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OPINION

The case for cytosolic NO_3^- heterostasis: a critique of a recently proposed model

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ABSTRACT

A model recently proposed by Siddiqi & Glass (*Plant, Cell, and Environment* 25, 1211–1217, 2002) attempts to reconcile discrepancies in the measurement of cytosolic nitrate concentrations ($[NO_3^-]_{cyt}$) in plant root cells, specifically between low (~ 4 mM) homeostatic values reported in studies using ion-specific microelectrodes on the one hand, and wide fluctuations in $[NO_3^-]_{cyt}$ reported in other studies, especially those using compartmental analysis by tracer efflux (CATE). Although Siddiqi & Glass concede that cytosolic NO_3^- homeostasis, as determined by microelectrodes, is at odds with certain experimental observations, they nevertheless promote a model that takes microelectrode readings at face value, and assert that the variations seen using CATE methodology are artefacts attributable to contributions from substantial, rapidly exchanging, and highly variable NO_3^- pools putatively residing in organelles such as plastids and the endoplasmic reticulum. We show here that such a model is not tenable, drawing upon experimental evidence from previous studies, and from a more comprehensive model that examines the characteristics and consequences of subcompartmented cytoplasmic exchange in root cells.

Key-words: compartmental analysis; cytoplasm; cytosol; efflux; heterostasis; homeostasis; microelectrodes; nitrate.

INTRODUCTION

Discrepancies in measurements of nitrate (NO_3^-) pools in compartments of plant root cells among various techniques have been a subject of controversy for at least a decade (King, Siddiqi & Glass 1992; Miller & Smith 1996; van der Leij, Smith & Miller 1998; Forde & Clarkson 1999; Britto & Kronzucker 2001; Miller *et al*. 2001; Siddiqi & Glass 2002). Estimates of cytosolic nitrate concentrations $[NO_3^-]_{cyt}$ using different methods can vary several-fold under the same conditions in the same plant system. In particular, studies using ion- and compartment-specific microelectrodes appear to show that the cytosolic nitrate pool is invariable (Zhen *et al*. 1991; Miller & Smith 1992, 1996; Miller, Walker & Smith 1995; van der Leij *et al*. 1998), in sharp contrast to all studies using other methods, including those that base pool size determinations on *in vivo* versus *in vitro* nitrate reductase activities (Robin *et al*. 1983; Belton, Lee & Ratcliffe 1985; King *et al*. 1992), on tracer influx profiles (Presland & McNaughton 1984), on nuclear magnetic resonance (NMR) signals (G. Ratcliffe, personal communication), and on a method utilized more extensively than any other technique in this context, compartmental analysis by tracer efflux [CATE (this term is more precise than the abbreviated form, 'CAE' used previously, e.g. by Siddiqi & Glass 2002) Lee & Clarkson 1986; Siddiqi, Glass & Ruth 1991; Devienne, Mary & Lamaze 1994; Kronzucker, Glass & Siddiqi 1995, 1999; Kronzucker, Siddiqi & Glass 1995; Kronzucker *et al*. 1999; Min *et al*. 1999; Britto & Kronzucker 2001]. Despite agreement among non-electrode methods that the cytosolic nitrate pool can vary considerably (up to 80-fold variations in the pool have been reported depending on plant N status and external conditions; see Britto & Kronzucker 2001), microelectrode readings are often taken as definitive, presumably because of their apparent directness. This trend is exemplified in a recent paper by Siddiqi & Glass (2002), who, despite their own leading work on this subject using CATE-based approaches (e.g. Siddiqi *et al*. 1991), 'accept the reported [microelectrode] values as valid measurements', and reject the idea that CATE measures the same parameter. Siddiqi & Glass propose a model that claims to reconcile discrepancies in absolute values and variability of $[NO_3^-]_{cyt}$ by classifying the major techniques into two groups, 'group 1 techniques' (including the microelectrode method) that quantify a purely cytosolic pool, and 'group 2 techniques' (including the CATE method) that quantify a combination of the cytosolic pool and other subcellular ('cytoplasmic') compartments such as plastids or the endoplasmic reticulum (ER). In fact, a new nomenclature was proposed by Siddiqi & Glass to distinguish 'cytosolic' from 'cytoplasmic' nitrate pools.

Unfortunately, although attractive, this hypothesis, and the classification and nomenclature associated with it, are unfounded. There is no factual basis, or *a priori* necessity, for the claim that techniques such as CATE fail to distinguish between cytosolic and organellar NO_3^- pools; never-

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theless, Siddiqi & Glass take this claim as the basic premise of their arguments. The implications of such a statement are substantial, and must be challenged here. Some of the important issues at stake are: the thermodynamic gradients of NO_3^- across the plasma membrane as well as across internal membranes; substrate availability for nitrate reductase activity (often considered the limiting step in nitrate acquisition); the regulatory role of subcellular N pools in transport activity; the understanding of unique cellular properties of nitrate acquisition such as the induction and de-induction of transport and assimilation systems; and the more general understanding of cellular ionic interactions, given that nitrate can be one of the most abundant anions in the plant cell.

In this article, we will show that the classification of techniques into 'group 1' and 'group 2' methods requires reconsideration. More importantly, we will also show why the central claim of Siddiqi & Glass, about the errors associated with CATE studies and their results pertaining to variations in cytosolic NO_3^-, is not only not substantiated, but also fundamentally untenable.

DISCUSSION

The grouping of techniques into those that measure a purely cytosolic NO_3^- pool (group 1), and those that measure a combination of subcellular pools (group 2), is questionable. Several major flaws in this classification proposal are apparent, and will be discussed in turn here.

1 Non-aqueous cell fractionation is considered by Siddiqi & Glass to be a group 2 technique, but, in fact, workers who use this technique go to substantial lengths to distinguish chemical pools in different subcellular organelles from one another, rather than presenting a combined reading. This is accomplished primarily by use of enzyme markers, following a well-established methodology (Martinoia, Heck & Wiemken 1981; Belton *et al*. 1985; Winter, Robinson & Heldt 1993, 1994). Surprisingly, in their only quantitative example, Siddiqi & Glass use 'cytosolic' values from one such study (in fact, these values are reported merely as 'detection limits' by personal communication in Winter *et al*. 1994), even after having stated that this technique, falling into group 2, does not distinguish 'cytosolic' from 'cytoplasmic' pools. It should be pointed out that for cellular compartments of small volume, such as the cytosol and the chloroplast stroma (as opposed to the vacuole) in many plant systems, even high nitrate concentrations would fall below the detection limits of this method (~30–46 mM in barley leaf cells), as would any variations in $[NO_3^-]_{cyt}$ occurring below this threshold. Detection limit values of this kind cannot be used in quantitative analyses such as presented by Siddiqi & Glass.

2 A study based on ^{14}N NMR methodology (Belton *et al*. 1985), on the other hand, was listed as using a group 1 technique. However, in this study, only a single NMR-spectrum peak for NO_3^- was observed in each plant system examined, and integration of this peak yielded values in excellent agreement with values for total tissue nitrate concentrations obtained from chemical analyses of plant tissue. Indeed, because of the large volume differences between cytosol and vacuole, cytosolic $[NO_3^-]$ in mature maize and barley roots (plant systems comparable or identical to those used in studies with CATE and microelectrodes) could be as high as those in the vacuole (i.e. 15–90 mM!) and still remain undetectable by this method. Thus, as in the case of cell fractionation, listing this method as yielding 'undetectable' cytosolic NO_3^- levels is misleading, as it reflects more a limitation of sensitivity in technique than the lack of a substantial, and potentially variable, cytosolic NO_3^- pool.

3 CATE is assumed by Siddiqi & Glass to be incapable of providing cytosolic ion concentration measurements that are unadulterated by the participation of other subcellular (i.e. organellar) ion pools in the tracer efflux signal, and hence is placed in group 2. It is proposed, at least in the case of nitrate, that labelled fluxes from such extracytosolic pools (e.g. the plastids) not only contribute to tracer elution signals, but also dominate cellular nitrate partitioning to the extent that any variations in 'cytosolic' NO_3^- pools reported in many such studies (Lee & Clarkson 1986; Siddiqi *et al*. 1991; Devienne *et al*. 1994; Kronzucker, Glass & Siddiqi 1995, 1999; Kronzucker, Siddiqi & Glass 1995, 1997; Kronzucker *et al*. 1999; Min *et al*. 1999; Britto & Kronzucker 2001) are attributable entirely to variations in these putative additional pools. There are several serious problems with this proposal (see also earlier dismissals of similar ideas by Cram 1968 and Hajibagheri *et al*. 1988):

A Siddiqi & Glass use detection-limit data reported in Winter *et al*. (1994; see above for a critique of this usage) to justify that the contribution of a plastidic nitrate pool could potentially double the estimates of what would be assumed to be a purely cytosolic pool obtained by CATE. However, although it is indeed well established that millimolar concentrations of NO_3^- can build up in chloroplasts (Schröppel-Meier & Kaiser 1988a, b), no such pools have been shown to exist in root proplastids (the pertinent compartment to be considering here), nor is there any reason to expect the existence of rapidly exchanging, high-capacity, nitrate storage functions in these organelles. Similarly, no such pools have been demonstrated to be associated with the endoplasmic reticulum, dictyosomes, nuclei, or any other major organelle in higher plant roots, other than the vacuole (Martinoia *et al*. 1981). Moreover, the requirement of the above proposal, that all variations in 'cytoplasmic' nitrate be due solely to variations in organellar pools, is unlikely to be true even in the case of chloroplasts; in fact, data from a significant number of studies estimating nitrate pools in the chloroplast, under various conditions, are remarkably consistent (typically 2–10 mM), and, importantly, these concentrations do not appear to vary as a function of external nitrate supply. In particular, Schröppel-Meier & Kaiser (1988a, b) have

shown in spinach that, even when the external nitrate concentration is stepped up from 15 to 300 mM, the internal concentration in chloroplasts isolated from this material appears homeostatically held at ~5 mM. This would render unlikely the possibility that plastids are responsible for the well-documented variability of 'cytoplasmic' nitrate pools, as determined by CATE and other methods. Interestingly, chloroplasts also act as a major storage pool for potassium, with stromal concentrations typically reaching double those found in the cytosol (Robinson & Downton 1984; Schröppel-Meier & Kaiser 1988a, b). Siddiqi & Glass use such considerations to explain why higher 'cytoplasmic' $[K^+]$ values were found by Memon, Saccomani & Glass (1985) using CATE with $^{86}Rb^+$ (a problematic tracer for K^+ – see Jeschke 1970; Jacoby, Abas & Steinitz 1973), than were found by Walker, Leigh & Miller (1996) using potassium-selective microelectrodes. However, our own extensive studies (unpublished), using CATE with $^{42}K^+$ (an appropriate tracer, not afflicted with isotope discrimination), contradict this, having yielded cytosolic $[K^+]$ values closely in line with those determined for the same species (barley) in the study by Walker *et al*. (1996; see also Hajibagheri *et al*. 1988).

B A consequence of the proposal by Siddiqi & Glass, although not discussed explicitly by the authors, is that the presence of substantial, rapidly exchanging, extra-cytosolic nitrate pools renders the accumulation and release of tracer to and from the cytosol a non-exponential set of processes. Figure 1 shows how the patterns of labelling and release for the cytosol and a putative extra-cytosolic compartment are deflected from simple exponentiality, assuming that the nitrate contents in the cytosol and this compartment are equal, and that the fluxes across the plasma membrane and across the membrane separating the cytosol from the extra-cytosolic compartment are also equal. Not only do none of the four processes of labelling or elution obey first-order kinetics (see, for instance, Fig. 1, inset), but the apparent exchange 'half-times' falsely derived from efflux plots in such situations could be substantially longer than the apparent 'half-times' of the labelling process. Consequently, estimates of the specific activity of compartments within the cell that are releasing tracer to the external medium could be severely erroneous and, in turn, the widely used practice of tracer efflux analysis to determine proper loading and washing time courses for influx experiments would be invalidated. Although the present authors do not endorse this view, because such deviations from exponentiality have never been experimentally observed, we wish to point out that it is a necessary outcome of the proposal by Siddiqi & Glass. Only in the presence of very large fluxes between the cytosol and the extra-cytosolic compartment(s) would an approximation to exponentiality, and an equality between apparent labelling and elution half-times, take place, a condition that we will now address.

C The reconciliation offered by Siddiqi & Glass between invariable 'cytosolic' readings by microelectrode methods, and variable 'cytoplasmic' readings by other methods, rests upon the assumption that any nitrate-pool variation observed occurs exclusively inside the extra-cytosolic compartment(s). Modelling the actual magnitude of variation seen experimentally (see Kronzucker, Glass & Siddiqi 1999 for a detailed data set on barley, used here for modelling purposes), this assumption requires that the apparent half-times of exchange made visible in tracer elution profiles must systematically change with external nitrate supply (and thus with the filling state of the extra-cytosolic compartment), unless the fluxes operating at the membranes bounding these extra-cytosolic compartments are of an unprecedentedly high magnitude (Fig. 2). Such changes in apparent 'cytoplasmic' NO_3^- half-times, however, have never been experimentally observed (see Britto & Kronzucker 2001; interestingly, the resilience of the half-time parameter across many conditions of NO_3^- provision is also acknowledged by Siddiqi & Glass). Comparing actual experimental values (from Kronzucker, Glass & Siddiqi 1999) for NO_3^- influx, half-time, and 'cytoplasmic' pool size in uninduced barley plants and plants cultured at a steady state of 10 mM NO_3^-, Fig. 2 shows that to account for the 38-fold increase in the 'cytoplasmic' pool size, fluxes of ~800 μmol g^{-1}(FW) h^{-1} would have to prevail across the extra-cytosolic compartment membranes to obtain a half-time commensurate with the one actually observed. Such large fluxes are without precedent in the plant ion transport literature, and, if they exist, should be the target of significant attention amongst transport physiologists in search of high-capacity NO_3^- membrane transporters capable of abstracting nitrate at enormously variable rates from a non-changing substrate pool (as implied by cytosolic NO_3^- homeostasis). However, until demonstrated, such flux events remain purely speculative.

4 Although Glass & Siddiqi place CATE in 'group 2' without any evidence to support this categorization, they also place microelectrode methods in 'group 1', again without substantiation. If it is indeed true that substantial NO_3^- pools exist in plant cell compartments other than the cytosol or vacuole, it is plausible that microelectrodes, which at present are designed to only distinguish cytosol from vacuole, might furnish readings from such other compartments.

CONCLUSION

It emerges clearly that, both on experimental and theoretical grounds, the proposal by Siddiqi & Glass, which attempts to reconcile NO_3^- pool measurements obtained by microelectrodes and by CATE, is not tenable, and alterna-

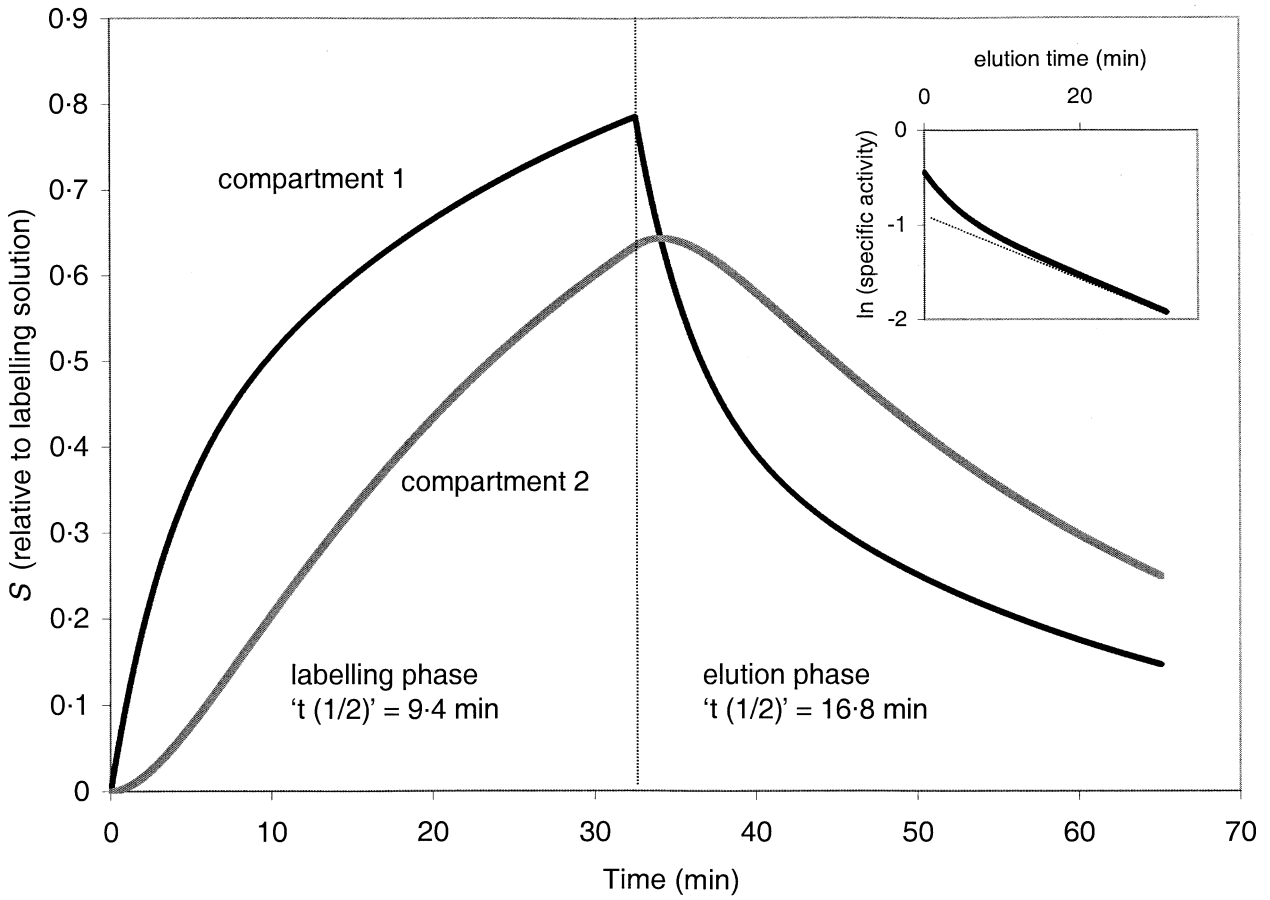

Figure 1. Model depicting the rise and fall of specific activity (*S*), relative to an external labelling solution, in both compartments of an initially unlabelled cellular system consisting of two compartments in series. Compartment sizes of 0·43 and 0·59 μmol g^{-1}(FW) were assigned, respectively, to compartments 1 and 2, following the example given in Siddiqi & Glass (2002; but see text for a caveat concerning such values), and moderate values of 3 μmol g^{-1}(FW) h^{-1} were assigned to chemical fluxes in both directions across the plasma membrane and across the intracellular membrane separating the two compartments. Tracer flux was calculated as the product of the chemical flux and the specific activity of the pool from which the transported material originated. To adequately model tracer reflux between the two compartments during labelling and elution, a spreadsheet was used to calculate 6500 iterations (each representing a duration of 0·01 min) resulting from the four simultaneous fluxes (one in each direction across each of the two membranes). The effect of this reflux can be seen in the continued rise of *S* in compartment 2 even after the elution of the whole system has begun; this rise persists until *S* in compartment 2 exceeds that of compartment 1. Apparent half-times of exchange (abbreviated 't (1/2)') are given for labelling and elution of compartment 1. Inset: semi-logarithmic plot of the elution phase of compartment 1, showing deviation from linearity (dashed line) and hence from first-order kinetics.

tive explanations of the reported discrepancies must be sought. We here suggest that the lack of variability of NO_3^- pools in the cytosol as measured by the microelectrode method, in the face of substantial variations found by all other major methods, should give rise to doubt as to the veracity of the method in this particular context. Although it is true that microelectrodes afford some confidence because of the directness of the measurements involved, and indeed have provided subcellular pool size values for some ions (e.g. K^+) that are in agreement with other methods, it should be kept in mind that electrical signals collected in such systems are not nearly as straightforward to interpret as is often suggested. Indeed, one leading expert in microelectrodes emphasized in a review on calcium electrodes, that work of this nature is 'notoriously difficult, and prone to many pitfalls' (Felle 1989). These problems include interference by ions other than the one of interest (Cuin *et al.* 1999; Carden, Diamond & Miller 2001), signal clarity and stability (Mertz & Higinbotham 1976; Beilby & Blatt 1986; see also Felle 1989), and calibration (Coombs, Miller & Sanders 1994; Miller & Smith 1996). Another problem, rarely considered, is the likelihood that the invasive nature of an electrode impalement will cause substantial ionic efflux from the cell compartment under observation (as is known for other kinds of physical perturbations; see Forde & Clarkson 1999), which could substantially influence the reading. The fact that NO_3^- readings obtained by microelectrodes appear to remain stable

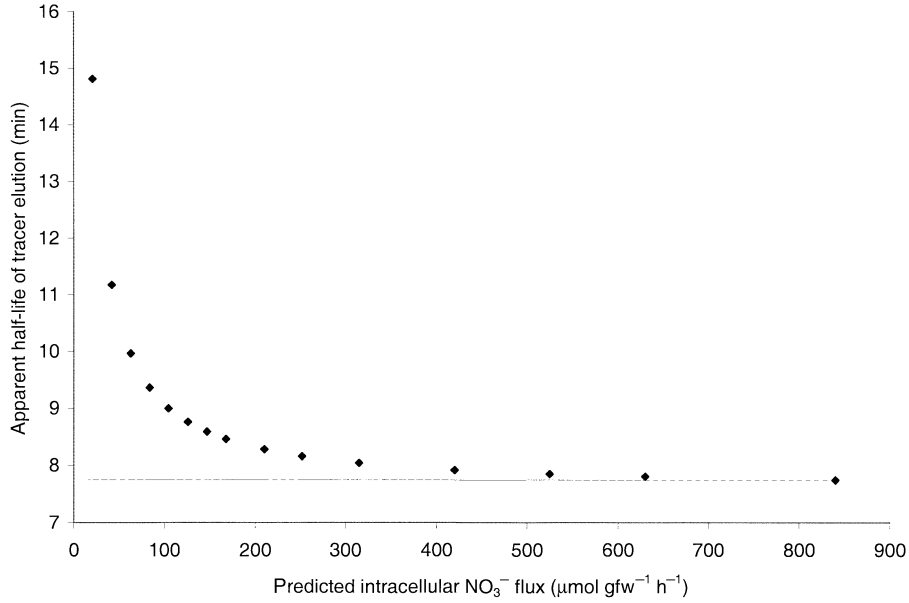

Figure 2. Model depicting expected apparent half-times of exchange that would be observed in tracer elution from a two-compartment system in which all changes in the size of the combined pool are accounted for by changes in the pool of the interior compartment (based on a data set from Kronzucker, Glass & Siddiqi 1999). Half-times are plotted as a function of the extent of fluxes across the membrane separating the two compartments, and also result from changes in plasma membrane fluxes in response to increased external provision of the traced ion. Apparent half-times were determined by linear regression of iterative plots generated in the same manner as that in Fig. 1 (inset). Dashed line indicates the half-life that was actually observed under these conditions (Kronzucker, Glass & Siddiqi 1999).

regardless of the level of NO_3^- supply (including the condition of prolonged NO_3^- withdrawal during which de-induction of nitrate transport and assimilation have been documented – see Siddiqi *et al*. 1989) adds additional uncertainty to nitrate-specific microelectrode methodology. This is particularly true given that the undiminished presence of NO_3^- as an induction signal, as implied by microelectrode results, challenges our understanding of the induction and de-induction of NO_3^- transport and assimilation (see, e.g. Forde & Clarkson 1999; Tischner 2000). CATE, on the other hand, has yielded eminently sensible results in this context (see, e.g. Kronzucker, Siddiqi & Glass 1997). It should also be noted that CATE has yielded compelling results regarding ecophysiological differences in nitrate acquisition among numerous plant species, especially trees (Kronzucker, Glass & Siddiqi 1995; Kronzucker, Siddiqi & Glass 1995, 1997; Min *et al*. 1999; Kronzucker *et al*. 2003), which reflect well-established differences in adaptations to ecological N niches. Until equally detailed studies are available from microelectrode work, dismissal of CATE appears premature, and is difficult to justify scientifically.

REFERENCES

Beilby M.J. & Blatt M.R. (1986) Simultaneous measurements of cytoplasmic K^+-concentration and the plasma-membrane electrical parameters in single membrane samples of *Chara corallina*. *Plant Physiology* **82,** 417–422.

Belton P.S., Lee R.B. & Ratcliffe R.G. (1985) A ^{14}N nuclear magnetic resonance study of inorganic nitrogen metabolism in barley, maize and pea roots. *Journal of Experimental Botany* **36,** 190–210.

Britto D.T. & Kronzucker H.J. (2001) Constancy of nitrogen turnover kinetics in the plant cell: insights into the integration of subcellular N fluxes. *Planta* **213,** 175–181.

Carden D.E., Diamond D. & Miller A.J. (2001) An improved Na^+-selective microelectrode for intracellular measurements in plant cells. *Journal of Experimental Botany* **52,** 1353–1359.

Coombs H.V., Miller A.J. & Sanders D. (1994) Disruptive effects of protein on performance of liquid membrane-based ion-selective microelectrode. *American Journal of Physiology – Cell Physiology* **36,** c1027–c1035.

Cram W.J. (1968) The control of cytoplasmic and vacuolar ion contents in higher plant cells. In *International Symposium on Transport and Distribution of Matter in Cells of Higher Plants* (ed. K. Mothe), pp. 117–126. Akademie-Verlag, Berlin, Germany.

Cuin T.A., Miller A.J., Laurie S.A. & Leigh R.A. (1999) Nitrate interference with potassium-selective microelectrodes. *Journal of Experimental Botany* **50,** 1709–1712.

Devienne F., Mary B. & Lamaze T. (1994) Nitrate transport in intact wheat roots. 1. Estimation of cellular fluxes and NO_3^- compartmental analysis from data of (NO_3^-) ^{15}N distribution using efflux. *Journal of Experimental Botany* **45,** 667–676.

Felle H. (1989) Ca^{2+}-selective microelectrodes and their application to plant cells and tissues. *Plant Physiology* **91,** 1239–1242.

Forde B.G. & Clarkson D.T. (1999) Nitrate and ammonium nutrition of plants: physiological and molecular perspectives. *Advances in Botanical Research Incorporating Advances in Plant Pathology* **30,** 1–90.

Hajibagheri M.A., Flowers T.J., Collins J.C. & Yeo A.R. (1988) A comparison of the methods of X-ray-microanalysis, compart-

mental analysis and longitudinal ion profiles to estimate cytoplasmic ion concentrations in 2 maize varieties. *Journal of Experimental Botany* **39,** 279–290.

Jacoby B., Abas S. & Steinitz B. (1973) Rubidium and potassium absorption by bean-leaf slices compared to sodium absorption. *Physiologia Plantarum* **28,** 209–214.

Jeschke W.D. (1970) On application of ^{86}Rb as a tracer for potassium, measurements of light-dependent ^{42}K/K-influx and ^{86}Rb/Rb-influx in *Elodea densa*. *Zeitschrift Fur Naturforschung Part B – Chemie Biochemie Biophysik Biologie und Verwandten Gebiete* **25b,** 624–670.

King B.J., Siddiqi M.Y. & Glass A.D.M. (1992) Studies of the uptake of nitrate in barley. 5. Estimation of root cytoplasmic nitrate concentration using nitrate reductase activity – implications for nitrate influx. *Plant Physiology* **99,** 1582–1589.

Kronzucker H.J., Siddiqi M.Y. & Glass A.D.M. (1995) Nitrate induction in spruce – an approach using compartmental analysis. *Planta* **196,** 683–690.

Kronzucker H.J., Siddiqi M.Y. & Glass A.D.M. (1997) Conifer root discrimination against soil nitrate and the ecology of forest succession. *Nature* **385,** 59–61.

Kronzucker H.J., Glass A.D.M. & Siddiqi M.Y. (1999) Inhibition of nitrate uptake by ammonium in barley. Analysis of component fluxes. *Plant Physiology* **120,** 283–291.

Kronzucker H.J., Siddiqi M.Y. & Glass A.D.M. (1995) Compartmentation and flux characteristics of nitrate in spruce. *Planta* **196,** 674–682.

Kronzucker H.J., Siddiqi M.Y., Glass A.D.M. & Britto D.T. (2003) Root ammonium transport efficiency as a determinant in forest colonization patterns: a hypothesis. *Physiologia Plantarum* in press.

Kronzucker H.J., Siddiqi M.Y., Glass A.D.M. & Kirk G.J.D. (1999) Nitrate-ammonium synergism in rice: a subcellular analysis. *Plant Physiology* **119,** 1041–1046.

Lee R.B. & Clarkson D.T. (1986) Studies of nitrate fluxes in barley roots.1. Compartmental analysis from measurements of ^{13}N efflux. *Journal of Experimental Botany* **37,** 1753–1767.

van der Leij Smith S.J. & Miller A.J. (1998) Remobilisation of vacuolar stored nitrate in barley root cells. *Planta* **205,** 64–72.

Martinoia E., Heck U. & Wiemken A. (1981) Vacuoles as storage compartments for nitrate in barley leaves. *Nature* **289,** 292–294.

Memon A.R., Saccomani M. & Glass A.D.M. (1985) Efficiency of potassium utilization by barley varieties – the role of subcellular compartmentation. *Journal of Experimental Botany* **36,** 1860–1876.

Mertz S.M. & Higinbotham N. (1976) Transmembrane electropotential in barley roots as related to cell type, cell location, and cutting and aging effects. *Plant Physiology* **57,** 123–128.

Miller A.J. & Smith S.J. (1992) The mechanism of nitrate transport across the tonoplast of barley root cells. *Planta* **187,** 554–557.

Miller A.J. & Smith S.J. (1996) Nitrate transport and compartmentation in cereal root cells. *Journal of Experimental Botany* **47,** 843–854.

Miller A.J., Cookson S.J., Smith S.J. & Wells D.M. (2001) The use of microelectrodes to investigate compartmentation and the transport of metabolized inorganic ions in plants. *Journal of Experimental Botany* **52,** 541–549.

Miller A.J., Walker D.J. & Smith S.J. (1995) Nitrate and potassium compartmentation in nitrogen- and potassium-starved barley root cells. In *Proceedings of the Second Stressnet Conference, Salsomaggiore* (eds R. A. Leigh & M. Blake-Kalff), pp. 263–268. EU Commission Press, Brussels, Belgium.

Min X., Siddiqi M.Y., Guy R.D., Glass A.D.M. & Kronzucker H.J. (1999) A comparative study of fluxes and compartmentation of nitrate and ammonium in early-successional tree species. *Plant, Cell, and Environment* **22,** 821–830.

Presland M.R. & McNaughton G.S. (1984) Whole plant studies using radioactive N-13. 2. A compartmental model for the uptake and transport of nitrate ions by *Zea mays*. *Journal of Experimental Botany* **35,** 1277–1288.

Robin P., Conejero G., Passama L. & Salsac L. (1983) Assessment of the nitrate metabolic pool by *in situ* assay of NO_3^- reduction. *Physiologie Vegetale* **21,** 115–122.

Robinson S.P. & Downton W.J.S. (1984) Potassium, sodium, and chloride content of isolated intact chloroplasts in relation to ionic compartmentation in leaves. *Archives of Biochemistry and Biophysics* **228,** 197–206.

Schröppel-Meier G. & Kaiser W.M. (1988a) Ion homeostasis in chloroplasts under salinity and mineral deficiency. 1. Solute concentrations in leaves and chloroplasts from spinach plants under NaCl or $NaNO_3$ salinity. *Plant Physiology* **87,** 822–827.

Schröppel-Meier G. & Kaiser W.M. (1988b) Ion homeostasis in chloroplasts under salinity and mineral deficiency. 2. Solute distribution between chloroplasts and extrachloroplastic space under excess or deficiency of sulfate, phosphate, or magnesium. *Plant Physiology* **87,** 822–832.

Siddiqi M.Y. & Glass A.D.M. (2002) An evaluation of the evidence for, and implications of, cytoplasmic nitrate homeostasis. *Plant, Cell, and Environment* **25,** 1211–1217.

Siddiqi M.Y., Glass A.D.M. & Ruth T.J. (1991) Studies of the uptake of nitrate in barley. 3. Compartmentation of NO_3^-. *Journal of Experimental Botany* **42,** 1455–1463.

Siddiqi M.Y., Glass A.D.M., Ruth T.J. & Fernando M. (1989) Studies of the regulation of nitrate influx by barley seedlings using $^{13}NO_3^-$. *Plant Physiology* **90,** 806–813.

Tischner R. (2000) Nitrate uptake and reduction in higher and lower plants. *Plant, Cell, and Environment* **23,** 1005–1024.

Walker D.J., Leigh R.A. & Miller A.J. (1996) Potassium homeostasis in vacuolate plant cells. *Proceedings of the National Academy of Sciences, USA* **93,** 10510–10514.

Winter H., Robinson D.G. & Heldt H.W. (1993) Subcellular volumes and metabolite concentrations in barley leaves. *Planta* **191,** 180–190.

Winter H., Robinson D.G. & Heldt H.W. (1994) Subcellular volumes and metabolite concentrations in spinach leaves. *Planta* **193,** 530–535.

Zhen R.-G., Koyro H.-W., Leigh R.A., Tomos A.D. & Miller A.J. (1991) Compartmental nitrate concentrations in barley root cells measured with nitrate-selective microelectrodes and by single-cell sap sampling. *Planta* **185,** 356–361.

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