Plant Physiol. (1987) 85, 0860–0864
0032-0889/87/85/0860/05/$01.00/0

Nitrogen Utilization in *Lemna*[1]

II. STUDIES OF NITRATE UPTAKE USING $^{13}NO_3^-$

Received for publication April 16, 1987 and in revised form August 6, 1987

BJÖRN INGEMARSSON*, PETTER OSCARSON, MAGNUS AF UGGLAS, AND CARL-MAGNUS LARSSON
Department of Botany, University of Stockholm, S-106 91 Stockholm, Sweden (B.I., P.O., C.-M.L.); *and Research Institute of Physics, Frescativ. 24, S-104 05 Stockholm, Sweden* (M.af U.)

ABSTRACT

^{13}N-labeled nitrate was used to trace short-term nitrate influx into *Lemna gibba* L. G3 in experiments where disappearance of both radioactivity and total nitrate from the incubation medium was measured continuously and simultaneously. In plants performing net nitrate uptake from an initial nitrate concentration of 40 to 60 micromolar, there was no discrepancy between net uptake and influx, irrespective of the N status of the plants, indicating that concomitant nitrate efflux was low or nil. Plants treated with tungstate to inactivate nitrate reductase were able to take up nitrate following induction of the uptake system by exposure to a low amount of nitrate. Also, in this case, net uptake was equivalent to influx. In tungstate-treated plants preloaded with nitrate, both net uptake and influx were nil. In contrast to these observations, a clear discrepancy between net uptake and influx was observed when the plants were incubated at an initial nitrate concentration of approximately 5 micromolar, where net uptake is low and eventually ceases. It is concluded that plasmalemma nitrate transport is essentially unidirectional in plants performing net uptake at a concentration of 40 to 60 micromolar, and that transport is nil when internal nitrate sinks (vacuole, metabolism) are eliminated. The efflux component becomes increasingly important when the external concentration approaches the threshold value for net nitrate uptake (the nitrate compensation point) where considerable exchange between internal and external nitrate occurs.

The kinetics of net NO_3^- uptake in plant tissue depends on the supply of NO_3^- to the plants during culturing, and can be drastically altered by changed NO_3^- availability during pretreatment or experiments (4, 18). Nitrogen starvation generally stimulates net NO_3^- uptake (20, 31). Influences of internal NO_3^- (2), accumulated Cl^- or $Cl^-+NO_3^-$ (3, 8, 11, 30), or N-metabolites other than NO_3^- or NH_4^+ (20) have been considered in relation to regulation of the rates of NO_3^- fluxes and net NO_3^- uptake.

There are contrasting opinions regarding the effect of N-availability and plant N-status on the unidirectional components of plasmalemma NO_3^- transport. Occurrence of significant efflux of NO_3^- has been demonstrated in several species by the use of $^{15}NO_3^-$ (2, 18, 24), $^{13}NO_3^-$ (21, 23, 26, 27), as well as the NO_3^- analog $^{36}ClO_3^-$ (6, 7, 10, 12). Models for efflux regulation of net NO_3^- uptake have been developed, involving NO_3^-/NO_3^- exchange and substrate cycling (5, 9). On the other hand, it has been concluded from experiments with ^{15}N- and ^{13}N-labeled NO_3^- that regulation of NO_3^- uptake mainly is related to modulation of NO_3^- influx (18, 22, 26).

General relations between net NO_3^- fluxes and NO_3^- assimilation in *Lemna* were considered in a preceding paper (16). In the present investigation, we have used $^{13}NO_3^-$ to study the unidirectional fluxes of NO_3^- in exponentially growing N-sufficient and N-limited *Lemna gibba*. Nitrogen-limited plants were also used for studies of fluxes of NO_3^- at the threshold concentration for net NO_3^- uptake, as well as in tungstate-treated plants with blocked nitrate reductase activity.

MATERIALS AND METHODS

Culturing and Pretreatments. *Lemna gibba* L. strain G3 was cultivated as previously described (16, 17). In the N-limited cultures, NO_3^- was added once daily in doses calculated to sustain a culture N increment $(R_N)^2$ of 0.20 d^{-1}. The NO_3^- concentration of the medium directly after the NO_3^- addition ranged from 70 to 350 μM, depending on culture density. The N-sufficient cultures were grown in a medium with an initial concentration of 1.8 mM NO_3^-, the medium changed regularly depending on culture density.

Tungstate treated N-limited plants were grown as above except that N was supplemented as NH_4^+, and that MoO_4^{2-} was replaced by WO_4^{2-} (300 μM). Plants treated in this way could be grown at the relative growth rate set by R_N for several weeks in constant light. Twenty-four h before the experiments the cultures were given NO_3^-, at final concentrations of either 10 μM (to induce the NO_3^- uptake system) or 1 mM (to both induce the uptake system and saturate the NO_3^- storage pool). *In vitro* nitrate reductase activity in the tungstate treated plants was measured as described previously (16), and was found negligible.

Experiments with plants performing net uptake were conducted 1 to 6 h after the daily NO_3^- addition, the intracellular NO_3^- level being between 1 and 6 μmol g^{-1} FW. Experiments with plants incubated at the threshold concentration for net NO_3^- uptake, *i.e.* when no net uptake was recorded, were performed 24 to 30 h after the last NO_3^- addition, the intracellular NO_3^- level being below 2 μmol g^{-1} FW. The experimental medium was the same N-free medium as used before (13) with the inclusion of KNO_3 to initial concentrations of either 11 or 100 μM. Prior to the experiments, the plants were weighed and placed in appropriate experimental medium for 20 to 30 min to avoid instant changes in the relations between the external NO_3^- concentration and the concentrations of exchangeable pools of the plants at the beginning of the experiment.

Production of $^{13}NO_3^-$. The $^{13}NO_3^-$ ($t_{1/2}$ = 10 min) was produced in the 225 cm cyclotron of the Research Institute of Physics, Stockholm, in the nuclear reaction ^{16}O (p, α) ^{13}N. About 2 GBq $^{13}NO_3^-$ was produced by bombarding a water cooled target of 14 ml pure water (18 MOhm cm^{-1}) with a proton beam

[1] Supported by the Swedish Natural Science Research Council.

[2] Abbreviations: R_N: relative nitrogen addition rate; eV, electron volt; FW, fresh weight; GM-tube, Geiger-Müller-tube.

of 1 to 2 μA (14.25 MeV). After irradiation, 9 to 11 ml of the target volume could be recovered and used for experiments. The sample was flushed with argon (2 × 20 ml) to remove gaseous impurities. The chemical purity, regarding incompletely oxidized ^{13}N-species ($^{13}NO_2^-$, $^{13}NH_4^+$), was checked by ion exchange chromatography using HPLC and >96% of the radioactivity was recovered as $^{13}NO_3^-$. Using the same type of system, Gersberg *et al.* (14) obtained >99.6% $^{13}NO_3^-$ without any measurable contamination by $^{13}NO_2^-$ or $^{13}NH_4^+$. The preparations were normally used without purification as corroborating results were obtained in control experiments with HPLC purified preparations.

The chemical purity was also checked by scanning logarithmic plottings of some samples for over 2.5 h. A straight line was obtained with a half-life of 10 min. Only at very low counting rates, near the detection limit for the system, a more long-lived component ($t_{1/2}$ = 1–3 h) could be noticed. It is to be expected that this contamination might be ^{18}F which has been reported to be produced with water target systems (22). More detailed information on the radiochemistry has appeared (1, 26).

Experimental Setup. The experimental system is schematically shown in Figure 1. The glass beaker contained initially 15 ml of experimental medium and 1 g FW plants. The medium was circulated from the beaker through a quartz flow-through cuvette in a spectrophotometer and back to the beaker again. The concentration of NO_3^- was continuously recorded as the difference in absorption between 202 and 250 nm and radioactivity continuously measured in the circulating medium, employing a GM-tube placed adjacent to the cuvette in the spectrophotometer.

Calculations. Net uptake was calculated from the change in medium NO_3^- concentration. After compensation for radioisotope decay, influx of NO_3^- was calculated from the relation between the rate of disappearance of radioactivity (mRem h^{-1} h^{-1}) from the medium and the specific activity (mRem h^{-1} μmol^{-1}). The rate of radiolabel uptake in each point was obtained from the slopes of the tangents to a curve fitted to the medium radioactivity values. Efflux is given by the difference between influx and net uptake.

RESULTS

Figure 2 shows data on net NO_3^- uptake and absorption of radioactivity in N-limited plants. The original data recordings are given in Figure 2A. The plants were incubated at an initial NO_3^- concentration of approximately 100 μM, and the depletion of NO_3^- in the solution was recorded until a stable uptake rate was attained, generally between 5 and 10 min after the start of the recording. The addition of the sample containing $^{13}NO_3^-$ to the experimental medium caused an approximately 1.5-fold dilution of the NO_3^- concentration to between 40 and 60 μM. Radioactivity and total NO_3^- were uniformly distributed in the experimental medium approximately 1 min after the addition of the radioactive sample. The rate of net NO_3^- uptake was essentially unaffected by the change in external concentration from initially 100 μM to approximately 30 μM during the course of each experiment.

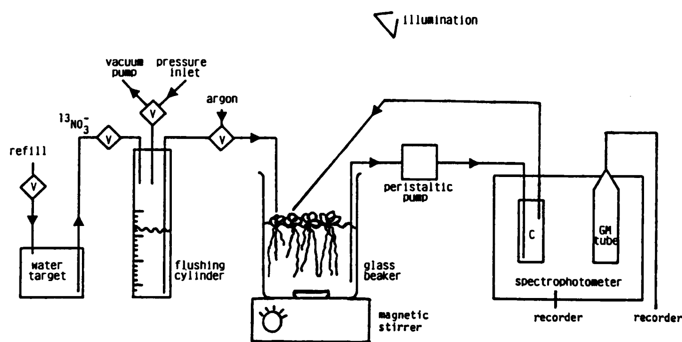

FIG. 1. Experimental system for continuous monitoring of radioactivity and NO_3^- concentration in the experimental medium. The radioactive sample was withdrawn from the target by vacuum and collected in a measuring cylinder where it was flushed with argon. Subsequently, the sample was transferred pneumatically to the glass beaker containing the plants. The GM tube placed in the spectrophotometer was protected from the bulk radioactivity in the glass beaker by lead/concrete shielding (not shown). Abbreviations: C, flow-through quartz cuvette (0.7 ml); GM, Geiger-Müller tube; V, pneumatically or manually operated valve.

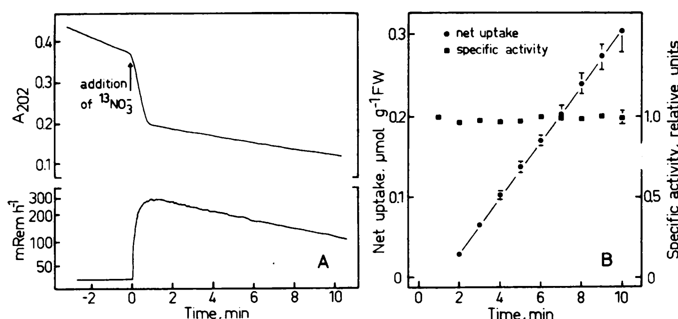

FIG. 2. Depletion of NO_3^- and radioactivity with time in experimental medium containing N-limited *Lemna*. A, Recordings of A_{202} (top) and radioactivity of the medium (bottom). The time of $^{13}NO_3^-$ addition is indicated by arrow. B, Specific activity of the experimental medium relative to the value at time 1 min and cumulative net NO_3^- uptake. Means of 3 experiments ± SD. Standard deviation bars within symbols if not indicated.

Cumulative net NO_3^- uptake together with the specific activity of the incubation medium is shown in Figure 2B. The data indicate that net NO_3^- uptake was practically identical to NO_3^- influx over the entire experimental period, as manifested by the completely constant specific activity of the medium. Any concomitant efflux of intracellular $^{14}NO_3^-$ should be detected as a decrease in specific activity, provided that the experimental resolution allows detection of this change, before isotopic equilibration is obtained (see "Discussion").

The way of maintaining the cultures with a 24 h periodicity in NO_3^- additions results in a fast depletion of medium NO_3^- to a concentration of 3 to 10 μM, followed by a phase where the plants rely solely on stored nitrogen (16, 17). Plants in this phase were transferred to experimental medium with an initial NO_3^- concentration of 11 μM, to which $^{13}NO_3^-$ was added after complete cessation of net uptake. The changes in rates of influx and net uptake, and in specific activity of the medium following $^{13}NO_3^-$ addition, are given in Figure 3. Addition of labeled NO_3^- induced a short phase of net uptake before a new threshold concentration (3–7 μM NO_3^-) for net NO_3^- uptake was attained. Initially the influx rate, calculated from the rate of disappearance of radioactivity and the specific activity of the medium, was considerably higher, but declined gradually after cessation of net uptake. The drop in specific activity of the medium points to a considerable exchange of NO_3^- between plant and medium.

Flux data obtained in plants with different N status are compiled in Table I. The highest influx rates were obtained with N-limited plants, regardless of whether the plants were incubated at 40 to 60 μM NO_3^- or whether they were incubated close to the threshold NO_3^- concentration for net uptake. The influx rate of N-sufficiently cultured plants was approximately 60% of the rate in N-limited plants. It should be pointed out that influx in the N-sufficient plants was assayed at an external concentration which is more than one order of magnitude lower than the

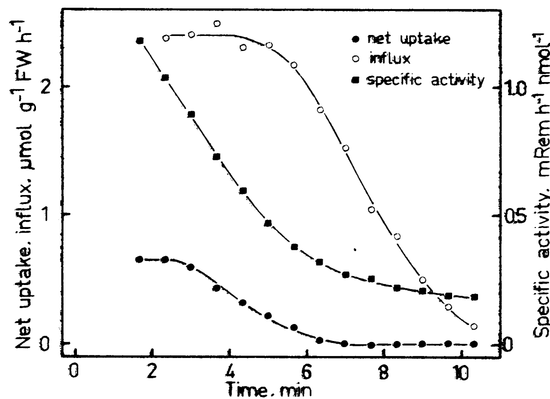

FIG. 3. Changes in rates of NO_3^- influx and net NO_3^- uptake with time in N-limited *Lemna* at low external nitrate concentrations. The NO_3^- concentration was initially 8.1 μM, and 4.5 μM after complete cessation of net NO_3^- uptake. Also plotted is the specific activity of the medium.

concentration during growth.

Fluxes were also measured in plants with blocked nitrate reductase activity due to treatment with tungstate (Table I). A small amount of NO_3^- (10 μM; 2.5 μmol g^{-1} FW) added 24 h before the assay was enough to induce the uptake system. The uptake rate was fairly low; however, there was no measurable discrepancy between influx and net uptake of NO_3^-, corroborating the observations in plants with functional nitrate reductase. Pretreatment with 1 mM NO_3^- (250 μmol g^{-1} FW) resulted in an appreciable intracellular NO_3^- accumulation, and also resulted in a total inhibition of plasmalemma NO_3^- transport. Since these plants were assayed at external NO_3^- concentrations much lower than during pretreatment, NO_3^- transport at higher external (≥1 mM) concentrations cannot be excluded.

No efflux was detected when the plants were incubated at 40 to 60 μM NO_3^-, regardless of pretreatment (Table I). Efflux was only observed close to the threshold concentration for net NO_3^- uptake.

DISCUSSION

The short half-times reported for the cytosolic NO_3^- pool, 1.5 to 18 min in corn roots (27) and 4 min (21) to 17 min (10) in barley roots, emphasizes the importance of the time resolution in experiments on plasmalemma NO_3^--tracer fluxes. Use of ^{13}N-labeled NO_3^- or the NO_3^--analog $^{36}ClO_3^-$ gives opportunities to trace short-term NO_3^- fluxes which can be attributed to plasmalemma NO_3^- transport (6, 7, 10, 12, 15, 21–23, 27), although the possibility of spatial separation of influx and efflux cannot be neglected (26). The strong radioactive emission of ^{13}N facilitates continuous monitorings of NO_3^- exchange, which further contributes to experimental resolution.

With the employed experimental approach we were, however, not able to detect any discrepancy between influx and net uptake of NO_3^- in *Lemna* at 40 to 60 μM NO_3^-, regardless of the plant N status (N-limited, N-sufficient, NO_3^--starved or NO_3^--loaded tungstate-treated plants). The lack of apparent efflux could, possibly, be caused by rapid label-equilibration of the exchangeable pool during the time of physical stabilization of the experimental system immediately after addition of the tracer. There are, however, some arguments against such interpretation. Even if we consider the shortest half-life of the exchangeable pool so far reported (1.5 min in corn roots at external NO_3^- concentrations >1 mM, [27]), it appears unlikely that an efflux component of any significance should remain undetected. The experimental system, furthermore, allowed detection of considerable efflux when the plants were incubated at NO_3^- concentrations close to the threshold value for net uptake (Fig. 3). The same system was also used in a previous study on pea, where clear discrepancies between influx and net uptake were recorded under similar nutritional conditions as used in the present study (26).

It can thus be concluded that plasmalemma NO_3^- flux in *Lemna* at 40 to 60 μM NO_3^- is essentially unidirectional, and that differences in net NO_3^- uptake related to the plant N status correspond solely to differences in plasmalemma NO_3^- influx. The external NO_3^- concentrations used for the influx experiments are within a range where net uptake is essentially concentration independent, and also encountered by the plants maintained at N-limitation. The N-sufficient or NO_3^- loaded plants

Table I. *Measured Rates of Influx and Net Uptake of NO_3^-, and Calculated Rates of NO_3^- Efflux in Plants Differing in N-status*

Values at the threshold concentration represents initial (100–140 s) rates. The assays were performed at the external NO_3^- concentrations indicated within brackets. Values represent means ± SD and the number of determinations (*n*) is indicated for each treatment.

Growth and Assay Conditions	Net Uptake	Influx	Efflux	Intracellular NO_3^-
		μmol g⁻¹ FW h⁻¹		*μmol g⁻¹ FW*
N-sufficient [40–60 μM] (*n* = 4)	1.12 ± 0.05	1.12 ± 0.11	−0.01 ± 0.08	10.6 ± 1.2
N-limited				
During net uptake [40–60 μM] (*n* = 4)	2.02 ± 0.19	1.96 ± 0.19	0.06± 0.06	1–6 (increasing)
At the threshold concentration [3–10 μM] (*n* = 5)	1.28 ± 0.34	1.92 ± 0.15	0.64 ± 0.24	<2
Tungstate-treated				
10 μM NO_3^- added 24 h before test [40–60 μM] (*n* = 3)	1.08 ± 0.06	1.07 ± 0.10	0.00 ± 0.06	2.8
1 mM NO_3^- added 24 h before test [65 μM] (*n* = 3)	0	0	0	30.8

were, however, pretreated at concentrations 10 times those used during experiment. Despite this, the relatively slow performance at 40 to 60 μM NO_3^- is strictly related to the influx rate and not to any stimulation of efflux.

The central role of influx capacity in regulating net NO_3^- uptake has also been demonstrated by the concentration kinetics of $^{13}NO_3^-$ influx in barley seedlings differing in N status, where increased net NO_3^- uptake in N-starved seedlings was accompanied by an increase in the maximum rate of NO_3^- influx (I_{max}) without any change in K_s (22). Interpretations based on other experiments with $^{13}NO_3^-$ (15) or $^{36}ClO_3^-$ (7, 9, 12), however, rather suggested that the main control of net NO_3^- uptake was related to modulation of NO_3^- efflux. The reasons for these discrepancies are obscure; however, some of the contradictions might be attributed to different pretreatment of the plant material (22).

A clear discrepancy between influx and net uptake of NO_3^- in *Lemna* only appeared near the threshold concentration for NO_3^- uptake (Fig. 3). At this concentration, influx initially proceeded at the same rate as during net uptake, although the rate of net NO_3^- uptake was low and eventually ceased. It is plausible that the rate of efflux is stimulated when the NO_3^- concentration of the medium decreases, due to net NO_3^- uptake. The subsequent decline in the influx rate is probably a consequence of NO_3^- exchange, which finally leads to isotopic equilibration between the external medium and the exchangeable pool. These results, thus, indicate that the threshold concentration for net uptake should be regarded as a compensation point. It can be assumed that efflux of NO_3^- is favored by the concentration gradient over plasmalemma, and could possibly be mediated by a NO_3^-/NO_3^- exchange or substrate cycling system as proposed by Deane-Drummond (5, 9). The control of the NO_3^- compensation point, if regulated, could be of vital importance for the ability of the plants to cope with low N concentrations, since it sets the ultimate affinity limit for net uptake, and may also affect the rate of NO_3^- diffusion to the membrane surface (28).

The absence of any NO_3^- efflux during net NO_3^- uptake in *Lemna* contrasts to results reported for other plants, *e.g.* pea (7, 26), dwarf bean (2), wheat (18), perennial ryegrass (24), barley (10, 12, 21). It must be stressed that NO_3^- efflux induced by a transient change in external NO_3^- concentration was avoided in the present study by pretreatment of the plants in the appropriate experimental medium for 20 to 30 min prior to each experiment. Furthermore, in *Lemna* there is a proximity between uptake and assimilation of NO_3^-, whereas a certain proportion of the NO_3^- taken up in root tissue of soil-growing plants is subject to long distance translocation. Interpretations of recent results on NO_3^- uptake and reduction in corn roots (25, 29), partially based on results on K^+ uptake in the same species (19), indicate that most NO_3^- uptake and reduction take place in epidermal cells at low external NO_3^- concentrations (0.2 mM), whereas cortical cells may be involved in uptake and reduction at high (50 mM) concentrations. It can be hypothesized that efflux of NO_3^- from the symplast to the apoplastic space during translocation occurs from cells with lower affinity to external NO_3^-, and that tissue age might be of importance. The less marked tissue heterogenity in *Lemna* may explain the low efflux in this species. It can further be hypothesized that hydroponic culture of normally soil-growing plants amplifies what would under normal conditions be a negligible efflux.

Concluding Remarks. Differences in net NO_3^- uptake in relation to N-status of the *Lemna* cultures are directly associated with alterations in NO_3^- influx, whereas no association to efflux is seen. These observations corroborate other studies with $^{13}NO_3^-$ (22, 26), but contradict the contention of efflux being a major regulator of net NO_3^- uptake, as proposed previously (5, 9, 12). However, control of efflux may be an important parameter when it comes to regulation of the NO_3^- compensation point. To what extent the observed variations in influx reflect regulation of activity of existing transport systems, or merely differences in the amount of transport systems cannot be concluded; elucidation of this problem probably requires a more molecular approach.

In an accompanying paper (16), the influence of net NO_3^- uptake on the rate of NO_3^- reduction in *Lemna* was established. Bearing the results of the present study in mind, it can be concluded that regulation of plasmalemma NO_3^- influx in *Lemna* performing net NO_3^- uptake is of vital importance also for the rate of NO_3^- reduction. The pattern is, however, less clear when the plants rely solely on the utilization of internal NO_3^- pools, *i.e.* at the NO_3^- compensation point, where considerable exchange between internal and external pools of NO_3^- occurs.

LITERATURE CITED

1. AF UGGLAS M, C-M LARSSON, B INGEMARSSON, P OSCARSON, M LARSSON, W FORSLING, I IOVA 1986 The use of ^{13}N in studies of regulation of membrane transport in plants. Bienn Rep 1984–1985 of Res Inst of Physics, Stockholm, pp 175–181
2. BRETELER H, P NISSEN 1982 Effect of exogenous and endogenous nitrate concentration on nitrate utilization by dwarf bean. Plant Physiol 70: 754–759
3. CRAM WJ 1973 Internal factors regulating nitrate and chloride influx in plant cells. J Exp Bot 24: 328–341
4. DEANE-DRUMMOND CE 1982 Mechanisms for nitrate uptake into barley (*Hordeum vulgare* L. cv Fergus) seedlings grown at controlled nitrate concentrations in the nutrient medium. Plant Sci Lett 24: 79–89
5. DEANE-DRUMMOND CE 1984 Mechanism of nitrate uptake into *Chara corallina* cells: lack of evidence for obligatory coupling to proton pump and a new NO_3^-/NO_3^- exchange model. Plant Cell Environ 7: 317–323
6. DEANE-DRUMMOND CE 1984 Nitrate transport into *Chara corallina* cells using $^{36}ClO_3^-$ as an analogue for nitrate. I. Interaction between $^{36}ClO_3^-$ and NO_3^- and characterization of $^{36}ClO_3^-/NO_3^-$ influx. J Exp Bot 35: 1289–1298
7. DEANE-DRUMMOND CE 1985 Characteristics of $^{36}ClO_3^-$ and $^{36}Cl^-$ uptake into *Pisum sativum* L. seedlings: limitations and uses of $^{36}ClO_3^-$ as an analogue for NO_3^-. Plant Sci 42: 99–105
8. DEANE-DRUMMOND CE 1986 A comparison of regulatory effects of chloride on nitrate uptake, and of nitrate on chloride uptake into *Pisum sativum* seedlings. Physiol Plant 66: 115–121
9. DEANE-DRUMMOND CE 1986 Nitrate uptake into *Pisum sativum* L. cv. Feltham First seedlings: commonality with nitrate uptake into *Chara corallina* and *Hordeum vulgare* through a substrate cycling model. Plant Cell Environ 9: 41–48
10. DEANE-DRUMMOND CE, ADM GLASS 1982 Nitrate uptake into barley (*Hordeum vulgare*) plants. A new approach using $^{36}ClO_3^-$ as an analogue for NO_3^-. Plant Physiol 70: 50–54
11. DEANE-DRUMMOND CE, ADM GLASS 1982 Studies of nitrate influx into barley roots by the use of $^{36}ClO_3^-$ as a tracer for nitrate. I. Interactions with chloride and other ions. Can J Bot 60: 2147–2153
12. DEANE-DRUMMOND CE, ADM GLASS 1983 Short term studies of nitrate uptake into barley plants using ion-specific electrodes and $^{36}ClO_3^-$. I. Control of net uptake by NO_3^- efflux. Plant Physiol 73: 100–104
13. ERICSSON T, C-M LARSSON, E TILLBERG 1982 Growth responses of *Lemna* to different levels of nitrogen limitation. Z Pflanzenphysiol 105: 331–340
14. GERSBERG R, K KROHN, N PEEK, CR GOLDMAN 1976 Denitrification studies with ^{13}N-labelled nitrate. Science 192: 1229–1231
15. GLASS ADM, RG THOMPSON, L BORDELEAU 1985 Regulation of NO_3^- influx in barley. Studies using $^{13}NO_3^-$. Plant Physiol 77: 379–381
16. INGEMARSSON B 1987 Nitrogen utilization in *Lemna*. I. Relations between net nitrate flux, nitrate reduction, and *in vitro* activity and stability of nitrate reductase. Plant Physiol 85: 856–859
17. INGEMARSSON B, L JOHANSSON, C-M LARSSON 1984 Photosynthesis and nitrogen utilization in exponentially growing nitrogen-limited cultures of *Lemna gibba*. Physiol Plant 62: 363–369
18. JACKSON WA, KD KWIK, RJ VOLK, RG BUTZ 1976 Nitrate influx and efflux by intact wheat seedlings: effects of prior nitrate nutrition. Planta 132: 149–156
19. KOCHIAN LV, WJ LUCAS 1983 Potassium transport in corn roots. II. The significance of the root periphery. Plant Physiol 73: 208–215
20. LEE RB, KA RUDGE 1986 Effects of nitrogen deficiency on the absorption of nitrate and ammonium by barley plants. Ann Bot 57: 471–486
21. LEE RB, DT CLARKSON 1986 Nitrogen-13 studies of nitrate fluxes in barley roots. I. Compartmental analysis from measurements of ^{13}N efflux. J Exp Bot 37: 1753–1767
22. LEE RB, MC DREW 1986 Nitrogen-13 studies of nitrate fluxes in barley roots. II. Effect of plant N-status on the kinetic parameters of nitrate influx. J Exp Bot 37: 1768–1779

23. McNaughton GS, MR Presland 1983 Whole plant studies using radioactive 13-nitrogen. I. Techniques for measuring the uptake and transport of nitrate and ammonium ions in hydroponically grown *Zea mays*. J Exp Bot 34: 880–892
24. Morgan MA, RJ Volk, WA Jackson 1973 Simultaneous influx and efflux of nitrate uptake by perennial ryegrass. Plant Physiol 51: 267–272
25. Morgan MA, WA Jackson, RJ Volk 1985 Concentration-dependence of the nitrate assimilation pathway in maize roots. Plant Sci 38: 185–191
26. Oscarson P, B Ingemarsson, M af Ugglas, C-M Larsson 1987 Short-term studies of NO_3^- uptake in *Pisum* using $^{13}NO_3^-$. Planta 170: 550–555
27. Presland MR, GS McNaughton 1984 Whole plant studies using radioactive 13-nitrogen. II. A compartmental model for the uptake and transport of nitrate ions by *Zea mays*. J Exp Bot 35: 1277–1288
28. Robinson D 1986 Limits to nutrient inflow rates in roots and root systems. Physiol Plant 68: 551–559
29. Rufty TW Jr, JF Thomas, JL Remmler, WH Campbell, RJ Volk 1986 Intercellular localization of nitrate reductase in roots. Plant Physiol 82: 675–680
30. Smith FA 1973 The internal control of nitrate uptake into excised barley roots with differing salt contents. New Phytol 72: 769–782
31. Ullrich WR, HD Schmitt, E Arntz 1981 Regulation of nitrate uptake in green algae and duckweeds. Effect of starvation and induction. *In* H Boethe, A Trebst, eds, Biology of Inorganic Nitrogen and Sulfur. Springer-Verlag, Heidelberg, pp 244–251