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***Review*:**

Cytosolic ion exchange dynamics: insights into the mechanisms of component ion fluxes and their measurement

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Abstract. The quantification of cellular pool sizes of ions is essential for the understanding of the energetics of metabolic and membrane transport processes. No less important is the quantification of ion fluxes into, out of, and within cells. Of the variety of analytical methods available, only one, compartmental analysis by tracer efflux (CATE), can be used to simultaneously determine subcellular ion pool sizes and resolve ion fluxes. Thus, this methodology can be used to provide steady-state isotherms for major flux processes not amenable to direct measurement, such as effluxes or xylem fluxes, and to develop hypotheses about mechanisms underlying them. The exchange half-time for an ion in a cellular compartment emerges as a key CATE parameter that relates pool sizes with fluxes, and is a term that can be used to estimate errors in a wide range of findings in plant ion relations, and verify their plausibility. Case studies involving the flux and compartmentation of Ca^{2+}, K^+, and inorganic N are presented.

Keywords: calcium, compartmental analysis, efflux, half-time, influx, ion transport, nitrogen, potassium, turnover.

Introduction

Our understanding of the ionic composition of plant cells has made substantial progress in recent decades. For instance, the tight control of calcium at low cytosolic concentrations, essential for contrast against oscillatory Ca^{2+} spikes catalysed by ion channels, is now a central element in discussions of signal transduction events in plants (Sanders *et al.* 2002). Nevertheless, many questions and controversies regarding the sequestration of ions within cellular compartments, and the fluxes that govern such localisation patterns, remain. In many cases, variant methods of determining subcellular pool sizes have led to fundamental disagreements about these parameters (Siddiqi and Glass 2002; Britto and Kronzucker 2003), and in other cases flux-determination protocols have failed to take into account important interactions between such pools and the fluxes in question (see Britto and Kronzucker 2001*a*). In this paper, we propose that a more comprehensive approach needs to be taken toward the mapping of ion pools and their contributing fluxes, as both of these parameters are necessary for the resolution of the underlying energetics of transport and metabolic processes in plant cells. Moreover, they are inextricably linked by the turnover or exchange half-time of the pool. We favour the use of compartmental analysis by tracer efflux (CATE) as an investigative tool, as it is the only one available that can be used for the delineation of all of these parameters. We show how this methodology can yield mechanistic and procedural insights that are essentially unavailable to other methods. Our own use of short-lived radiotracers has lent itself to the analysis of more rapidly exchanging cellular compartments, in particular the cytosol, but the rationale provided here can be directly extended to other compartments such as the vacuole, when the latter are studied with longer-lived or stable tracers.

Discussion

Flux-turnover equation

A CATE experiment involves the labelling of a plant (usually via the root system), under steady-state nutritional conditions, with a tracer suitable for the ion in question (for methodological and mathematical details pertaining to CATE, see for example Walker and Pitman 1976; Lee and Clarkson 1986; Siddiqi *et al.* 1991; Kronzucker *et al.* 1995*a*). Suitable tracing should preclude the use of mere radioactive analogues such as $^{86}Rb^+$ as a K^+ tracer, or $^{14}CH_3NH_3^+$ for NH_4^+, which would invalidate the steady-state requirement for CATE. After a sufficiently long labelling period, the exponentially changing rate of tracer efflux from the system is monitored (see Fig. 1, *inset*, for an example of a tracer-release-rate plot), against a background of ‘cold’ solution (i.e. one not containing tracer). At the end

Abbreviations used: CATE, compartmental analysis by tracer efflux; NMR, nuclear magnetic resonance.

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of the experiment, the amount of tracer remaining in the root and/or shoot systems is measured. Once the various tracer-releasing compartments within the system are identified, a multiplicity of parameters becomes available with respect to the traced ion: its influx, efflux, net flux (or 'quasi-steady' flux to vacuole and metabolism), and flux to the shoot, its pool sizes in a variable number of intra- and extracellular compartments, and the turnover times of those pools.

The pool size, influx into the pool, and its turnover are related by the equation:

$$Q = t_{1/2}\phi_{oc}\Omega, \quad (1)$$

in which Q is the pool size, ϕ_{oc} is the flux into the pool, Ω is a proportionality constant, and $t_{1/2}$ is the exchange half-time of the pool (see Mathematical appendix for further details).

Cytosolic turnover of inorganic ions I: methodological critique and the cases of nitrate and calcium exchange

Inspection of eqn 1 shows that, for a given pool size, the influx into the pool under investigation, and its turnover, are inversely proportional. The question of whether cytosolic nitrate pools are held constant at approximately 4 mM (Zhen *et al.* 1991; Miller and Smith 1996; van der Leij 1998), or vary according to NO_3^- provision (Siddiqi *et al.* 1991; Kronzucker *et al.* 1995*a*), provides a good example of the utility of this basic principle for the verification of hypotheses concerning cellular ion relations. Because it is well established that steady-state unidirectional NO_3^- influx to the root cytosol from an external medium increases substantially as the concentration in the medium increases (Siddiqi *et al.* 1990, 1991; Devienne *et al.* 1994; Kronzucker *et al.* 1995*a*, 1999*a*), an unchanging cytosolic $[NO_3^-]$ must entail an ever-shortening exchange half-time of cytosolic NO_3^- exchange, as depicted in Fig. 1. However, such changes have never been observed for NO_3^-, even over several orders of magnitude in external $[NO_3^-]$ variations. On the contrary, the turnover times of inorganic N pools in the cytosol appear to be the only parameters in cellular N relations that are highly resistant to changes in N supply (Britto and Kronzucker 2001*b*; Fig. 1, *inset*). This constancy of the half-time parameter, then, argues strongly against the constancy of cytosolic $[NO_3^-]$.

The variability of the cytosolic nitrate pool has also been confirmed using measurements of nitrate reductase activity (Robin *et al.* 1983; Belton *et al.* 1985; King *et al.* 1992), and has been seen in nuclear magnetic resonance (NMR) data (see Britto and Kronzucker 2003). This raises the question, then, of why intracellular, ion-specific, microelectrode techniques have failed in the case of nitrate, even though they appear to give results consistent with other methods for a number of ions, including K^+ (Walker *et al.* 1996), Ca^{2+} (Felle *et al.* 1989), H^+ (Walker *et al.* 1996), and Cl^- (Felle 1994). In this context, it has to be noted that several potential pitfalls may apply to microelectrode approaches, and results are often not as straightforward as they appear (see Felle 1989, for a discussion of such pitfalls in the case of Ca^{2+} measurements). In the case of NO_3^-, an examination of the original data reported in some of the seminal papers on the subject reveals variations at least as large as 3- to 8-fold in NO_3^- signals from both vacuole and cytosol, collected under supposedly identical conditions by microelectrodes (see for example Fig. 2 in van der Leij *et al.* 1998); this extraordinary amount of variation is what remains even following selection criteria as outlined in Zhen *et al.* (1991) and Walker *et al.* (1995). In this light, it appears difficult to use such evidence to draw rigorous conclusions about the constancy of the cytosolic nitrate pool. The extent of such variations under identical physiological conditions is far smaller when CATE methodology is used (e.g. Siddiqi *et al.* 1991). Questions also arise as to the extent of disturbance to a cell that has been impaled with an electrode, the dimensions of which are sizeable relative to the surface area of the cell. In the case of nitrate, such impalement is expected to result in substantial efflux from the cell (see Forde and Clarkson 1999) via plasma-membrane transporters, in addition to possible leaks prior to the achievement of the required high-resistance seal between electrode and membrane. Further, interferences from other ions or macromolecules, abundant in the cytosol, but not typically contained in electrode calibration media, with ostensibly ion-selective resins or cocktails, are well known (Zhen *et al.* 1992; Coombs *et al.* 1994; Cuin *et al.* 1999; Carden *et al.* 2001) and must be taken into consideration as possible explanations for discrepancies between measurement techniques for cytosolic pool sizes.

Cytosolic nitrate (and nitrate flux) readings obtained using CATE are in better agreement not only with other

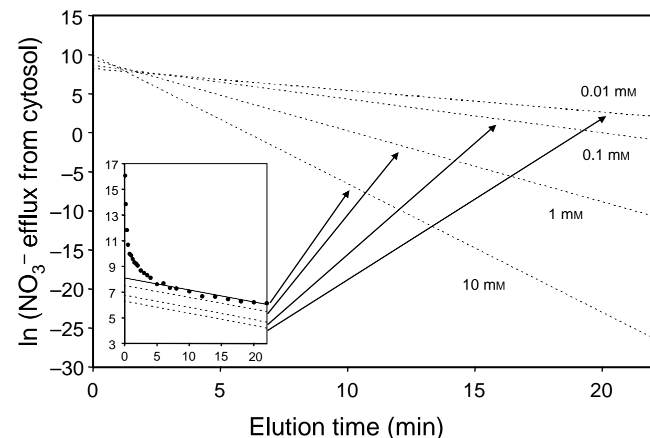

Fig. 1. Tracer-efflux lines (slopes calculated using eqn 1) predicted for NO_3^- release from the cytosol of root cells, based on variable influxes at different external $[NO_3^-]$ (indicated on figure), and assuming a constant cytosolic $[NO_3^-]$ of 4 mM. *Inset*, efflux traces as actually measured under the same external $[NO_3^-]$ conditions (Britto and Kronzucker 2001*b*).

measurement techniques for pool sizes, they also are consistent with a large number of key observations concerning the physiology of plant nitrate relations, as detailed elsewhere (Britto and Kronzucker 2003). Several qualifying statements, however, need to be made regarding CATE methodology and its results. In particular, tissue heterogeneity is a concern, given that CATE integrates tracer signals over a large number of individual cells whose individual flux and pool size characteristics may differ to varying extents, and provides values for these parameters which are averaged over the tissue examined. In essence, therefore, the technique accomplishes what many other well-established techniques in plant physiology (and numerous other disciplines) often achieve — the presentation of values averaged over a large number, and wide range, of cells. Such methods include measurements of chlorophyll fluorescence, gas-exchange, tracer influx, net ion flux (depletion), NMR signals, enzyme kinetic analyses, and even protein and mRNA extraction. Rather than the multicellular averaging of these methods detracting from the relevance and applicability of such results, however, such approaches are indeed desirable when the cellular basis of whole-plant or ecophysiological phenomena is under investigation (Kronzucker *et al.* 1997; Britto *et al.* 2001). Moreover, the extent of heterogeneous behaviour between cell types appears to often be overestimated (see, for instance, Leigh 2001), given that excellent agreement has been seen between single-cell techniques and CATE-based cell averaging in important cases such as K^+ compartmentation (Pitman and Saddler 1967; Davis and Higinbotham 1976; Rona *et al.* 1982; Memon *et al.* 1985; Walker *et al.* 1996; Kronzucker *et al.* 2003). In addition to cellular heterogeneity at the tissue level, the possibility that intracellular heterogeneity, that is, the presence of organelles such as mitochondria and chloroplasts, could confound the interpretation of efflux traces has been raised (Siddiqi and Glass 2002), but, in practice, no significant contribution from such compartments has been verified (Britto and Kronzucker 2003).

The validity of any CATE approach assumes that a number of basic requirements have been met (Bell *et al.* 1994; Kronzucker *et al.* 1995*a*, *b*); in particular, that the plant tissue be under steady-state with respect to the fluxes and pool sizes being measured, and that the tracer-releasing compartments be rigorously identified. The first of these requirements is relatively easy to fulfil, by cultivating and making measurements with plants under constant growth conditions in terms of temperature, light intensity, humidity, and, most importantly, nutrient provision. Also, perturbation associated with plant transfers during CATE experiments (which are, nevertheless, far less drastic than, for example, the impalement of a cell with an electrode, or tissue or organ excision) must be minimised. The second of these requirements, that is, the verification of compartment identity in assigning tracer efflux phases, requires more experimental innovation and effort, and has, thus, often been disregarded by many workers in the field (Macklon 1975; Macklon and Sim 1981; Macklon *et al.* 1990; Bell *et al.* 1994), to the detriment of the understanding of plant-cell ion relations. Such tests, however, have been conducted by others, and have included protocols involving the disruption of membrane integrity by high-temperature or detergent treatment (Siddiqi *et al.* 1991; Kronzucker *et al.* 1995*a*; Britto *et al.* 2002), the inhibition of cytosolic enzymes (such as cytosolic glutamine synthetase, Kronzucker *et al.* 1995*b*), comparisons between uninduced and induced states for the nitrate-assimilation pathway (Kronzucker *et al.* 1995*c*), the transitory effects of inhibitory substances on efflux (Kronzucker *et al.* 1999*a*, *b*) and the steady-state suppressions of ion contents and fluxes in various compartments by various long-term treatments (Kronzucker *et al.* 1995*a*–*d*; Kronzucker *et al.* 2003). Finally, a less direct, but nevertheless compelling, means of testing the veracity of CATE measurements is the agreement of these results with key parameters obtained independently, such as by influx or net flux determinations, or pool size estimates by means of methods such as X-ray microanalysis, NMR, cell fractionation, ion-profile analysis, and microelectrodes (see above; also see Britto and Kronzucker 2003).

It is sometimes mistakenly stated that CATE protocols are inherently flawed because they do not consider metabolic or long-distance (root-to-shoot) ion fluxes (Miller and Smith 1996). This criticism fails to take into account that modern approaches using CATE include two important features: first, that intact plants are used, allowing for measurements of tracer and (in the case of non-metabolised ions) chemical fluxes to the shoot; and second, that the rate of tracer release from the labelled tissue, rather than the counts remaining in the tissue, is measured and analysed. When experiments are conducted on intact plants, then, the changing kinetics of this release inherently takes into account such internal fluxes to metabolism and to the shoot (as well to any other ionic sinks within the plant).

Another, rather different, example of the utility of eqn 1 is in the demonstration of the feasibility of measuring influxes into very small cytosolic pools, such as that of calcium. This is illustrative of a situation that is not amenable to CATE methodology for the simple reason that the cytosolic exchange half-time for Ca^{2+} is, at most, about 3 s. Under most conditions it is very much shorter, given two input values for eqn 1: first, that $[Ca^{2+}]$ in the cytosol is typically ~ 0.1 μM (Williamson and Ashley 1982; Plieth *et al.* 1998); and second, that fluxes of Ca^{2+} into the plant are substantial (Macklon and Sim 1981; Glass and Siddiqi 1984). This analysis reveals two essential points. First, the half-times reported in CATE-based surveys (Macklon and Sim 1981; Glass and Siddiqi 1984) of Ca^{2+} exchange with the presumed cytosolic compartment (1380–4500 s) are far in excess of what must be the case given extremely low

cytosolic [Ca^{2+}] values, illustrating the fundamental errors that can be made when compartment identification (see above) does not accompany the execution of CATE experiments. Second, as shown in Fig. 2, it emerges that unidirectional Ca^{2+} fluxes across the plasma membrane are rendered unmeasurable by tracer techniques, owing to the very rapid equilibration of tracer in the cytosol, and simultaneous efflux of tracer during labelling and desorption procedures (see Britto and Kronzucker 2001*a*). Figure 2 shows three scenarios of efflux comprising 10, 50, or 90% of influx under the two conditions of $t_{1/2}$ assuming values of 2.94 s or 0.017 s [derived using eqn 1, from fluxes reported by Macklon and Sim (1981), a cytosolic [Ca^{2+}] of 0.1 µM, and the employment of eqn 1]. The analysis reveals that net flux is achieved within 30 s during labelling (or much faster, depending on which $t_{1/2}$ prevails), a period much shorter than practical labelling and desorption protocols permit. The issue is further complicated by two problems: first, that only maximal $t_{1/2}$ values can be deduced (because only *minimal* unidirectional influx is quantifiable); and second, that accurate knowledge of the ratios of efflux to influx presupposes knowledge of the very parameter whose determination is the subject of investigation. In short, the constraints of eqn 1 pose the experimenter with an insoluble problem.

Cytosolic turnover of inorganic ions II: *relationships between cytosolic pool sizes and component fluxes*

The simultaneous appraisal of pool sizes and ion fluxes in intact plant systems, made possible by CATE but inaccessible by other means, renders possible an investigation of the kinetic relationship between the size of the cytosolic pool and the fluxes from that pool, such as efflux to the external medium (ϕ_{co}), and the flux to the xylem (ϕ_{xylem}). In other words, CATE methodology allows the construction of steady-state isotherms (i.e. the determination of fluxes from substrate concentrations to which the plant has become acclimatised, as distinct from perturbational isotherms, which show immediate responses to changes in substrate concentration). In Figs 3 and 4 we show such kinetic relationships for these two flux parameters, drawing upon a number of CATE studies on nitrate, ammonium and potassium. It has been proposed several times (Clarkson 1986; Ter Steege *et al.* 1999) that influx and efflux, in particular for nitrate, operate in a 'pump-and-leak' fashion, that is, that efflux is essentially an unregulated function of the cytosolic concentration of the ion in question. However, Fig. 3 shows

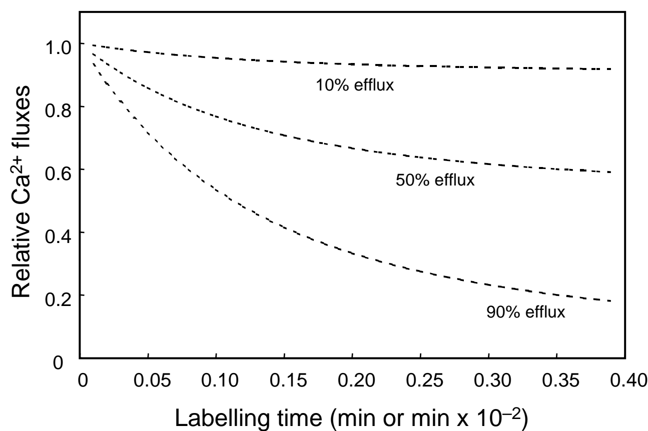

Fig. 2. Changes in measured influx as a function of labelling time, due to variable tracer efflux occurring simultaneously with tracer uptake. Six scenarios are presented, with three representative ratios of efflux to influx, and two half-times of cytosolic ion exchange, 2.94 s or 0.017 s. The longer half-time corresponds to the *x*-axis representing min, while the shorter corresponds to the *x*-axis representing min × 10^{-2}.

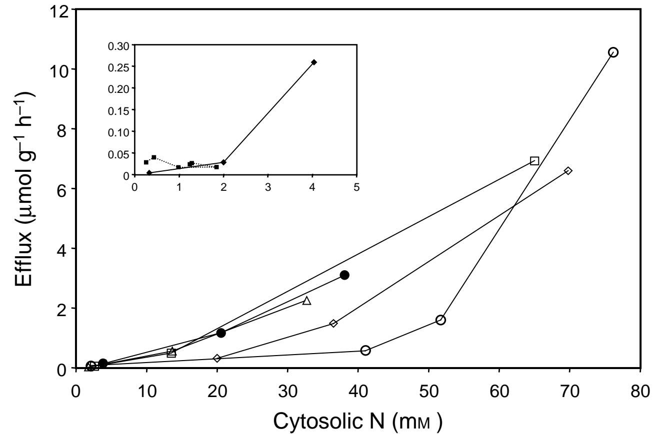

Fig. 3. Efflux of NO_3^- or NH_4^+ from cytosol to external medium, as a function of cytosolic pool size. (◇) NO_3^- in barley roots (Siddiqi *et al.* 1991), (□) NH_4^+ in wheat leaf slices (Britto *et al.* 2002), (△) NH_4^+ in spruce roots (Kronzucker *et al.* 1995*d*), (●) NH_4^+ in rice roots (Wang *et al.* 1993), (○) NO_3^- in barley roots (Kronzucker *et al.* 1999*b*). *Inset*, NO_3^- efflux *vs* cytosolic [NO_3^-] in roots of white spruce, in fully induced seedlings (solid line; Kronzucker *et al.* 1995*a*), or during a 3-d course of induction (dashed line; Kronzucker *et al.* 1995*c*).

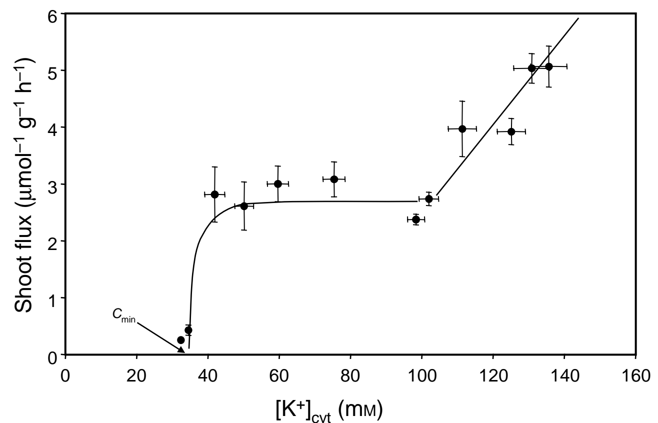

Fig. 4. Xylem flux (ϕ_{xylem}) of K^+ as a function of cytosolic [K^+] (Kronzucker *et al.* 2003). For calculation of ϕ_{xylem}, see Mathematical appendix.

that, for the two major inorganic nitrogen ions NO_3^- and NH_4^+, this is not the case. Rather, a 'J-curve' pattern is observed for nitrogen efflux as a function of cytosolic pool size. The absence of a simple linear relationship suggests that more sophisticated regulatory mechanisms govern efflux than a simple pump-and-leak process. This is most readily evident in the case of the time-course of induction of the NO_3^- acquisition pathway in white spruce, shown in the inset to Fig. 3 (dashed line). In this case, efflux is unresponsive to changes in cytosolic [NO_3^-]. Importantly, the right-hand tails of the J-curves seen in Fig. 3 represent conditions where efflux begins to approximate influx; under these conditions, efflux becomes a more direct function of pool size. Particularly in the case of NH_4^+ at higher levels of external NH_4^+, efflux achieves values of 90%, or even higher, of influx (Britto *et al.* 2001, 2002), resulting in the futile cycling of this ion. As efflux approaches 100% of influx, the shape of steady-state isotherms for influx (as a function of external supply), therefore, will be identical to that of efflux, as well as that of pool size; the latter result will be true as long as half-time constancy for cytosolic NH_4^+ or NO_3^- is maintained (Britto and Kronzucker 2001*b*; also see eqn 1). The regulation and kinetics of efflux transporters, it should be noted, is often overlooked, but of clear importance, in efforts to maximise the efficiency of nutrient capture in crop plants. CATE provides unique opportunities for insight into these poorly understood processes.

Similarly cogent insights can be gained from an analysis of the response of xylem flux to pool size (see Mathematical appendix for details of ϕ_{xylem} calculations). Figure 4 shows how CATE information, in the case of potassium, yields eminently sensible, albeit surprising, isotherm patterns for ϕ_{xylem}. In striking resemblance to well-known patterns of K^+ influx, both a saturable pattern, and a linear one, at higher cytosolic [K^+], can be seen for the shoot translocation term. It appears as though long-distance translocation of K^+ from root to shoot is mediated by a minimum of two distinct transport systems, one of which is binding-site-limited (saturable), while the other displays channel-type characteristics. Like those catalysing efflux to the external medium, the transporters responsible for shoot translocation, differ from most other transport systems hitherto studied, in that they have clearly adapted to high substrate concentrations. In the case of ϕ_{xylem} for K^+ in particular, a minimum concentration of about 30 mM (C_{min}) appears to be necessary before any sizeable shoot translocation can occur, suggesting that a root-priority regulatory mechanism exists (see Kronzucker *et al.* 1998), operating at the level of the cytosol. It is interesting to note that the linear portion of the isotherm begins at a cytosolic [K^+] of ~100 mM, which has been shown to be a typical resting value for this parameter (Memon *et al.* 1985; Walker *et al.* 1996).

Conclusion

Our analysis highlights the importance of obtaining, simultaneously, ion pool size and unidirectional flux parameters, as well as turnover times, so that the following become possible.

The nature of the homeostatic mechanism(s) underlying cellular ion relations can be delineated. Homeostasis can manifest itself in a number of ways, in the maintenance of compartmental pool sizes, ion fluxes, half-times of compartmental ion exchange, or any combination of these. CATE, if judiciously applied, is a particularly appropriate method by which to undertake such investigations, as it renders all of these types of homeostases amenable to analysis. In the present work, we have emphasised the interconnection of fluxes, pool sizes, and exchange half-times, which can be elegantly summarised mathematically (eqn 1).

Once the nature of the homeostatic mechanism(s) is determined, this information can be used to evaluate the relative veracities of findings obtained by various methods, and to resolve differences in key parameters, and in the concepts that derive from them, resulting from these different methods. One such example is the controversy regarding cytosolic [NO_3^-]. Another is the practicality of tracer-based measurements of unidirectional Ca^{2+} fluxes, and of cytosolic [Ca^{2+}].

Effluxes from the cytosol to the external medium, as well as to the xylem, can be analysed as a function of cytosolic pool size, and be shown to display kinetics more complex than previously realised. Integrative analyses such as those performed here provide insight into possible regulatory mechanisms underlying these phenomena. This must be taken into account in initiatives to improve nutrient-capture efficiency in plants.

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Mathematical appendix

The use of tracer flux data to calculate pool size

A starting point for the use of the relationship:

$$Q = t_{1/2}\phi_{oc}\Omega, \quad (1)$$

to determine cytosolic pool sizes in CATE protocols, is the observation that tracer efflux from the radiolabelled collective cytosol of a plant system to the external medium generally obeys first-order exponential decline kinetics (MacRobbie 1971; Walker and Pitman 1976; Behl and Jeschke 1982; see Fig. 1, *inset*, for an example of this phenomenon). These kinetics can be used to determine the total amount of tracer residing in the releasing compartment (in this discussion, the cytosol), which can in turn be corrected for specific activity, to determine the chemical content of the pool. The release of tracer from the fully labelled cytosol (ϕ^*_{co}, the tracer fluxes and pools being denoted by an asterisk, *), at any time t after labelling, can be described by a first-order equation of the form:

$$\phi^*_{co(t)} = \phi^*_{co}e^{-kt},$$

where k is the exponential decay constant for this process, obtained directly from the slope of the semi-logarithmic plot of tracer efflux (Fig. 1, *inset*), and ϕ^*_{co} is the maximal tracer efflux from the cytosolic phase, which can be obtained from the intercept of the same line, given sufficient labelling time. In practice, however, maximal labelling might not occur, such as when a short-lived radioisotope is used (e.g. ^{13}N, which has a half-life of 9.98 min). In such a case, the intercept value would have to be corrected to account for a specific activity in the cytosol (S_c) that is less than that of the external medium (S_o), according to the relationship:

$$S_c = S_o[1-e^{-kt(L)}],$$

where $t(L)$ is labelling time. The situation can be further complicated if there are substantial non-labelled fluxes of the traced ion occurring internal to the cell (such as the flux from the vacuole to the cytosol, ϕ_{vc}, see Britto and Kronzucker 2001*a*). Another effect of internal fluxes being large, relative to influx from the outside, is that they exert influences on the exchange half-time of the pool independent of plasma-membrane influx, and under such circumstances reduce the validity of eqn 1. In such a case, the more comprehensive equation:

$$Q = t_{1/2}(\Sigma\, \phi_c)\Omega,$$

becomes appropriate; in this case $\Sigma\, \phi_c$ is the sum of all fluxes into the cytosolic pool. In general, however, such potentially confounding factors appear to be minor, given the characteristic agreement between flux values determined using CATE, and those found using independent methods (Memon *et al.* 1985; Siddiqi *et al.* 1991; Wang *et al.* 1993; Kronzucker *et al.* 1995*a–c*).

The total amount of tracer that would be captured were tracer efflux to proceed to completion can then be found by integrating this equation from $t = 0$ to $t = \infty$ (in practice, 'five half-times of exchange' are used as the integration interval, approximating 97% of complete exchange; see, e.g. Siddiqi *et al.* 1991):

$$\int_0^\infty \phi^*_{co(t)}\, dt = \int_0^\infty \phi^*_{co}\, e^{-kt}\, dt = \left[-\frac{\phi^*_{co}}{k}\, e^{-kt}\right]_0^\infty = \frac{\phi^*_{co}}{k}.$$

To obtain the total amount of tracer initially residing in the cytosol, this resulting quantity has to be corrected for tracer removed from the cytosol by other fluxes, such as fluxes to the vacuole, to metabolism (where appropriate, e.g. with ions such as NO_3^-, NH_4^+, and SO_4^{2-}), and to the xylem stream. This correction is accomplished by multiplying the above result by the ratio of efflux to all fluxes removing tracer from the cytosol; the sum of all removal fluxes, under steady-state conditions, is equal to tracer influx (ϕ^*_{oc}), a quantity measured in the course of a CATE experiment (Siddiqi *et al.* 1991). For instance, if efflux amounts to 20% of all removal fluxes (i.e. 20% of influx), this quantity would be multiplied by five. More generally, the corrected tracer quantity Q^* is found by:

$$Q^* = \left\{ \frac{\phi^*_{co}}{k} \bigg/ \frac{\phi^*_{co}}{\phi^*_{oc}} \right\} = \frac{\phi^*_{oc}}{k}.$$

This equation can then be converted to chemical quantities, yielding eqn 1, by dividing by the specific activity S_o, by converting the exponential decay constant to a half-time value using the relationship $t_{1/2} = (\ln 2)/k$, and by factoring in a proportionality constant Ω (Britto and Kronzucker 2001*a*).

Calculation of ion flux to the xylem

The k value describing cytosolic exchange for a non-metabolised ion can also be used to calculate the flux to the xylem according to the following rationale. Radioactivity (cpm) recovered in shoots accumulates over the course of the CATE experiment as a result of two oppositely changing isotopic fluxes: I — the flux from a rising specific activity in the xylem-loading (cytosolic) pool during the labelling period; and II — the flux from a declining specific activity in this pool during the elution period. Mathematically, these isotopic fluxes can be expressed in the following terms:

$$\text{I: } \phi_{\text{xylem}}^* = \phi_{\text{xylem}} S_o (1 - e^{-kt})$$

$$\text{II: } \phi_{\text{xylem}}^* = \phi_{\text{xylem}} S_o (1 - e^{-kt(L)}) e^{-kt}$$

where ϕ_{xylem}^* is the radioisotopic flux from the cytosol to the xylem, t is time, and $t(L)$ is labelling time. The sum of these terms, when integrated, respectively, over $t(L)$ and over elution time $t(E)$, is equal to the total tracer remaining in the shoot, and this relationship can be used to solve for ϕ_{xylem} in the following way:

$$\begin{aligned} \textit{cpm remaining } g^{-1} &= \int_0^{t(L)} \phi_{\text{xylem}} S_o (1 - e^{-kt})\, dt + \int_0^{t(E)} \phi_{\text{xylem}} S_o (1 - e^{-kt(L)})\, e^{-kt}\, dt \\ &= \phi_{\text{xylem}} S_o \left\{ \left(\int_0^{t(L)} dt - \int_0^{t(L)} e^{-kt}\, dt \right) + (1 - e^{-kt(L)}) \int_0^{t(L)} e^{-kt}\, dt \right\} \\ &= \phi_{\text{xylem}} S_o \left\{ \left[t + \frac{1}{k} e^{-kt} \right]_0^{t(L)} - \left[\frac{1 - e^{-kt(L)}}{k} e^{-kt} \right]_0^{t(E)} \right\}. \end{aligned}$$

Evaluating the integral and solving for ϕ_{xylem} gives the equation used to determine the shoot fluxes as plotted in Fig. 4:

$$\phi_{\text{xylem}} = \frac{\textit{cpm remaining } g^{-1}}{t(L)S_o - \dfrac{S_o}{k}\left(1 - e^{-kt(L)}\right)\left(e^{-kt(E)}\right)}\ .$$

It is important to note that, for metabolised ions, such a flux cannot be calculated, given that newly synthesised compounds containing tracer may contribute to the shoot tracer accumulation, but the specific activities of these pools cannot, in practice, be determined easily.