Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport

Dev T. Britto and Herbert J. Kronzucker

Department of Life Sciences, University of Toronto, 1265 Military Trail, Toronto, ON, M1C 1A4, Canada

Plant nutrient acquisition from concentrated soil solutions is governed by low-affinity transport systems in the plasma membranes of root cells. In this Opinion article, we illustrate that for six major nutrient ions, in addition to influx mediation by low-affinity transporters, high rates of ion cycling at the plasma membrane are a hallmark of nutrient transport at elevated external concentrations. This phenomenon is characterized by high rates of ion influx and concurrent high efflux of the same ion, resulting in efflux:influx ratios close to 1. Prolonged maintenance of futile cellular ion cycling can be energetically unfavorable and detrimental to plant growth and survival. We discuss how ion cycling can contribute to the toxicities of ions such as Na^+ and NH_4^+ in the low-affinity range of ion provision. We also argue that cellular ion cycling makes the reliable measurement of ion influxes in the low-affinity range a formidable challenge.

Introduction

Plants are remarkably plastic in their cellular responses to diverse soil environments. By changing the patterns of expression and regulation of membrane-bound transport proteins, roots can tolerate soil nutrient concentrations that vary over orders of magnitude. Transport systems catalyzing ion influx across the plasma membrane of root cells fall into two broad categories: high-affinity transport systems (HATS) that mediate uptake from relatively dilute solutions at relatively low rates, and low-affinity transport systems (LATS) that operate at higher rates and higher external concentrations [1,2]. These categories reflect the dual mechanisms of ion transport that were characterized in the pioneering work of Emanuel Epstein and colleagues [1], who used radiotracers to resolve the kinetic patterns of unidirectional influx. The most consistent and widely recognized feature of HATS is the saturable concentration dependence of influx, contrasting with LATS, which show linear responses to substrate concentrations [1,2]. The transition concentration that determines the switch between HATS and LATS depends on the ion in question, but typically occurs around 1 mM for macronutrient ions [1,2]. This concentration is also species- and genotype-dependent. For instance, NH_4^+ influx in white spruce (*Picea glauca*) is still dominated by HATS when external concentrations of NH_4^+ are 1.5 mM, whereas in Douglas-fir

(*Pseudotsuga menziesii*) the influx of NH_4^+ ions is predominantly mediated by LATS at this concentration [3].

In general, HATS have been much more thoroughly investigated than LATS, partly because of the view that plants rarely encounter soil nutrient concentrations that would result in substantial engagement of LATS. Notwithstanding such a view, a survey of many different agricultural soils [4] showed that 50% of the sites surveyed had K^+ concentrations >1 mM. Likewise, the concentrations of inorganic nitrogen (NO_3^- and NH_4^+) in the upper soil horizons of agricultural soils and natural ecosystems are often >1 mM [5]. Furthermore, Na^+ and Cl^- concentrations in salinized soils are well within the LATS range for these ions [6]. Indeed, it is estimated that >400 million hectares (6% of the land area of the world and 20% of the irrigated land) are affected by salinity [6]. Thus, investigating the characteristics of LATS is of fundamental importance for understanding ion transport physiology and plant performance in the field.

It has long been established that the influx capacity of LATS can be many times greater than that of HATS [1–3,5]. However, it has not been widely recognized that the high rates of LATS-mediated influx are, in many cases, concurrent with nearly equivalent rates of efflux. Together, these opposing unidirectional fluxes create a condition of futile ion cycling at the plasma membrane of plant cells. In this Opinion article we summarize what is known about the unidirectional fluxes of the LATS (efflux and influx) of six major nutrient ions (K^+, Cl^-, NH_4^+, NO_3^-, Na^+ and SO_4^{2-}), drawing on a variety of experimental approaches, and introduce a model of low-affinity transport that fundamentally incorporates the concept of futile cellular ion cycling. We present a generally applicable energetic analysis of futile cycling at the plasma membrane, based upon accepted models of ion transport, and discuss its consequences for the development of ion toxicities, such as those of Na^+ and NH_4^+. In addition, we discuss how cellular ion cycling has significant implications for measuring ion fluxes in plant cells and tissues.

Mechanisms of influx and efflux in the LATS range

As in most organisms, the plasma membranes of plant cells maintain a negative electrical charge towards the interior of the cell, with values that typically range between −50 to −140 mV [7], but can exceed −200 mV [8,9]. In plants, this is achieved predominantly via

Corresponding author: Kronzucker, H.J. (herbertk@utsc.utoronto.ca).
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the electrogenic activity of plasma-membrane (P-type) H^+-ATPases, which extrude protons against their electrochemical potential from the cytosol to the outside medium at the expense of ATP. The number of H^+ ions pumped per ATP molecule hydrolyzed is fixed at or near 1 at the plasma membrane [9]. In the LATS range, this polarized membrane condition, in conjunction with the extent to which major ions accumulate in the cytosol [10], entails cations entering plant cells passively [1,2,5–8,11–15], following their electrochemical potential gradient across the plasma membrane. However, for cation efflux, cations must be actively pumped out of the cell [6,7,11–16]. By contrast, anions enter against an electrochemical potential gradient, via active transport, when external concentrations are high [5,10,17,18], but can, under these same conditions, leak back passively into the external solution [5,17–20]. Figure 1 summarizes the general mechanisms of influx and efflux operating in the LATS range (and their functional connection to the H^+-ATPase) for univalent cations and anions (the flux of di- or polyvalent ions involves an additional proton for each additional charge). Current models suggest that cation influx in the LATS range is catalyzed by electrogenic uniport mechanisms (channels) and that cation efflux uses the electrochemical potential of the trans-plasma-membrane proton gradient by engaging cation–H^+ exchange (antiport) mechanisms that possess 1:1 stoichiometry (i.e. are electroneutral) [16,21]. By contrast, anion influx in the LATS (and HATS) range occurs via proton-coupled, electrogenic symport mechanisms (of general $A^-/2H^+$ stoichiometry, Ref. [10] and references therein), whereas anion efflux in LATS (and HATS) is catalyzed by anion channels, which are electrogenic [17,19,20]. The H^+-ATPase is centrally implicated in re-establishing membrane potential deviations caused by all electrogenic fluxes, and in re-establishing the proton gradient across the plasma membrane [9]. In this way, no ion flux is a truly passive affair. The functional and/or gene candidates that are known to be responsible for the influx and efflux events of the six major nutrient ions discussed here are summarized in Table 1. Experimental and modeling work has shown that cation channels
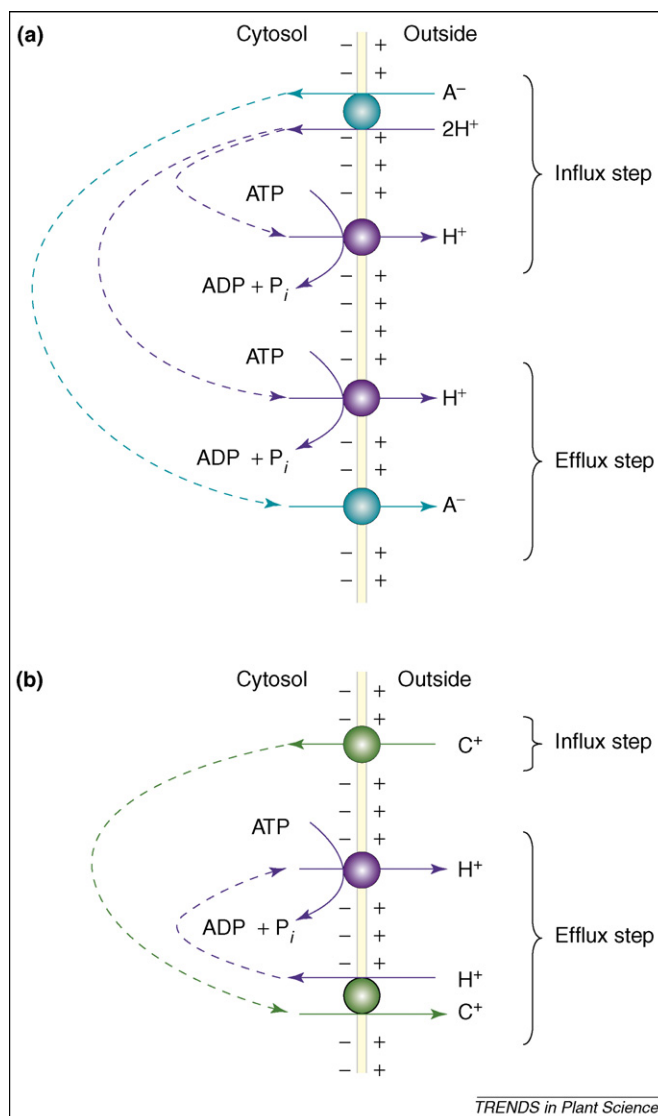

Figure 1. Generalized model of univalent anion **(a)** and cation **(b)** cycling at the plasma membrane. Active transport steps involve symport (for anion influx) and antiport (for cation efflux) of protons. Plus- and minus-signs indicate electrical polarization of the membrane. The involvement of the proton ATPase ensures the electroneutrality and the pH-neutrality of the complete transport cycle.

Table 1. Mechanisms and gene or functional candidates identified as responsible for influx and efflux of six major nutrient ions in the low-affinity transport systems range

Ion	Flux	Flux mechanism	Functional or gene candidates[a]	Refs
K^+	Influx	Channel	K^+ inward-rectifying channels KIRC (*AKT1*, cyclic nucleotide gated channels CNGC), *LCT1*, nonselective cation channels NSCC, *KUP/KT/HAK*	[6,17,24]
	Efflux	Proton antiport (K^+/H^+)	*KEA/CPA*, *CHX*	[6,16,21,24]
NH_4^+	Influx	Channel (NH_4^+, NH_3)	*AMT1;2*, *AQP/TIP*, KIRC, NSCC	[5,12,17,38]
	Efflux	Proton antiport (NH_4^+/H^+), channel (NH_3)	*CHX*, *AQP/TIP*	[5,11,12,16]
Na^+	Influx	Channel	*LCT1*, KIRC (CNGC), NSCC, *HKT1*	[6,13,14,15,17,24]
	Efflux	Proton antiport (Na^+/H^+)	*SOS1*, *CHX*	[6,16]
Cl^-	Influx	Proton symport (Cl^-/2 H^+)	Not known	[17,53]
	Efflux	Channel	*Arabidopsis* root anion channel ARAC, Al^{3+}-activated anion channels AIAAC	[17,19]
NO_3^-	Influx	Proton symport (NO_3^-/2 H^+)	*NRT1.1*, *NRT1.2*, *NRT1.4*	[5]
	Efflux	Channel	ARAC, AIAAC, *AQP/TIP*	[19,25]
SO_4^{2-}	Influx	Proton symport (SO_4^{2-}/3 H^+)	*SULTR* (group 2?)	[54]
	Efflux	Channel	ARAC, AIAAC	[19,20]

[a]Green indicates functional designations (genes not necessarily identified at present); blue indicates known genes or gene groups; purple indicates putative genes or gene groups.

can switch on or off in response to external concentrations that either rise above or fall below a threshold concentration [22,23]; this work could provide a clue to the onset, and control, of LATS flux activity as external ion concentrations rise. Nevertheless, under conditions of inhibited HATS transport, some LATS transporters are capable of conducting influx even at low external ion supply [8]; other transporters, in the cases of K^+ and NO_3^-, possess dual affinities (i.e. the ability to operate in HATS and LATS ranges) and to switch transport modality as a result of protein modifications such as phosphorylation and dephosphorylation [5,24]. Many LATS transporters, in particular channels, lack refined ion specificity but possess the ability to transport several ions, which further complicates the information presented in Table 1. This condition is particularly evident in the case of anion channels that can transport organic acids (Cl^-, NO_3^- and SO_4^{2-} [19]), of non-selective cation channels (NSCC) that can mediate Na^+, NH_4^+ and K^+ entry [6,17,24], and of 'aquaporins' (AQP) that have been implicated in the transport of water, glycerol, urea, nitrate [25] and, more recently, ammonium and ammonia [5]. Similarly, some cation or proton antiporters might be able to transport several ionic species [16]. Interestingly, changing the sequence of a transporter by only a few amino acids can change the selectivity properties from generic to specific, or from one ion to another [26].

Futile cycling at the plasma membrane

It is universally accepted that ion acquisition is accompanied by a certain degree of ion efflux. This contribution of efflux to plasma membrane transport has been quantified by many laboratories, using a variety of experimental approaches. These include: (i) monitoring the decline of apparent tracer influx or phases of tracer accumulation as functions of tracer exposure time [13–15,27–29]; (ii) determining the difference between unidirectional influx (measured over short time periods) and net flux (measured over long time periods) [13–15,30–34]; (iii) quantifying isotope ratios during isotopic dilution of an unlabeled bathing medium by labeled plants (or, in reverse, of a labeled bathing medium by unlabeled plants) over short periods [31,35–39]; (iv) monitoring tracer washout kinetics from labeled plants under steady-state conditions, either by complete, periodic exchange of the external medium, or by subsampling this medium [11,14,15,18,20,27,30,33,40–42].

Drawing upon concentration-dependence data from these four methods, an important pattern emerges: that efflux becomes progressively pronounced, approaching the rates of influx, as external ion concentrations rise, and resulting in increased futile nutrient cycling at the plasma membrane (Figures 2 and 3). With the possible exception of phosphate [43], this trend is evident for all major ions examined to date. Figure 2 shows that cellular ion cycling of cations and anions occurs to a similar extent and,
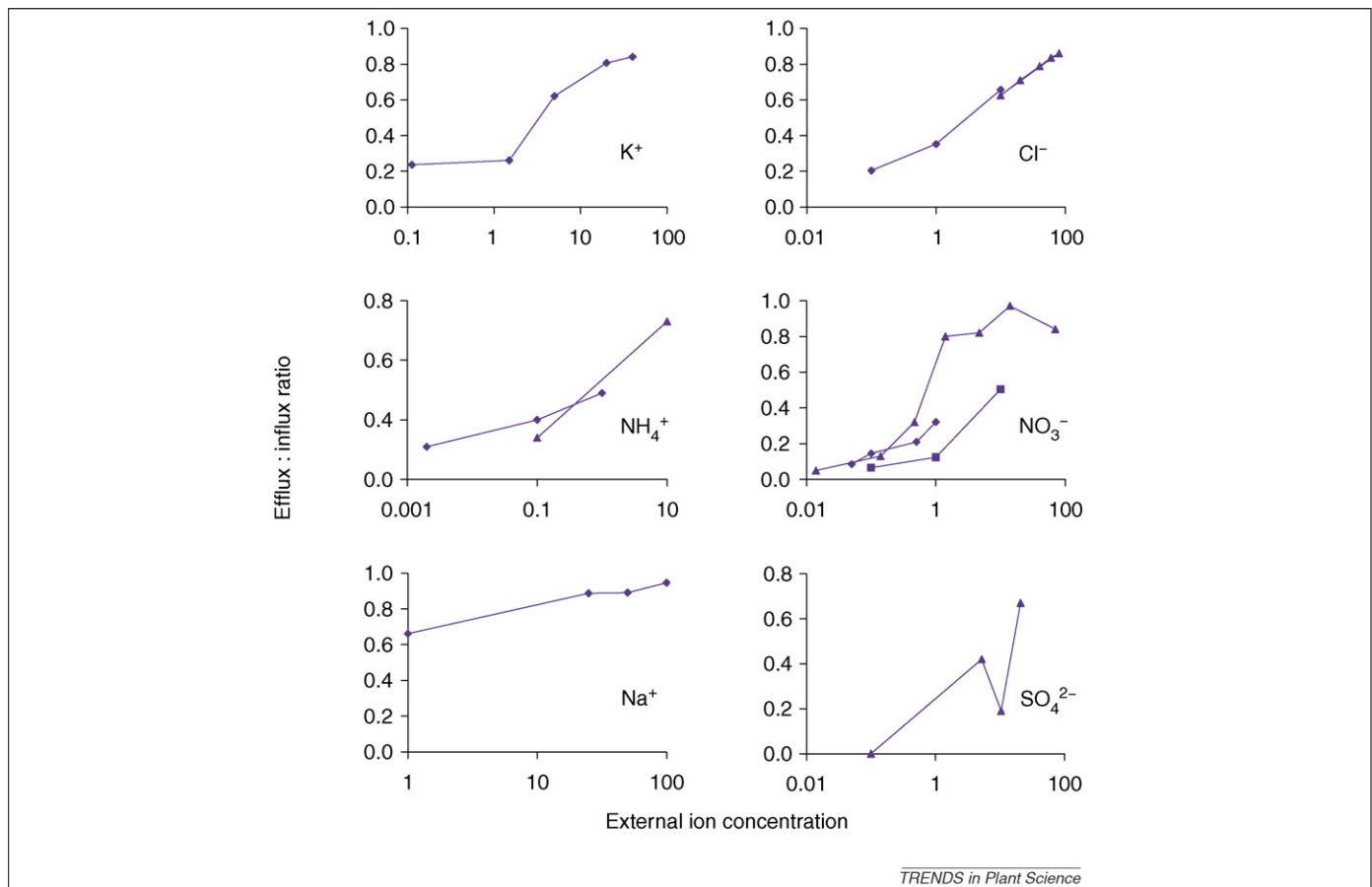

Figure 2. Ratios of efflux to influx across the plasma membrane for six major nutrient ions, replotted from ten previous studies. In nearly all cases, the flux ratio increases progressively with external ion concentration. References are as follows: potassium (K^+) [7]; ammonium (NH_4^+) – diamonds [33], triangles [11]; sodium (Na^+) [55]; chloride (Cl^-) – diamonds [30], triangles [18]; nitrate (NO_3^-) – diamonds [31], triangles [28], squares [41]; sulfate (SO_4^{2-}) [20].
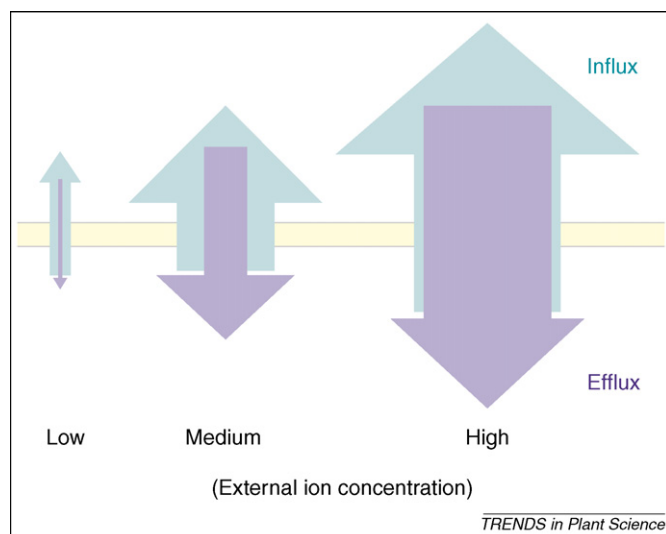
Figure 3. Increases in efflux and influx, and the ratio of efflux to influx of nutrient ions at the plasma membrane as the external ion concentration increases.

contrary to previous proposals [11,12], is not, *per se*, a hallmark of ion toxicities at elevated ion supply, as it is in the cases of NH_4^+ and Na^+, but a hallmark of low-affinity transport in general. Figure 3 summarizes this trend, showing the increase in the ratio of efflux to influx with increasing external ion concentration, as well as the increasing magnitude of both fluxes. Whether this condition constitutes a maladaptation of the ion transport machinery of the plant at high levels of ion supply or is of adaptive significance under certain environmental conditions remains obscure but will be discussed below.

Energetics of cellular cycling
Because unidirectional fluxes of both anions and cations are, according to accepted models, mechanistically committed to the stoichiometries outlined above (Figure 1), it is possible to analyze the energy costs of futile ion cycling. From the outset, it is clear that either influx or efflux must be energetically active, except under the rare condition in which a thermodynamically passive distribution (at the Nernst potential of the ion) is achieved [11,12,44]. In the low-affinity transport range, plasma membrane fluxes become increasingly large in both directions (Figure 3) and, therefore, the energetic cost of powering the active flux must become increasingly large too. In parallel with increasing fluxes and their associated energy requirements, root respiration rates and carbon delivery from shoot to root are known to increase [45–49].

Hans Lambers and co-workers [34,48] developed a model to calculate the flux of ATP and respiratory O_2 involved in primary ion acquisition. They showed that (assuming an efflux:influx ratio of 0.5) as much as 76% of total root respiration can go towards supporting low-affinity plasma-membrane NO_3^- fluxes [48]. Here we present generalized equations (equations 1 and 2) to calculate the amount of ATP and the oxygen fluxes associated with the futile cycling of univalent cations and anions, according to the mechanisms and stoichiometries shown in Figure 1.

For futile cycling of cations:

$$\phi_{O_2} = (P/O_2)^{-1} \phi_{ATP} = (P/O_2)^{-1} \phi_e \qquad \text{(Eqn 1)}$$

For futile cycling of anions:

$$\phi_{O_2} = (P/O_2)^{-1} \phi_{ATP} = 2 (P/O_2)^{-1} \phi_e \qquad \text{(Eqn 2)}$$

where ϕ_{O_2} and ϕ_{ATP} are the fluxes of oxygen and ATP (respectively) coupled to membrane fluxes, and ϕ_e is the unidirectional efflux of the ion. P/O_2, the phosphorylation efficiency, is typically near 5 when the cytochrome pathway is fully engaged [12,48]. The equations show that twice as much energy per cycle is required for anions because of the additional proton involved in futile anion cycling (Figure 1). Furthermore, the equations show how the energy cost of futile ion cycling can be calculated directly from efflux, without the need for precise knowledge of either the ion distribution across the plasma membrane, or the electrical potential of the membrane.

The utility of the equations can be seen in an analysis of NH_4^+ cycling in barley (*Hordeum vulgare*) roots. A previous study [11] showed that O_2 consumption increased by 7.5 μmol g(fw)$^{-1}$ h^{-1} (a 41% increase) when barley roots were exposed to high concentrations (10 mM) of NH_4^+, which was associated with an increase in active NH_4^+ efflux of 47.5 μmol g(fw)$^{-1}$ h^{-1}. This yields a ratio of increased NH_4^+ efflux to increased O_2 consumption of 6.3, close to published values of P/O$_2$, as predicted in the cation equation above (rearranged as $P/O_2 = \phi_e/\phi_{O_2}$). This result differs from a previously published analysis of this study [12], which indicated that the measured O_2 influx was tenfold higher than the predicted O_2 flux. However, that analysis was based not on the proton-gradient model proposed here, but upon an NH_4^+-gradient analysis that used estimates of cytosolic NH_4^+ pools, external NH_4^+ concentrations and plasma-membrane electrical potentials. The difference between these two results illustrates that the commitment to the mechanisms depicted in Figure 1 entails energy inefficiencies that become particularly pronounced in the low-affinity transport range.

Because of the potentially large energy cost associated with futile ion cycling, we propose that it must have significant implications for plant performance in the field, particularly under conditions where growth is energy-limited. Thus, we expect the greatest impacts on plant survival to occur with the futile cycling of ions such as NH_4^+ and Na^+ that have toxic effects on photosynthesis and the supply of energy to the roots [6,12,49]. By contrast, in the case of high K^+ supply, which does not compromise photosynthetic capacity or energy delivery to the root, futile cycling, even though energetically costly, does not appear to be detrimental to growth [7]. It is intriguing to speculate whether under conditions of high irradiance, which are common in agricultural and early-successional settings, the futile cycling of ions constitutes an energy sink that helps to alleviate the over-energization of the photosynthetic apparatus.

Implications for flux measurement and mechanistic inference
In a paper on a study of the sodium LATS, Pauline Essah *et al.* [13] suggested that 'almost all published data

measuring $^{22}Na^+$ accumulation into higher plant tissues are made over time periods that are too long to ensure they are not significantly reduced by efflux of $^{22}Na^+$...which could result in misattribution of function to particular proteins or protein families.' Essah *et al*. contrasted their data on Na^+ influx obtained by 2-min exposure to a radiotracer with those of other groups [42,50] who had used the same plant system but longer labeling times ($\geq$10 min) and/or long desorption times following labeling, with estimates of influx as much as 18-fold lower than those obtained with 2-min measurements. The conclusion was that the drastic influence of efflux because of rapid cellular cycling of Na^+ rendered standard protocols of influx determination invalid. Similarly, we have shown that, for potassium, steady-state influx in the LATS range can be as much as sixfold higher than estimated, mostly because of efflux [51]. Such underestimates have consequences for the energy analyses of flux processes, as seen above.

The widespread phenomenon of futile cellular ion cycling indicates that widely used flux protocols, and the data and conclusions based on them must be treated with considerable caution when applied in the LATS range. Typically, reported LATS fluxes, and the pivotal system parameters (e.g. V_{max} and K_m [1]) deduced from them, are the result of substantial bidirectional flux activity and, thus, cannot be taken at face value [51]. In general, analyses of influx isotherms have not usually considered the rising contribution of efflux at elevated nutrient concentrations. This might warrant a re-investigation of, for example, recent proposals about the possible dual-affinity nature of certain transporters, such as those catalyzing the influx of K^+ [24] and NO_3^- [5]. Furthermore, understanding the response of transporters to feedback signals [2,5] and other regulators such as divalent cations [13,15], counterions [1], and cyclic nucleotides [50], requires knowledge of possible modulations of efflux as a result of the imposed treatments. In the absence of such measurements, influx comparisons could lead to inaccurate models of transport.

Concluding remarks

In this Opinion article we have illustrated the substantial role of ion efflux in the acquisition of nutrients by plants growing under low-affinity transport conditions. For six major ions, the ratio of efflux to influx increases with increasing external concentration, reaching values that approach 1, at which point an apparently futile cycling of nutrient ions occurs (Figure 2). Together with the rise in efflux:influx ratios, the magnitude of these fluxes and the energy costs associated with them rise substantially. A complete picture of the transport processes in the LATS range requires the identification and characterization of efflux transporters at the molecular level. Biotechnological modification of these proteins, in conjunction with the modification of influx transporters, might alleviate problems associated with LATS in nutrient-rich or salinized soils. In particular, the energy-intensive nature of futile cycling of ions such as NH_4^+ and Na^+, and the compromising effects of these ions on photosynthate production and supply to the roots, might be the main reason for the NH_4^+ and Na^+ toxicities seen in plants. Modifying influx

transporters, such that their activities are restricted in the LATS range, might diminish or prevent toxicity. This approach could be feasible because there is natural variation in the primary influx of potentially toxic ions among plant genotypes [3,11,15,52]. Finally, the discovery of the apparent universality of the futile cycling of the LATS-range indicates that the kinetic patterns of influx in this range need to be re-evaluated, which might ultimately lead to a revision of the fundamental models of plant ion transport.

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