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Source and Magnitude of Ammonium Generation in Maize Roots[1]

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Studies with ^{15}N indicate that appreciable generation of NH_4^+ from endogenous sources accompanies the uptake and assimilation of exogenous NH_4^+ by roots. To identify the source of NH_4^+ generation, maize (*Zea mays* L.) seedlings were grown on $^{14}NH_4^+$ and then exposed for 3 d to highly labeled $^{15}NH_4^+$. More of the entering $^{15}NH_4^+$ was incorporated into the protein-N fraction of roots in darkness (approximately 25%) than in the light (approximately 14%). Although the $^{14}NH_4^+$ content of roots declined rapidly to less than 1 μmol per plant, efflux of $^{14}NH_4^+$ continued throughout the 3-d period at an average daily rate of 14 μmol per plant. As a consequence, cumulative $^{14}NH_4^+$ efflux during the 3-d period accounted for 25% of the total ^{14}N initially present in the root. Although soluble organic ^{14}N in roots declined during the 3-d period, insoluble ^{14}N remained relatively constant. In shoots both soluble organic ^{14}N and $^{14}NH_4^+$ declined, but a comparable increase in insoluble ^{14}N was noted. Thus, total ^{14}N in shoots remained constant, reflecting little or no net redistribution of ^{14}N between shoots and roots. Collectively, these observations reveal that catabolism of soluble organic N, not protein N, is the primary source of endogenous NH_4^+ generation in maize roots.

Short-term studies with $^{13}NH_4^+$ have provided estimates of NH_4^+ influx, efflux, and cytoplasmic concentration in spruce (Kronzuker et al., 1995a, 1995b). As the NH_4^+ concentration in the external solution increased from 10 to 1500 μM, cytosolic NH_4^+ levels increased from 2 to 33 mM and efflux increased from 10% to 35% of influx. Similar rates were reported for rice: as external NH_4^+ levels increased from 2 to 1000 μM, cytosolic NH_4^+ levels increased from 3 to 38 mM and efflux rose from 11% to 29% of influx (Wang et al., 1993). The half-lives of cytosolic NH_4^+ were 8 and 15 min for rice and spruce, respectively.

During short-term exposure of cereal seedlings to $^{15}NH_4^+$ solution, efflux of endogenous $^{14}NH_4^+$ exceeded the total $^{14}NH_4^+$ initially present in the root tissues (Morgan and Jackson, 1988a, 1988b). Moreover, after a 5-h pretreatment in $^{15}NH_4^+$ solution, the subsequent efflux of $^{15}NH_4^+$ to the ambient $^{14}NH_4^+$ solution was greater than the initial NH_4^+ content of the root (Morgan and Jackson, 1989). Thus, appreciable generation of NH_4^+ from endogenous organic N sources accompanies concurrent uptake and assimilation of NH_4^+ by roots.

The efflux of NH_4^+ provides only a minimal estimate of endogenous NH_4^+ generation because part of the NH_4^+ is likely reassimilated. In support of this possibility, when NH_4^+ assimilation via Gln synthetase was blocked with Met sulfoxamine, NH_4^+ generation in maize roots was estimated to be 50% faster than concurrent NH_4^+ uptake (Jackson, et al., 1993). The potential for substantial generation, recycling, and efflux of endogenous NH_4^+ in roots is thus indicated.

A crucial question raised by these observations is whether protein turnover is the source of endogenous NH_4^+ generation, or if recycling of intermediates of the NH_4^+ assimilation pathway, such as Gln, is the source. To address this question, maize (*Zea mays*) seedlings that had been grown on $^{14}NH_4^+$ were exposed to highly labeled $^{15}NH_4^+$ for 3 d. It was hypothesized that if protein turnover is the source of NH_4^+ and if part of this NH_4^+ is subject to efflux and translocation to the shoot, a decline in endogenous ^{14}N protein in the root should occur as new protein is synthesized from the entering $^{15}NH_4^+$.

The fact that $^{15}NH_4^+$ was applied during six diurnal periods also permitted us to (a) directly measure of the diurnal pattern of NH_4^+ fluxes into and out of roots, (b) compare $^{15}NH_4^+$ uptake and assimilation by roots during successive light and dark periods, and (c) determine the relationship of the latter processes to carbohydrate levels in shoots and roots.

MATERIALS AND METHODS

Plant Culture

Maize (*Zea mays* L. cv Pioneer 3320) caryopses were germinated at 30°C in contact with 0.1 mM $CaSO_4$. After 30 h, uniform seedlings were selected and their seminal roots excised. Cultures of eight seedlings each were transplanted into 160 L of basal nutrient solution, pH 6.0, containing 0.125 mM $(NH_4)_2SO_4$, 1.25 mM K_2SO_4, 0.25 mM $Ca(H_2PO_4)_2$, 1 mM $CaSO_4$, 46 μM B, 9 μM Mn, 0.8 μM Zn, 0.3 μM Cu, 0.1 μM Mo, and 54 μM Fe as ferric diethylenetriamine pentaacetate. The solution was aerated with compressed air that had been washed with H_2SO_4 and water to remove ambient NH_4^+. A combination of sodium vapor and metal halide lamps provided 1140 $\mu E\ m^{-2}\ s^{-1}$ illumination at canopy height during a 14-h photoperiod (7 AM to 9 PM). The average air temperature during the experiment was 23.4°C ± 1.6°C. Both pH and $[NH_4^+]$ of the nutrient

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solution were checked daily and maintained at pH 6.0 and 0.25 mM, respectively, by continuous injection of $Ca(OH)_2$ and $(NH_4)_2SO_4$.

Experimental Procedure

At the beginning of the dark period on the 7th d after imbibition, four cultures were harvested and the rest were transferred to fresh basal nutrient solution containing 0.22 mM NH_4^+ labeled with 96.1 atom % ^{15}N. An initial sample of $^{15}NH_4^+$ nutrient solution was taken for subsequent analysis. Four additional cultures were harvested and a solution sample was taken at the beginning (7 AM) and end (9 PM) of each light period on the 8th, 9th, and 10th d. The pH and [NH_4^+] of the nutrient solution were checked daily and maintained as described above by continuous injection of $Ca(OH)_2$ and $(^{15}NH_4)_2SO_4$ containing 99.8 atom % ^{15}N.

At harvest, the roots were dipped five times in 0.1 mM $CaSO_4$, excised, blotted lightly, and weighed. After weighing the shoots, which included the remaining seed pieces, all tissue samples were freeze dried, weighed, ground, and mixed thoroughly.

N and ^{15}N Analysis

Shoots, roots, and nutrient solutions were analyzed for NH_4^+ and its ^{15}N enrichment (Jackson et al., 1993). In addition, shoots and roots were analyzed for soluble and insoluble N and their respective ^{15}N enrichments. These fractions were separated by extraction with acidified (pH 3.0) 80% (v/v) ethanol. Organic N in the extract and residue was converted to NH_4^+ by Kjeldahl digestion (McKenzie and Wallace, 1954), and the NH_4^+ was quantified by spectrophotometric analysis (Smith, 1980). NH_4^+ was recovered by diffusion, converted to dinitrogen gas by a freeze-layer procedure (Volk and Jackson, 1979), and analyzed for ^{15}N enrichment by MS.

Total N and ^{15}N analyses were used to calculate the tissue contents of six isotopic N species: $^{14}NH_4^+$, $^{15}NH_4^+$, soluble ^{14}N, soluble ^{15}N, insoluble ^{14}N, and insoluble ^{15}N. Because $^{14}NH_4^+$ and $^{15}NH_4^+$ were subtracted from soluble ^{14}N and soluble ^{15}N, these fractions represent soluble organic N constituents. Protein is the primary constituent of the insoluble-N fraction.

Carbohydrate Analysis

To obtain comparable tissue samples for nonstructural carbohydrate analysis, the experiment was repeated and the seed pieces were discarded at harvest. Insoluble and soluble carbohydrates of shoots and soluble carbohydrates of roots were assayed by enzymatic and spectrophotometric procedures after separation by extraction with 80% (v/v) ethanol (Jones et al., 1977).

RESULTS

Changes in Endogenous ^{14}N Fractions

An appreciable increase in insoluble ^{14}N in shoots (Fig. 1C) was balanced by an equivalent decline in the $^{14}NH_4^+$ and soluble-^{14}N fractions (Fig. 1, A and B). Thus, the total endogenous ^{14}N in shoots remained constant during the 3-d exposure to $^{15}NH_4^+$ (Fig. 1D). By contrast, the insoluble ^{14}N of roots remained relatively constant (Fig. 2C), even though $^{14}NH_4^+$ and soluble ^{14}N decreased appreciably (Fig. 2, A and B). As a consequence, total ^{14}N in the root declined during the 3-d exposure to $^{15}NH_4^+$ (Fig. 2D). On a whole-plant basis, the changes in ^{14}N (Fig. 3) reflected those in the shoot, which contained more than 75% of the total ^{14}N in the plant.

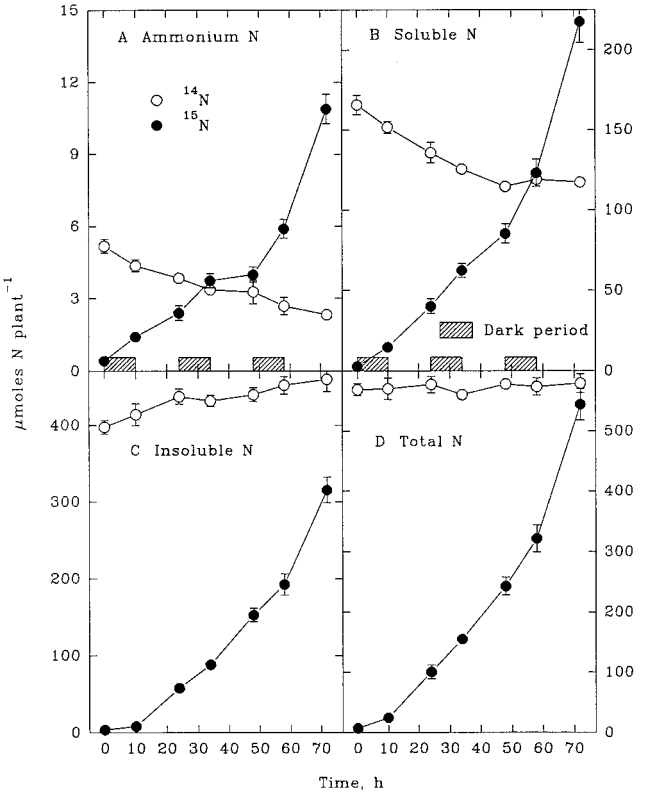

Figure 1. Shoot contents of NH_4^+ (A), soluble N (B), insoluble N (C), and total N (D) derived from endogenous (^{14}N) sources and from exogenously supplied NH_4^+ (^{15}N) during a 3-d continuous exposure of maize seedlings to highly labeled $^{15}NH_4^+$. Each value is the mean of four replicates ± SE.

Estimation of NH_4^+ Fluxes into and out of Roots

During each diurnal period a complete balance sheet of the changes in $^{14}NH_4^+$ and $^{15}NH_4^+$ in the uptake solution was compiled. The procedure is illustrated in Table I using data from the initial 10-h dark period. Even though an appreciable quantity of 99.8 atom % ^{15}N was injected to maintain the [NH_4^+], the ^{15}N enrichment of the nutrient solution declined from 96.1 to 93.5 atom % ^{15}N, reflecting a release of $^{14}NH_4^+$ from the root. The net rate of release, 4.3 μmol plant^{-1} h^{-1}, is less than the actual rate because of concurrent uptake of $^{14}NH_4^+$ from the nutrient solution. The latter can be estimated from the fact that $^{14}NH_4^+$ and $^{15}NH_4^+$ are taken up in proportion to their average molar concentrations in the nutrient solution during any given

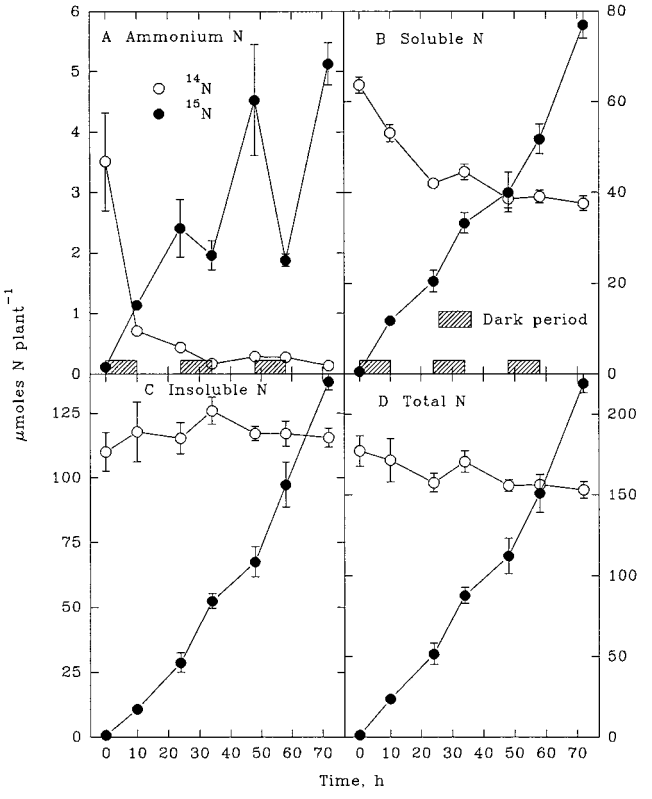

Figure 2. Root contents of NH_4^+ (A), soluble N (B), insoluble N (C), and total N (D) derived from endogenous (^{14}N) sources and from exogenously supplied NH_4^+ (^{15}N) during a 3-d continuous exposure of maize seedlings to highly labeled $^{15}NH_4^+$. Each value is the mean of four replicates ± SE.

period. The calculation (Table I, line G) reveals that $^{14}NH_4^+$ was taken up at a rate of 2.6 μmol plant^{-1} h^{-1}. This rate, when added to the net rate of $^{14}NH_4^+$ release, provides an estimate of "true" $^{14}NH_4^+$ release (Table I, line J). Similar calculations were made throughout the experiment to quantify $^{14}NH_4^+$ release from the root in successive diurnal periods (Table II). Both the uptake and release of NH_4^+ were greater in light than in darkness. NH_4^+ release remained appreciable even in the final light period, when it accounted for 4.8% of the total ^{14}N initially present in roots harvested on the 7th d. As a consequence, cumulative $^{14}NH_4^+$ release during the 3-d period was equivalent to 25% of the total ^{14}N initially present in the root.

It is important to note that the data in Table II cannot be compared directly with the data in Figure 2. Table II shows the true release of $^{14}NH_4^+$ from roots, which consists of measured net release of $^{14}NH_4^+$ plus the concurrent, calculated uptake of $^{14}NH_4^+$. By contrast, Figure 2 portrays the net change in root ^{14}N, which reflects only net release of $^{14}NH_4^+$ from the root, provided that no interchange of ^{14}N between roots and shoots occurs.

Uptake and Assimilation of Applied $^{15}NH_4^+$

The rate of $^{15}NH_4^+$ uptake by maize seedlings was similar during light and dark periods (Fig. 3D). A small but measurable amount of the entering $^{15}NH_4^+$ accumulated as NH_4^+ in the shoot, and the amount increased appreciably as the plants developed (Fig. 1A). By contrast, $^{15}NH_4^+$ accumulation in the root exhibited a diurnal pattern (Fig. 2A). Except for the initial dark period, root $^{15}NH_4^+$ increased during illumination and declined in darkness.

Most of the entering $^{15}NH_4^+$ was assimilated into the soluble-^{15}N and insoluble-^{15}N fractions (Fig. 3, B and C). Although the patterns of accumulation in shoots (Fig. 1, B and C) and roots (Fig. 2, B and C) were similar to those in the whole plant, diurnal differences in assimilation and translocation were evident (Fig. 4). During each dark period a greater percentage of the entering $^{15}NH_4^+$ was retained in the root and incorporated into the insoluble-^{15}N fraction. Conversely, during each light period a greater percentage of the entering $^{15}NH_4^+$, or metabolites thereof, was translocated to the shoot and incorporated into the shoot insoluble-^{15}N fraction.

Diurnal Changes in Dry Matter and Carbohydrate

The dry weight of maize shoots generally increased during light periods and declined during dark periods (Fig. 5). By contrast, root weight increased at least as rapidly in darkness as in light. Although the carbohydrate content of

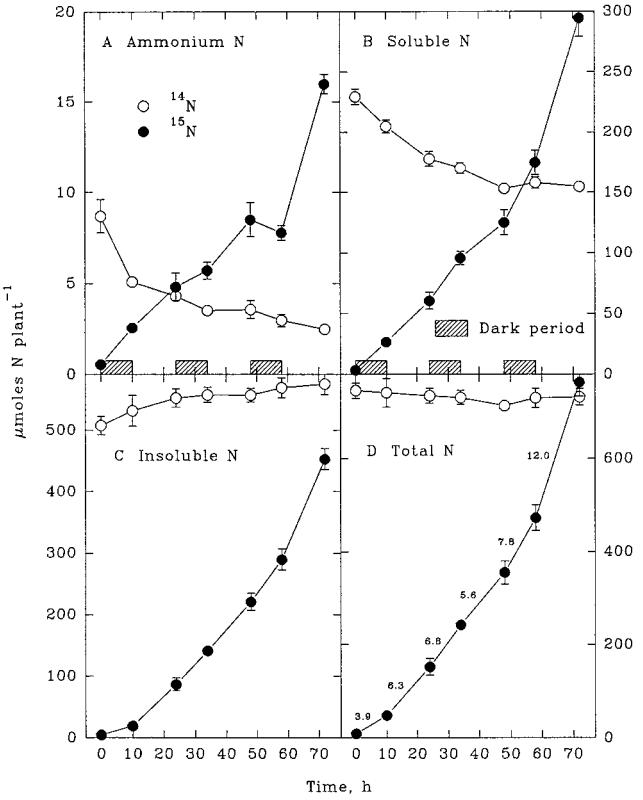

Figure 3. Whole-plant contents of NH_4^+ (A), soluble N (B), insoluble N (C), and total N (D) derived from endogenous (^{14}N) sources and from exogenously supplied NH_4^+ (^{15}N) during a 3-d continuous exposure of maize seedlings to highly labeled $^{15}NH_4^+$. Each value is the mean of four replicates ± SE. The diurnal rates of $^{15}NH_4^+$ uptake (in micromoles per gram fresh weight per hour) are noted in D.

Table I. *Calculation of NH_4^+ fluxes into and out of maize roots*

Rates of $^{15}NH_4^+$ uptake, $^{14}NH_4^+$ uptake, and $^{14}NH_4^+$ release by 227 maize seedlings were calculated from [$^{15}NH_4^+$] and [$^{14}NH_4^+$] in the nutrient solution at the beginning (9 PM) and end (7 AM) of the first 10-h dark period, during which 97.56 mL of 113 mM $^{15}NH_4^+$ (99.8 atom % ^{15}N) was injected to maintain the total [NH_4^+] close to its initial level (0.223 mM).

Measurement	Volume	[NH_4^+]	Atom % ^{15}N	I $^{14}NH_4^+$	II $^{15}NH_4^+$	Rate
	L	*mM*	*%*	*μmol 160 L^{-1}*		*μmol plant^{-1} 10 h^{-1}*
A. Initial (9 PM)	160	0.223	96.10	1392	34,288	
B. Injection	0.09756	113	99.80	22	11,002	
C. Final (7 AM)	160	0.231	93.55	2384	34,576	
D. Change (C − A − B)				+970	−10,714	
E. Mean concentration (A + C)/2				1888	34,432	
F. Net $^{15}NH_4^+$ uptake (DII/227 plants)						47.2
G. $^{14}NH_4^+$ uptake[a] (F[EI/EII])						2.6
H. Total NH_4^+ uptake (F + G)						49.8
I. Net $^{14}NH_4^+$ release (DI/227 plants)						4.3
J. "True" $^{14}NH_4^+$ release[b] (I + G)						6.9

[a] $^{14}NH_4^+$ uptake can be estimated from net $^{15}NH_4^+$ uptake because the two isotopic species are taken up in proportion to their mean molar concentrations in the nutrient solution during the uptake period. [b] Net $^{14}NH_4^+$ release = true $^{14}NH_4^+$ release − $^{14}NH_4^+$ uptake. Therefore, true $^{14}NH_4^+$ release = net $^{14}NH_4^+$ release + $^{14}NH_4^+$ uptake.

both shoots and roots exhibited a typical diurnal pattern (Fig. 6), the pattern in roots was attenuated considerably.

DISCUSSION

Source of $^{14}NH_4^+$ Generation in Roots

Morgan and Jackson (1988a, 1988b, 1989) demonstrated that significant generation of NH_4^+ occurs in roots during uptake and assimilation of exogenously supplied NH_4^+. They suggested that organic N degradation, NH_4^+ assimilation, and NH_4^+ influx and efflux can be modified by environmental and nutritional conditions that alter the pool size of NH_4^+ in roots.

Throughout the 3-d exposure of maize seedlings to $^{15}NH_4^+$, during which appreciable synthesis of insoluble ^{15}N occurred in roots, the endogenous insoluble ^{14}N of roots remained relatively constant. However, $^{14}NH_4^+$ and soluble ^{14}N in roots declined significantly. There was evidence of ^{14}N-protein synthesis in the shoot, apparently at the expense of the pool of soluble ^{14}N in the shoot. The ultimate source of ^{14}N for protein synthesis in the shoot remains tentative, however, because of the likelihood of soluble-^{14}N interchange between the root and shoot as a consequence of amino acid cycling (Lambers et al., 1982; Cooper and Clarkson, 1989). In spite of this possibility, no measurable net transport of endogenous ^{14}N from roots to shoots occurred during the 3-d exposure to $^{15}NH_4^+$. Collectively, these data reveal that soluble organic N, rather than protein, is the primary source of endogenous NH_4^+ generation in maize roots.

Release of $^{14}NH_4^+$ from Roots

During the first 24 h of exposure to $^{15}NH_4^+$ the release of endogenously derived $^{14}NH_4^+$ from the root into the nutrient solution was equivalent to 10.7% of the initial ^{14}N content of the root (Table II). A slower but measurable release of $^{14}NH_4^+$ continued throughout the subsequent 2-d period. This occurred even though the content of $^{14}NH_4^+$ in the root was less than 1 μmol after 24 h of exposure to exogenous $^{15}NH_4^+$ (Fig. 2A), indicating a continual generation of $^{14}NH_4^+$ from endogenous ^{14}N pools. Thus, it is clear that the source of $^{14}NH_4^+$, presumably the soluble-^{14}N pool of the root, was not replaced completely

Table II. *Diurnal rates of $^{15}NH_4^+$ uptake, $^{14}NH_4^+$ uptake, and $^{14}NH_4^+$ release by maize roots*

Roots of maize seedlings were exposed continuously to highly labeled $^{15}NH_4^+$ for 3 d. NH_4^+ flux rates were calculated from periodic analysis of the uptake solution, as illustrated in Table I.

Day	Diurnal Period	Length	Total ($^{15}NH_4^+$ + $^{14}NH_4^+$) Uptake	True $^{14}NH_4^+$ Release	True Root ^{14}N Release[a]
		h	*μmol plant^{-1}*	*period^{-1}*	*%*
7–8	Dark	10	49.8	6.9	4.0
8	Light	14	103.1	11.6	6.7
8–9	Dark	10	63.9	0.2	0.1
9	Light	14	177.3	11.1	6.4
9–10	Dark	10	90.6	5.2	3.0
10	Light	14	193.3	8.3	4.8
Total			678.0	43.3	25.0

[a] $^{14}NH_4^+$ release of the total ^{14}N present in roots harvested on the 7th d (174 μmol ^{14}N root^{-1}).

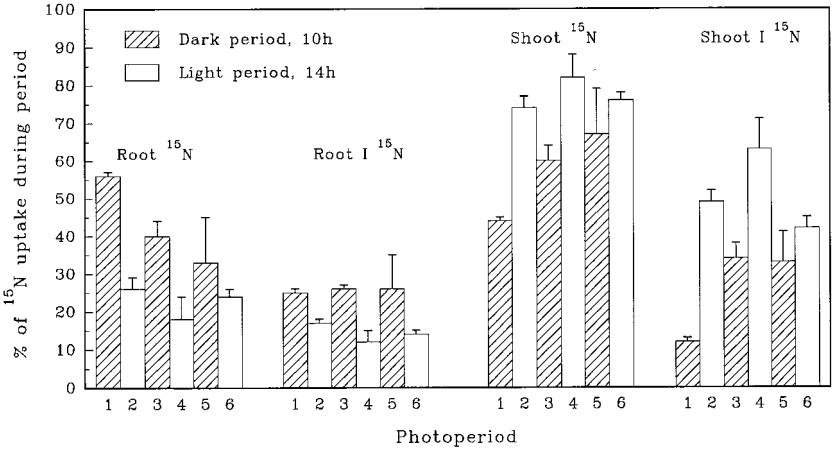

Figure 4. Diurnal increments of total ^{15}N and insoluble ^{15}N (I ^{15}N) in shoots and roots of maize seedlings as percentages of ^{15}N uptake during each of six successive photoperiods. Each value is the mean of four replications. SE values are indicated by vertical bars.

by synthesis of soluble ^{15}N from entering $^{15}NH_4^+$. In support of this premise are the observations that the root soluble-^{14}N pool declined only 22 μmol root^{-1} (34%) during the first 24 h, and remained relatively constant at 40 μmol root^{-1} thereafter (Fig. 2B). This occurred even though the root soluble-^{15}N pool increased from near 0 to more than 200 μmol root^{-1} during the 3-d period. One interpretation of these observations is that the endogenous soluble-^{14}N pool, the putative source of $^{14}NH_4^+$ generation in roots, occupies a different cellular or intracellular "compartment" than that of recently synthesized soluble ^{15}N. For example, synthesis of soluble ^{15}N might occur primarily in the root tip (0–2 cm), where NH_4^+ uptake is maximal (Cruz et al., 1995), whereas NH_4^+ generation might occur in the more mature regions of the root.

Diurnal Use of Exogenous $^{15}NH_4^+$

Although effective absorption and assimilation of $^{15}NH_4^+$ occurred in both dark and light periods, diurnal differences in utilization were observed (Fig. 4). More of the entering ^{15}N was retained by the root in the dark (33%–56%) than in the light (18%–26%). A similar retention of NH_4^+ was reported for perennial ryegrass by Ourry et al. (1996). We also observed that more of the entering $^{15}NH_4^+$ was incorporated into root insoluble ^{15}N in the dark (approximately 25%) than in the light (approximately 14%). Conversely, the synthesis of shoot insoluble ^{15}N was enhanced by light.

In contrast to the data reported here, Ourry et al. (1996) found that the rate of NH_4^+ uptake by perennial ryegrass declined during darkness. However, the plants were exposed to a lower light intensity (500 μmol m^{-2} s^{-1}) and a lower [NH_4^+] (20 μM) than were used in our study. Restricted rates of NH_4^+ uptake in the dark were also reported for both fescue and timothy grass when grown at 20 μM NH_4^+ (Macduff et al., 1997). Finally, the rate of NH_4^+ uptake in the dark by barley grown under light-limited conditions (350 μmol m^{-2} s^{-1}) was only 50% of the rate in the light (Rigano et al., 1996), perhaps reflecting a limitation of NH_4^+ uptake by carbohydrate supply.

Several lines of evidence suggest that both the uptake and assimilation of NH_4^+ are regulated by carbohydrate supplied from the shoot. First, Massimino et al. (1981) reported that NH_4^+ uptake by maize declined within 2 h after lowering the light intensity to restrict photosynthesis. Second, after bean plants had been ringed, NH_4^+ uptake and root soluble carbohydrate content declined concurrently (Michael et al., 1970). When exogenous Suc was supplied to the roots of ringed plants, however, the rate of NH_4^+ uptake exceeded that of intact plants. Third, Lewis et al. (1987) found that a much higher proportion of ^{14}C derived from photosynthetic CO_2 fixation was allocated to the root when N was supplied as NH_4^+ rather than as NO_3^-.

Based on these observations we hypothesized that diurnal differences in retention and incorporation of NH_4^+ into macromolecules were related to changes in the diurnal supply of carbohydrate to the root. To examine this possibility, the changes in carbohydrate contents of the shoot and roots in a duplicate experiment were compared with $^{15}NH_4^+$-assimilation rates in the original experiment (Table III). It was assumed that (a) the carbohydrate changes in the replicate experiment were comparable to those in the

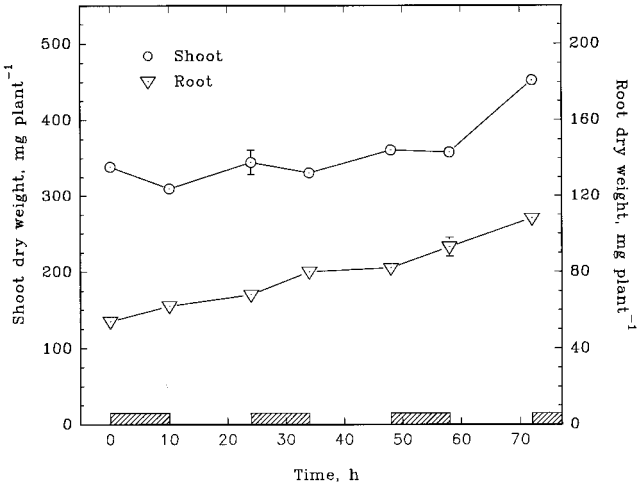

Figure 5. Dry weights of maize shoots and roots harvested at the beginning and end of each photoperiod during a 3-d exposure to highly labeled $^{15}NH_4^+$. Each value is the mean of four replications. SE bars are shown when they are larger than the symbols.

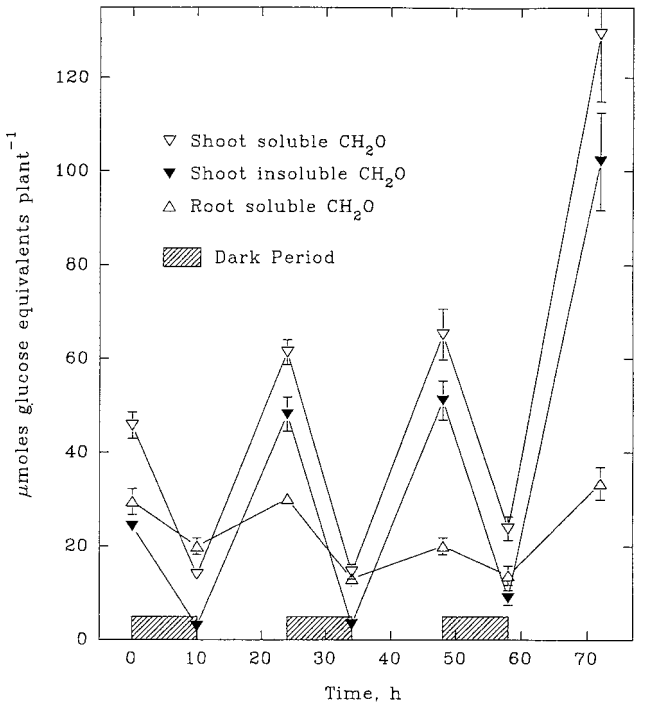

Figure 6. Soluble and insoluble carbohydrate contents of shoots and soluble carbohydrate contents of roots during a 3-d continuous exposure of maize seedlings to $^{14}NH_4^+$. Each value is the mean of four replicates ± SE.

original study, (b) assimilation of the entering $^{15}NH_4^+$ occurred exclusively in the root, (c) the initial product of NH_4^+ assimilation in the root was Gln, and (d) the assimilation of 1 mol of $^{15}NH_4^+$ into Gln requires 0.505 mol of Glc equivalents to supply the necessary reductant, ATP, and C skeletons (Schubert, 1980). Using these assumptions, the C required for net $^{15}NH_4^+$ assimilation during each diurnal period was estimated.

As plants developed, the C required to support measured $^{15}NH_4^+$ assimilation increased from 117 μmol plant^{-1} in the first dark period to 872 μmol plant^{-1} during the final light period (Table III). Yet the carbohydrate content of the roots at the beginning of each dark period varied from 120 to 200 μmol plant^{-1} (i.e. 20–33 μmol Glc equivalents plant^{-1}; Fig. 6). These data reveal that with the exception of the first dark period, there were insufficient carbohydrate reserves in the root to support assimilation of the $^{15}NH_4^+$ absorbed. However, there were adequate reserves in the shoot. Thus, appreciable transport of carbohydrate from the shoot to the root must have occurred during both dark and light periods.

Minimal estimates of the transport of C to the root required to support $^{15}NH_4^+$ assimilation can be calculated by adding the total C required for $^{15}NH_4^+$ assimilation to the accumulation of C by the root (Table III). Such estimates ranged from 60 μmol plant^{-1} during the first dark period to 990 μmol plant^{-1} during the final light period (Table III). It is of particular interest to note that during the three successive dark periods, $^{15}NH_4^+$ assimilation accounted for 19%, 31%, and 65%, respectively, of the decline in shoot carbohydrate. This reflects the facts that carbohydrate reserves in the root at the beginning of each dark period were relatively constant (12–19 μmol Glc equivalents plant^{-1}), whereas the rate of $^{15}NH_4^+$ uptake and assimilation during the three successive dark periods increased from 3.9 to 7.8 μmol g^{-1} fresh weight root h^{-1} (Fig. 3D).

The increasing proportion of C reserves required to sustain the assimilation of entering NH_4^+ supports the concept that carbohydrate availability is involved in the regulation of NH_4^+ uptake and assimilation by the root. If so, the dark-enhanced assimilation of $^{15}NH_4^+$ into the insoluble-^{15}N fraction of the root indicates that C availability is higher during dark periods relative to $^{15}NH_4^+$ uptake. This possibility is consistent with the diurnal pattern of $^{15}NH_4^+$ accumulation in roots (Fig. 2A). Although the $^{15}NH_4^+$ content of roots was low (<5 μmol), fluctuations likely reflect the changing diurnal equilibria between uptake and assimilation of $^{15}NH_4^+$. With the exception of the first dark period, the $^{15}NH_4^+$ content of roots declined in darkness and increased during the light. This suggests that in darkness the assimilation of $^{15}NH_4^+$ usually exceeded its

Table III. *Carbohydrate required for $^{15}NH_4^+$ assimilation by maize roots*

The diurnal assimilation of $^{15}NH_4^+$ was measured during a 3-d exposure of maize seedlings to highly labeled $^{15}NH_4^+$. The minimal transport of C from shoot to root required to support $^{15}NH_4^+$ assimilation was calculated from the theoretical C requirement for $^{15}NH_4^+$ assimilation and the changes in tissue carbohydrate. Each value is the mean of four replicates ± SE.

Day	Photoperiod	$^{15}NH_4^+$ Assimilation	Carbohydrate			
			Requirement[a]	Change in shoot	Change in root	Transport to root[b]
		μmol N plant^{-1}	μmol C plant^{-1}			
7–8	Dark	39 ± 4	117 ± 13	−319 ± 7	−57 ± 10	60 ± 15
8	Light	102 ± 18	310 ± 53	+556 ± 35	+61 ± 7	371 ± 55
8–9	Dark	91 ± 11	275 ± 34	−550 ± 12	−103 ± 6	172 ± 37
9	Light	110 ± 24	333 ± 74	+590 ± 67	+42 ± 11	375 ± 64
9–10	Dark	120 ± 28	365 ± 84	−501 ± 24	−38 ± 13	327 ± 97
10	Light	288 ± 30	872 ± 91	+1193 ± 147	+118 ± 21	990 ± 108

[a] Calculation of the total C requirement for $^{15}NH_4^+$ assimilation is based on the assumption that assimilation of entering $^{15}NH_4^+$ occurs in the root, and that the initial product of NH_4^+ assimilation is Gln, which requires 0.505 mol of Glc equivalents mol^{-1} NH_4^+ to provide the necessary reductant, ATP, and C skeletons (Schubert, 1982). [b] Minimal C transport to the root required to support $^{15}NH_4^+$ assimilation is equal to the total C required for $^{15}NH_4^+$ assimilation plus the change in root C content.

uptake, indicating an adequate supply of carbohydrate. During illumination, however, the $^{15}NH_4^+$ content of roots increased, suggesting that the supply of carbohydrate was insufficient to assimilate all of the entering $^{15}NH_4^+$. Yet, measured carbohydrate levels in roots increased during illumination. Additional studies are in progress to examine this apparent anomaly.

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