

# Nitrate Absorption and Assimilation in Ryegrass as Influenced by Calcium and Magnesium<sup>1</sup>

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## ABSTRACT

The absorption and assimilation patterns of  $^{15}\text{NO}_3^-$  supplied as the  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  salts to intact ryegrass (*Lolium perenne*) seedlings were compared. No statistically significant effect of ambient cation on the amounts of  $^{15}\text{NO}_3^-$  absorbed was observed in the initial six hours, but during the subsequent six hours, absorption from  $\text{Ca}(^{15}\text{NO}_3)_2$  exceeded that from  $\text{Mg}(^{15}\text{NO}_3)_2$ .

Lower rates of  $^{15}\text{NO}_3^-$  assimilation were found in roots exposed to  $\text{Mg}(^{15}\text{NO}_3)_2$  than in those exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$ . It was proposed that  $\text{Mg}^{2+}$  initiated a restriction in  $^{15}\text{NO}_3^-$  reduction in roots, probably as a consequence of a  $\text{Mg}^{2+}$ -induced physiological  $\text{Ca}^{2+}$  deficiency. Lower  $^{15}\text{N}$  translocation rates were also observed from  $\text{Mg}(^{15}\text{NO}_3)_2$ . These effects of  $\text{Mg}^{2+}$  in depressing  $^{15}\text{NO}_3^-$  assimilation and translocation occurred prior to an effect on  $^{15}\text{NO}_3^-$  uptake.

In shoots, larger amounts of reduced  $^{15}\text{N}$  products occurred with  $\text{Ca}(^{15}\text{NO}_3)_2$  than with  $\text{Mg}(^{15}\text{NO}_3)_2$ . It was concluded that this was due to enhanced translocation of  $^{15}\text{NO}_3^-$  (and possibly its reduced products) in presence of  $\text{Ca}^{2+}$  rather than to specific cation effects on  $^{15}\text{NO}_3^-$  assimilation in the shoots.

and assimilation by roots, and the possibility of  $\text{Ca}^{2+}$  modifying these processes, prompted the present investigation. We reasoned that  $\text{Ca}^{2+}$ , or  $\text{Mg}^{2+}$ , or both, might affect  $\text{NO}_3^-$  reduction by roots by: (a) influencing the rate of  $\text{NO}_3^-$  absorption, thereby influencing both the amount of  $\text{NO}_3^-$  available for reduction and the amount of nitrate reductase (a substrate-inducible enzyme); (b) directly altering the *in vivo* activities of  $\text{NO}_3^-$  reduction and assimilatory enzymes; and (c) modifying the translocation of  $\text{NO}_3^-$  to the shoots, thereby indirectly altering the amount of  $\text{NO}_3^-$  available for reduction in the roots. We report here results of two experiments with perennial ryegrass (*Lolium perenne*). The first was conducted to determine the general pattern of  $^{15}\text{N}$  uptake from  $\text{Ca}(^{15}\text{NO}_3)_2$  and its relative distribution between roots and shoots. The more detailed second experiment compared the effects of ambient  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  on the  $^{15}\text{N}$  absorption, assimilation, and translocation patterns of plants supplied with highly enriched  $^{15}\text{NO}_3^-$ . The data indicate that absence of  $\text{Ca}^{2+}$  in the ambient medium resulted in alterations in the products of  $^{15}\text{NO}_3^-$  assimilation prior to the time that net  $^{15}\text{NO}_3^-$  uptake was affected significantly.

## MATERIALS AND METHODS

**Experiment I, 30-Day-Old Plants.** Seeds of perennial ryegrass (*L. perenne*) were surface-sterilized with 5%  $\text{H}_2\text{O}_2$  and germinated in opaque polyethylene cups with bottoms of stainless steel screen. Developing roots thus grew through the screen into aerated nutrient solution. Each cup (one culture) contained 25 seedlings after thinning. Forty cultures were placed in 13 liters of aerated nutrient solution and the plants were grown in a chamber maintained at 24 to 25 C during the light period (16 hr, 75.6 hectolux at leaf surface) and at 18 to 19 C during the dark period. The nutrient solution contained 50  $\mu\text{M}$   $\text{K}_2\text{SO}_4$ , 50  $\mu\text{M}$   $\text{MgSO}_4$ , 0.1 mM  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 0.25 mM  $\text{NaNO}_3$ , 1 mg iron per liter as  $\text{FeEDTA}$ , and one-fifth the trace elements supplied by Hoagland's solution (8). Solutions were changed every other day. At 28 days after sowing,  $\text{NaNO}_3$  was deleted from the solution and 2 days later the experiment was initiated.

Immediately prior to application of the treatment solution roots were rinsed by dipping 10 times in each of two 15-liter tanks of distilled water and extraneous water allowed to drain off. Roots were then exposed to an aerated solution containing 0.25 mM  $\text{Ca}(^{15}\text{NO}_3)_2$  enriched to 46.7 atom %  $^{15}\text{N}$ . Light intensity was 54.0 hectolux and temperature  $28 \pm 1$  C. Except for the initial 30 min when the acidity rose to pH 5.9, solution acidity was maintained at  $\text{pH } 6.5 \pm 0.3$  by periodic addition of dilute  $\text{H}_2\text{SO}_4$  or  $\text{NaOH}$ . Duplicate cultures were harvested at 0, 0.5, 1, 2, 4, 8, 12, and 16 hr after exposure to  $^{15}\text{N}$ ; a single culture was harvested after 24 hr. After rinsing the roots in redistilled water and blotting dry, roots and shoots were weighed separately, freeze-dried, reweighed, and ground in a Wiley mill to pass a 40-mesh screen. The tissue was then

Beneficial effects of ambient  $\text{Ca}^{2+}$ , relative to  $\text{Mg}^{2+}$ , on  $\text{NO}_3^-$  absorption by nitrogen-depleted wheat seedlings have been reported (18), but whether this effect invariably occurs is not known. Similarly, the suggestion (17) that ambient  $\text{Ca}^{2+}$  enhances  $\text{NO}_3^-$  translocation also requires further documentation.

Relatively little direct information is available regarding the extent to which absorbed  $\text{NO}_3^-$  is reduced in root tissue of intact plants. Nitrate reductase activities generally are lower in roots than in leaves (2) although sizable activities have been observed in the former (14-16, 27). Moreover, the presence of amides, amino acids, and ureides in the bleeding xylem sap of decapitated plants exposed to  $\text{NO}_3^-$  indicates a significant capacity for  $\text{NO}_3^-$  reduction in the intact root tissue of some species (3, 22, 23, 29, 30). Whether  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are directly involved in  $\text{NO}_3^-$  assimilation by roots has not been examined, although the accumulation of  $\text{NO}_2^-$  in shoots (7, 24), and lack of carbohydrates in roots of  $\text{Ca}^{2+}$ -deficient plants (11, 20) suggest such an involvement for  $\text{Ca}^{2+}$ .

The paucity of information on  $\text{NO}_3^-$  absorption, reduction,

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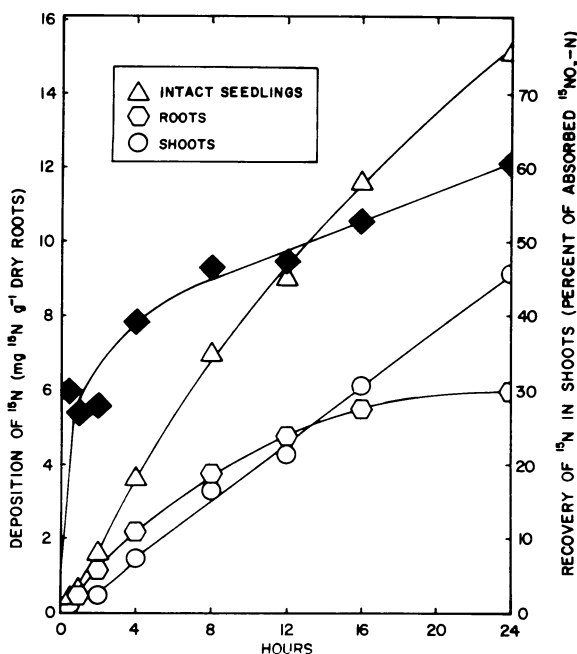


FIG. 1. Deposition of  $^{15}\text{N}$  in roots, shoots, and intact seedlings (open symbols) and percentage of total absorbed  $^{15}\text{NO}_3^-$  recovered in the shoots (closed symbols) of 30-day-old seedlings exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$ .

analyzed for total nitrogen (21) using Devarda's alloy to reduce  $\text{NO}_3^-$  and for  $^{15}\text{N}$  enrichment by mass spectrometric methods (26).

**Experiment II, 21-Day-Old Plants.** Cultures contained 45 to 50 seedlings per cup after thinning. Plants were grown in a chamber at  $22 \pm 1$  C during the light period (16 hr, 238 hectolux) and at  $18 \pm 1$  C during the dark period. Four cultures were placed in 1-liter plastic beakers containing 820 ml of aerated nutrient solution, containing 0.1 mM  $\text{KH}_2\text{PO}_4$ , 0.25 mM  $\text{Ca}(\text{NO}_3)_2$ , and 0.25 mM  $\text{MgSO}_4$ . Iron and other trace elements were supplied as in experiment I. These solutions were replaced every other day, and 2 days prior to the experiment the  $\text{Ca}(\text{NO}_3)_2$  concentration was doubled.

Two experimental treatments were applied 1 hr after the beginning of the light period on the 21st day after sowing. Following thorough rinsing and draining of the roots as previously described, plants were placed in the aerated single salt solutions of  $\text{Ca}(^{15}\text{NO}_3)_2$  or  $\text{Mg}(^{15}\text{NO}_3)_2$ , both applied at 0.75 mM and 97.5 atom %  $^{15}\text{N}$  enrichment. During the 12-hr absorption period, illumination was maintained at 238 hectolux and air temperature at  $24 \pm 1$  C. Initial solution acidity was pH 5.0, but it rose rapidly to 6.8. Four replicates of two cultures each were harvested after 0-, 3-, 6-, and 12-hr exposure to each treatment solution. Root and shoot samples were then treated as outlined for experiment I.

Tissue nitrogen was separated into four fractions. Weighed samples were extracted with cold, 70% ethanol in an Omnimixer and the insoluble residue was separated by Buchner filtration. The ethanol-soluble portion was evaporated to dryness by means of a warm air stream and partitioned into chloroform-soluble and water-soluble constituents. Following acidification of the latter to pH 2.7, Dowex 50W-X8 resin in hydrogen form was used to separate  $\text{NO}_3^-$  from reduced nitrogenous compounds, which were eluted with 1.2 N  $\text{K}_2\text{SO}_4$ .

The  $\text{NO}_3^-$  content of the treatment solutions was determined by ultraviolet absorption at 207 nm (1). Tissue  $\text{NO}_3^-$  was determined by the method of Johnson and Ulrich (12). Total nitrogen in the other three fractions was determined by Cope's

procedure (5). The  $^{15}\text{N}$  enrichment of all fractions was determined by mass spectrometry (26).

All data were calculated on the basis of a unit weight of dry root tissue and analyzed statistically by the analysis of variance technique. Appropriate LSD values are indicated in the figures by the ordinate scale values of vertical lines.

## RESULTS

The patterns of  $^{15}\text{NO}_3^-$  absorption and  $^{15}\text{N}$  translocation by the 30- and 21-day-old plants exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$  were surprisingly similar in view of their different ages, different growing conditions, and the different experimental conditions during the absorption period. In both instances, a decline in the rate of absorption occurred after the first few hours (Figs. 1 and 2A) as a consequence of decreasing accumulation of  $^{15}\text{N}$  in root tissue. During this period the deposition of  $^{15}\text{N}$  in shoots occurred at nearly constant rates ( $\sim 420$  and  $490 \mu\text{g } ^{15}\text{N}$  per g dry roots per hr for the 30- and 21-day-old plants, respectively). With 21-day-old plants,  $^{15}\text{NO}_3^-$  absorption during the first 6 hr was unaffected by the accompanying cation (Fig. 2A). By 12 hr, however, significantly less ( $p \leq 0.01$ )  $^{15}\text{NO}_3^-$  had been absorbed from  $\text{Mg}(^{15}\text{NO}_3)_2$ , due primarily to a diminished accumulation of  $^{15}\text{N}$  in the roots (Fig. 2B).

Accumulation of  $^{15}\text{NO}_3^-$  in the roots was significantly greater ( $p \leq 0.01$ ) during the 3- to 6-hr period when  $\text{Mg}(^{15}\text{NO}_3)_2$ , rather than  $\text{Ca}(^{15}\text{NO}_3)_2$ , was applied (Fig. 3). In the shoots, however, the  $\text{Mg}(^{15}\text{NO}_3)_2$  treatment resulted in less accumulation of  $^{15}\text{NO}_3^-$ , particularly between 6 and 12 hr ( $p \leq 0.01$ ). In contrast to  $^{15}\text{NO}_3^-$ , amounts of  $^{15}\text{N}$ -labeled water-soluble reduced constituents (amino acids, amides) in the shoots of both treatments exceeded those in the roots (Fig. 4) after the 3rd hr. In both roots and shoots,  $\text{Ca}(^{15}\text{NO}_3)_2$  rather than  $\text{Mg}(^{15}\text{NO}_3)_2$ , resulted in greater accumulation of  $^{15}\text{N}$  in the water-soluble reduced N fraction (Fig. 4). The difference was evident in the roots by the 3rd hr ( $p \leq 0.05$ ) and became highly significant ( $p \leq 0.01$ ) by the 6th hr. In spite of sizable variation in the  $^{15}\text{N}$  of this fraction in the shoots, the advantage for the  $\text{Ca}(^{15}\text{NO}_3)_2$  treatments was evident ( $p \leq 0.05$ ) by the 6th hr. On the other hand, accumulation of  $^{15}\text{N}$  in the ethanol-insoluble fraction of both roots and shoots was not significantly affected during the first 6 hr (Fig. 5). Between 6 and 12 hrs, however, significantly greater ( $p \leq 0.01$ )  $^{15}\text{N}$  accumulation in the ethanol-insoluble fraction occurred in both tissues of the  $\text{Ca}(^{15}\text{NO}_3)_2$  treatment compared to  $\text{Mg}(^{15}\text{NO}_3)_2$ . Chloroform-soluble  $^{15}\text{N}$  constituted a relatively small proportion of the total  $^{15}\text{N}$  absorbed (data not presented). No significant influence of the ambient cation on this fraction was detectable in the root tissue; in the shoots the advantage for  $\text{Ca}^{2+}$  was statistically significant ( $p \leq 0.01$ ) only by the 12th hr.

## DISCUSSION

**Influence of  $\text{Ca}^{2+}$  versus  $\text{Mg}^{2+}$  on  $^{15}\text{NO}_3^-$  Absorption.** It has been suggested that ambient  $\text{Ca}^{2+}$  enhances anion uptake by neutralizing negative charges in the boundary region of absorbing cells (10, 25), while  $\text{Mg}^{2+}$  is less effective (9). In the present studies, however, no significant difference between the two cations was observed in net  $^{15}\text{NO}_3^-$  uptake until after 6 hr (Fig. 2A). Since 1 hr was sufficient for  $\text{Mg}(\text{NO}_3)_2$  solutions to displace the peripheral  $\text{Ca}^{2+}$  from roots of ryegrass similar to those used here (19), the initial displacement of  $\text{Ca}^{2+}$  from the roots did not alter the net  $^{15}\text{NO}_3^-$  absorption rates. The decrease in uptake from the  $\text{Mg}(^{15}\text{NO}_3)_2$  treatment after 6 hr was associated with a smaller amount of  $^{15}\text{N}$  reaching the shoots (Fig. 2B). Significant ambient cation effects on the magnitude of the  $^{15}\text{NO}_3^-$  (Fig. 3) and water-soluble reduced  $^{15}\text{N}$  (Fig. 4) pools occurred prior to the effect on net  $^{15}\text{NO}_3^-$  absorption (Fig. 2A).

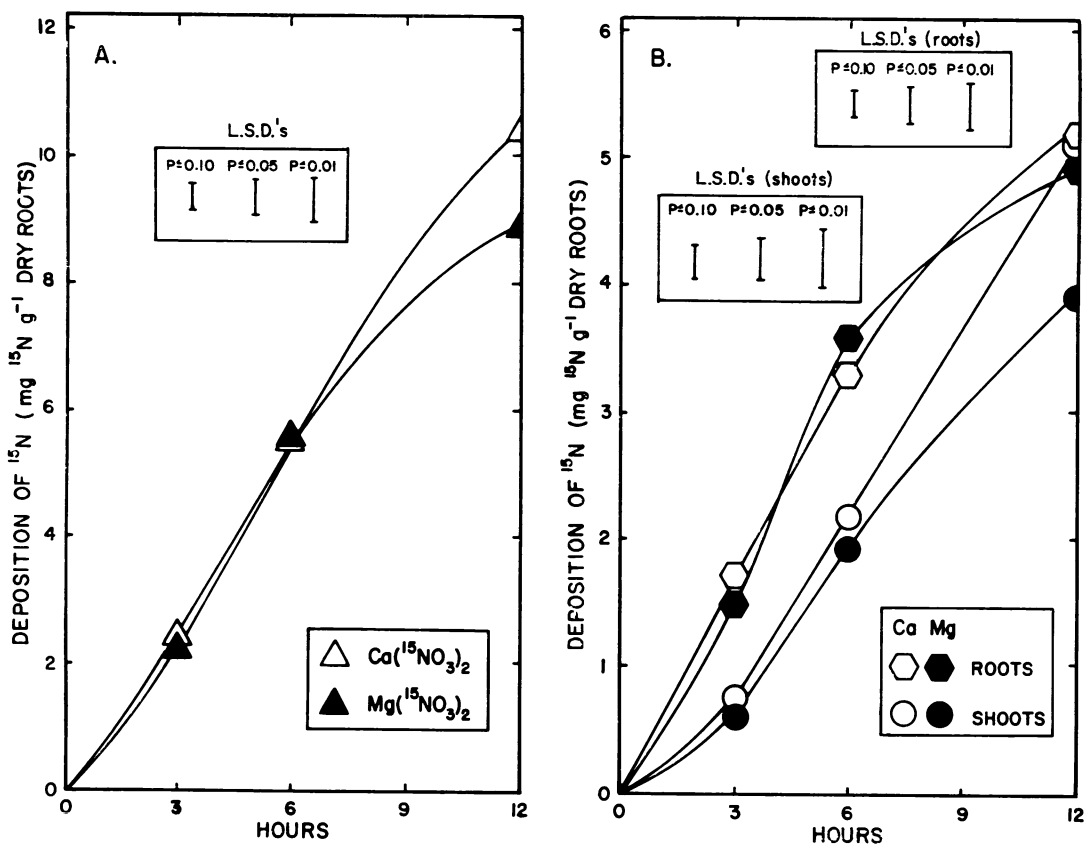


FIG. 2. Deposition of  $^{15}\text{N}$  in intact seedlings (A) and total  $^{15}\text{N}$  accumulation in roots (B) and shoots (B) of 21-day-old plants exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$  or  $\text{Mg}(^{15}\text{NO}_3)_2$ .

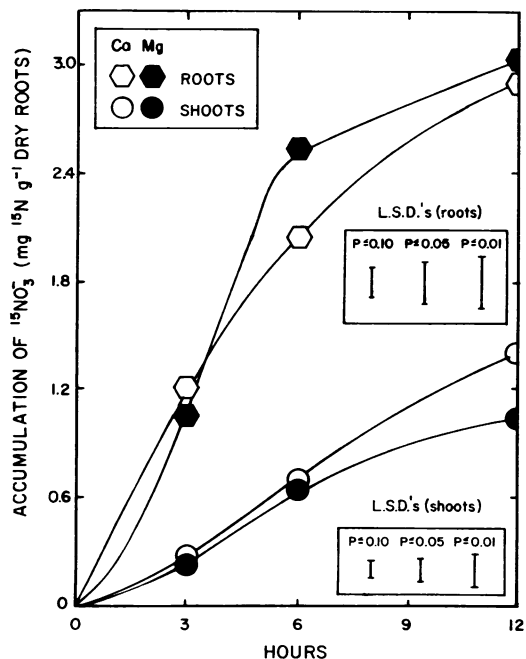


FIG. 3. Accumulation of  $^{15}\text{NO}_3^-$  in roots and shoots of 21-day-old seedlings exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$  or  $\text{Mg}(^{15}\text{NO}_3)_2$ .

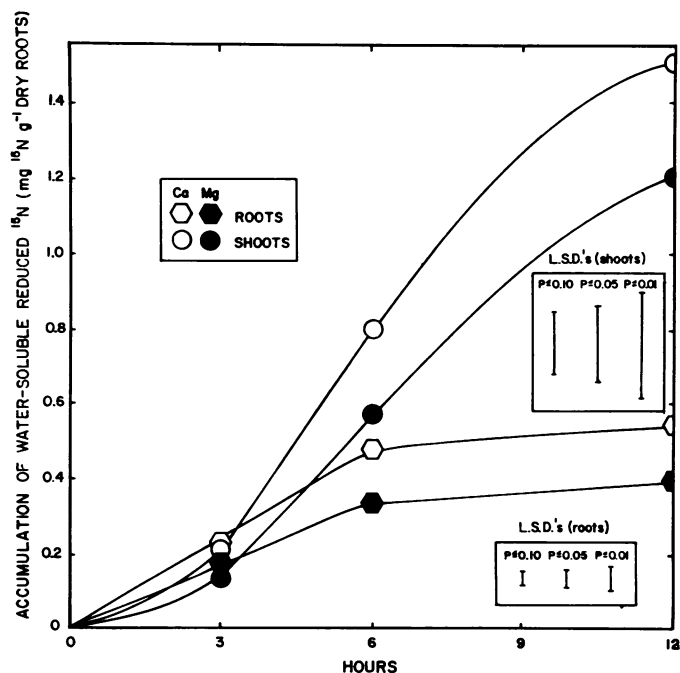


FIG. 4. Accumulation of water-soluble reduced  $^{15}\text{N}$  in roots and shoots of 21-day-old seedlings exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$  or  $\text{Mg}(^{15}\text{NO}_3)_2$ .

The data therefore indicate an influence of the ambient cation on nitrogen assimilation reactions prior to an influence on net  $^{15}\text{NO}_3^-$  uptake.

**Influence of  $\text{Ca}^{2+}$  versus  $\text{Mg}^{2+}$  on  $^{15}\text{N}$  Translocation.** After the first 2 or 3 hr, translocation of absorbed  $^{15}\text{N}$  to the shoots oc-

curred at nearly constant rates when 30-day-old or 21-day-old plants were exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$ . The constant rate occurred in spite of a decreasing rate of accumulation of  $^{15}\text{N}$  in the root tissue (Figs. 1 and 2B). Even during the first 3 hr the amount of  $^{15}\text{N}$  reaching the shoots constituted nearly 30% of the amount

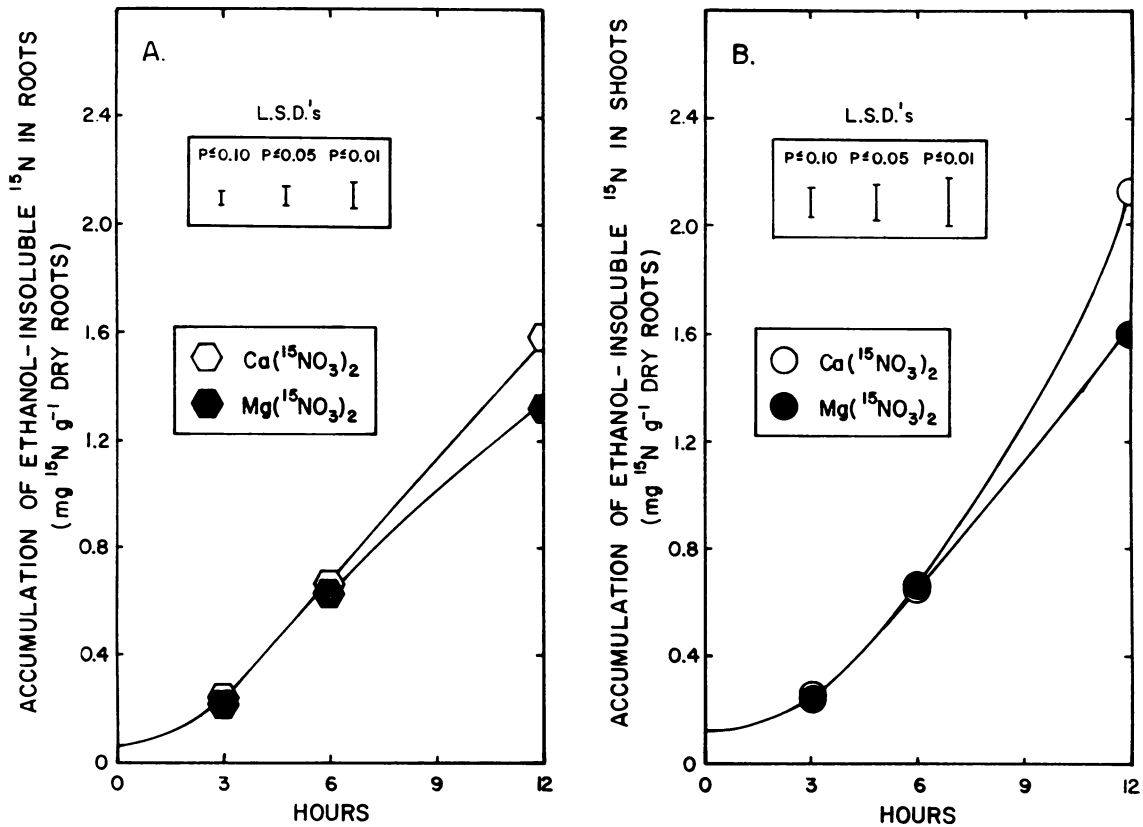


FIG. 5. Accumulation of ethanol-insoluble  $^{15}\text{N}$  in roots (A) and shoots (B) of 21-day-old seedlings exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$  or  $\text{Mg}(^{15}\text{NO}_3)_2$ .

absorbed. It therefore appears that the rate of  $^{15}\text{N}$  translocation was not closely dependent upon the rate of  $^{15}\text{N}$  accumulation in root tissue, and that an efficient translocation system was operating in concert with the  $^{15}\text{NO}_3^-$  accumulation and assimilation systems in the root tissue.

Exposure to  $\text{Mg}(^{15}\text{NO}_3)_2$ , rather than  $\text{Ca}(^{15}\text{NO}_3)_2$ , decreased the translocation of  $^{15}\text{N}$ , particularly after 6 hr (Fig. 2B). Calculations of the proportion of total absorbed  $^{15}\text{N}$  which was recovered in the shoots (percentage translocation) reveal a significantly ( $p \leq 0.01$ ) higher value (39.7%) for  $\text{Ca}(^{15}\text{NO}_3)_2$  than for  $\text{Mg}(^{15}\text{NO}_3)_2$  (34.7%) by the 6th hr although the total amount of  $^{15}\text{NO}_3^-$  absorbed (Fig. 2A) was nearly identical for the two treatments at this time.

Concentrations of  $\text{NO}_3^-$  and water-soluble reduced N compounds in the xylem translocation stream can vary considerably (22, 23, 29, 30). It is therefore not possible to decide for certain whether the lower  $^{15}\text{N}$  translocation in the  $\text{Mg}(^{15}\text{NO}_3)_2$  treatment was due entirely to lower translocation of  $^{15}\text{NO}_3^-$ , entirely to lower translocation of water-soluble reduced  $^{15}\text{N}$ , or a combination of the two effects. The data do indicate, however, that  $^{15}\text{NO}_3^-$  translocation was depressed. A minimal value for  $^{15}\text{NO}_3^-$  translocated during a specific time is given by the increase in  $^{15}\text{NO}_3^-$  in the shoots during that time. For the 6- to 12-hr period, the increase in  $^{15}\text{NO}_3^-$  was considerably greater for the  $\text{Ca}(^{15}\text{NO}_3)_2$  treatment (698  $\mu\text{g}$   $^{15}\text{N}$  per g dry roots) than for the  $\text{Mg}(^{15}\text{NO}_3)_2$  treatment (392  $\mu\text{g}$   $^{15}\text{NO}_3^-$ -N per g dry roots; cf. Fig. 3). The total reduced  $^{15}\text{N}$  in the shoots during this period increased by a greater amount with  $\text{Ca}(^{15}\text{NO}_3)_2$  than with  $\text{Mg}(^{15}\text{NO}_3)_2$  (2250 versus 1603  $\mu\text{g}$   $^{15}\text{N}$  per g dry roots, respectively, Fig. 6) which indicates that  $^{15}\text{NO}_3^-$  reduction in the shoots was not impaired by  $\text{Ca}^{2+}$  relative to  $\text{Mg}^{2+}$ . Hence, the increase in  $^{15}\text{NO}_3^-$  in the shoots indicates an advantage for  $\text{Ca}^{2+}$  relative to  $\text{Mg}^{2+}$  in the process of  $^{15}\text{NO}_3^-$  translocation. A bene-

ficial influence of ambient  $\text{Ca}^{2+}$  on  $\text{NO}_3^-$  translocation was suggested earlier by Minotti *et al.* (17).

**Influence of  $\text{Ca}^{2+}$  versus  $\text{Mg}^{2+}$  on  $^{15}\text{NO}_3^-$  Assimilation in Roots.** Reduced  $^{15}\text{N}$  continued to accumulate in the roots throughout the 12-hr absorption period with a maximal rate in the 3- to 6-hr period (Fig. 6A). The decline after 6 hr was associated with decreased rates of accumulation of  $^{15}\text{N}$  in the water-soluble reduced fraction (Fig. 4). Accumulation of the ethanol-insoluble fraction continued nearly linearly to 12 hr (Fig. 5). We assume that translocation of water-soluble reduced  $^{15}\text{N}$  from roots to shoots occurred at a greater rate than the reverse process (23). The quantities of all reduced  $^{15}\text{N}$  in the roots at each harvest (Fig. 6A) therefore represent only minimal values of the actual amounts reduced there. For the  $\text{Ca}(^{15}\text{NO}_3)_2$  treatment, these minimal rates of reduction in the root tissue during the 0- to 3-, 3- to 6-, and 6- to 12-hr time periods were 163, 251, and 181  $\mu\text{g}$   $^{15}\text{N}$  per hr per g dry roots, respectively. The corresponding values for the  $\text{Mg}(^{15}\text{NO}_3)_2$  treatment were 143, 216, and 138  $\mu\text{g}$   $^{15}\text{N}$  per hr per g dry roots, which indicates less efficiency in reduction of the absorbed  $^{15}\text{NO}_3^-$ . During the 3- to 6-hr period, when the minimal rate of  $^{15}\text{NO}_3^-$  reduction clearly became greater with  $\text{Ca}(^{15}\text{NO}_3)_2$ , the accumulation rates of  $^{15}\text{NO}_3^-$  were 279 and 493  $\mu\text{g}$   $^{15}\text{N}$  per hr per g dry roots for the  $\text{Ca}(^{15}\text{NO}_3)_2$  and  $\text{Mg}(^{15}\text{NO}_3)_2$  treatments, respectively (Fig. 3). The greater reduction rate with  $\text{Ca}^{2+}$  was therefore not due to a higher substrate concentration. The advantage for  $\text{Ca}^{2+}$  in total amount of reduced  $^{15}\text{N}$  present in the roots was highly significant ( $p \leq 0.01$ ) by the 6th hr (Fig. 6A). Similarly, the proportion of total  $^{15}\text{N}$  in the roots present in reduced forms was also significantly ( $p \leq 0.01$ ) greater for  $\text{Ca}(^{15}\text{NO}_3)_2$  by the 6th hr (Fig. 7). Since the  $\text{Mg}(^{15}\text{NO}_3)_2$  did not increase the amount of reduced  $^{15}\text{N}$  in the shoots (Fig. 6B) or increase the relative proportion of  $^{15}\text{N}$  present there in reduced form (Fig. 7), this treat-

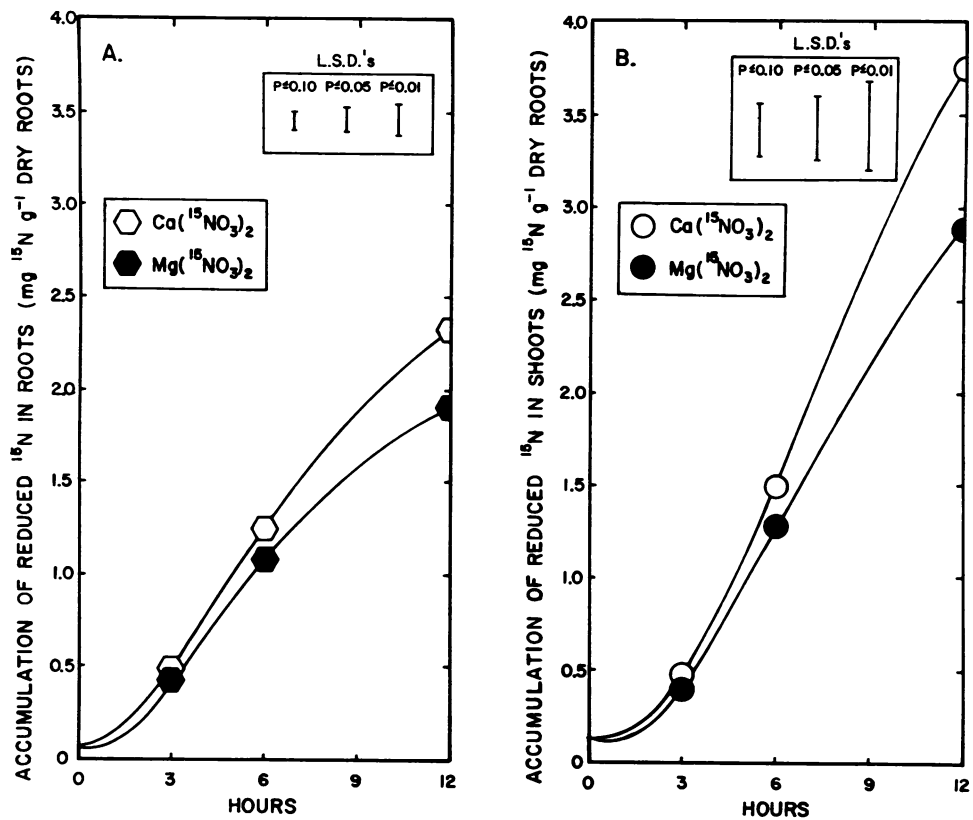


FIG. 6. Accumulation of reduced  $^{15}\text{N}$  in roots (A) and shoots (B) in 21-day-old seedlings exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$  and  $\text{Mg}(^{15}\text{NO}_3)_2$ .

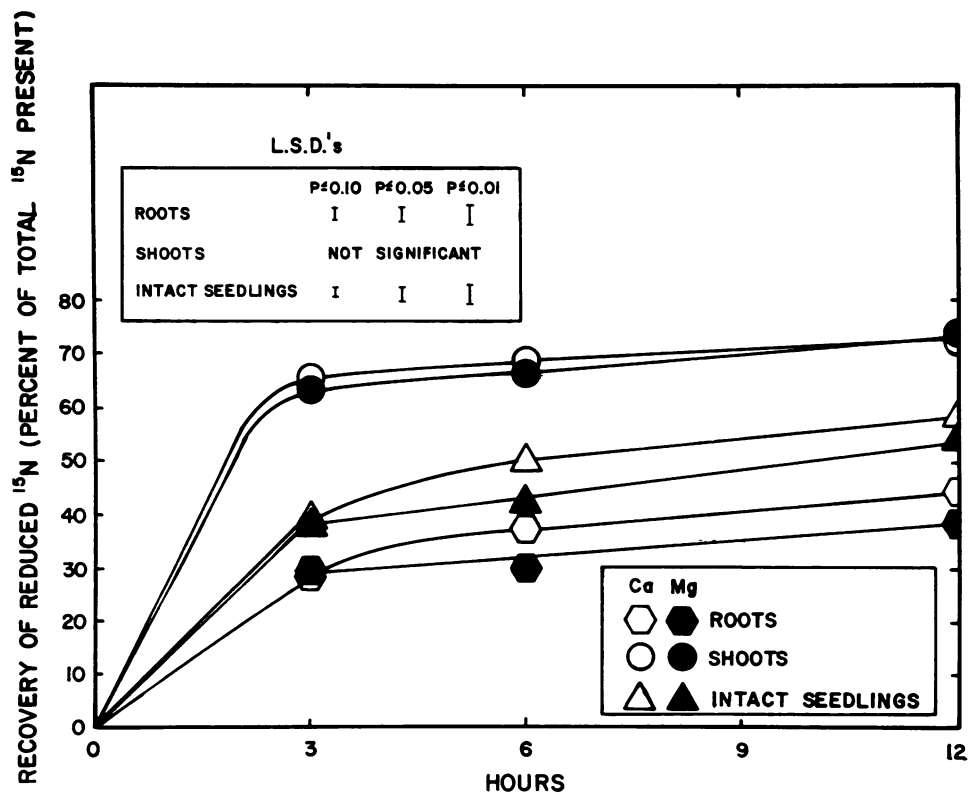


FIG. 7. Percentage of total absorbed  $^{15}\text{NO}_3^-$ -N in roots, shoots, and intact seedlings recovered in the reduced N fraction of 21-day-old seedlings exposed to  $\text{Ca}(^{15}\text{NO}_3)_2$  and  $\text{Mg}(^{15}\text{NO}_3)_2$ .

ment resulted in a restriction in the rate of  $^{15}\text{NO}_3^-$  reduction in the roots. Furthermore, this restriction apparently occurred during the 3- to 6-hr period, *i.e.*, prior to a restriction in  $^{15}\text{NO}_3^-$  absorption (*cf.* Fig. 2A).

The consistency of  $\text{Ca}^{2+}$  versus  $\text{Mg}^{2+}$  in promoting higher amounts of reduced  $^{15}\text{N}$  in the roots indicates that during exposure to  $\text{Mg}(\text{}^{15}\text{NO}_3)_2$  there was a restriction in the reduction of  $^{15}\text{NO}_3^-$  to ammonium, or a restriction in the amination reactions leading to the formation of amino acids, or both. Failure of transport of the intermediates in these reactions to the appropriate reaction centers, as well as decreased concentrations or activities of the appropriate enzymes, could account for the depressed rates. The onset of the restriction in  $^{15}\text{NO}_3^-$  reduction of the roots exposed to  $\text{Mg}(\text{}^{15}\text{NO}_3)_2$  must have been fairly rapid because statistically significant ( $p \leq 0.05$ ) cation effects were exerted on the water-soluble reduced  $^{15}\text{N}$  fraction of the roots by the 3rd hr (Fig. 4). We propose that the restriction resulted from suboptimal cell surface  $\text{Ca}^{2+}$  in the  $\text{Mg}(\text{}^{15}\text{NO}_3)_2$  treatment. The roots contained sizeable  $\text{Mg}^{2+}$  concentrations prior to exposure to the  $^{15}\text{NO}_3^-$  solutions and were not depleted of  $\text{Mg}^{2+}$  appreciably during exposure to  $\text{Ca}(\text{}^{15}\text{NO}_3)_2$  (19). Upon exposure to  $\text{Mg}(\text{}^{15}\text{NO}_3)_2$ , however, two distinct  $\text{Ca}^{2+}$  fractions in the roots were clearly delineated, the largest of which was rapidly displaced within the 1st hr (19). The second fraction was only very slowly displaced for at least 12 hr. Thus, ryegrass roots exposed to  $\text{Mg}(\text{}^{15}\text{NO}_3)_2$  for more than 1 hr were probably deprived of physiologically sufficient amounts of  $\text{Ca}^{2+}$ . Although  $\text{Ca}^{2+}$  deficiency did not influence the efficiency of nitrate reduction in wheat roots (4, 17), a physiological deficiency of  $\text{Ca}^{2+}$  in our ryegrass roots seems the best explanation for the restriction in reduction of  $^{15}\text{NO}_3^-$  when  $\text{Mg}(\text{}^{15}\text{NO}_3)_2$ , rather than  $\text{Ca}(\text{}^{15}\text{NO}_3)_2$ , was applied.

Increased vacuolization (28) is one of the changes (13) in root cells resulting from low ambient  $\text{Ca}^{2+}$ . Accordingly, greater compartmentalization of  $^{15}\text{NO}_3^-$  away from reaction centers could have occurred with  $\text{Mg}(\text{}^{15}\text{NO}_3)_2$  compared to  $\text{Ca}(\text{}^{15}\text{NO}_3)_2$ , the result being an increased  $^{15}\text{NO}_3^-$  accumulation (Fig. 3) and decreased  $^{15}\text{NO}_3^-$  reduction (Fig. 7). Since membrane turnover rates are rapid, possibly approaching 5 min (6), there appears to have been ample opportunity for the cation treatments to have exerted differential influences on membrane synthesis and reconstruction in the present experiments.

**Influence of  $\text{Ca}^{2+}$  versus  $\text{Mg}^{2+}$  on  $^{15}\text{NO}_3^-$  Assimilation in Shoots.** By the 6th hr, the total quantities of reduced  $^{15}\text{N}$  in the shoots exceeded that in the roots (Fig. 6) and as early as the 3rd hr the proportion of total  $^{15}\text{N}$  in the shoots occurring in reduced form was substantially greater than in the roots (65 and 39%, respectively; Fig. 7). The water-soluble reduced  $^{15}\text{N}$  fraction of the roots tended to become saturated by the 6th hr but  $^{15}\text{N}$  continued to accumulate at sizable rates in this fraction of the shoots (Fig. 4). It is possible that the upward translocation process was partially responsible for the continued accumulation of  $^{15}\text{N}$  in the water-soluble reduced  $^{15}\text{N}$  fraction of the shoots (Fig. 4) while removal from this fraction into ethanol-insoluble components (Fig. 5) was proceeding vigorously in the shoot tissue. Because of uncertainty in the magnitude of the upward translocation of water-soluble reduced  $^{15}\text{N}$ , the present data do not permit an accurate estimate of the relative efficiencies of the roots and shoots in  $^{15}\text{NO}_3^-$  reduction and assimilation.

Incorporation of  $^{15}\text{NO}_3^-$  into the reduced components of the shoots was consistently greater with  $\text{Ca}(\text{}^{15}\text{NO}_3)_2$  than with  $\text{Mg}(\text{}^{15}\text{NO}_3)_2$  (Figs. 4 and 5). The larger quantities of  $^{15}\text{N}$  present in the shoots with  $\text{Ca}(\text{}^{15}\text{NO}_3)_2$  (Fig. 2B) were due, in part at least, to the greater rate of  $^{15}\text{NO}_3^-$  translocation discussed previously. Calculations of the proportion of total  $^{15}\text{N}$  present which

was recovered in reduced components of the shoots indicates, in contrast to the roots, no statistically significant difference between the cation treatments (Fig. 7). Hence, the enhanced  $^{15}\text{N}$  assimilation in the shoots with  $\text{Ca}(\text{}^{15}\text{NO}_3)_2$  was probably due to the greater translocation rates of  $^{15}\text{NO}_3^-$  (and possibly water-soluble reduced  $^{15}\text{N}$ ) than to any direct cation effect on the assimilation processes in shoots.

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