Short title: Targeting root uptake kinetics

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Targeting root ion uptake kinetics to increase plant productivity and nutrient use efficiency

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One-sentence summary: Root ion uptake kinetics is presented as a promising target for boosting nutrient acquisition by addressing knowledge gaps, meta-analysis of kinetics across species, and providing future prospects.

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Abstract

Root system architecture has received increased attention in recent years; however, significant knowledge gaps remain for physiological phenes, or units of phenotype, that have been relatively less studied. Ion uptake kinetics studies have been invaluable in uncovering distinct nutrient uptake systems in plants with the use of Michaelis-Menten kinetic modelling. This review outlines the theoretical framework behind ion uptake kinetics, provides a meta-analysis for macronutrient uptake parameters, and proposes new strategies for using uptake kinetics parameters as selection criteria for breeding crops with improved resource acquisition capability. Presumably, variation in uptake kinetics is caused by variation in types and numbers of transporters, assimilation machinery, and anatomical features that can vary greatly within and among species. Critically, little is known about what determines transporter properties at the molecular level or how transporter properties scale to the entire root system. A meta-analysis of literature containing measures of crop nutrient uptake kinetics provides insights about the need for standardization of reporting, the differences among crop species, and the relationships among various uptake parameters and experimental conditions. Therefore, uptake kinetics parameters are proposed as promising target phenes that integrate several processes for functional phenomics and genetic analysis, which will lead to a greater understanding of this fundamental plant process. Exploiting this genetic and phenotypic variation has the potential to greatly advance breeding efforts for improved nutrient use efficiency in crops.
Key Words:

Ion uptake kinetics, nutrient acquisition, root, transporter, macronutrients, nitrate, phosphate, potassium, Michaelis-Menten kinetics.
Abbreviations:

- MIUK, multiple-ion uptake kinetics;
- HATS, high-affinity transport system;
- LATS, low-affinity transport system;
- \( C \), concentration of solute;
- \( I_n \), net influx;
- \( I_{\text{max}} \), maximal influx rate;
- \( K_m \), Michaelis constant;
- \( C_{\text{min}} \), minimum solute concentration at which net influx can occur.
Sustainable crop production will be an ever-increasing challenge as the world population and global food demand continue to rise (Hunter et al., 2017). Improvements in food production need to be made despite threats from climate change and competition for land, water, and energy (Godfray et al., 2010; Parry and Hawkesford, 2010). A particular concern is the dependency of modern agriculture on chemical fertilizers that require finite fossil fuels and mineral reserves for their manufacture (Cordell et al., 2009). The world cannot be considered food secure until dependency on fertilizers is minimized. In order to mitigate these risks, the development of new cultivars that are more efficient in water and nutrient uptake is essential. During the first “Green Revolution”, above-ground phenes, or elemental units of phenotype (sensu York, 2013), were the target of crop selection in breeding programs. Roots were largely ignored; however, indirect selection leading to changes in crop root systems over time may have occurred due to direct selection of above-ground phenes and yield in high-input and high plant-density farming systems (York et al., 2015; Waines and Ehdaie, 2007). Selection of crops based on the root system is inherently challenging, but is predicted to pave the way for a second “Green Revolution” to help attain food security (Lynch, 2007).

The root system of a plant provides vital functions including resource uptake, storage, and anchorage in the soil, and is an interface between the plant and the soil microbiome. As soil resources are spatially and temporally heterogeneous, adaptations in the root system can be particularly important for survival (Wang et al., 2012). For plant growth and development, the elements nitrogen (N), sulphur (S), phosphorus (P), magnesium (Mg), calcium (Ca), and potassium (K) are required in the greatest amounts (Hawkesford, 2011). Of these macronutrients, N, P, and K are often at limiting quantities for crop yield in agriculture and are applied as fertilizers (Fageria, 2009). Chemical fertilizers are widely used to enrich soils and enhance crop productivity, but also add a significant cost to food production and represent major environmental pollutants (Bumb and Baanante, 1996). Fertilizer utilization in agriculture is neither sustainable nor efficient, with as little as 50% of applied fertilizer being captured by the roots of crops (Pask et al., 2012). In addition, selection of crops in non-limiting nutrient environments may have limited gains in nutrient acquisition efficiency. Hence, understanding the mechanisms involved in plant nutrient uptake is of key importance for improving worldwide agricultural production and mitigating environmental risks.
With the development of image-based root phenotyping, significant advances in characterizing root system architecture have been made in recent years (Bucksch et al., 2014; Atkinson et al., 2015; Colombi et al., 2015; Rellán-Álvarez et al., 2015; York and Lynch, 2015). However, whereas the importance of where roots are located and how they are arranged as determined by root system architecture is relatively well-known, functional processes such as root cortical senescence, root respiration, and root nutrient uptake have received much less attention. The advent of functional phenomics offers one path forward by combining high-throughput phenotyping, physiology, multivariate statistics, and simulation modelling (York, 2019). Nutrient uptake kinetics is the study of localized uptake rates of ions from external solutions by roots and the use of mathematical models to summarize uptake rate dependence on environmental and physiological conditions.

The aims of this review are to (1) describe the fundamentals of ion movement processes in the soil, the mechanisms involved with plant uptake of ions, and the popular mathematical models of nutrient uptake, (2) evaluate current experimental approaches used for ion uptake kinetics and provide a comprehensive meta-analysis of kinetics parameters reported in the literature across multiple crop species and nutrients, and (3) discuss future prospects for ion uptake studies in advancing plant science and the incorporation of findings in crop breeding programs.
Theoretical framework

Processes of ion movement in the soil environment

Root uptake of mineral nutrients from the soil solution is of major importance for plant growth. Nutrients in soil must be intercepted by the root surface through either nutrient movement or root growth to be taken up by the plant. Plants themselves also influence the physical structure of the soil and resource availability by root-driven soil displacement and increased porosity and the release of root exudates (Tisdall and Oades, 1982; Helliwell et al., 2017). Solute movement and availability at the root surface are mediated by the processes of mass flow and diffusion (Barber, 1962). Mass flow is the process of an ion moving across a water potential gradient which occurs while the plant is transpiring. Nitrate ($\text{NO}_3^-$) is an example of an ion with high solubility, and therefore has greater uptake at peak plant transpiration (Le Bot and Kirkby, 1992). The solubility of nitrate also affects its soil availability, as nitrate typically rapidly leaches into the deeper soil layers and ground water, is lost through surface run off, or is temporarily immobilized during drought (Bray, 1954). When mass flow does not saturate the root uptake capacity, ions will be depleted from the surrounding soil environment. This depletion from the soil causes a concentration gradient in which ions move from a high concentration to a lower concentration passively by diffusion. The effective diffusion rate in the soil is influenced by multiple parameters, including the electrochemical gradient and charge of the nutrient, the ion exchange capacity of the soil, and the moisture content (York, Carminati, et al., 2016). Phosphate is relatively immobile in soil for this reason, and therefore small depletion zones form around roots. Despite phosphate being relatively immobile, it also contributes to eutrophication of lakes, rivers, and marine environments via attachment to soil particles during erosion. Soil nutrient mobility and availability play a large part in determining the optimal root system architecture, but little is known about how optimal uptake kinetics are determined by nutrient mobility.

Physical mechanisms of plant ion uptake

The soil environment to which a root is exposed can vary greatly within a field where nutrient concentrations are often lower than the internal concentrations of the root cells (Barber, 1995; Lark et al., 2004). Therefore, plants have evolved mechanisms to passively facilitate and regulate ion transport down favourable electrochemical gradients as well as actively move...
ions against these gradients. Primary active transport uses ATP to move a substrate across a membrane against its gradient, whereas secondary active transport utilizes a gradient previously established by primary active transport. For both nutrient anions and cations, secondary active transport across the plasma membrane is mediated by H+-ATPases that establish electrical and proton gradients that drive uptake (Reid and Hayes, 2003).

Plant genomes encode many types of membrane-bound transport proteins that are important for nutrient uptake and mobilization in the plant (Zelazny and Vert, 2014). Transporters often exhibit specificity to particular nutrients in their most common chemical forms. This specificity is important for a plant to preferentially absorb ions that are in demand and to block those that are not needed or toxic. Individual nutrient chemical species are known to have sub-types of transporters responsible for their uptake with unique functional properties and regulatory patterns (O’Brien et al., 2016). For instance, a high-affinity transport system (HATS) and a low-affinity transport system (LATS) for nitrate have transporter proteins encoded by the NPF (also known as NRT1 and PTR) and NRT2 gene families, respectively, in Arabidopsis (Tsay et al., 2007). Whereas these genes are most commonly studied in Arabidopsis, analogues in cereals are known. In rice (Oryza sativa), OsNPF6.5 (NRT1.1B) was found to be sustainably induced by nitrate and showed dual-affinity nitrate transport affinity (Hu et al., 2015). In maize (Zea mays L.), the analogous ZmNrt1 and ZmNrt2 genes correspond to differences in uptake relating to expression levels (Quaggiotti et al., 2003; Quaggiotti, 2004; Trevisan et al., 2008). Although nitrate is generally regarded as the nutrient most commonly limiting growth, other macronutrients are also of interest, especially in nutrient-poor soils. Transporters have been identified for ammonium (Howitt and Udvardi, 2000; Sohlenkamp et al., 2000), phosphate (Raghothama, 2000), potassium (Coskun et al., 2013), and sulfate (Takahashi et al., 2012). Adding to the complexity, transporters have been demonstrated to exhibit cross regulation with multiple nutrients at the tissue and whole-plant levels as well as to facilitate uptake of phytohormones (Krouk et al., 2010; Medici et al., 2019). The genetics and transcriptional regulation of transporters have been identified in many cases but our understanding of the physical mechanisms as to how ions are intercepted and shuttled across the membrane by individual transporters is relatively poor.

Little is known about transporter-level uptake kinetics or how it scales up to root segments or whole-root systems (discussed in York, Silberbush and Lynch, 2016). This knowledge gap is exacerbated because of the primitive understanding of the physical and molecular
mechanisms involved in how an ion approaches the cell membrane, is bound by a transporter, and then shuttled by the transporter changing conformations from the external to the internal environment (Figure 1). Recent research has supported proton-coupled transport of nitrate by \textit{NPF6.3} (also known as \textit{CHL1} and \textit{NRT1.1}) and an alternating access mechanism where a central binding site reorients to alternatively expose the bound nitrate from the external to internal solution (Parker and Newstead, 2014). When \textit{NPF6.3} is phosphorylated, the nitrate affinity becomes greater, yet maximum uptake rate is decreased (Parker and Newstead, 2014; Sun \textit{et al.}, 2014). The fact that both the maximum uptake rate and the affinity can be post-translationally modified in a single transporter type is a strong indicator that the specific molecular form of a transporter influences kinetics. It follows that there may be different alleles within a species or homologs among species that have different transporter properties that influence uptake capability. Understanding the scaling of transporter-level uptake to the whole-root system must begin with a deeper understanding of these molecular foundations of individual transporters, yet even less is known about how transporters and other molecular machinery behave in groups and combinations.

The numbers and types of transporters found in the root epidermis must logically scale to root segment-level uptake. A linear scaling was showing for transporter abundance with uptake for the prokaryotic CIC transporter (Garcia-Celma \textit{et al.}, 2013). However, to our knowledge, no study has quantified the numbers of specific nutrient transporters in a given root surface area, i.e. transporter density. How the types of transporters and their density modulate scaling of transporter-level dynamics to the root level is not known. Whereas several studies have shown a relationship between transporter transcript abundance and uptake rates at single external concentrations (Quaggiotti, 2004; Trevisan \textit{et al.}, 2008; Garnett \textit{et al.}, 2015), these studies typically do not quantify $I_{\text{max}}$ and $K_m$. These studies have also not revealed the relationship between transcript abundance and transporter density at the root surface. Therefore, how uptake kinetics scale from transporter to root segment remains a knowledge gap. A complete physical mechanistic model at the molecular and cellular level is highly desirable as a foundation for understanding these dynamics, yet modelling of uptake kinetics in plants has generally been accomplished using Michaelis-Menten kinetics at the root-segment or whole root-system level.

The activity of many nutrient transporters is linked to the establishment of hydrogen ion gradients using ATPase to pump H+ outside of the cell (Figure 1C). For example, the
phosphate and nitrate transporters are symporters where the anion nutrient and a proton together move into the cell. Like transporters, little is known about how the numbers or types of ATPase proteins influence the electrochemical gradient or uptake rate. For iron acquisition, concerted action among ATPases, reductases, and iron transporters is required, which highlights the importance of spatial distribution of these proteins (Gayomba et al., 2015). Therefore, one of the most crucial conceptual gaps to address before utilizing nutrient uptake to increase plant productivity is how the specific types and numbers of transporter, ATPase proteins, and assimilation machinery influence root segment-level and whole root system-level uptake (Figure 1D, also discussed conceptually in York, Silberbush and Lynch, 2016 and more formally in Le Deunff et al., 2019).

**Michaelis-Menten kinetics**

The active uptake of nutrients by plants is most often modelled using the Michaelis-Menten theoretical framework of enzyme-substrate saturation kinetics (see Equation 1, Figure 2). Epstein and Hagen (1952) first used Michaelis-Menten kinetics to model uptake of nutrients by roots. Using this framework, Epstein et al. (1963) uncovered two distinct root uptake systems for potassium in barley (*Hordeum vulgare* L.) that respectively operate at low and high concentration ranges (>250 μM and <250 μM). Since then, there have been numerous studies identifying distinct uptake systems for other ions in multiple plant species (Siddiqi et al., 1990; Rao et al., 1997). In Michaelis-Menten formulation, as the external concentration of a solute (C) increases, the chance of binding to the respective transporter protein also increases, resulting in a greater net uptake rate or influx (I_n). When this external concentration continues to increase, a plateau is reached with a maximal uptake rate (I_{max}) as the carrier binding sites are saturated. The affinity between the carrier and ion is represented by the Michaelis constant (K_m), which is the half saturation constant where the solute concentration is ½I_{max}. Less reported is the minimum substrate concentration at which net influx can occur (C_{min}).

**Equation 1.** Michaelis-Menten kinetic model.

\[ I_n = \frac{I_{max} (C - C_{min})}{K_m + (C-C_{min})} \]

**Alternatives to Michaelis-Menten modelling**
Use of Michaelis-Menten modelling for uptake by root segments or whole-root systems presupposes that the behaviour of the entire system of multiple transporters, assimilation, and uptake into vacuoles can be aggregated by a single mathematical function. However, this assumption limits our understanding of what may cause variation in uptake kinetics that could be used for breeding because the formalism does not include transporter-level properties, scaling, or accounting for cellular external and internal concentrations. Alternatives to the enzyme-substrate model for nutrient uptake include the Porter-Diffusion model, which has more flexibility at the cellular level, and the Flow-Force interpretation, which is more realistic at the macroscopic scale (Le Deunff et al., 2019).

The Porter-Diffusion model framework was developed for modelling nutrient uptake by aquatic phytoplankton (reviewed in Shaw et al., 2015). The original work coupled the Michaelis-Menten model with the concept of a diffusion boundary surrounding cells to accurately predict nutrient concentration at the cell surface containing the transporters (Pasciak and Gavis, 1974). Over time, this Porter-Diffusion modelling framework has led to various refinements that allow additional flexibility with incorporation of cell and transporter characteristics such as cell size, number of transporters, transporter area, and substrate handling time by transporters as well as physical properties such as diffusion and convection (Dugdale, 1967; Aksnes and Egge, 1991; Armstrong, 2008; Aksnes and Cao, 2011). Combined with Michaelis-Menten formalism, a Porter-Diffusion model can be scaled between a cell and colonies of cells for phytoplankton modelling (Fiksen et al., 2013).

Because the Porter-Diffusion framework is more mechanistic at the molecular and cellular levels, it may be an ideal starting place for incorporation into plant models and for guiding empirical research. As an example, the following equation from Aksnes and Egge (1991) determines finite uptake rate ($V$) as a function of the number of transporters ($n$), the ‘ion-catch’ area ($A$), ion handling time ($h$), external nutrient concentration ($S$), and the mass transfer coefficient or velocity ($v$).

$$V = \frac{nAvS}{1 + hAvS}$$

At the macroscopic scale, the Flow-Force model first proposed by Thellier (1970) operates at the whole root-system level for ion flux and root conductance (see Equation 3). Flow-Force differs from Michaelis-Menten kinetics in that it is based on thermodynamic absorption of
ions rather than enzyme activity. Therefore, Flow-Force modelling disregards transporter assumptions and does not allow deduction of plant transporter affinities along ion concentration ranges (Le Deunff and Malagoli, 2014). The Flow-Force framework is promising because it describes the macroscopic conductance of the whole root for the substrate and introduces more flexibility to use for physiological plasticity, root aging, or differences among root classes and positions along roots (Le Deunff et al., 2019). Flow-Force models may be suited for scaling from root systems to cropping systems by including total uptake at a given concentration ($J_j$), root conductance ($L_j$; based on thermodynamic considerations), the external concentration ($c^e_j$), and the external concentration at which no uptake occurs ($c^o_j$).

Equation 3. Flow-force model.

$$J_j = L_j \ln \left( \frac{c^e_j}{c^o_j} \right)$$

Models inherently have limitations and therefore should be interpreted with consideration. Being able to summarize and link nutrient uptake to physiological phenes and transporter regulation is challenging because these occur at different spatial and temporal scales. Combination of Porter-Diffusion modelling and Flow-Force modelling is one such proposition that could enable new models that retain flexibility at different biological scales (Le Deunff et al., 2019). This spatial and temporal aspect has been explored with nutrient and water uptake across differential root tissue types and developmental zones (Foster & Miklavcic., 2013; Sakurai et al., 2015); however, these models do not include active transport which is the focus of this review. Other key improvements would be to include multiple ions and transporters, incorporate electrochemical gradients, and model how multiple ions compete or facilitate uptake. At present, an operable and simple nutrient uptake model that incorporates spatial and temporal scales is still lacking.
Experimental approaches for studying root ion uptake kinetics

There are many experimental approaches that have been employed for studying ion uptake kinetics in roots, but most are hydroponics-based so lack the physical and chemical properties of soil. Ion uptake studies generally quantify the net influx, which is the difference of gross influx and efflux, of a particular ion into the root from an external solution (BassiriRad, 2000). Measurements of uptake kinetics have spanned from root segments to the whole-root system. A full kinetics assessment would provide \( I_{\text{max}} \), \( K_m \), and \( C_{\text{min}} \) values; however, measuring influx rate for a single external concentration and time point can provide a snapshot of the plant performance at a particular concentration. Singular influx rate experiments are more easily scaled to large populations and allow the comparison of more experimental conditions. The uptake kinetic capabilities of a plant are highly dependent on the growth and measurement environments. Differences in laboratory protocols for growing plants and uptake assay methods may explain the variability observed for \( I_{\text{max}} \), \( K_m \), and \( C_{\text{min}} \) values in the literature. In this section, ion uptake kinetic protocols and variables in experimental setups are discussed.

Depletion vs accumulation

Experimental setups for ion uptake kinetics fall into two main categories, specifically measurement of ion depletion from a nutrient solution and measurement of ion accumulation in the plant, with the latter method employing either stable isotopes, such as \( ^{15}\text{N} \), or radiotracers, such as \( ^{32}\text{P} \). In general, the depletion-based method is convenient because no radioactive material is used and only standard analytical chemistry instruments are needed. In addition, depletion studies are non-destructive because only the solution is sampled as opposed to sampling all plant tissue in accumulation studies, which enables temporal ion uptake kinetics studies for characterizing the same plants across multiple plant ages and plasticity responses to nutrient availability. However, depletion studies using non-labelled nutrients cannot distinguish influx from efflux and therefore only allow quantification of net influx. Techniques for quantifying ion depletion of a solution include ion chromatography, colorimetric assays, and ion selective electrodes. Stable isotopes and radiotracers, however, are useful when available, as short accumulation measures can be made at both very low and high nutrient concentrations (Lee and Drew, 1986). Labelled nutrients are the only method for determining absolute influx and can be used for tracking accumulation through the plant.
In addition, they are convenient for determining ion uptake rates in the field and foraging
capacity at depth (Kristensen and Thorup-Kristensen, 2004). Promising technologies such as
microdialysis and ion selective electrodes, which measures ion concentrations from the soil
solution based on passive diffusion, are opening further opportunities for ion kinetic
depletion studies in soil without the use of tracers; however, at present, these approaches are
low-throughput (Oyewole et al., 2014; Shaw et al., 2014; Shabala et al., 2013; Hawkins et al.,
2008).

Depletion over concentration ranges vs depletion over time

Plants have different mechanisms for uptake across nutrient concentration ranges. Passive
diffusion occurs across the cell membrane when nutrients are found in high external
concentrations. When nutrients are scarce, ATP-mediated active transport is likely to occur,
and therefore measured ion influx rates will reflect the plant capabilities in that environment.
For example, nitrate has three main transport systems: constitutive and inducible high-affinity
transport systems (cHATS and iHATS, respectively) characterized by low $K_m$ and $I_{max}$
values ($K_m$ of 6–20 µM and 20–100 µM respectively); and a constitutive LATS system, the
latter of which dominates transport activity and uptake at concentrations greater than 250 µM
and fails to saturate at concentrations as high as 50 mM (Crawford and Glass, 1998; Ho et al.,
2009; Hu et al., 2009). Therefore, the sampling strategy for plant ion influx experiments is
important to consider as the ion concentrations tested provide information on the respective
transport mechanisms and their working ranges.

There are two main depletion-based methods for estimating kinetic parameters for nutrient
uptake. The first method will be referred to as ‘depletion over concentration ranges’ and
relies on characterizing nutrient uptake of different individual plants placed in different
concentrations of nutrient that represent the range of relevant concentrations (originally in
Epstein and Hagen, 1952). The second method will be referred to as ‘depletion over time’
and relies on measuring nutrient uptake by a single plant starting at a high nutrient
concentration and measuring depletion from the same solution over time at set intervals
(originally in Claassen and Barber, 1974).

The depletion over concentration ranges experimental design is advantageous in that it allows
a broad external concentration range to be tested in a short space of time (5–60 min).
However, this entails the use of multiple plants and vessels and, therefore, introduces
interplant variability. By contrast, for depletion over time experiments, the concentration range tested is typically narrower and starts at a lower concentration (<100 µM) to allow for ion depletion within hours to avoid diurnal effects. In order to characterize a plant across a wide concentration range while minimizing the number of experimental plants, these methods could be combined. Depletion over time can be used to calculate depletion rates at lower concentrations that can be depleted to $C_{min}$ within a few hours and depletion over concentration ranges can provide rates at concentrations too high for full depletion but that allow for accurate measurement of $I_{max}$.

**Intact vs excised roots**

Preparation of root samples for ion uptake kinetics varies among studies, with either intact or excised roots being used. Using intact root systems minimizes disruption to the plant so presumably provides more representative ion uptake kinetic measurements. Once a root is excised, the carbon and nutrient balance maintained in roots is irreversibly altered with carbohydrate exhaustion of root tissues, falling respiration rates, and nutrient movement through the plant disrupted (Farrar, 1985; BassiriRad et al., 1999; Jones et al., 2005). Using excised roots is often necessary; however, for field-grown plants, excavation of intact plants from soil is challenging, and thus excision studies should be conducted as quickly as possible to avoid disruption to uptake processes (BassiriRad et al., 1999).

Excised root studies have been valuable for in depth analysis of ion influx along root zones, classes, and ages. Rao et al., (1997) demonstrated that there is variation in nitrate uptake rates along the maize primary root. In another study, Sorgonà et al., (2011) showed that there is spatial and temporal heterogeneity in nitrate uptake along maize root axes, with a significantly lower $I_{max}$ in apical compared to basal regions but no detectable difference when fully induced. More recently, individual root segment-specific chambers were used to demonstrate variation in $I_{max}$ and $K_m$ among root classes in maize without excising the roots by using short PVC chambers sealed around root segments (York, Silberbush and Lynch, 2016). These segment-level measures are valuable, because the uptake kinetics measured when using the intact entire root system are aggregated across root positions and classes, whereas research has shown that uptake kinetics vary along a root of a particular class (i.e., seminal) and also among classes (i.e., seminal, nodal, or lateral). However, since lateral roots dominate the root system by length, root system-level uptake kinetics may largely reflect
lateral-root uptake kinetics. These new advances greatly improve the validity of the kinetic
parameters reported, and therefore root excision should be avoided where possible due to
possible wound responses (Gronewald and Hanson, 1980).

Deprivation & induction

The expression and activity of ion transport proteins in plants are highly dependent on the
environment and plant nutrient status. Depriving a plant of a focal nutrient followed by
exposure to high nutrient concentration for a period of time has been widely reported to
substantially increase plant influx rates. For example, nitrate influx rates were shown to be
significantly greater in N-starved plants compared to replete plants (Siddiqi et al., 1990;
Raman et al., 1995). In addition, influx rates can be further increased by a nitrate induction
period after deprivation (Lee and Drew, 1986; Hole et al., 1990). Therefore, deprivation and
induction steps are commonly used in kinetic studies for determining the genetic potential of
a genotype with measurements conducted when the plant is operating at near maximum
uptake capacities.

A key question is whether deprivation and induction steps provide representative ion uptake
rates for kinetics experiments compared to soil. These steps directly affect plant nutrient
balance and demand, which in turn affects ion transporter expression and therefore should be
critically considered when using uptake rates as selection criteria for plant breeding. The
effects of induction and deprivation steps on ion uptake rates are concentration, time, and
species dependent, and whether plants experience such conditions in the field has not been
explored (Maeck and Tischner, 1986; Siddiqi et al., 1989). Deprivation and induction steps
can eliminate plant plasticity responses and the uptake rates measured may not be
representative of those in the field. These experimental manipulations are used because they
maximize $I_{\text{max}}$ and may represent the maximum potential uptake rate for a plant species or
variety. In the case of deprivation only studies where no nutrient is supplied, it is possible that
internal cellular concentrations decline substantially and the concentration gradient drives
much of the observed increased uptake. Future research should address the relevance of these
methodologies.
The most recent meta-analysis for nutrient kinetics dates to Raman et al., (1995) for nitrate. They found that literature values for $K_m$ varied more than the $I_{max}$ values, speculating that this may reflect both the sensitivity of $K_m$ estimates to measurement artefacts and an inherent variability due to the complexity of the uptake process. In addition, they implied that the $K_m$ values were relatively low so nitrate can be reduced to very low concentrations by plants. Here, a new meta-analysis is presented for uptake kinetics across multiple crop species and for multiple nutrient types: nitrate, phosphate, and potassium. The meta-analysis data and statistical analysis code are available (Griffiths and York, 2020, http://doi.org/10.5281/zenodo.3561113).

To summarize the current state of crop ion uptake kinetic research, maize is the most widely characterized crop for ion uptake kinetics, with approximately half of all studies focusing on maize; however, there are also a substantial number of studies for barley and rice (Figure 3A). By comparison, wheat (Triticum aestivum L.), sorghum (Sorghum bicolor L.), soybean (Glycine Max L.), and rapeseed (Brassica napus L.) combined account for only 8 of the 50 collated studies. The nutrients investigated in these studies were distributed among nitrate, phosphate, and potassium, with the greatest number of studies focussed on nitrate (20 of 50 studies). Two-thirds of ion uptake studies were with seedlings 0–21 days old, with the oldest specified plant age being 60 days in rice (Teo et al., 1992, Figure 3B). Most studies used intact root systems, with one-quarter of studies using excised root samples (Figure 3C). For the plant pre-treatment process, there was no clear preferred method with deprivation only and no pre-treatment most widely used in 21 and 20 studies respectively (Figure 3D).

With the collated meta-analysis data for ion uptake kinetics, global trends can be observed. Results are given for nitrate because other nutrient data were relatively sparse. At the species level, Figure 4E shows the variation for nitrate $I_{max}$ with maize and rice having greater uptake rates compared with wheat and barley. For $K_m$, there were no substantial differences among species and high variability (Figure 4F). The influences of experimental conditions upon kinetics parameters were also determined. Interestingly, $I_{max}$ weakly correlated with influx rate at 50 µM, demonstrating the importance for maximum uptake capability to determine uptake even at low concentrations. (Figure 4A). However, $I_{max}$ was not correlated to $K_m$, which supports Michaelis-Menten formalism compared to some Porter-Diffusion models that indicate a possible dependency (Figure 4B). In addition, the ranking of studies by
highest uptake rate strongly predicted $I_{\text{max}}$ rank, which supports the use of single uptake rates at high concentrations in order to approximate relative $I_{\text{max}}$ for high-throughput phenotyping of mapping populations, since full uptake curves would be more difficult to achieve at that scale. Analyzing influx rates across nutrient ranges, however, are important for determining the exact $I_{\text{max}}$ saturation point, and $K_m$ could not be predicted from other easier-to-measure values such as uptake at low concentrations. Another benefit of determining ion influx rates across nutrient ranges is that Michaelis-Menten curves have wider utility for comparing to other studies that use similar concentration ranges. Considering the inconvenience of converting literature kinetic parameters to a common standard (Supplementary 1), uptake rate on a root mass basis (specific uptake rate, $\mu$m g dw$^{-1}$ h$^{-1}$) should be used for future reporting of ion uptake rates while also reporting on root length and volume.

Ion uptake kinetics research so far illustrates that there is substantial genetic and phenotypic variation in plants that could be used to improve nutrient uptake efficiency. However, there are very few studies comparing genotypic variation within the same species (Supplementary 1). The greatest number of genotypes compared is 16, occurring twice in studies for maize and rice (Pace and McClure, 1986; Hasegawa and Ichii, 1994). These studies illustrate that there is variation for ion uptake phenes among cultivars within a species. In addition, to our knowledge there has been only a single study reporting multiple ion uptake parameters across multiple genotypes (Baligar and Barber, 1979). In that study, potassium, phosphate, calcium, and magnesium $I_{\text{max}}$ and $K_m$ were determined from the excised roots of 12 maize genotypes. Single-ion studies are more abundant, however, to date there are still no mapping population-sized studies for single-ion or multiple-ion uptake kinetics. A key issue is that current experimental approaches are still both challenging and time-consuming for high-throughput phenotyping of ion uptake.
Future prospects

Uptake kinetics for advancing plant research

This review reported on the current state of research on ion uptake kinetics by roots through outlining the background concepts, describing fundamental methods, and providing a comprehensive meta-analysis of parameters reported in the literature. Ion uptake kinetics have not been sufficiently studied in plant research and significant knowledge gaps remain about the genetic, physical, and molecular mechanisms involved in nutrient uptake by roots (see Outstanding Questions).

In general, the study of nutrient uptake has focused on a single nutrient at a time, often from solution containing only that nutrient while ignoring the interplay among substrates. Whereas this approach is useful for a fundamental understanding of the uptake kinetics for a specific nutrient, it complicates the translation of experimental results to real-world conditions where soil solutions generally contain a complex complement of nutrients in varying concentrations. Since electrochemical gradients influence plant nutrient uptake, the uptake of a nutrient is influenced by other nutrients with the same as well as opposite charges (Cox and Reisenauer, 1973). Although most relevant under field conditions, this area of nutrient uptake kinetic interactions is critically understudied.

In addition to characterising ion transporters, ion uptake kinetic studies have been valuable in determining the function and contribution of physiological phenes and environmental factors that relate to ion uptake. Factors that influence ion uptake kinetics include plant species, cultivars, plant developmental age, root class, and position along a root (Baligar and Barber, 1979; Rao et al., 1997; BassiriRad et al., 1999; Jungk & Barber 1975; Garnett et al., 2013; Sorgonà et al., 2011; York, Silberbush and Lynch, 2016), in addition to environmental conditions including external ion concentration, nutrient distribution, temperature, and pH (Carter and Lathwell, 1967; Claassen and Barber, 1974; Rao and Rains, 1976; Kochian and Lucas, 1982). However, there are many unexplored phenes that could significantly affect ion uptake rates and uptake efficiencies, such as electrochemical gradient establishment, root system architecture, root anatomy, root respiration efficiency, photosynthesis, and transpiration (Figure 5). An additional unexplored area of complexity is potential variation among genotypes for transporter types, transporter densities, and assimilation processes that may drive uptake by developing a chemical gradient. Characterization of ion transporter
properties may explain the variation observed among species for $I_{\text{max}}$ and illustrate why $K_m$ varies less among species (Figure 4EF). In general, $I_{\text{max}}$ is supposed to scale positively with increased transporter density whereas $K_m$ may not be influenced. Conceptualizing and modelling the optimal uptake configuration necessitates more information about the relative benefits and costs of transporter abundance, types, maintaining electrochemical gradients, and assimilation, as well as their interactions.

Whereas the benefits of more and faster transporters seem intuitive, knowledge gaps surrounding the costs of synthesis, maintenance, and activity of these transporters need to be filled to determine an optimal uptake configuration. Synthesis costs depend on the protein size and the energy required for transcription, translation, and mobilization to the membrane. Synthesis and maintenance costs include the opportunity costs of maintaining a maximum uptake rate configuration when soil nutrient concentrations are low and unable to saturate the uptake machinery rather than investing in other processes such as root growth. Activity costs during uptake are largely due to the synthesis of ATP for maintaining electrochemical gradients with the H+ ATPase pumps (Sze et al., 1999). In maize, 20% of total plant respiration was estimated to be devoted to nitrate uptake (Veen, 1980). In barley, 5% of root respiration was determined to be devoted to nitrate absorption and 15% to assimilation (Bloom et al., 1992). Maintaining a uniformly high $I_{\text{max}}$ capacity throughout an entire root system regardless of soil nutrient levels may be extremely costly and inefficient.

Nutrient concentrations in soils are heterogeneously located in time and space (Dunbabin et al., 2004) due to fertilizer and residue placement. Therefore, plants may routinely construct roots with less uptake capacity that are capable of increasing uptake capacity when a patch of high nutrient concentration is encountered, which could be cost-saving strategy. The plasticity in maximum uptake rate is often termed physiological plasticity in the literature as opposed to morphological plasticity, or proliferation of roots in patches (Hodge, 2004). Simulation studies have shown that ion uptake plasticity may be especially important for exploiting patches of nitrate and has a positive but smaller effect for less mobile phosphate (Jackson and Caldwell, 1996). In studies that placed roots from the same root system in either high phosphate or distilled water, phosphate uptake was found to be almost double for the roots previously exposed to the high-phosphate treatment (Jackson et al., 1990). This may explain why root tips are often found to have greater $I_{\text{max}}$, because as roots deplete soil nutrients and grow into new regions, less uptake capacity is required in the mature root zones.
remaining. In fact, the deprivation and induction methods used for kinetics assays actually use uptake rate plasticity in order to maximize uptake rates. Plasticity is likely not governed by transporter type or constitutive abundance, but rather by the capability of the plant to differentially transcribe and translate transporter genes or other molecular machinery then locate them to the epidermal membrane. Ion uptake plasticity may be under independent genetic control with the transcript levels of transporter genes responding to nutrient supply dependent on crop genotype (El-Kereamy et al., 2011; Garnett et al., 2015). Further research is needed on how to measure ion uptake plasticity, its genetic diversity, and its functional ramifications in various soils.

Ion kinetics research is key for parameterization of holistic root models including OpenSimRoot (Postma et al., 2017), ROOTMAP (Diggle, 1988; Dunbabin et al., 2003), and R-SWMS (Javaux et al., 2008). Incorporation of ion kinetics data for more crops, root types, and developmental stages would greatly improve the validity and representativeness of the models. Using such models that simulate plant growth and environmental conditions dynamically can be used to compare the relative importance of kinetic parameters in a variety of simulated environments. In addition, a theoretical ideotype could be devised by simulating all combinations of ion kinetic parameters and root architectural phenes in a variety of nutrient limiting and replete environments. The value of this approach was demonstrated in a previous study (York, Silberbush and Lynch, 2016), where nitrate uptake parameters from maize were used to parameterize the holistic model SimRoot (now OpenSimRoot). By factorially combining kinetic parameters and root system architectural phenes, additive effects were observed between beneficial nitrate kinetics states stacked with favourable root architectural phene states to improve plant performance. Through the use of simulations, Dunbabin et al., (2004) also found that the benefits of increased uptake capacity depended on theoretical highly branched (herringbone) or sparsely branched (dichotomous) root systems. Parametrization with a greater number of species and populations would be valuable in determining strategies and ideotypes in a variety of environments and management scenarios. In addition, modelling of root plasticity responses is currently limited with little research on the responses of $I_{max}$ and $K_m$ to non-uniform resource supply.

Incorporation of ion uptake kinetics into crop pre-breeding programs

Ion uptake kinetics studies have been invaluable in uncovering distinct nutrient uptake systems in plants and have the potential to greatly advance breeding efforts. However, pre-
breeding activities will be required to better understand the underlying physiology and
genetics, and to transfer novel traits into breeding lines. As illustrated in the meta-analysis,
there is substantial variation among species and cultivars for uptake parameters, making it a
viable target for breeding efforts (Figure 4). To our knowledge, the most complete
documentation of intraspecific variation is in the work by Pace and McClure (1986) that
found 2.3-fold variation in $I_{\text{max}}$ and 12.8-fold variation for $K_m$ among maize inbred lines.
The potential for variation is also supported by work that shows uptake variation along roots
and among root classes (such as York et al., 2015). Whereas different transporter gene
families for the same nutrient have been discovered, potential allelic variation for uptake
kinetics has rarely been considered. One notable example that demonstrates direct utility of
uptake kinetics is that allelic variation in a nitrate transporter and differential uptake
capability was found when comparing two sub-species of rice (Hu et al., 2015). Together,
this research demonstrates that there is genetic and phenotypic potential available that may be
harnessed for plant breeding.

Ion uptake kinetics by roots is very difficult to measure, especially in field conditions.
Potential aboveground proxies, such as leaf nutrient content or nutrient use efficiency, could
accelerate plant breeding if tightly coupled to uptake kinetics. A common agronomic
definition for nutrient use efficiency described by Moll et al. (1982) is the ratio of yield (or
biomass) to fertilizer input (or nutrient availability in soil). This agronomic use efficiency can
be subcategorized into nutrient uptake efficiency, specifically the ratio of nutrient in biomass
to nutrient availability, and nutrient utilization efficiency, specifically the ratio of yield to
nutrient in biomass. Utilization efficiency is determined by many processes that are not
directly related to root function, such as photosynthetic capability and nutrient
remobilization. Uptake efficiency is generally taken to be largely determined by root
processes. Root system architecture determines the overall exploration and potential for local
exploitation of soil resources. Specific uptake rates or kinetics parameters would also
influence uptake efficiency and use efficiency, but these relationships become more indirect
because of the interaction of many phenes (York et al., 2013). Similarly, multiple-ion uptake
is partly addressed by the study of the plant ionome, meaning the contents and concentrations
of elements (Salt, 2004). Ionomics has uncovered numerous genetic components for both
macro- and micronutrient accumulation (Baxter et al., 2008; Baxter et al., 2010; Pinson et al.,
2015; Segura et al., 2012; Yang et al., 2018). Components linking uptake with accumulated
tissue content have been shown in maize for nitrate (Dechorgnat et al., 2018); however, due
to the influence of many interacting processes, shoot nutrient content may not be a reliable proxy for ion uptake kinetics by roots and needs further research.

Nutrient uptake efficiency is suggested to explain most of the variation in nutrient use efficiency and genetic progress in yield, so root traits are viable breeding targets (Li et al., 2015; Hirel et al., 2007; Nehe et al., 2018). Identified loci that associate with uptake, assimilation, biomass, and grain yield simultaneously will be especially important for breeding higher-yielding and nutrient-efficient crops (Yang et al., 2018). Even though breeding has historically been based on yield- or biomass-based crop selection, comparing historic varieties with modern varieties of wheat indicated that selection for yield also indirectly selected for higher total nitrogen uptake over total root length with smaller root systems (Aziz et al., 2017), which could be due to both more efficient soil exploration and specific nitrogen uptake capacity. In most studies, nutrient use efficiency and nutrient content will be completely correlated because the nutrient supply is the same for all genotypes tested. For example, Li et al. (2015) found only moderate correlations among root system architectural traits and nitrogen content in maize under high or low nitrogen supply, and even those relationships may be due to plant vigour and allometry. Including nutrient uptake kinetics in such analyses may improve the explanatory power of the statistical models. Based upon a previous observation that a less-vigorous wheat genotype acquired as much nitrogen as more vigorous genotypes in the field, Pang et al. (2015) determined that the less-vigorous genotype had greater $I_{\text{max}}$ and lower $K_m$ for nitrate. These results illustrate that an understanding of and breeding for ion uptake kinetics is possible.

Ideotype, or trait-based, breeding supposes that understanding and selecting phenes known to influence plant physiology can accelerate gains relative to selection on yield alone (Donald, 1968). Ideotypes have been proposed for root architecture and these could be further improved by utilizing uptake parameters (Lynch, 2013; Zhan and Lynch, 2015; Morris et al., 2017). A crop ideotype in respect to uptake kinetics would be a plant that has a high $I_{\text{max}}$, low $K_m$, and low $C_{\text{min}}$, depending on the associated costs, possible trade-offs, and the respective nutrient soil mobility and availability. High $I_{\text{max}}$ would be particularly important for high-input agricultural systems or mobile nutrients when the rate of mass flow is sufficient to supply the respective nutrient in great enough concentrations to sustain the $I_{\text{max}}$ uptake rate. For low-input agricultural systems, soils susceptible to nutrient leaching, or immobile nutrients, breeding for a low $K_m$ and $C_{\text{min}}$ should take priority as it would allow
greater uptake when external ion concentrations are below that needed to attain $I_{\text{max}}$, and it also increases the pool of nutrients available to the plant for net uptake. Model sensitivity analysis by Silberbush and Barber (1983) indicated that increased $I_{\text{max}}$ would not benefit plant growth in low-phosphate conditions due to its relative immobility, and this was partially confirmed in work that overexpressed phosphate transporters in barley with no effect on uptake (Rae et al., 2004). Ion uptake kinetic parameters change greatly across a plant lifecycle, and this will also have to be carefully considered in crop selection so that uptake characteristics are beneficial in that particular environment (Garnet et al., 2013; Jungk & Barber 1975). Additionally, ion uptake parameters can be used to select species for multiple-species systems such as cover crops where ecological niches exhibit complementarity and reduce intraspecific competition within monocultures (Wendling et al., 2017).

The greatest technological challenges for ion uptake kinetics as a breeding target are developing high-throughput phenotyping for mapping populations and verifying uptake capacity in soil. Using an automated hydroponic platform with chamber-specific control of nutrient concentrations and a sensor system for logging ion depletion across time, ion uptake kinetics could be scaled up to population-sized studies. A genome-wide association mapping study would for the first time provide a significant genetic insight into ion uptake kinetics. Identification of genes and pathways involved in uptake kinetics could allow selection of crops with improved uptake efficiency. Not only would known transporter genes be confirmed, but new allelic variations and completely novel components with important contributions to the molecular machinery of ion uptake could be discovered while simultaneously addressing knowledge gaps. In the long term, knowledge gained about uptake capacity will have to be integrated with knowledge of soil microbes, soil properties, exudations, and soil water content in order to understand the holistic rhizosphere (York Carminati, et al., 2016). Confirmation of uptake capacity in soil would be required using techniques like excavation of living roots or the use of microdialysis. Considering ion uptake kinetics parameters or specific uptake rates as breeding targets provides an exciting opportunity to combine basic plant biological research with applied agronomy and breeding in order to design sustainable agroecosystems to address food insecurity.
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Data Availability

Data and statistical programming code are publicly available at:

http://doi.org/10.5281/zenodo.3561113
Figure legends

Figure 1. The scaling from the soil environment to transporter-level dynamics to harness the potential of root uptake kinetics. Soil nutrient mobility and bioavailability determine interception of the root surfaces with nutrient ions (A). Ions may travel across the root through the symplastic pathway through cells or the apoplastic pathway around cells to reach the xylem for transport throughout the plant (B) (Heymans et al., 2019). Nutrient ions may enter the root from the soil solution through a variety of mechanisms as depicted (C). Whereas general mechanisms are known, the state of knowledge is too limited to address questions about how transporter-level properties may be influenced by genetic variation and how these properties can be utilized to breed or engineer more nutrient acquisition-efficient crops (D).

Figure 2. Example of an ion uptake kinetics graph showing the relationship of external ion concentration to ion influx rate. The curve and highlighted derived parameters are calculated using nonlinear least squares to fit the Michaelis-Menten model.

Figure 3. Summary of ion uptake kinetic studies for nitrate, phosphate, and potassium in major crops. (A) Study count by plant species and nutrient of interest. (B) Plant age distribution (days). (C) Root sample type used for ion uptake rate determination. (D) Plant pre-treatment process used before ion uptake rate determination.

Figure 4. Meta-analysis of kinetic parameters for nitrate uptake in different crop species. Data are for barley (●), wheat (▲), rice (■), and maize (+). The relationship of $I_{max}$ (A) with $K_m$, (B) uptake rate at 50 µM, (C) highest uptake rate reported, and (D) highest concentration used in study. (E) Nitrate $I_{max}$ and (F) $K_m$ variability by species.

Figure 5. The aggregative hierarchy for uptake kinetics showing how underlying phenes create the emergent property measured as uptake kinetics. Nutrient uptake is determined by the integration of underlying properties, and ultimately influences nutrient use efficiency and yield.
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ADVANCES

- For specific nutrient transporters, a number of genes and protein structures have been characterized, and the molecular mechanisms of a nitrate transporter were recently determined.
- Individual root segment-specific chambers facilitate more precise measurements of kinetic parameters along root axes and among root classes.
- The Porter-Diffusion model offers good approximation of how molecular processes scale to uptake kinetics, whereas the Flow-Force model allows flexible scaling for uptake across tissues.
- Root simulation models predict interactions between beneficial ion uptake states stacked with favourable root architectural phenes to improve plant performance.
- Differences in uptake abilities among maize lines and a transporter allele that confers greater nitrate uptake in rice illustrate the genetic variation that can be harnessed to increase nutrient acquisition.
OUTSTANDING QUESTIONS

- What is the complete mechanistic description of how an ion is intercepted by the transporter, shuttled across the membrane, and released into the cytoplasm?
- What specific transporter properties influence the transport stages above and which are the most useful to target for engineering?
- How is active ion transport influenced by the electrochemical gradient?
- What are the construction and energetic costs of increasing nutrient uptake?
- Are there homologous genes among species or alleles within species that have different uptake parameters, and what would this reveal about mechanisms?
- How do the types and numbers of transporters, along with processes such as assimilation and translocation, scale to the root-segment and whole root-system levels?
- Could genetic variation for ion uptake and plasticity be harnessed for crop improvement?
Figure 1. The scaling from the soil environment to transporter-level dynamics to harness the potential of root uptake kinetics. Soil nutrient mobility and bioavailability determine interception of the root surfaces with nutrient ions (A). Ions may travel across the root through the symplastic pathway through cells or the apoplastic pathway around cells to reach the xylem for transport throughout the plant (B) (Heymans et al., 2019). Nutrient ions may enter the root from the soil solution through a variety mechanisms from (C). While general mechanisms are known, the state of knowledge is too limited to address questions about how transporter-level properties may be influenced by genetic variation and how these properties can be utilized to breed or engineer more nutrient acquisition efficient crops (D).
Figure 2. Example of an ion uptake kinetics graph showing the relationship of external ion concentration to ion influx rate. The curve and highlighted derived parameters are calculated using nonlinear least squares to fit the Michaelis-Menten model.
Figure 3. Summary of ion uptake kinetic studies for nitrate, phosphate and potassium in major crops. (A) Study count by plant species and nutrient of interest. (B) Plant age distribution (days). (C) Root sample type used for ion uptake rate determination. (D) Plant pre-treatment process used before ion uptake rate determination.
Figure 4. Meta-analysis of kinetic parameters for nitrate uptake for barley (●), wheat (▲), rice (■) and maize (+). (A) $I_{\text{max}}$ and $K_m$ no significant relationship ($R = 0.02$). (B) $I_{\text{max}}$ positive relationship with uptake rate at 50 µM ($R = 0.47$). (C) $I_{\text{max}}$ positive relationship with highest uptake rate reported ($R = 0.92$). (D) $I_{\text{max}}$ positive relationship with highest concentration used in study ($R = 0.21$). (E) Nitrate $I_{\text{max}}$ variability by species. (F) $K_m$ variability by species.
**Figure 5.** The aggregative hierarchy for uptake kinetics show how underlying phenes create the emergent property measured as uptake kinetics. Nutrient uptake will be determined by the integration of underlying properties, and will finally influence nutrient use efficiency and yield.
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