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ORIGINAL ARTICLE

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Ion fluxes and cytosolic pool sizes: examining fundamental relationships in transmembrane flux regulation

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Abstract The relationships among cellular ion fluxes, ion compartmentation, and the turnover kinetics of cytosolic ion pools are crucial to the understanding of the regulatory mechanisms and thermodynamic gradients that determine plasma membrane ion fluxes. We here provide an analysis of published data to quantify these relationships for the two major nutrient elements in plants, nitrogen and potassium. We discuss the implications of these relationships for plant ion fluxes in general, and focus more specifically on problems associated with the accurate measurement of fluxes to and from rapidly exchanging pools, particularly the cytosolic calcium pool.

Keywords Compartmentation · Cytosolic pool size · Efflux · Exchange half-time · Ion transport · Turnover

Introduction

The trafficking of ions between and within plant cells is a complex process, and the movement of an ionic species into and out of subcellular compartments generally involves multiple, interdependent membrane and metabolic fluxes. Nevertheless, this complexity is integrated by the living cell into coherent, tightly regulated flux behavior. One way of visualizing such coherence is to observe the continuous turnover of an ionic pool within the cell, by measuring changes in the efflux of an isotopic tracer that has been used to label the pool. Under steady-state conditions, the kinetics of decline in the tracer signal is expected to, and generally does, conform to first-order kinetics (MacRobbie and Dainty 1958; Walker and Pitman 1976; Britto and Kronzucker 2001a), yielding an exchange half-time for the pool that is the result of the simultaneous action of all processes introducing and removing ions from that pool. In this paper, we will show how attention to this summary term can be used to verify or reject claims about ion fluxes and pool sizes, and their regulation, as arrived at through the use of different measurement systems. We also present a correlation analysis of individual fluxes and pools in several higher-plant systems, providing further insight into regulatory and mechanistic elements underlying transport and compartmentation in plant cells.

It should be noted that the ideas in this paper are contingent upon the plant system(s) in question being examined under steady-state conditions, a term which is not meant to imply the lack of growth or net nutrient fluxes, but rather a situation in which the fluxes into and out of the plant cell are essentially constant, as is the concentration of the ionic pool under consideration. Such a situation is certainly realistic for many plant systems over the duration of isotopic labelling experiments (Kronzucker et al. 1995d; Britto et al. 2002), as long as environmental conditions are held constant throughout growth and experimental phases. Conversely, these ideas will not directly apply to perturbed systems, in which these parameters are changing (e.g. influx isotherms; see Britto and Kronzucker 2001a).

It should also be pointed out that the methods of compartmental analysis drawn upon here yield values for particular subcellular compartments, averaged over the entire plant root. While this methodology cannot therefore show distinctions among various cell types or regions of maturation in the root, distinctions which no doubt exist and have physiological importance, its integrative approach allows for substantial insight concerning questions of whole-root or whole-plant ion relations, and ecophysiological questions that examine differences among potentially competing plant species (Kronzucker et al. 1997). The same characteristics (both positive and negative) apply to the interpretation of diverse forms of plant analysis that are routinely used to

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determine cellular or subcellular parameters via tissue-level examinations, from tracer influx measurements to fluorescence readings to respiratory traces.

Cytosolic turnover of inorganic ions: implications for fluxes and pools

The turnover of any ionic pool in the cytosol is related to the pool size, and to the flux into the pool, which, under steady-state conditions, is equal to the flux from the pool (with the addition of a small net flux to the cytosol which is diluted by growth, and considered to be negligible). This relationship can be generalized in the equation

$$Q = t_{1/2}\left(\sum \varphi_c\right)\Omega \qquad (1)$$

in which Q is the pool size, $\Sigma\ \phi_c$ is the sum of all fluxes into the pool, Ω is a proportionality constant, and $t_{1/2}$ is the exchange half-time of the pool. Equation 1 is the solution of a first-order differential equation describing steady-state fluxes into a pool of constant size (for mathematical details, see MacRobbie 1971; Walker and Pitman 1976), modified by the substitution of the half-time term for the exponential decay constant k ($t_{1/2} = \ln 2/k$). This substitution provides a more straightforward understanding of the meaning of the equation, since the half-time is an expression of pool turnover, and is the time required for 50% of the pool to be replaced by incoming quantities of the ionic species comprising it. Such a situation can be further visualized, in both theoretical and practical contexts, by considering the nature of isotopic labelling of an ion pool. If this pool is isotopically pure, and is then replaced by means of an ionically identical, but isotopically different, flux into that pool, then the time course of replacement will obey the exponential kinetics described in Eq. 1. Q and $t_{1/2}$ can be determined by various methods whose results may not always agree (see below), although the relationship in Eq. 1 must always hold. A more specific form of Eq. 1 applies when influx across the plasma membrane (ϕ_{oc}) is large compared to other fluxes (such as efflux from the vacuole) that deliver non-traced ions to the pool:

$$Q = t_{1/2}\varphi_{oc}\Omega \qquad (2)$$

The broad validity domain of this equation is illustrated by the excellent agreement between influx determinations arrived at by efflux analysis and by other, more direct, means, in studies that have comprehensively applied these methods to the same plant system (Siddiqi et al. 1991; Wang et al. 1993a; Kronzucker et al. 1995a, 1995b, 1995c, 1998, 2000). Therefore, Eq. 2 forms the basis for the arguments presented below.

In a previous study (Britto and Kronzucker 2001b), we showed that, in the case of inorganic nitrogen (nitrate and ammonium), the turnover of the cytosolic pool, as measured by tracer efflux kinetics, is maintained within extremely narrow limits, even though steady-state nitrogen influx (and the fate of the incoming nitrogen) varies enormously as external N supply changes. Acceptance of this surprising finding, however, demands the conclusion that there is an equally high (and positively correlated) variability in the cytosolic pool size of inorganic nitrogen, as inspection of Eq. 2 clearly shows. The half-time constancy inherent in inorganic N turnover in the cytosolic compartment of plant cells translates into a linear relationship between pool size and influx (Fig. 1), as determined by analysis of tracer efflux and retention in numerous studies (Siddiqi et al. 1991; Wang et al. 1993a; Kronzucker et al. 1995a, 1995b, 1995c; Britto et al. 2002). An important application of this linearity is that, once the exchange half-times have been ascertained, cytosolic pool sizes of inorganic N can be calculated simply from direct influx measurements. In all cases where such linearity is observed, patterns of

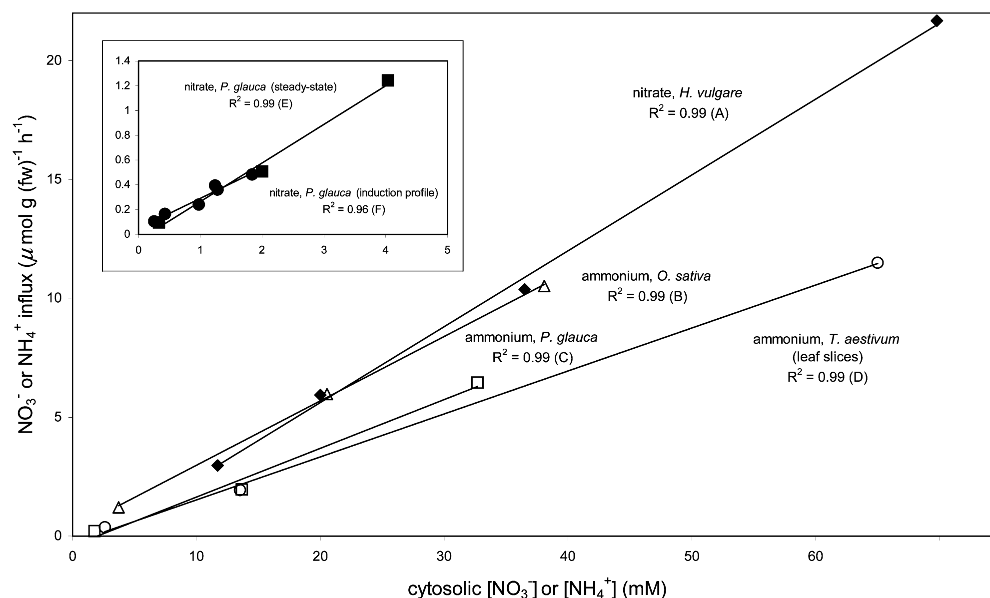

Fig. 1 Linear relationship between cytosolic pool sizes of NO_3^- or NH_4^+ and plasma membrane influxes of the respective ions, determined using compartmental analysis by efflux of ^{13}N. *Closed symbols* refer to NO_3^- studies, *open symbols* to NH_4^+ studies. The *inset*, pertaining to white spruce (*Picea glauca*), was used because NO_3^- fluxes and pools in this species are generally very low (axes refer to the same units as larger graph). Correlation coefficients (R^2) are given for each data set. Sources for the data are as follows: *A*, Siddiqi etal. 1991, Britto and Kronzucker 2001b; *B*, Wang et al. 1993a; *C*, Kronzucker et al. 1995c; *D*, Britto et al. 2002; *E*, Kronzucker et al. 1995b; *F*, Kronzucker et al. 1995a

steady-state influx isotherms (see, e.g., Glass and Siddiqi 1984a) therefore translate into identical patterns of cytosolic pool size (illustrated for NO_3^- and NH_4^+ in Fig. 2). The pronounced differences in the isotherms for cytosolic pool size of the two N sources shown in Fig. 2 are the result of fundamental differences in the regulation of the fluxes governing the compartmentation of the two ions, particularly at higher external N concentrations (Britto et al. 2001; also see next section).

Unfortunately, the conclusion that concentrations of inorganic N in the cytosol varies with external N provision (and with steady-state influx values) has not met with consensus among researchers, and therefore warrants further discussion here. In particular, more invasive studies using nitrate-selective microelectrodes appear to show that cytosolic concentrations of nitrate ($[NO_3^-]_{cyt}$) are invariable (at approximately 3–5 mM) under a wide range of conditions (Zhen et al. 1991; Miller and Smith 1996; Van der Leij et al. 1998), including time courses over which the induction states of nitrate transport and metabolism are known to vary widely (Minotti et al. 1969; Clarkson 1986; Siddiqi et al. 1989; Kronzucker et al. 1995a). These findings have been taken at face value by many workers in the field, despite the fact that they contradict key observations, especially of the widely recognized phenomenon that nitrate itself serves as the signal for the induction of its own transport and metabolism (MacKown and McClure 1988; Tischner et al. 1993; Crawford 1995; Tischner 2000), given that it is likely that this signalling occurs intracellularly, as evidenced by a recent study in *Chlamydomonas* (Rexach et al. 2002; however, Unkles et al. 2001 have suggested the possibility of an extracellular nitrate sensor in yeast, and there remains the further, if remote, possibility that an intracellular sensor could be non-cytosolic—see Britto and Kronzucker 2003). Such a function is difficult, if not impossible, to reconcile with a proposed constancy of $[NO_3^-]_{cyt}$ prevailing during all stages of induction and de-induction, even long after external nitrate has been withdrawn, and influx has returned to non-induced levels (Siddiqi et al. 1989). By contrast, in a study using white spruce, which has a sufficiently long induction period (3 days) that its subcellular nitrate fluxes and compartmentation may be studied using ^{13}N tracer efflux analysis over this period, Kronzucker et al. (1995a) showed that $[NO_3^-]_{cyt}$ varies considerably during the nitrate induction process, in agreement with previous proposals (Clarkson 1986), and consistent with the idea that NO_3^- itself is a signal for induction.

In addition, the claim of $[NO_3^-]_{cyt}$ constancy is incompatible with the constancy of cytosolic NO_3^- turnover under conditions of changing fluxes into the pool (Britto and Kronzucker 2001b; also see Fig. 1), as such a situation must entail changing exponential rate constants of ^{13}N or ^{15}N release, which have never been experimentally observed (Lee and Clarkson 1986; Siddiqi et al. 1991; Devienne et al. 1994; Kronzucker et al. 1995a, 1995b; Min et al. 1999; Britto and Kronzucker 2001b). Drawing upon data sets from Siddiqi et al. (1991) and Britto and Kronzucker (2001b) that span a comprehensive range of external NO_3^- concentrations, Table 1 shows the exchange half-times that would be predicted were a constant cytosolic $[NO_3^-]$ of 4 mM to prevail, and the half-times that were actually observed using radiotracing with ^{13}N (importantly, similar results are obtained using stable isotope tracing with ^{15}N—see Devienne et al. 1994). The absence of variability in exchange half-times is further grounds for rejecting a model claiming cytosolic $[NO_3^-]$ homeostasis, and results obtained with microelectrodes require alternative explanations, such as the well-documented propensity

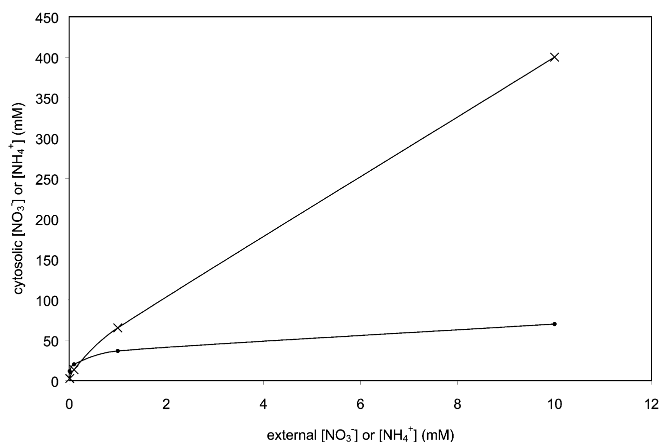

Fig. 2 Representative steady-state cytosolic pool size "isotherms" for inorganic N as a function of external concentration. Because of the constancy of cytosolic exchange half-times for inorganic N, such isotherms are congruent with steady-state influx isotherms. *Dots* refer to NO_3^- treatments with barley (Siddiqi et al. 1991; Britto and Kronzucker 2001b); *crosses* refer to NH_4^+ treatment with leaf slices of wheat (Britto et al. 2002)

Table 1 Half-times of cytosolic nitrate exchange, as predicted from ion-selective microelectrode measurements, which place the concentration of the cytosolic nitrate pool at an essentially invariant value of approximately 4 mM (Miller and Smith 1996) and from NO_3^-influxes measured using ^{13}N tracer analysis by Siddiqi et al. (1991) and Britto and Kronzucker (2001b). Half-time predictions were calculated from the relationship $t_{1/2} = Q/\phi_{oc}\ \Omega$ (see Eq. 2 in text). The value of Ω used here is 0.48 g h mmol min^{-1} l^{-1} µmol^{-1}, used to interconvert fluxes expressed in µmol g^{-1}h^{-1}, exchange half-times in minutes, and concentrations expressed in mM; it also takes into account the conversion from half-time to first-order rate constant, and the assumption that tissue volume occupied by the cytosol is 5% (see Lee and Clarkson 1986). In comparison to these predicted half-times are the half-times actually measured. Similar half-times have been reported in other studies (see Devienne et al. 1994)

External $[NO_3^-]$(mM)	Reported influx (µmol g^{-1} h^{-1})	Cytosolic exchange half-times (min)	
		Predicted	Reported
0.01	3.62	2.5	7.2
0.1	5.69	1.6	7.2
1	11.9	0.76	7.5
10	21.67	0.42	7.4

for artifacts produced by interference with other ions (Cuin et al. 1999; Carden et al. 2001), electrical noise and signal stability (Mertz and Higinbotham 1976; Beilby and Blatt 1986; see also Felle 1989), and the difficulties in appropriately calibrating electrodes to measure ion activities within compartments whose chemical composition is the very object of study (Coombs et al. 1994; Miller and Smith 1996).

A different scenario develops when extending the above analysis to the case of potassium, for which cytosolic concentrations are typically found to be held constant (Leigh and Wyn Jones 1984; Memon et al. 1985; Walker et al. 1996; Maathuis and Amtmann 1999). Because of this constancy, Eq. 2 predicts that for cytosolic K^+, exchange half-times must change inversely with steady-state membrane fluxes, which can differ widely under varying K^+ supply conditions (Glass and Siddiqi 1984a; Memon et al. 1985). Importantly, unlike in the case of NO_3^-, such changes in turnover have indeed been observed (Memon et al. 1985; our own unpublished results). For instance, when Memon et al. (1985) increased the K^+ supply to barley plants (cv. Fergus) from 0.01 to 0.1 mM, the cytosolic half-time for K^+ decreased from 93 min to 34 min, while K^+ influx increased from 1.88 to 5.23 μmol g^{-1} h^{-1}. Similar trends were observed in this study for two other cultivars of barley. More generally, Fig. 3 illustrates the expected changes in $t_{1/2}$ for various steady-state influxes of K^+, given a constant cytosolic pool of about 100 mM. In this graph, Eq. 2 was used to calculate these half-times; for instance, the approximate tripling in influx between 2and 20 μM external [K^+] (Glass and Siddiqi 1984a) is accompanied by a shortening of the half-time to a third of that at 2 μM. Without such commensurate adjustments in these parameters, maintenance of the cytosolic pool near 100 mM could not occur. Interestingly, in the case of K^+, pool size estimates obtained by electrodes agree well with those obtained by compartmental analysis, and by other methods, and this consistency is reflected in the agreement between exchange half-time predictions and measurements of such half-times. Clearly, a recognition of the relationship between exchange half-times, influx and pool size (as in Eq. 2) can lead to the identification of the relative accuracies of irreconcilable data sets as in the case of inorganic N, and in the corroboration of the cross-methodological convergence of results, as evident in the case of K^+.

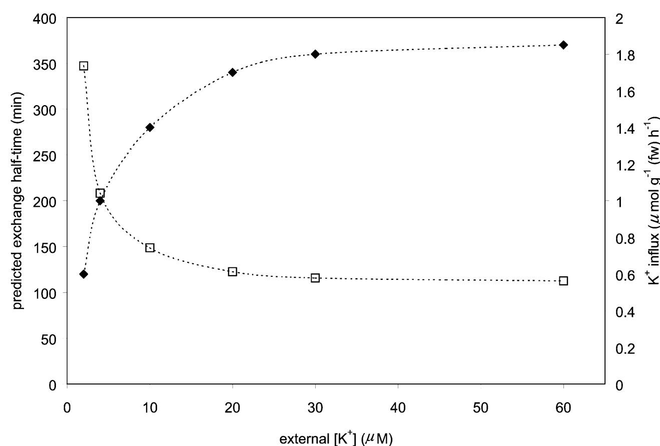

Fig. 3 Exchange half-times of cytosolic K^+ (*closed symbols*) as a function of external [K^+], predicted from steady-state influxes of K^+ (*open symbols*) (Glass and Siddiqi 1984a), and from an assumed cytosolic [K^+] of 100 mM (see Eq. 2, and Table 1)

An analysis of the $t_{1/2}$ relationships between influx and pool size also has important, and previously unrecognized, implications for the measurement of fluxes of ions such as calcium (Ca^{2+}), whose cytosolic pools turn over very rapidly. An essential aspect of the function of the cytosolic Ca^{2+} pool in signal transduction is the maintenance of a very low background level of Ca^{2+} against which transient and periodic rises in cytosolic [Ca^{2+}] provide contrast (Williamson and Ashley 1982; Plieth et al. 1998; Berridge et al. 2000). Given that this background is typically near 100 nM, and that Ca^{2+} influx has been reported in the case of intact barley roots to be 1.67 μmol g^{-1} h^{-1} (Glass and Siddiqi 1984b), Eq. 2 can be used to calculate an exchange half-time for the cytosolic pool of about 7 ms, which, interestingly, is in good agreement with the timescale of the more rapid cytosolic Ca^{2+} modulation events (Oberwinkler and Stavenga 2000; Soeller and Cannell 2002). Similarly short half-times can be calculated in the same manner from data obtained using excised onion roots (Macklon and Sim 1981; Macklon 1984). However, given such a rapid turnover of Ca^{2+} in the cytosol, the direct estimation of this parameter by tracer efflux analysis becomes impossible in practice. Indeed, Table 1 shows that predicted half-times for cytosolic Ca^{2+} are 1,300 to 184,000 times lower than values estimated using tracer analysis. Clearly, these studies involved the incorrect assignment of the cytosol as the compartment releasing tracer to the external medium (verification of the tentative compartmental assignment was not provided in these studies), and because of this yielded cytosolic [Ca^{2+}] estimates many orders of magnitude in excess of what has been firmly established using fluorescence imaging techniques (Williamson and Ashley 1982; Plieth et al. 1998).

An important consequence of rapid cytosolic Ca^{2+} turnover is that measurements of Ca^{2+} influx across the plasma membrane made by any tracer method, whether by use of short-term exposure of plant tissue to a Ca^{2+} tracer (Deane-Drummond and Glass 1983), or by use of tracer efflux and retention data (Macklon and Sim 1981; Glass and Siddiqi 1984b), can be severely underestimated due to Ca^{2+} efflux unaccounted for during loading and elution times, which typically are many orders of magnitude longer than the half-times of the pool (Cram 1969; Lee and Ayling 1993; Britto and Kronzucker 2001a). The situation is exacerbated by the fact the exchange half-times estimated in Table 2 were calculated using such influx underestimates; correct influx measurements would therefore further shorten the

Table 2 Half-times of cytosolic calcium exchange, as predicted from a background cytosolic [Ca^{2+}] value of 100 nM (Williamson and Ashley 1982) and from Ca^{2+} influxes measured using ^{45}Ca by Macklon and Sim (1982) and by Glass and Siddiqi (1984b). Fluxes from Macklon and Sim were converted to μmol g^{-1} h^{-1} from nmol m^{-1} s^{-1} based on conversion factors given by Cram (1983). For further calculation details, see Table 1. In comparison to the predicted half-times are those reported in the two studies, for a putative cytosolic phase of tracer efflux

External [Ca^{2+}] (mM)	Reported influx (μmol g^{-1} h^{-1})	Cytosolic exchange half-times (s)	
		Predicted	Reported
10	0.755	0.0166	2,580
1	0.59	0.0212	3,420
0.5[a]	1.67	0.00749	1,380
0.1	0.335	0.0373	3,240
0.01	0.03	0.417	4,500
0.001	0.00425	2.94	3,720

[a]Data from Glass and Siddiqi (1984a)

half-time estimates to potentially much less than 7 ms. It must be concluded that the rapidity of cytosolic Ca^{2+} turnover, and the potentially very high ratio of efflux to influx of Ca^{2+} across the plasma membrane, render the measurement of unidirectional Ca^{2+} influx essentially impossible, at least by isotopic methods in higher plants. Even ostensibly simpler systems such as those involving ^{45}Ca measurements in giant algal cells (Spanswick and Williams 1965; MacRobbie and Banfield 1988; Reid and Smith 1992a, 1992b; Reid et al. 1993, 1995) must be beset by similar problems, given that the similarly very low values for cytosolic [Ca^{2+}] in these cells (Williamson and Ashley 1982; Plieth et al. 1998), in combination with even the minimal fluxes estimated in these experiments, again indicate that turnover of the cytosolic pool is so rapid that measurement of true unidirectional fluxes across the plasma membrane cannot be amenable to such tracer-based approaches. This is a discouraging, but inescapable, conclusion, and must be heeded in future discussions of such experimental data sets.

Regulatory functions of the cytosolic pool upon ion fluxes

It has long been known that ion fluxes are subject to positive and negative regulation, although the regulatory agents involved remain difficult to ascertain due to lack of information about the compartmentation of putative agents and changes in pool sizes of such agents under various growth conditions. In cases where cytosolic pools are held constant, such as Ca^{2+} and K^+, such pools can clearly exert no such differential regulatory influences, although regulation might occur via vacuolar pools or by changes in external supply (Leigh and Wyn Jones 1984; Clarkson 1985; King et al. 1993). In the cases of NO_3^- and NH_4^+, however, the plasticity inherent in cytosolic pool sizes potentially provides a means of flux regulation. It has been suggested by several authors that cytosolic pools of inorganic N themselves may control, in a negative feedback fashion, their own influx across the plasma membrane (Siddiqi et al. 1989; King et al. 1993; Rawat et al. 1999). Although, as shown in Fig. 1, the highest influx values for inorganic N are observed when the highest pool sizes are achieved, it must be pointed out that each measurement within a given data set in the graph, except those for NO_3^- induction in spruce (Kronzucker et al. 1995a), was made at a unique external N concentration, and hence is not directly comparable to the others within the set. Indeed, it is well established that increasing external [N] generally results in a downregulation of the influx of NO_3^- at any given external concentration (Siddiqi et al. 1990), as well the influx of NH_4^+ when external [NH_4^+] is low to intermediate, i.e. in the high-affinity range (Wang et al. 1993b; Cerezo et al. 2001; Glass et al. 2002).

Because these downregulatory events are associated with increases in the cytosolic pool sizes of the respective substrates, these pools might be considered plausible candidates for regulatory activity. This is particularly true in the case of NO_3^-, which appears able to regulate its own influx even in plants that lack N metabolites commonly invoked as potential regulatory agents (Cooper and Clarkson 1989; Lee et al. 1992; Forde 2002). Evidence for this comes from unaltered regulatory events observed in mutants deficient in nitrate reductase (King et al. 1993), and in plants whose N metabolism was blocked using tungstate (Mattsson et al. 1991) or methionine sulfoximine (MSX; King et al. 1993). The role of NO_3^- itself as a negative feedback agent is further supported by the well-documented observation that the downregulation of NO_3^- influx significantly lags behind peaks in nitrate reduction and further assimilation (Zhang and MacKown 1993; Kronzucker et al. 1995a). Nevertheless, none of these studies was able to link regulation of NO_3^- influx specifically to changes in the *cytosolic* pool of root NO_3^-. On the contrary, the study of NO_3^- induction in white spruce by Kronzucker et al. (1995a) showed that, against a background of unchanging external [NO_3^-], the highest NO_3^- influxes were observed when the cytosolic NO_3^- pool sizes were also the highest, which strongly suggests that the cytosolic NO_3^- pool is not a regulatory agent, at least over the time frame of induction. The possibility remains that the vacuolar NO_3^- pool could serve such a regulatory function, as was suggested above for ions like Ca^{2+} and K^+ whose cytosolic pool sizes do not vary. In addition to root vacuolar pools, shoot nitrate contents may also play a strong regulatory role, especially in the longer term (see Forde 2002 for a review of this topic).

A special case exists for NH_4^+ fluxes, where increases in external concentration above approximately 1 mM are associated not with a downregulation, but with an *increase* in NH_4^+ influx (Wang et al. 1993b; Rawat et al. 1999; Min et al. 2000; Cerezo et al. 2001; Glass et al. 2002), which may become so pronounced that it can result in the toxic uptake of NH_4^+ (Britto et al. 2001).

This surprising finding argues against the putative role of the cytosolic NH_4^+ pool as a feedback regulator of NH_4^+ influx, at least at higher external NH_4^+ concentrations. This likelihood is also supported by experiments using methionine sulfoximine in this concentration range (Kronzucker et al. 1995d; and references therein), which resulted in an increase in cytosolic [NH_4^+], and a roughly commensurate increase in NH_4^+ influx, rather than the decrease which would be expected were NH_4^+ to be a negative feedback agent. With NH_4^+ turnover, as with NO_3^- turnover, it is important to remember that the parameter that is held constant to the highest degree is the exchange half-time, a phenomenon which dictates that influx and pool size will change in tandem with each other.

Finally, it is interesting to examine the relationship of the efflux of a substrate to its pool size in the cytosol. It has often been proposed that plasma-membrane ion transport operates in a 'pump-and-leak' manner (Elbrink and Bihler 1975; Glass and Siddiqi 1984a), and specifically that nitrate efflux is determined by the size of the cytosolic pool (Clarkson 1986; Ter Steege et al. 1999), which is plausible given that this flux is likely to be passive under most circumstances. Indeed, there is abundant evidence that efflux of NO_3^-, and many other ions, increases as tissue levels of these ions increase (Glass and Siddiqi 1984a; Siddiqi et al. 1991; Wang et al. 1993a; Zhang and MacKown 1993; Kronzucker et al. 1995b, 1995c). Again, however, the issue of subcellular compartmentation of such putative regulatory pools is unresolved. Figure 4 provides some insight into this matter by showing a particularly interesting case, that of nitrate induction in spruce. It is clear from this figure that no strong relationship between cytosolic [NO_3^-] and NO_3^- efflux can be reasonably assumed. Clearly, ϕ_{co} is regulated independently of $[NO_3^-]_{cyt}$, a principle that is easy to accept in cases of ions such as K^+ or Ca^{2+} (or even H^+), where cytosolic pool sizes are held more or less constant, but appears to apply as well to pools of NO_3^-, which are highly variable. This analysis refutes the tempting, and therefore often proposed, argument for simple pump-leak models of ionic efflux. Interestingly, a vectorial reversal of this model might apply in the case of NH_4^+ efflux at high external [NH_4^+], which is mediated by an active transport mechanism, while influx occurs passively. This "leak-and-pump" scenario also exists in the cases of Ca^{2+} and H^+ fluxes, where ATPases drive efflux and passive pathways mediate influx, although with these ions the fluxes appear to be tightly regulated, while the "leak" component (i.e. influx) in the case of NH_4^+ appears to be unregulated in some species, resulting in an abnormally (and, possibly, toxically) high energetic requirement for the efflux-pump component of the transmembrane ammonium distribution (Britto et al. 2001; Kronzucker et al. 2001). Surprisingly, even though the efflux of NH_4^+ is active under these conditions, there is a strong correlation (R^2 = 0.97–0.99, determined from data in Wang et al. 1993a; Kronzucker et al. 1995c; Britto et al. 2002) between NH_4^+ efflux and cytosolic [NH_4^+]. This high correlation directly reflects the observation that the ratio of efflux to influx of NH_4^+ tends to approach unity as external NH_4^+ supply increases. Given constancy of exchange half-time for NH_4^+, Eq. 2 will now dictate that efflux and pool size also become linearly related as the flux ratio approaches one.

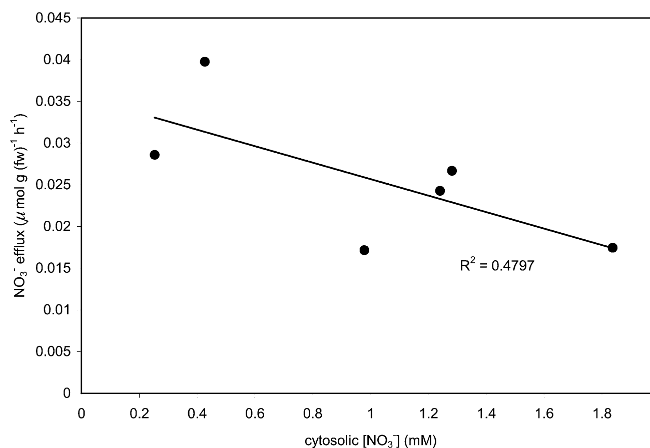

Fig. 4 NO_3^- efflux from the cytosol of roots of white spruce seedlings undergoing induction of NO_3^- transport and assimilation as a function of cytosolic NO_3^- pool size. Both parameters were measured over the course of the 3-day induction period using compartmental analysis by ^{13}N efflux. Data from Kronzucker et al. 1995a

Conclusion and outlook

It should be clear from the above considerations that the cytosolic exchange half-time for nutrient ions is not a parameter of esoteric interest to researchers conducting compartmental analysis, but is a term pivotal to the understanding of ion flux relations in plant cells. Because the half-time is a term which describes the overall dynamic state of ion pools within cell compartments, the information it reveals provides an important, integrative, context for many of the finer-scale manipulations that new technologies are making possible, but which tend, by their very nature, to be fragmentary. The variability or constancy of the half-time differs for given ions, but predictions using this term, based on Eq. 2, can be very useful in determining the veracity of conflicting claims regarding the magnitudes of pools and fluxes of ions across plant cell membranes. Only once these input parameters are reasonably established can questions of regulatory agents and mechanisms, as discussed in the second part of this paper, be adequately addressed. Our analysis of the regulation of inorganic N fluxes shows that the cytosolic pool size of the substrate cannot be a major regulatory force feeding back upon influx or determining efflux; rather, it is more an outcome than a determinant of a regulatory system of which it is, at best, only a minor component. The regulatory system governing inorganic N acquisition appears to draw flexibly

upon the substrate pools in various compartments, as well as metabolites derived from the substrates. Clearly, more work is required to elucidate the regulatory rules of these pools, as well as regulatory elements of other ions. Our analysis shows that key concepts in cellular ion relations need revisiting, and an integrative approach of the kind developed here will be essential to identify new directions in the field.

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