydrin-reactive constitutents (Table III) also occurred but the quantities of these components recovered in solution were small compared to the magnitude of $^{14}NO_3^-$ efflux (Fig. 1).

Replacement of the original solutions at hourly intervals in the second experiment permitted a more critical examination of the various NO_3^- fluxes. At the end of each hourly period, a decrease in the ^{15}N-enrichment of the $Ca(^{15}NO_3)_2$ and $Mg(^{15}NO_3)_2$ solutions from their initial value of 97.5% was found, and appropriate calculations revealed significant and continual $^{14}NO_3^-$ efflux to the $^{15}NO_3^-$ solutions (Figs. 2 and 3). Cumulative $^{15}NO_3^-$ influx, the best estimate of NO_3^- uptake capacity, substantially exceeded $^{14}NO_3^-$ influx with both Ca^{2+} and Mg^{2+} salts (Figs. 2 and 3). These differences were clearly evident within the 1st hr. After 6-hr exposure to $Ca(^{15}NO_3)_2$, cumulative $^{15}NO_3^-$ influx reached 9.2 mg ^{15}N g^{-1} roots, whereas only 5.7 mg ^{14}N g^{-1} roots were taken up from $Ca(^{14}NO_3)_2$ (Fig. 2). Throughout the experiment $^{14}NO_3^-$ influx from both $Ca(^{14}NO_3)_2$ and $Mg(^{14}NO_3)_2$ occurred at only 60 to 65% of the capacity of the roots for NO_3^- uptake as estimated from $^{15}NO_3$ influx. Influx of $^{15}NO_3^-$ was slightly larger from $Mg(^{15}NO_3)_2$ than from $Ca(^{15}NO_3)_2$ (Figs. 2 and 3).

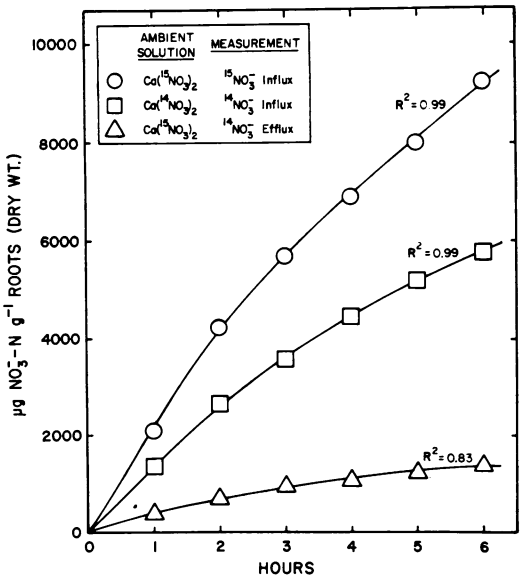

FIG. 2. Time course of cumulative NO_3^- influx and efflux by seedlings during exposure to solutions of $Ca(^{15}NO_3)_2$ or $Ca(^{14}NO_3)_2$ in experiment 2.

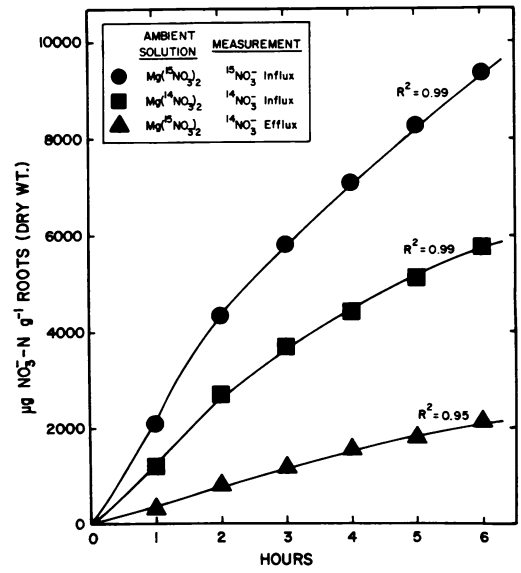

FIG. 3. Time course of cumulative NO_3^- influx and efflux by seedlings during exposure to solutions of $Mg(^{15}NO_3)_2$ or $Mg(^{14}NO_3)_2$ in experiment 2.

Efflux of $^{14}NO_3^-$ to $Ca(^{15}NO_3)_2$ solutions was also observed in experiment 3. The higher ambient $^{15}NO_3^-$ concentration elicited a more rapid $^{14}NO_3^-$ efflux during phase A (Fig. 4). Seedlings which had been exposed to 1.4 meq $Ca(^{14}NO_3)_2$ l^{-1} for a total of 9 hr (treatment I, phase B) prior to exposure to $Ca(^{15}NO_3)_2$ exuded more $^{14}NO_3^-$ than those receiving only the 3-hr pretreatment (treatment II, phase A).

Following absorption from $Ca(^{15}NO_3)_2$ solutions containing either 1.4 meq l^{-1} or 2.8 meq l^{-1}, $^{15}NO_3^-$ efflux to $^{14}NO_3^-$ solutions was also demonstrated (Fig. 5). The $^{15}NO_3^-$ efflux rate was greater at the higher concentration of $^{14}NO_3^-$ in the ambient solution.

DISCUSSION

Proposed Model for NO_3^- Uptake. It is necessary to account for the simultaneous occurrence of the influx and efflux phenomena observed in these experiments. Based in part on the model proposed by Oertli (20) in describing the absorption isotherm for Rb^+ uptake, a schematic representation of a proposed mechanism for NO_3^- uptake is shown in Figure 6. The salient features of the proposed mechanism are:

1. A NO_3^- pumping mechanism (P) located at the outer unstirred layer-plasmalemma interface, the operation of which is dependent on aerobic metabolism (9, 11, 19). No specific type of active mechanism is assigned to the pump. It is, however, assumed to be inwardly directed only and is, therefore, shown as a diode. It is assumed that P operates at, or close to, its maximal capacity.
2. A passive leak mechanism whose exit (or entry) through

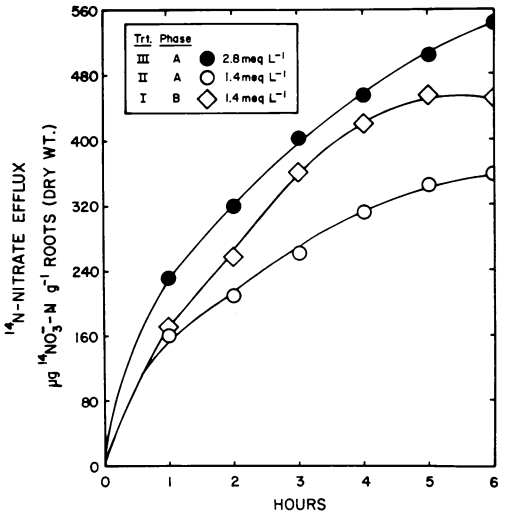

FIG. 4. Time course of cumulative $^{14}NO_3^-$ efflux to $Ca(^{15}NO_3)_2$ solutions of two concentrations, experiment 3. The open squares are for seedlings that had absorbed $^{14}NO_3^-$ under the exact conditions of the experiment for 6 hr prior to exposure to $^{15}NO_3^-$ (Table I).

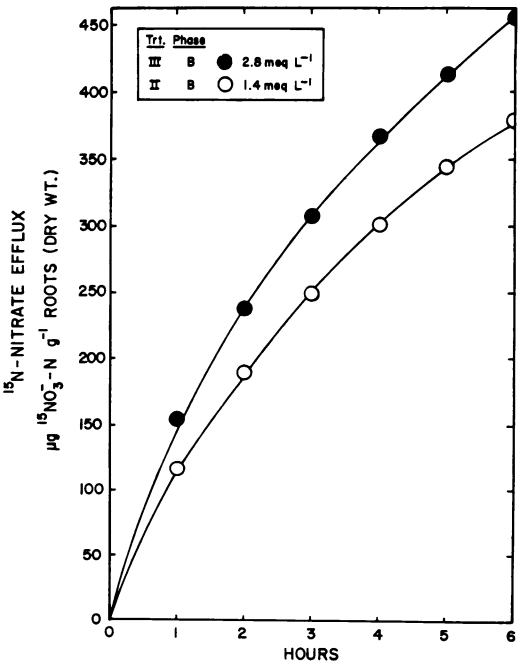

FIG. 5. Time course of cumulative $^{15}NO_3^-$ efflux to $Ca(^{14}NO_3)_2$ solutions of two concentrations following a previous 6-hr exposure to $^{15}NO_3^-$, experiment 3 (Table I).

the plasmalemma (M) is designated L. The leak may function either toward the ambient solution (S) or the cytoplasm (C), depending on the direction of the concentration gradient existing between the internal and external surfaces of the plasmalemma. The bidirectional nature of the leak seems justified since on the one hand, passive NO_3^- influx has been previously reported (11, 19) while on the other, efflux in general is a common feature of ion-root relationships (*cf.* "Introduction"). It is envisaged that L and P are quite adjacent, each representing areas along the plasmalemma which may, in fact, overlap. For an individual root cell, a number of such L-P associations exist.

3. A recycling pathway along which NO_3^- diffusion from the cytoplasm (via L) to P may occur.

4. An outer (O) and inner (I) unstirred layer whose physical and chemical characteristics are quite different from those of the solution (S) and cytoplasm (C) with which they are in contact (7).

5. A series of resistances (R) indicated by subscripts referring to the regions within which the resistances are operative. It is assumed that the magnitude of the various resistances to NO_3^- diffusion is the same for all isotopes of the same ion and that R_o is greater than R_{lp}.

The fate of exogenous NO_3^- on entry into root cells is also indicated in Figure 6 and, at any instant, the NO_3^- concentration within the root cytoplasm is the net result of the indicated reaction sequence. Passive NO_3^- fluxes in the root-solution system, then, are postulated to arise from the NO_3^- concentration gradients between (*a*) C and L, (*b*) L and P, and (*c*) L and S.

Application of Model. In this section, the proposed model for NO_3^- uptake is applied to the results obtained. The following definitions and nomenclature apply: (*a*) $[X]_l$, $[X]_p$, $[X]_s$, or $[X]_c$ = the concentration of X at L, P, S, and C respectively, where X represents either $^{14}NO_3^-$ or $^{15}NO_3^-$; (*b*) leakage = diffusion of $^{14}NO_3^-$ or $^{15}NO_3^-$ from C to L; (*c*) recycling = diffusion of $^{14}NO_3^-$ or $^{15}NO_3^-$ from L to P; (*d*) efflux = diffusion of $^{14}NO_3^-$ or $^{15}NO_3^-$ from L to S; (*e*) hence, efflux = leakage − recycling.

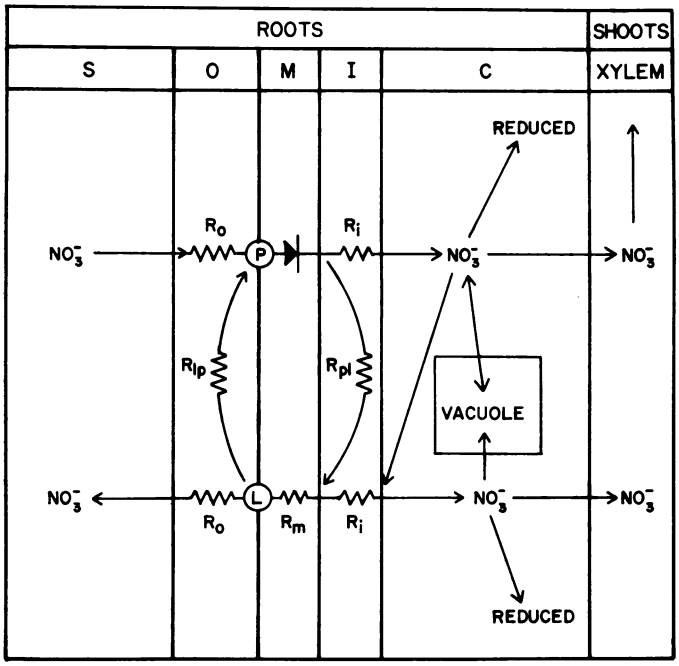

FIG. 6. A generalized model for NO_3^- uptake by root cells and possibilities for NO_3^- reaction within the cytoplasm: S = solution; O = outer unstirred layer; M = plasmalemma; I = inner unstirred layer; C = cytoplasm.

The plants were grown in $^{14}NO_3^-$ so $[^{15}NO_3^-]_p$ and $[^{15}NO_3^-]_c$ may be assumed to have been vanishingly low when they were first exposed to the treatment solutions. On the other hand, $[^{14}NO_3^-]_c$ would be high and greater than $[^{14}NO_3^-]_p$. Upon exposure to the $Ca(^{14}NO_3)_2$ or $Mg(^{14}NO_3)_2$ treatments of experiment 2, $^{14}NO_3^-$ would diffuse from S to P. Simultaneously there would be occurring leakage of $^{14}NO_3^-$ from C to L ($[^{14}NO_3^-]_c > [^{14}NO_3^-]_l$) and a recycling of these leaked $^{14}NO_3^-$ ions to P since $R_o > R_{lp}$. If, as a result, $^{14}NO_3^-$ accumulated at P, a restriction in the diffusion rate of $^{14}NO_3^-$ from S to P would develop, leading to a lower $^{14}NO_3^-$ influx rate than had previously been the case. In this view the $^{14}NO_3^-$ influx rate would be directly or indirectly dependent on (*a*) the extent of recycling and (*b*) the activity of the absorption mechanism (P). In contrast, when roots were exposed to $^{15}NO_3^-$ solutions, $[^{15}NO_3^-]_p$ would be essentially nil while the initial $[^{14}NO_3^-]_p$ would have been finite, since it was being supplied continuously from endogenous $^{14}NO_3^-$ via the leak and recycling pathway. Hence, in response to a $^{15}NO_3^-$ concentration gradient considerably greater than that of $^{14}NO_3^-$, the diffusive flux of $^{15}NO_3^-$ from S to P would be greater than that of $^{14}NO_3^-$ from an otherwise identical ambient solution (Figs. 2 and 3).

The appearance of $^{14}NO_3^-$ in solution on exposure to $^{15}NO_3^-$ in experiments 1 and 2 (Figs. 1, 2, and 3) and the subsequent reabsorption of the previously exuded $^{14}NO_3$ after the 3rd hr when the ambient solution was not continually replaced (Fig. 1) are consistent with the proposed mechanism. As $^{15}NO_3^-$ influx continued, $^{14}NO_3^-$ and $^{15}NO_3^-$ would leak from C to L in proportion to their respective concentration gradients between the inner surface of the plasmalemma and L. Because of the presence of the inner unstirred layer (I) complete mixing of $^{15}NO_3^-$ with $^{14}NO_3^-$ in the cytoplasm need not occur prior to its diffusion to L.

In the early stages, $^{14}NO_3^-$ would be the dominant species undergoing leakage since $[^{14}NO_3^-]_c$ would be high and $[^{15}NO_3^-]_c$ low. As the endogenous $^{14}NO_3^-$ accessible to the leakage channels became depleted during continual exposure to $^{15}NO_3^-$ (through enzymatic reduction, translocation, transport to vacuoles, or prior efflux), $^{14}NO_3^-$ leakage and recycling would decline. In plants for which the ambient solutions were not continually replaced, the diffusion gradients ultimately would shift such that the $^{14}NO_3^-$ which had previously exuded to the external solution would subsequently diffuse back again either to P or through the leakage channels into the cytoplasm (Fig. 1). Since $[^{14}NO_3^-]_s$ was minimized in experiment 2 by periodically replacing the $Ca(^{15}NO_3)_2$ and $Mg(^{15}NO_3)_2$ solutions, $^{14}NO_3^-$ efflux would continue to occur (Figs. 2 and 3). Nonetheless, it appears that the amount of endogenous $^{14}NO_3^-$ available for efflux was approaching depletion as evidenced by the decrease in rates of $^{14}NO_3^-$ efflux.

The greater rate of $^{14}NO_3^-$ efflux to the higher concentration of $^{15}NO_3^-$ during phase A (Fig. 4) and the greater rate of $^{15}NO_3^-$ efflux to the higher concentration of $^{14}NO_3^-$ during phase B (Fig. 5) of experiment 3 also support the proposed model in that the higher ambient solution concentrations should result in a greater proportion of the ambient isotopic species (relative to those undergoing leakage from the cytoplasm) at P. Hence, a greater tendency should exist for the tissue ions to continue to diffuse to the external solution.

Curvilinearity of the Fluxes. Prediction equations for the cumulative values of each flux over the 6-hr period were computed for experiment 2. All patterns obeyed quadratic functions of the type $y = a + bt + ct^2$ where y = cumulative uptake and t = time in hours. As noted by the R^2 values in Figures 2 and 3, the prediction equations described the data quite accurately. For each individual set of observations, the

use of orthogonal polynomials resulted in independent linear and quadratic coefficients, thus permitting direct comparison of coefficients in any two equations. A consequence of the number of time variables employed (*i.e.*, seven) and of the use of orthogonal polynomials was that the linear coefficients reflect the slopes of the curves at 3 hr. The relative magnitudes of the linear and quadratic coefficients are presented in Table IV. The linear component of NO_3^- uptake is envisaged to be that rate which would obtain if absorption occurred from a NO_3^- pool into a sink whose components did not limit the uptake rate; an attendent assumption here is that ambient NO_3^- concentrations not be depleted in such a way that they become rate-limiting. In this view, linear rates reflect the capacity of the tissue to absorb either $^{14}NO_3^-$ or $^{15}NO_3^-$. It is seen in Table IV that such capacity was smaller for $^{14}NO_3^-$ uptake than for $^{15}NO_3^-$ uptake whether the ambient cation was Ca^{2+} or Mg^{2+}. In contrast, the quadratic components for $^{14}NO_3^-$ influx tended to exceed those for $^{15}NO_3^-$ influx.

It is unlikely that the quadratic nature of the influx patterns (Figs. 2 and 3) resulted from progressively limiting ambient NO_3^- concentration because the removal of solution $^{15}NO_3^-$ never exceeded 12% of that originally offered. We suggest that the progressively decreasing rate of influx was due to increased recycling. Alternatively, the experimental conditions may have resulted in an impairment of the absorption mechanism after about 2 hr, although we have no reason to expect this to have happened.

Effect of Ambient Cation. Figure 7, combines the $^{15}NO_3^-$ influx and $^{14}NO_3^-$ efflux for the $Ca(^{15}NO_3)_2$ and $Mg(^{15}NO_3)_2$ treatments shown in Figures 2 and 3. Also included in Figure 7 is net NO_3^- influx ($^{15}NO_3^-$ influx minus $^{14}NO_3^-$ efflux) for the two treatments. The relative sizes of the coefficients of these fluxes are shown in Table V. Although the patterns with the two divalent cations were very similar (Fig. 7), $^{15}NO_3^-$ influx in the presence of Mg^{2+} significantly exceeded that in the presence of Ca^{2+}. This stimulation arose from enhanced linear uptake only (Table 5) and is consistent with the general observation of enhanced anion uptake from single salt solutions containing a rapidly absorbed cation *versus* a slowly absorbed cation. In this context Mg^{2+} is commonly absorbed by roots more rapidly than Ca^{2+} (12, 17). Moreover, results of experiments to be published elsewhere showed that $^{28}Mg^{2+}$ was absorbed more rapidly than $^{45}Ca^{2+}$ by ryegrass plants similar to those used here.

The largest effect of cation moiety was on $^{14}NO_3^-$ efflux which was stimulated substantially when Mg^{2+}, rather than Ca^{2+}, was the counter ion (Figs. 1 and 7; Table V). The direct consequence of this Mg^{2+}-stimulated $^{14}NO_3^-$ efflux was to depress the net influx of NO_3^- from $Mg(^{15}NO_3)_2$ compared to $Ca(^{15}NO_3)_2$ (Fig. 7, Table V).

Table IV. *Comparison of Linear and Quadratic Coefficients of $^{14}NO_3^-$ Influx from Ca^{2+} or Mg^{2+} Salts with Those of $^{15}NO_3^-$ Influx*

Experiment 2, linear comparisons refer to the 3rd hour.

Comparison	Ambient Cation	Relationship	
		Linear coefficient	Quadratic coefficient
$^{15}NO_3^-$ influx *vs.* $^{14}NO_3^-$ influx	Ca^{2+}	$^{15}NO_3^- > {}^{14}NO_3^-$ **	$^{14}NO_3^- > {}^{15}NO_3^-$ *
$^{15}NO_3$ influx *vs.* $^{14}NO_3^-$ influx	Mg^{2+}	$^{15}NO_3^- > {}^{14}NO_3^-$ **	$^{14}NO_3^- > {}^{15}NO_3^-$ **

* $P \leq 0.05$.
** $P \leq 0.01$.

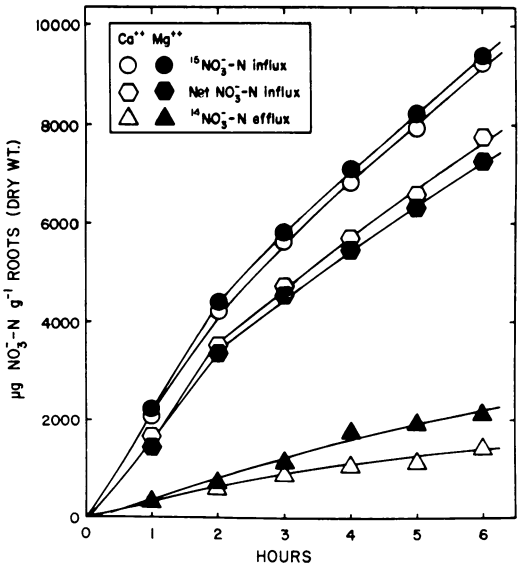

FIG. 7. Time course of cumulative NO_3^- influx and efflux by seedlings during exposure to solutions of $Ca(^{15}NO_3)_2$ (open symbols) and $Mg(^{15}NO_3)_2$ (closed symbols), experiment 2. Influx of $^{15}NO_3^-$ and efflux of $^{14}NO_3^-$ are replotted from Figures 2 and 3; net NO_3^- influx is calculated as the difference between these two values.

Table V. *Comparison of the Linear and Quadratic Coefficients of NO_3^- Influx and Efflux as Affected by Ambient Cation*

Experiment 2, linear comparisons refer to the 3rd hour.

Flux Parameter	Relationship	
	Linear coefficient	Quadratic coefficient
$^{15}NO_3^-$ influx	$Mg^{2+} > Ca^{2+}$ *	NS
Net influx†	$Ca^{2+} > Mg^{2+}$ **	NS
$^{14}NO_3^-$ influx	NS	NS
$^{14}NO_3^-$ efflux	$Mg^{2+} > Ca^{2+}$ **	NS

† Net influx = $^{15}NO_3^-$ influx − $^{14}NO_3$ efflux (to the $^{15}NO_3^-$ solution).
* $P \leq 0.10$.
** $P \leq 0.01$.

The fact that cumulative $^{14}NO_3^-$ efflux, as well as amino-N efflux, were restricted by the presence of ambient Ca^{2+} relative to Mg^{2+} (Fig. 1 and 7; Table III) suggests decreased membrane permeability in the presence of Ca^{2+} (22, 24). However, it is also possible that the enhanced $^{14}NO_3^-$ efflux in the absence of ambient Ca^{2+} resulted from a more favorable $^{14}NO_3^-$ concentration gradient between the cytoplasm and the solution. Such a situation could arise if ambient Mg^{2+} compared to ambient Ca^{2+} resulted in a relatively greater accumulation of $^{14}NO_3^-$ in the root tissue (18).

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