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Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. cv. T-5) grown under ammonium or nitrate nutrition

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ABSTRACT

Studies that quantify plant $\delta^{15}N$ often assume that fractionation during nitrogen uptake and intra-plant variation in $\delta^{15}N$ are minimal. We tested both assumptions by growing tomato (*Lycopersicon esculentum* Mill. cv. T-5) at NH_4^+ or NO_3^- concentrations typical of those found in the soil. Fractionation did not occur with uptake; whole-plant $\delta^{15}N$ was not significantly different from source $\delta^{15}N$ for plants grown on either nitrogen form. No intra-plant variation in $\delta^{15}N$ was observed for plants grown with NH_4^+. In contrast, $\delta^{15}N$ of leaves was as much as 5·8‰ greater than that of roots for plants grown with NO_3^-. The contrasting patterns of intra-plant variation are probably caused by different assimilation patterns. NH_4^+ is assimilated immediately in the root, so organic nitrogen in the shoot and root is the product of a single assimilation event. NO_3^- assimilation can occur in shoots and roots. Fractionation during assimilation caused the $\delta^{15}N$ of NO_3^- to become enriched relative to organic nitrogen; the $\delta^{15}N$ of NO_3^- was 11·1 and 12·9‰ greater than the $\delta^{15}N$ of organic nitrogen in leaves and roots, respectively. Leaf $\delta^{15}N$ may therefore be greater than that of roots because the NO_3^- available for assimilation in leaves originates from a NO_3^- pool that was previously exposed to nitrate assimilation in the root.

Key-words: Lycopersicon esculentum Mill. cv. T-5; tomato; ammonium; isotope discrimination; nitrate; nitrogen isotopes.

INTRODUCTION

Quantification of natural variation in the isotopic composition of nitrogen is common in studies of plant nitrogen dynamics. The isotopic composition of plant-available nitrogen can vary between forms available for uptake (NH_4^+, NO_3^-, organic N and atmospheric N_2) and with soil depth (Shearer & Kohl 1986); thus, it is hypothesized that inter-plant differences in isotopic composition result from acquisition of nitrogen from different sources. Differences in the absolute abundance of ^{15}N are small, so nitrogen isotope composition is expressed using δ notation in parts per thousand as

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000‰, \quad (1)$$

where $\delta^{15}N$ is the isotopic ratio of the sample relative to a standard, and R_{sample} and $R_{standard}$ are the molar $^{15}N/^{14}N$ ratios of the sample and standard, respectively. The standard for nitrogen is atmospheric nitrogen ($R = 0 \cdot 0036765$).

Natural variation in $\delta^{15}N$ occurs because ^{15}N and ^{14}N react at different rates, causing the product of a reaction to have a different isotopic composition from the substrate. The ratio of the rate constants for the two isotopic species (k^{14}/k^{15}) is defined as the 'isotope effect' and is equivalent to the isotope ratio of the substrate divided by that of the product (R_s/R_p). Discrimination (Δ) is the deviation of the isotope effect (α) from unity,

$$\Delta = \alpha - 1. \quad (2)$$

Discrimination can also be expressed in relation to the $\delta^{15}N$ of the substrate and product as

$$\Delta = \frac{\delta^{15}N_s - \delta^{15}N_p}{1 + \delta^{15}N_p}. \quad (3)$$

$\delta^{15}N_p$ in Eqn (3) is much less than 1 and can be ignored, so discrimination is calculated as

$$\Delta = \delta^{15}N_s - \delta^{15}N_p. \quad (4)$$

In studies of plant nitrogen uptake, the $\delta^{15}N$ of the plant and inorganic nitrogen are equivalent to $\delta^{15}N_p$ and $\delta^{15}N_s$, respectively. Positive discrimination occurs when ^{14}N reacts more readily than ^{15}N, causing the $\delta^{15}N$ of the plant to be less than the $\delta^{15}N$ of the nitrogen source.

The hypothesis that differences in plant $\delta^{15}N$ are caused by acquisition of nitrogen from different sources assumes that no discrimination occurs during uptake, so whole-plant $\delta^{15}N$ is identical to the nitrogen source. Leaf material is most often collected for analysis, so a second common assumption is that leaf $\delta^{15}N$ reflects whole-plant $\delta^{15}N$.

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Both assumptions are important, because, while $\delta^{15}N$ values in the environment vary between −10 and +20‰ (Ehleringer & Rundel 1988), the range among co-occurring plants is much smaller (0 to 5‰), and biologically significant differences are often <1‰ (Handley & Raven 1992).

The validity of both assumptions may depend on whether plants use predominately NH_4^+ or NO_3^- as an inorganic nitrogen source. Relatively little is known about fractionation during uptake and intra-plant $\delta^{15}N$ variation for plants grown with NH_4^+ as their nitrogen source, even though NH_4^+ is the major source of inorganic nitrogen in many environments (Bloom 1988; Raven *et al.* 1992). In the only study to date, Yoneyama *et al.* (1991) observed that discrimination for two varieties of *Oryza sativa* L. ranged from 4·1–4·6‰ for plants grown on 1·4 mol m^{-3} NH_4^+ to 11·2–12·6‰ for plants grown on 7·2 mol m^{-3} NH_4^+. Intra-plant variation was <2‰. If the substantial discrimination observed by Yoneyama *et al.* (1991) is characteristic of NH_4^+ nutrition in general this clearly complicates interpretation of plant $\delta^{15}N$ values in environments where NH_4^+ is the primary nitrogen source.

Substantial discrimination has been observed when NO_3^- is the sole nitrogen source (Kohl & Shearer 1980; Mariotti *et al.* 1980, 1982; Bergersen *et al.* 1988). The amount of discrimination is often positively correlated with the NO_3^- concentration of the growth medium and negatively correlated with plant age. For example, Mariotti *et al.* (1982) observed that discrimination increased from 0·0‰ to as much as 3·3‰ for two species of *Pennisetum* when external NO_3^- concentrations were increased from 0·5 to 12·0 mol m^{-3}. Other studies, all conducted at external NO_3^- concentrations of greater than 1·0 mol m^{-3}, have measured discrimination from 0·0 to 9·5‰ (Kohl & Shearer 1980; Mariotti *et al.* 1980, 1982; Bergersen *et al.* 1988; Yoneyama & Kaneko 1989). Variation in discrimination with plant age was initially reported by Kohl & Shearer (1980), who found that discrimination decreased from 2·4–6·5‰ for three species at 23–31 d old to 1·7–3·7‰ for the same species at 65–101 d old. Mariotti *et al.* (1982) and Bergersen *et al.* (1988) subsequently found decreases in discrimination of up to 9·5‰ with increasing plant age in *P. americanum* and *Glycine max* (L.) Merrill, respectively. Intra-plant variation in $\delta^{15}N$ can also occur when NO_3^- is the sole nitrogen source. Shoots often have significantly greater $\delta^{15}N$ than roots; Bergersen *et al.* (1988) found that the $\delta^{15}N$ of roots was almost 2‰ less, and leaf $\delta^{15}N$ from 2 to 4‰ greater, than the NO_3^--N source. Yoneyama & Kaneko (1988) noted similar results, with the $\delta^{15}N$ of leaves being 3·3–6·9‰ greater than that of roots.

Fractionation during uptake of NH_4^+ or NO_3^- and intra-plant variation in $\delta^{15}N$ could introduce significant error in studies that quantify $\delta^{15}N$ to elucidate plant use of different nitrogen sources. However, in studies that have examined these processes plants have been grown using nitrogen concentrations from 1 to 12 mol m^{-3} even though soil inorganic nitrogen concentrations are usually less than 500 mmol m^{-3} (Haynes 1986), and concentrations adjacent to the root surface are even lower due to localized depletion zones (Nye & Tinker 1977). The use of nitrogen concentrations in excess of those found in the soil is significant, because in many species the mechanisms of uptake differ at relatively high (>1 mol m^{-3}) and low (<500 mmol m^{-3}) external concentrations for NO_3^- (reviewed in Glass 1988; Ullrich 1992) and NH_4^+ (Wang *et al.* 1993b): uptake at low concentrations is by a high-affinity, saturable transport system, while uptake at high concentrations is by a low-affinity, non-saturable transport mechanism. Therefore, experiments using inorganic nitrogen concentrations greater than 1 mol m^{-3} may not accurately reflect mechanisms operating under field conditions. We compared discrimination with uptake as well as intra-plant variation in $\delta^{15}N$ for plants grown under NH_4^+ or NO_3^- nutrition. Plants were grown in an open nutrient system that allows precise control of the nitrogen concentration of the growth medium at levels more closely approximating field conditions.

MATERIALS AND METHODS

Growth conditions

Seeds of *Lycopersicon esculentum* Mill. cv. T-5 were surface-sterilized and germinated in rolled germination paper saturated with 1 mol m^{-3} $CaSO_4$. Newly germinated seedlings were transferred to an open nutrient system. The system and growth methods are described in detail by Smart & Bloom (1993). The system consists of a 120 dm^3 stainless steel tank housed in a temperature-controlled water bath. Solution temperatures were maintained at 20 ± 0·1°C. Microbial growth was prevented by continuously circulating the solution through an ultraviolet light source, and by constructing all parts of the system in contact with the solution out of stainless steel, Teflon or high-density plastics to decrease the availability of carbon sources for microbial activity (Smart & Bloom 1993). The nutrient solution was continuously aerated with an air stream that was first passed through an acid trap (200 mol m^{-3} H_2SO_4) to collect atmospheric NH_3.

Forty plants were grown on 50 mmol m^{-3} NH_4^+ or NO_3^-. Concentrations of other nutrients are described in Smart & Bloom (1993). Discrimination may occur, but will not be observed due to conservation of mass, if the inorganic nitrogen concentration in the nutrient solution is allowed to fall to zero. NH_4^+ or NO_3^- concentration and pH were continuously monitored by passing a sample of the tank solution through a series of ion-selective electrodes (see Bloom 1989), and neither NH_4^+ nor NO_3^- concentrations varied more than 10% from the 50 mmol m^{-3} setpoint. NH_4^+ and NO_3^- concentrations were maintained by adding nitrogen from 1 mmol m^{-3} KNO_3 or 1 mmol m^{-3} NH_4Cl stock solutions. A single large reservoir of stock solution was used for each nitrogen source for the duration of the experiment. The $\delta^{15}N$ of the stock solutions did not change throughout the experiment, and the mean values were 2·7 ± 0·0‰ and

1·8 ± 0·0‰ for NH_4^+ and NO_3^-, respectively. The pH was maintained at 6·0 throughout each experiment through the addition of either H_2SO_4 or KOH.

The observed discrimination may also be negligible due to conservation of mass if plants are allowed to assimilate most of the inorganic nitrogen that is supplied to the tank. Therefore, the large volume of nutrient solution within the tank was replaced each week by diverting the solution through a Barnstead water purification system consisting of carbon filters and ion exchange cartridges. The specific conductivity of the solution was measured to ensure completeness of the filtration process, and fresh solution was supplied once filtration was complete. The $\delta^{15}N$ of the inorganic nitrogen within the tank was measured immediately before filtration throughout the experiment and in no instance had it been altered by plant uptake (NH_4^+, 2·9 ± 0·2‰; NO_3^-, 1·8 ± 0·1‰).

Growth and nitrogen accumulation

Five randomly chosen plants were harvested 20, 28 and 36 d after planting. Three plants were harvested on day 45 for plants grown with NO_3^- as their nitrogen source. In harvesting, the roots were briefly rinsed with deionized-distilled water to remove nitrogen from extra-cellular spaces, and then the plant was separated into roots, stems and leaves. All components were then frozen in liquid nitrogen and lyophilized. Dry biomass was measured after freeze-drying. All tissues were then finely ground using a mortar and pestle. Relative growth rate (RGR) was calculated following Hunt (1990).

The total nitrogen concentration of each fraction was measured using a CHN analyser (Perkin Elmer Model 2400). NH_4^+ was measured by combining 15 mg of dried sample with 4 cm^3 of hot water and measuring NH_3 using a gas-sensing electrode. NO_3^- was extracted by combining 15 mg of dried tissue with 1 cm^3 of hot water in a centrifuge tube. The sample was vortexed and centrifuged, and then the supernatant was transferred to a second tube. The procedure was repeated with an additional 1 cm^3 of hot water. Finally, the supernatant was filtered and analysed for NO_3^- by HPLC (Thayer & Huffaker 1980).

Isotopic composition

Tissue $\delta^{15}N$ was analysed following Evans & Ehleringer (1994). Nitrogen within tissues was converted to N_2 by combusting each sample at 850 °C in evacuated Vycor tubes with CuO and Cu for at least 6 h (Fiedler & Proksch 1975; Minagawa *et al.* 1984). The N_2 gas within the sample was purified cryogenically, and the $\delta^{15}N$ measured on a mass spectrometer (Finnigan MAT model Delta S).

The $\delta^{15}N$ of NO_3^- within leaves and roots was measured for plants grown with NO_3^- as their sole nitrogen source. Measurements were made for plants harvested on day 45. The methodology for $\delta^{15}N$ measurements was modified from Volk *et al.* (1979). NO_3^- within leaves was extracted with hot water as described before. The extract was lyophilized to dryness within a 9 mm Vycor tube, and 1·0 cm^3 of 18 mmol m^{-3} H_2SO_4 and 0·1 cm^3 of Hg added. The tube was frozen in a dry ice-EtOH slurry and the open end of the tube sealed under vacuum. The sealed tube was then agitated in an ultrasonic cleaner overnight at room temperature. The NO resulting from the reaction was collected by cracking the tube under vacuum and passing the gas through a liquid-N trap. The NO was then condensed into a Vycor tube containing Cu and CuO. The sample was subsequently prepared for $\delta^{15}N$ analysis as described previously. The $\delta^{15}N$ values for KNO_3 standards (4.6 ± 0·1‰, $n = 6$) analysed using this method were 4·5 ± 0·1‰ ($n = 3$).

All data were analysed as a completely randomized or factorial experimental design with a 5% level of significance (Neter, Wassermann & Kutner 1985).

RESULTS

Growth and nitrogen accumulation

NH_4^+ as a nitrogen source

Growth occurred throughout the experiment for plants grown with NH_4^+ (Fig. 1a). The RGR of plants grown with NH_4^+ was 0·06 g g^{-1} d^{-1} between days 20 and 28; this increased to 0·14 g g^{-1} d^{-1} between days 28 and 37. The RGR of leaves and roots was identical (0·14 g g^{-1} d^{-1}) over the latter time period. The nitrogen content of plants grown with NH_4^+ increased from 0·3 ± 0·1 to 4·3 ± 1·1 mmol N $plant^{-1}$ from days 20 to 37 (Fig. 1). Leaf nitrogen content increased from 0·2 ± 0·1 to 2·8 ± 0·7 mmol N $leaf^{-1}$, and root nitrogen content from 0·1 ± 0·0 to 1·1 ± 0·3 mmol N $root^{-1}$ over the same time period. The total nitrogen concentration of all organs was greatest on day 37 (Table 1), and nitrogen

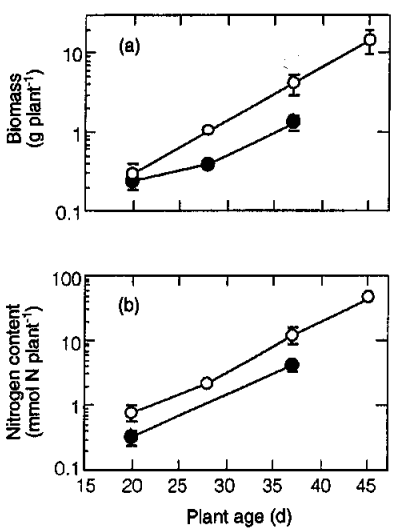


Figure 1. Biomass (a) and nitrogen (b) accumulation for tomato plants grown in an open nutrient system with either 50 mmol m^{-3} NH_4^+ (●) or NO_3^- (○) as their sole nitrogen source. Each point represents the mean of five observations, except when three plants were measured on day 45 for plants grown on NO_3^-.

Table 1. NH_4^+-N and total N concentration (mean ± SE) for tomato plants grown with NH_4^+ as the sole nitrogen source (nd: not determined)

	Leaf		Stem		Root	
Age (d)	NH_4^+-N ($\mu mol\ g_{dw}^{-1}$)	Total N ($mmol\ g_{dw}^{-1}$)	NH_4^+-N ($\mu mol\ g_{dw}^{-1}$)	Total N ($mmol\ g_{dw}^{-1}$)	NH_4^+-N ($\mu mol\ g_{dw}^{-1}$)	Total N ($mmol\ g_{dw}^{-1}$)
20	8 ± 1	1·63 ± 0·09	7 ± 2	0·96 ± 0·05	9 ± 3	1·81 ± 0·07
28	91 ± 26	nd	37 ± 10	nd	113 ± 17	nd
37	100 ± 10	3·82 ± 0·09	20 ± 2	1·86 ± 0·09	37 ± 9	2·82 ± 0·08

Table 2. NO_3^--N and total N concentration (mean ± SE) for tomato plants grown with NO_3^- as the sole nitrogen source

	Leaf		Stem		Root	
Age (d)	NO_3^--N ($\mu mol\ g_{dw}^{-1}$)	Total N ($mmol\ g_{dw}^{-1}$)	NO_3^--N ($\mu mol\ g_{dw}^{-1}$)	Total N ($mmol\ g_{dw}^{-1}$)	NO_3^--N ($\mu mol\ g_{dw}^{-1}$)	Total N ($mmol\ g_{dw}^{-1}$)
20	55 ± 13	2·07 ± 0·13	96 ± 34	1·21 ± 0·11	227 ± 42	2·51 ± 0·21
28	78 ± 16	2·13 ± 0·13	69 ± 21	1·04 ± 0·03	260 ± 34	2·84 ± 0·10
37	349 ± 22	3·15 ± 0·04	638 ± 34	2·29 ± 0·12	408 ± 29	2·72 ± 0·05
45	119 ± 38	2·52 ± 0·11	308 ± 45	1·79 ± 0·18	401 ± 80	2·22 ± 0·09

concentrations were greatest in the leaves. The NH_4^+ concentration was greatest for leaves on day 37, but the highest concentration for roots was observed on day 28.

NO_3^- as a nitrogen source

The biomass of plants grown with NO_3^- increased throughout the experiment (Fig. 1a). RGR varied between 0·14 and 0·17 $g\ g^{-1}\ d^{-1}$ when the plants were 20 to 45 d old. RGR did not differ by more than 0·03 $g\ g^{-1}\ d^{-1}$ between leaves and roots. Nitrogen absorption also continued throughout the experiment (Fig 1b). The nitrogen content of plants increased from 0·8 ± 0·2 to 46·4 ± 3·6 mmol N $plant^{-1}$ from days 20 to 45. Nitrogen accumulation occurred in both leaves and roots from days 20 to 45; leaf nitrogen content increased from 0·4 ± 0·2 to 21·2 ± 7·7 mmol N $leaf^{-1}$, and root nitrogen increased from 0·2 ± 0·1 to 8·9 ± 0·5 mmol N $root^{-1}$. The total nitrogen concentration of roots was not significantly different between dates (Table 2). Leaf and stem nitrogen concentration were greatest at age 37. The NO_3^- concentration of all organs increased from 20 to 37 d of age (Table 2). NO_3^- concentrations were greatest in the root, except on day 37 when values were significantly greater for stems.

Isotopic composition

NH_4^+ as a nitrogen source

The $\delta^{15}N$ of plants grown with NH_4^+ was only measured on day 37 because of the small plant size. No significant differences were detected between whole-plant $\delta^{15}N$ (3.2 ± 0.2‰) and that of the nitrogen source (2·9‰; Table 3). The $\delta^{15}N$ values for the different organs were also not significantly different.

NO_3^- as a nitrogen source

Whole-plant $\delta^{15}N$ (2·5 ± 0·04‰) did not change significantly throughout the experiment and was not significantly different from that of the nitrogen source (1·8‰) on any date ($P > 0.05$) (Fig. 2). Leaf $\delta^{15}N$ was significantly greater than whole-plant and root $\delta^{15}N$ on all harvest dates. Leaf values decreased from 4·2 ± 0·3‰ on day 28 to 3·3 ± 0·6‰ on day 45. Root $\delta^{15}N$ was significantly less than whole-plant $\delta^{15}N$, and values increased from −1·6 ± 0·2‰ on day 28 to −0·1 ± 0·4‰ on day 45.

The $\delta^{15}N$ of NO_3^--N within leaves was 11·3‰ greater than organic-N values on day 45 (Table 4). The difference between the two nitrogen pools was 12·9‰ in roots. The $\delta^{15}N$ of both nitrogen pools was greater in the leaves than in the roots. The $\delta^{15}N$ of the leaf organic N was 4·5‰ greater than root organic N. Leaf NO_3^--N had $\delta^{15}N$ 2·9‰ greater than root NO_3^--N.

DISCUSSION

Fractionation with uptake

Fractionation did not occur with root NH_4^+ or NO_3^- uptake; whole-plant $\delta^{15}N$ was not significantly different from the $\delta^{15}N$ of either nitrogen source throughout the

Table 3. $\delta^{15}N$ (‰) (mean ± SE) for tomato plants grown with NH_4^+ as the sole nitrogen source

Total	Leaf	Stem	Root
3·2 ± 0·2	3·3 ± 0·3	2·4 ± 0·2	3·1 ± 0·2

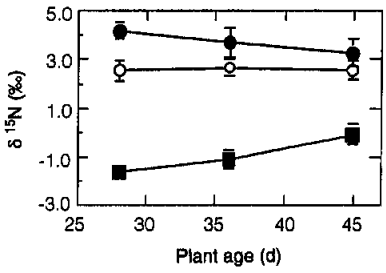
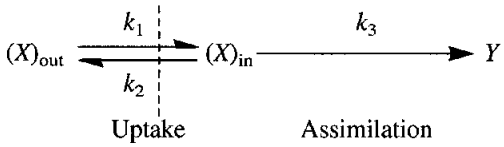


Figure 2. Total (○), leaf (●) and root (■) $\delta^{15}N$ values for plants grown in an open nutrient system with 50 mmol m^{-3} NO_3^- as their sole nitrogen source. Total $\delta^{15}N$ was calculated from the $\delta^{15}N$ and nitrogen concentration of leaves, roots and stems. Each point represents the mean of five observations, except when three plants were measured on day 45 for plants grown on NO_3^-. Total $\delta^{15}N$ was not significantly different ($P > 0.05$) from source $\delta^{15}N$ throughout the experiment, while differences between total, leaf, root and stem $\delta^{15}N$ were significant ($P < 0.05$) on each date.

experiments. Mariotti *et al.* (1982) proposed that discrimination during uptake can be described by the model



where $(X)_{out}$ and $(X)_{in}$ are the inorganic nitrogen pools outside and inside the cell, respectively, and k_1, k_2 and k_3 are the rate constants associated with influx, efflux and assimilation, respectively. At steady state, $k_1 = (k_3 + k_2)$. Nitrate reductase and glutamine synthetase both discriminate against ^{15}N during assimilation, causing an increase in the $\delta^{15}N$ of $(X)_{in}$ compared to $(X)_{out}$ (Mariotti *et al.* 1982; Yoneyama & Kaneko 1989; Hoch *et al.* 1992). Differences in $\delta^{15}N$ between a plant and the nitrogen source will occur if fractionation occurs during influx, or if significant efflux occurs from the enriched $(X)_{in}$ pool. Previous studies of fractionation during influx have used nitrogen concentrations corresponding to uptake by the low-affinity transport mechanism. The lack of discrimination observed in this study for either nitrogen form suggests that fractionation does not occur with influx at nitrogen concentrations associated with the high-affinity transport mechanisms of NH_4^+ and NO_3^-.

Our results for plants grown with NH_4^+ as their sole nitrogen source are in contrast to the findings of Yoneyama *et al.* (1991), who observed discrimination of 4·1 to 12·6‰ for rice grown on 1·4 or 7·2 mol m^{-3} NH_4^+. Discrimination would be possible with NH_4^+ uptake if efflux of NH_4^+ occurred from a pool within the root with a relatively high $\delta^{15}N$. Generally, plants assimilate NH_4^+ in the root, perhaps to avoid toxicity (Bloom 1988; Wang *et al.* 1993a). Presland & McNaughton (1986) did not observe NH_4^+ efflux in roots of maize, but efflux has been observed in other species (Morgan & Jackson 1989). For tomato grown under conditions similar to those used here, NH_4^+ efflux was negligible (Kosola & Bloom 1994). Whole-plant $\delta^{15}N$ may also be different from the $\delta^{15}N$ of the NH_4^+ source if nitrogen enters the root as NH_3, rather than NH_4^+, because the equilibrium isotope effect for $(NH_4^+ \leftrightarrow NH_3 + H^+)$ is 20‰ (Shearer & Kohl 1986), with NH_4^+ being enriched compared to NH_3. However, Wang *et al.* (1993b, 1994) recently concluded, on the basis of electrophysiological evidence and the insensitivity of influx to pH, that this is not likely in rice. The similarity between NH_4^+ and whole-plant $\delta^{15}N$ in this study suggests that NH_4^+ does not enter the root as NH_3.

Discrimination was not observed during NO_3^- uptake in this study, suggesting that efflux was not occurring from a nitrogen pool that had been exposed to nitrate reductase. Efflux of NO_3^- is often correlated with internal NO_3^- concentration (Teyker *et al.* 1988), but substantial efflux has been measured at external nitrate concentrations as low as 30–60 mmol m^{-3} (Larsson *et al.* 1992). Species differ in their patterns of nitrate assimilation; nitrate reductase is present in both roots and shoots of many species, but is present only in the leaves of some tropical and subtropical annuals and perennials (Andrews 1986). If efflux occurs, then discrimination should be observed in species with nitrate reductase present in the root; the enzyme is localized in the cytoplasm of epidermal cells (Rufty *et al.* 1986; Siddiqi *et al.* 1991; King *et al.* 1992), and thus all NO_3^- entering the plant would enter a pool that is subject to assimilation. However, substantial efflux could occur and not be accompanied by discrimination in those species that assimilate NO_3^- predominantly in the leaf. The elevated $\delta^{15}N$ of root NO_3^- observed in this study, in conjunction with a lack of discrimination, suggests that assimilation is occurring in the root, but that efflux of enriched NO_3^- is not occurring. This is consistent with previous findings that, for tomatoes grown in this manner, NO_3^- efflux is negligible (A.J. Bloom, unpublished results). Yoneyama & Kaneko (1989) and Mariotti *et al.* (1982) also observed large differences between source and root $\delta^{15}N$ without discrimination during uptake, suggesting that efflux was minimal.

Table 4. The $\delta^{15}N$ (‰) (mean ± SE) of organic N, NO_3^--N, and total N within leaves and roots of tomato plants grown with NO_3^- as the sole nitrogen source

	Total N	Organic N	NO_3^--N
Leaf	3·4 ± 1·0	2·7 ± 0·5	14·0 ± 4·6
Root	0·1 ± 0·7	−1·8 ± 0·5	11·1 ± 1·7

Intra-plant variation

Nitrogen source (NH_4^+ versus NO_3^-) strongly influenced intra-plant patterns of $\delta^{15}N$. Intra-plant variation in $\delta^{15}N$ can be caused by reallocation of nitrogen during growth (Shearer & Kohl 1986), or by differences in assimilation patterns between NH_4^+ and NO_3^-. Reallocation of nitrogen can cause differences in $\delta^{15}N$ between nitrogen sources

and sinks because most chemical reactions discriminate against ^{15}N (Shearer & Kohl 1986), so that nitrogen exported from a source is often depleted in ^{15}N compared to the sink. For example, Shearer *et al.* (1983) found that trunkwood of the woody perennial *Prosopis glandulosa* had $\delta^{15}N$ values 3·4‰ less than those of leaves. It is unlikely the differences observed in this experiment were the result of reallocation because nitrogen accumulation continued in roots and leaves throughout the experiment, and no abscission of leaves was observed.

The observed intra-plant variation in $\delta^{15}N$ is more likely to result from differences in assimilation patterns between NH_4^+ and NO_3^-. NH_4^+ is likely to be assimilated immediately after entering the root to prevent toxic accumulation within the plant (Bloom 1988). Organic nitrogen within the leaf and that within the root are both products of this single assimilation event and therefore have similar $\delta^{15}N$ values. We did observe NH_4^+ accumulation in leaves of 37-d-old plants (Table 1), but the primary source of NH_4^+ in leaves is photorespiration (Lea *et al.* 1992). Intra-plant variation could occur if the substrate or products of photorespiration were subsequently exported out of the leaf, yet the lack of intra-plant variation in $\delta^{15}N$ indicates that these compounds are recycled within the leaf.

In contrast to NH_4^+, primary NO_3^- assimilation can occur in both the root and the leaf. The NO_3^- not assimilated in the root would be enriched in ^{15}N because it originates from the NO_3^- pool that was exposed to nitrate reductase in the root epidermis (Yoneyama & Kaneko 1989). Therefore the $\delta^{15}N$ of NO_3^- available for assimilation is greater in the leaf than in the root. The relative assimilation of NO_3^- in leaves versus roots often shifts to leaves over time (Andrews 1986). This could cause the observed convergence of root and shoot $\delta^{15}N$ over time as increasingly greater amounts of organic nitrogen originate from a single assimilation event in the leaves.

In summary, discrimination did not occur in tomato plants grown under NH_4^+ or NO_3^- levels characteristic of those found in the soil. Differences in assimilation patterns for NH_4^+ and NO_3^- did affect intra-plant patterns of $\delta^{15}N$. Organic nitrogen originated from a single assimilation event in the root for NH_4^+, so intra-plant variation in $\delta^{15}N$ was negligible. In contrast, NO_3^- assimilation occurred in both the root and the leaves, so the leaves had relatively high $\delta^{15}N$. The absence of discrimination on a whole-plant basis suggests a lack of efflux from the enriched NO_3^- pool in the root.

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