Plant Nitrogen Transport and Its Regulation in Changing Soil Environments

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SUMMARY. In this chapter, we shall review plant responses to changes in supply of the two inorganic N sources nitrate (NO_3^-) and ammonium (NH_4^+), with an emphasis on primary mechanisms of transport, and their regulation. The topics discussed here include: soil N resources and their ecological implications for plant life; parallel responses in plant growth and N transport activity as functions of N supply; transport kinetics;

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inducibility and downregulation of NO_3^- and NH_4^+ acquisition; plant sensing of NO_3^-; and low-affinity N transport. Throughout, we attempt to identify areas that are controversial, and those in need of further examination. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <http://www.HaworthPress.com> © 2005 by The Haworth Press, Inc. All rights reserved.]*

KEYWORDS. Nitrate, ammonium, nutrient deficiency, nutrient toxicity, ion transport, feedback regulation, nitrate induction, plant growth, soil nitrogen

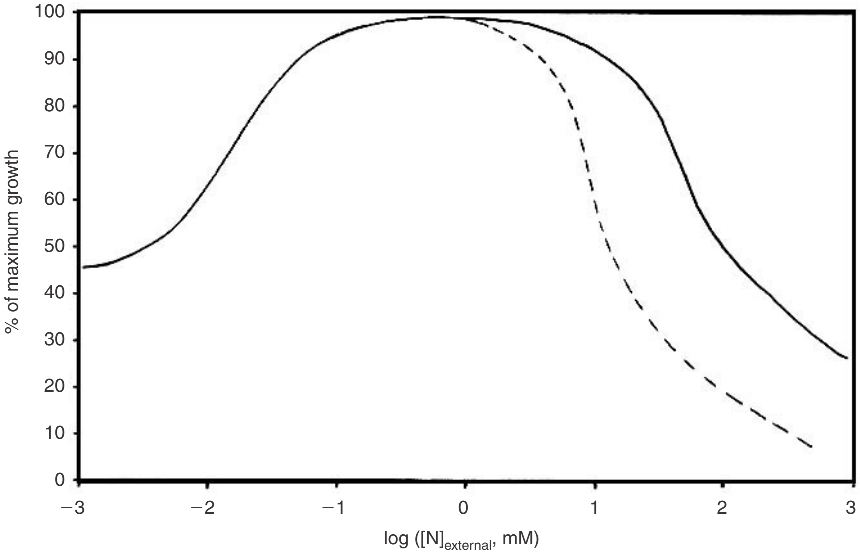
INTRODUCTION

Plants and Nitrogen in an Ecological Context

Nitrogen (N) limitation in the environment, and its consequences for plant growth and development, have long been of interest to agronomists and plant physiologists, while more recently, issues of excess N supply have been gaining attention, particularly among ecologists. Plant responses to high and low soil N are both important, as inorganic nitrogen concentrations in soil solution can range over many orders of magnitude, from barely detectable (~1 μM), to over 1 M (Magistad et al., 1945; Clement et al., 1978; Vitousek et al., 1982; Jackson and Caldwell, 1993; Wolt, 1994; Nesdoly and Van Rees, 1998). Accordingly, plants have evolved complex uptake systems that acquire N from even very dilute soil solutions, enabling them to grow at rates comparable to plants growing in soils with much higher N (Figure 1; Clement et al., 1978; Forde and Clarkson, 1999; von Wiren et al., 2000; Glass et al., 2002). At the other end of the scale, certain plants have evolved mechanisms by which they may survive exposure to potentially toxic soil-[N] concentrations, particularly those of NH_4^+ (Britto et al., 2001; Kronzucker et al., 2001; Britto and Kronzucker, 2002).

In well-aerated soils of agricultural and natural ecosystems (especially disturbed, early-successional ones), most of the nitrogen is typically present as nitrate (NO_3^-) (Crawford and Glass, 1998). In some soils, however, for example those of late-successional temperate and boreal forest environments, ammonium (NH_4^+) becomes the major or sole inorganic N source (Vitousek et al., 1982; Blew and Parkinson, 1993; Pearson and Stewart, 1993; van Cleve et al., 1993; Bijlsma et al.,

FIGURE 1. Typified response of plant growth as a function of externally provided nitrate (solid line) and ammonium (dashed line). Adapted from Clement et al., 1978.



2000). NH_4^+ is also commonly found at low soil pH and in soils of colder climates, heathlands, and the irrigated rice fields of the world (Kronzucker et al., 1997, 2000). Usually, the two N sources coexist in soils at various ratios, and therefore it is not surprising that most plants appear to be able to acquire both NO_3^- and NH_4^+.

At the low end of the N-provision scale, growth tends to be similar for most plants on either N source (Britto and Kronzucker, 2002). However, there are some interesting exceptions to this tendency among plants that have evolved in NH_4^+-rich habitats. Trees of late-successional forests demonstrate superior growth and more efficient N capture with NH_4^+ relative to NO_3^- (McFee and Stone, 1968; Van den Driessche, 1971; Marschner et al., 1991; Lavoie et al., 1992). In the case of white spruce (*Picea glauca*), this N-source preference is reflected in a high flux and compartmentation capacity for NH_4^+, together with an apparent atrophy in the uptake of NO_3^- (Kronzucker et al., 1997). Because soil [NH_4^+] tends to increase relative to [NO_3^-] over the time-course of ecological succession (Lodhi, 1977), and because species with corresponding N preferences also succeed each other over this time frame, we have proposed that soil N dynamics could be a driving force in forest succession (Kronzucker et al., 1997, 2003a). Plants belonging to the heather family (Ericaceae) are another example of plants that grow on acidic, ammonium-rich soils, and are likewise considered to be "ammonium specialists," to the extent that NH_4^+ appears to be obligatory for the growth of at least one member of this family, the cranberry (*Vaccinium macrocarpum*; Greidanu et al., 1972). Finally, rice (*Oryza sativa*), is a particularly interesting example of a plant that can thrive in an ammonium-rich environment. However, while it has long been regarded as an exemplary ammonium specialist, recent evidence has shown that rice has very high N-acquisition and yield potentials when growing on NO_3^-, possibly superior to those obtained in NH_4^+-dominant paddy soils. This difference could have important consequences for issues of world hunger (Kronzucker et al., 2000).

Despite these instances of NH_4^+ tolerance and even preference by plants, however, toxicity symptoms emerge for most species at increased levels of NH_4^+ supply (Pearson and Stewart, 1993; Kronzucker et al., 1997, 2003a; Bijlsma et al., 2000; Britto and Kronzucker, 2002). NH_4^+ over-supply can result in a decrease in plant yield of 15 to 60% in crops such as tomato and bean (Woolhouse and Hardwick, 1966; Chaillou et al., 1986), and results in plant mortality and even species extirpation in some cases (Gigon and Rorison, 1972; Magalhaes and Wilcox, 1983, 1984; Pearson and Stewart, 1993; de Graaf et al., 1998). It is also ecologically significant that high NH_4^+ can inhibit seed germination and seedling establishment (Cooke et al., 1962; Hunter and Rosenau, 1966; Megie et al., 1967; Barker et al., 1970; Westwood and Foy, 1999), and that both NH_4^+ and NO_3^- oversupply can significantly reduce the extent of mycorrhizal associations (Boxman et al., 1991; Lambert and Weidensaul, 1991; van Breemen and van Dijk, 1988; van der Eerden, 1988; Boukcim et al., 2001; Hawkins and George, 2001). This reduction can be particularly problematic, for instance, on phosphate-limited soils (Bolan et al., 1991). In general, plant responses to excessive inorganic nitrogen depend strongly on nitrogen source, dose, and genetic predisposition (Givan, 1979; Magalhaes and Huber, 1991; Britto and Kronzucker, 2002).

With the increasing use of fertilizer globally (nearly a 10-fold increase between 1950 and 2000; Brown, 2001), the impact of excess reactive nitrogen in agricultural and natural environments has become a subject of considerable recent attention (Nihlgard, 1985; de Graaf et al., 1998; Galloway et al., 2002). Although it should be noted that most forest ecosystems, at least in North America, remain N-limited (Fenn et al., 1998), [NH_4^+] values in forest-floor soil solutions can range as high as 0.4 to 4 mM [NH_4^+] in boreal and temperate forest ecosystems (based on Robertson, 1982; Vitousek et al., 1982; and Bijlsma et al., 2000; Kronzucker et al., 2003a). In agricultural soils, [NH_4^+] can be substantially higher, often ranging from 2 to 20 mM (Wolt, 1994), and even atmospheric deposition of NH_3/NH_4^+ from agricultural sources is known to cause nitrogen eutrophication in natural ecosystems adjacent to farmland (Nihlgard, 1985).

As membrane fluxes constitute the primary step in N acquisition for plants, they strongly determine growth potential at low ambient concentrations of N. On the other end of the soil-[N] scale, they provide (or restrict) the entry pathway for excessive, potentially toxic, quantities of nitrogen. This chapter will focus specifically on the membrane transport of inorganic nitrogen into the plant cell, and its regulation within the context of these temporally and spatially dynamic distributions of soil N.

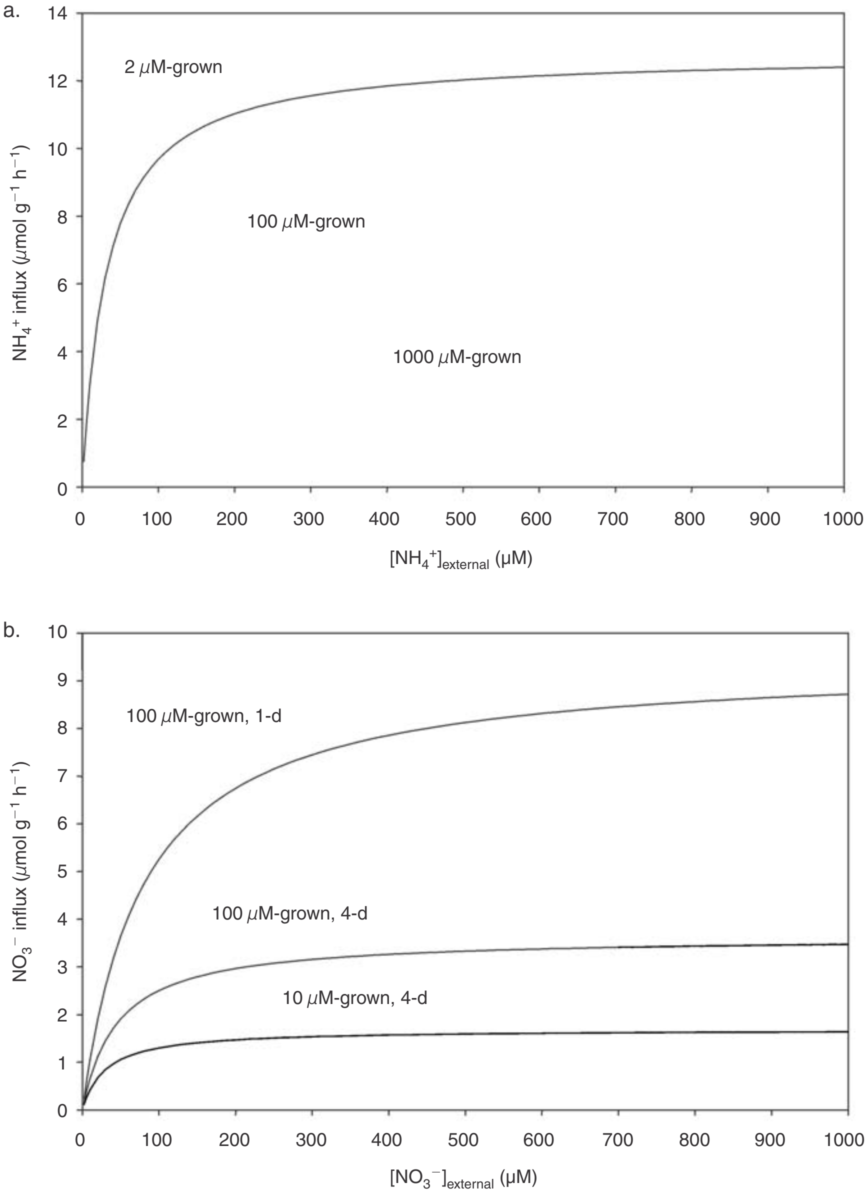
GROWTH RESPONSES ON VARIABLE N

Figure 1 shows a generalized growth-response curve for plants provided with varying supplies of NO_3^- and NH_4^+. As with other nutrients, a broad plateau of growth is usually observed with N nutrition (note logarithmic *x*-axis in Figure 1), over which growth rates and tissue N contents are largely unaffected by changes in N provision (Clement et al., 1978). This plateau is flanked to the left by a region of N-limited growth, and to the right by a region of growth suppression caused by N toxicity. The threshold concentration at which toxic effects of NH_4^+ become pronounced tends to be much lower than that for NO_3^- (Figure 1), although this value varies substantially with plant species and other nutritional factors, particularly K^+ supply (Britto and Kronzucker, 2002; Kronzucker et al., 2003b). Some evidence indicates that the soil [NH_4^+] at which toxicity occurs is lower for slower-growing species such as poplar and Douglas-fir trees, relative to faster-growing species such as grasses (Britto et al., 2001; Kronzucker et al., 2003a). Because of its high [NO_3^-] threshold, nitrate toxicity in plants is not an issue of great agronomic importance, although some reports have documented symptoms of this condition, such as chlorosis and growth inhibition (Reddy and Menary, 1990; Bennett et al., 1992). Nevertheless, high nitrate contents in plant tissues can become a toxicity problem in humans, livestock, fish, and other animals, when plant-derived nitrate is reduced to nitrite, creating the risk of methemoglobinemia (Bruningfann and Kaneene, 1993a,b). Therefore, preventing excessive NO_3^- accumulation in crop species is of substantial practical interest.

HIGH-AFFINITY N TRANSPORT: N-DEPENDENT CHANGES IN V_{MAX} AND K_M

Ion transport proteins situated in the membranes of plant cells typically operate with saturable, Michaelis-Menten-type kinetics when transporting inorganic-N substrates in the low-concentration, or "high-affinity transport system" (HATS), range (typically < 1 mM; Figure 2). These kinetics are well established for inorganic N, and variations in K_M and V_{max} values for N influx, as a function of N supply, illustrate substantial phenotypic plasticity and reflect the growth responses seen in Figure 1. For example, in one cultivar of rice (*cv*. M202), as the steady-state external NH_4^+ concentration is increased from 2, to 100, to 1000 μM, the V_{max} for high-affinity transport decreases from 12.8, to 8.2, to 3.4 μmol g^{-1} h^{-1}, respectively, while the K_M values increase from 32, to 90, to 188 μM (Figure 2a; Wang et al., 1993b). Thus, as the substrate becomes limiting, both the maximum flux and the substrate affinity of the system increase. Changes in K_M and V_{max} have also been observed for high-affinity influx of NO_3^-, in trempling aspen (*Populus tremuloides*) and lodgepole pine (*Pinus contorta*) (Min et al., 2000). However, concentration-dependent changes in K_M are not universally observed either for NH_4^+ uptake (Kronzucker et al., 1998a; Rawat et al., 1999; Min et al., 2000) or NO_3^- uptake (Siddiqi et al., 1990). In Figure 2b, for instance, a comparison of NO_3^- influx in barley plants grown on 100 μM or 10 mM external $[NO_3^-]$ for four days shows a suppression of the V_{max} for NO_3^- influx under the high-N condition, but no systematic change in the K_M is observed as this acclimation proceeds. Clearly, the relationships between substrate availability and substrate affinity for the enzyme are not always simple; these biological complexities are compounded by the difficulties in ascertaining reliable K_M values in heterogeneous cellular systems such as root tissues, and with difficulties arising from use of kinetic data transformation (i.e., linearization of rectangular hyperbolae; see Kronzucker et al., 1995d). Kinetic data are further complicated by the influx-protocol problems that arise from efflux occurring concurrently with influx (Britto and Kronzucker, 2001a, 2003), and by the likelihood of the concurrent operation of products of several members of the same gene family, kinetic distinctions among

FIGURE 2. Isothermic patterns of nitrogen influx in the high-affinity concentration range, as influenced by nitrogen source and supply. **a.** NH_4^+ influx (adapted from Wang et al., 1993b); **b.** NO_3^- influx (adapted from Siddiqi et al., 1990).



which are below the detection limits of the established methodology (Glass et al., 2001).

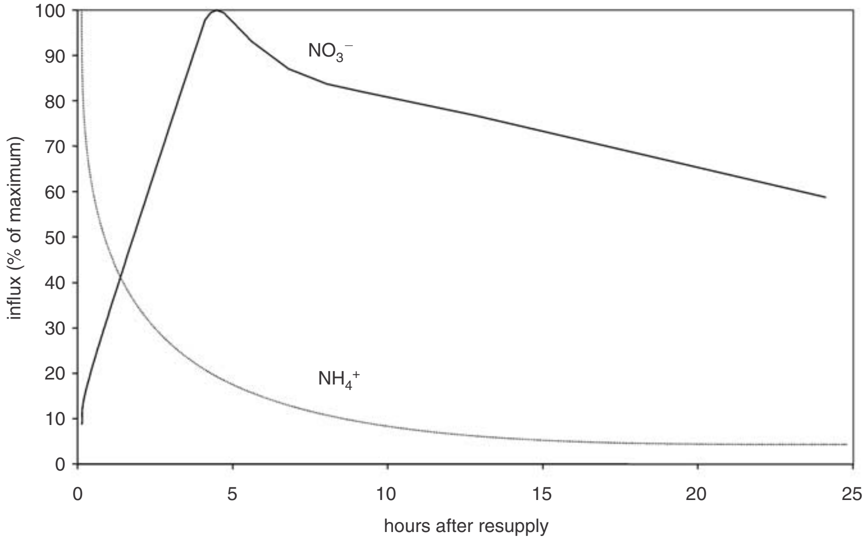
Notwithstanding uncertainties in K_M, changes in V_{max} are a consistent hallmark of plant root adaptation to changing environmental N, suggesting that, physiologically, V_{max} is the more important of the two kinetic parameters. Evidence from molecular studies, in which putative transporter gene expression and transport function are often fairly well correlated (Krapp et al., 1998; Zhou et al., 1999; Rawat et al., 1999; but see below for exceptions), supports the idea that the flexibility in V_{max} reflects the variable synthesis of transport proteins, rather than the modification (e.g., allosteric, or via phosphorylation) of a constitutively-expressed pool of transporters. The result of this flexibility is a situation in which influx, when measured at the specific substrate concentration to which the plant has become acclimated, is uniform over a plateau similar to the growth plateau in Figure 1 (Peuke and Tischner, 1991). As with other nutrients (e.g., phosphate and potassium), it is clear that inorganic nitrogen fluxes, at least in the low concentration range (but see below), respond inversely to external supply.

INDUCTION OF NITROGEN TRANSPORT

A crucial element of NO_3^- nutrition is that nitrate acts as a signal to induce the synthesis of proteins involved in its own acquisition and assimilation. This is true not only for the inducibility of key catabolic enzymes such as nitrate and nitrite reductases, but also of the transporter proteins mediating high-affinity NO_3^- influx into root cells (Crawford and Glass, 1998; Forde, 2000). In addition to inducible transporters, a class of high-affinity (but very low-capacity) nitrate transporters operates constitutively in root systems, permitting initial entry of NO_3^- into the cell without prior exposure of the plant to NO_3^-. Reception of NO_3^- (probably within the cell; but see below) stimulates the expression of the inducible systems at transcriptional and functional levels, resulting in much higher NO_3^- fluxes after a few hours in many herbaceous plants (e.g., a 30-fold increase after 24 h in barley, as reported by Siddiqi et al., 1990), and after periods of several days in conifers such as white spruce (Kronzucker et al., 1995d) and lodgepole pine (Min et al., 1998). Typically, however, maximal NO_3^- fluxes achieved during the induction process are not sustained, and a downregulation ensues, as depicted in Figure 2a for barley plants exposed to 100 μM NO_3^- for one to four days. The general pattern for transport induction and downregulation in the high-affinity [NO_3^-] range is shown in Figure 3. This behavior is sharp contrast to the reverse condition seen with NH_4^+ nutrition, in which the maximal rates of high-affinity uptake are found in plants with no prior exposure to NH_4^+ (Figure 3). Upon introduction of NH_4^+, a rapid downregulation of high-affinity NH_4^+ influx is generally observed, which is fundamentally different from the rapid induction of NO_3^- transport that is followed by a slower downregulation to a steady-state level, which remains higher than the uninduced level.

A few reports, nevertheless, have suggested an inductive effect of NH_4^+ on its own transport (Goyal and Huffaker, 1986; Morgan and Jackson, 1988; Jackson and Volk, 1992; Mäck and Tischner, 1994). The largest increase in NH_4^+ transport following provision of the ion was reported to be only about 3-fold higher than the pre-treatment value, in wheat plants (Goyal and Huffaker, 1986), a modest change compared with values typically seen with NO_3^- induction. Moreover, in that study, the NH_4^+ concentration in the medium was allowed to deplete, allowing the plant to adapt to a situation of increasing N deprivation, which could alternatively explain the reported rise in NH_4^+ uptake

FIGURE 3. Typified time course of changes in NO_3^- (solid line) and NH_4^+ (dashed line) from influx as the corresponding ions are resupplied to plants previously deprived of these nutrients.



over time. More generally, the moderate, transient, upregulation sometimes observed may be explained in terms of increased N transport to the shoot upon NH_4^+ provision (Kronzucker et al., 1998b). It should also be noted that NH_4^+ is an intermediate in a wide variety of biochemical reactions (Joy, 1988), and can accumulate within plant tissues to substantial levels (Wang et al., 1993a; Kronzucker et al., 1995a, b; Husted et al., 2000) even when not provided externally (Olsen et al., 1995), to the extent that emissions of gaseous ammonia (NH_3) from the plant can be observed (Mattsson and Schjoerring, 1996). Because of this, it is difficult to conceive that NH_4^+ can act as a signal for its own acquisition, except in the unlikely case of it acting as such only in quantities exceeding a high baseline concentration. Finally, the functional significance of the proposed induction is questionable, in that the steady-state flux in the HATS range is actually lower than that observed prior to NH_4^+ exposure (Figure 3).

The exact nature of the signal transduction mechanism by which NO_3^- initiates the upregulation of its own acquisition pathway (along with numerous other biochemical pathways, including photosynthesis and respiration–see Scheible et al., 2004) is still undetermined in higher plants. Indeed, it is not known whether the initial NO_3^- sensor is situated on the external face of the plasma membrane, or is intracellular, requiring that induction be triggered by NO_3^- transport via constitutive transport systems. While it has been shown that induction of prokaryotic NO_3^- acquisition in *E. coli* involves an external sensor (Parkinson and Kofoid, 1992), the situation for plants and algae is equivocal. Redinbaugh and Campbell (1991) proposed the idea of an external NO_3^- sensor for higher plants, observing that plants with an uninduced NO_3^- assimilation pathway can nevertheless have significant tissue NO_3^- content. However, if tissue NO_3^- is sequestered in the vacuole, a NO_3^- sensor may still operate in the cytosolic compartment of the cell (Siddiqi et al., 1989). Forde and Clarkson (1999) dismiss this latter possibility, citing evidence from NO_3^--selective microelectrodes (van der Leij et al., 1998) that suggests that the NO_3^- concentration in the cytosol is held constant at ~4 mM, even under conditions in which the plant is uninduced. This evidence, however, is contradicted by demonstrations of substantial variability in cytosolic $[NO_3^-]$ as determined by other methods, particularly compartmental analysis by tracer efflux (Lee and Clarkson, 1986; Siddiqi et al., 1991; Devienne et al., 1994; Kronzucker et al., 1995c; 1999a,b; Min et al., 1999; Britto and Kronzucker, 2001b, 2003), as well as comparisons of *in vivo* and *in vitro* nitrate reductase (NR) activities (Robin et al., 1983; Belton et al., 1985;

King et al., 1992), tracer influx profiles (Presland and MacNaughton, 1984), and NMR signals (G. Ratcliffe, personal communication). Contrary to Forde and Clarkson (1999), then, the plausible variability of the cytosolic NO_3^- pool makes the proposal that this pool functions as a modulator of the induction state of the cell a reasonable one. Other recent evidence that the NO_3^- sensor might be intracellular has come from manipulations of cytosolic NO_3^- levels, either by the suppression of NO_3^- uptake in barley by supplying plants with ammonium or amino acids (Aslam et al., 2001), or by use of mutant strains of *Chlamydomonas* with impaired nitrate reductase and nitrate transport activities (Rexach et al., 2002). Recently, however, Unkles et al. (2001) have proposed that the induction of nitrate reductase observed in *Aspergillus nidulans* mutants lacking NO_3^- uptake also argues for an external NO_3^- sensor, although the authors point out that NO_3^- might have entered the mutant cells in sufficient quantities to trigger induction, at rates below the threshold of detection.

AGENTS OF DOWNREGULATION

While it is clear that high-affinity NH_4^+ and NO_3^- transport decreases with increasing plant N status, the chemical nature of the agents responsible for this decrease has been another area of controversy. It has been widely proposed that end-product downregulation of N-assimilation pathways (including transport functions) by amino acid pools is the major means of control for high-affinity NO_3^- and NH_4^+ uptake (Breteler and Siegerist, 1984; Lee and Rudge, 1986; Cooper and Clarkson, 1989; Lee et al., 1992; Imsande and Touraine, 1994; Barneix and Causin, 1996; Rawat et al., 1999; Zhou et al., 1999; Forde, 2000; Vidmar et al., 2000; Aslam et al., 2001; Pal'ove-Balang and Mistrik, 2002; Gessler et al., 2004). In the case of NO_3^-, several lines of evidence support this idea, including the inverse correlation between tissue amino acid concentrations and NO_3^- uptake, suppression of NO_3^- uptake by exogenous application of amino acids (e.g., via solution culture to roots, or via foliar feeding), and enhancement of NO_3^- uptake by inhibitors of amino acid synthesis such as methionine sulfoximine (MSX). Use of aminotransferase inhibitors such as aminooxalacetic acid (AOA) and azaserine (AZA), which increase tissue levels of glutamate and glutamine, respectively, have indicated the high feedback potency of these particular amino acids (Zhou et al., 1999), although there are indications that arginine, which possesses an exceptionally high N:C ratio, may be even more important (Forde, 2000). Some caution, however, should be exercised in the interpretation of studies investigating these questions, which usually involve quantification of the total tissue content of putative regulatory agents, but fail to localize them to specific subcellular compartments. In addition, the exogenous application of amino acids is complicated by the possibility of their rapid interconversion within the plant (Glass et al., 2001), as well as their differential, and poorly characterized, uptake rates (Lipson and Näsholm, 2001).

Substantial evidence indicates that the inorganic transport substrates (NO_3^- and NH_4^+) themselves might be regulatory agents feeding back negatively on their own uptake. With NO_3^-, the use of mutants deficient in nitrate reductase activity (King et al., 1993; Wieneke, 1994), and the use of tungstate (WO_4^-) to block nitrate reductase (Ingemarrson et al., 1987; King et al., 1993; Wieneke, 1994), resulted in lowered tissue amino acid and (where measured) increased tissue NO_3^- pools, but downregulation of NO_3^- influx was nevertheless observed. At the level of genetic analysis, it is of further interest that while the use of WO_4^- in barley plants resulted in an increase in transcript abundance of a high-affinity nitrate transporter (Krapp et al., 1998; Vidmar et al., 2000), the actual measured influx under this condition was ~55% lower than in control plants (Vidmar et al., 2000). This result suggests that NO_3^- may act as a post-transcriptional inhibitor of its own transport (e.g. via allostery, phosphorylation, or thermodynamic means), rather than as a repressor (like some amino acids) of the transcription of transport-encoding genes (Forde and Clarkson, 1999). More generally, the discrepancy between abundance of transport-protein mRNA and actual transport indicates that the physiological interpretation of northern blots is not always as straightforward as it might appear.

Ambiguities are also seen in the regulation of NH_4^+ transport. Applications of MSX to plant roots, for instance, which raise [NH_4^+] and lower amino acid concentrations in tissue, have resulted in some instances in which NH_4^+ influx is reduced (Feng et al., 1994; Kronzucker et al., 1995a; Rawat et al., 1999), and others in which it is increased (Jackson et al., 1993; Lee and Ayling, 1993; Feng et al., 1994; Wieneke and Roeb, 1998). The first group of studies suggest that NH_4^+ is a potent inhibitor of its own influx, or perhaps that MSX, being a glutamine analog, itself acts as an agent of negative feedback. The latter possibility suggests that, instead of NH_4^+, the controlling agents are the amino acid concentrations in the tissue.

In general, the controversies discussed here might be explained by the likelihood that ammonium and nitrate uptake and metabolism have multiple points of regulation. It should, for instance, be considered that the uptake of inorganic N by root cells can be controlled by the influence of the shoot on root-cell pools of regulatory agents via long-distance transport. Split-root experiments have shown that nitrogen cycling via the xylem and phloem can exert powerful effects on N uptake (Cooper and Clarkson, 1989; Imsande and Touraine, 1994). N acquisition can also be modulated via changes in the extent of efflux (Siddiqi et al., 1991). The overall pattern of flux dynamics in the plant may thus be maintained by multiple mechanisms within the dictates of the growth and development pattern of the plant; this pattern may be linked to a timing mechanism that governs in cytosolic organic N turnover in the cytosolic compartment of plant cells (Britto and Kronzucker, 2001b).

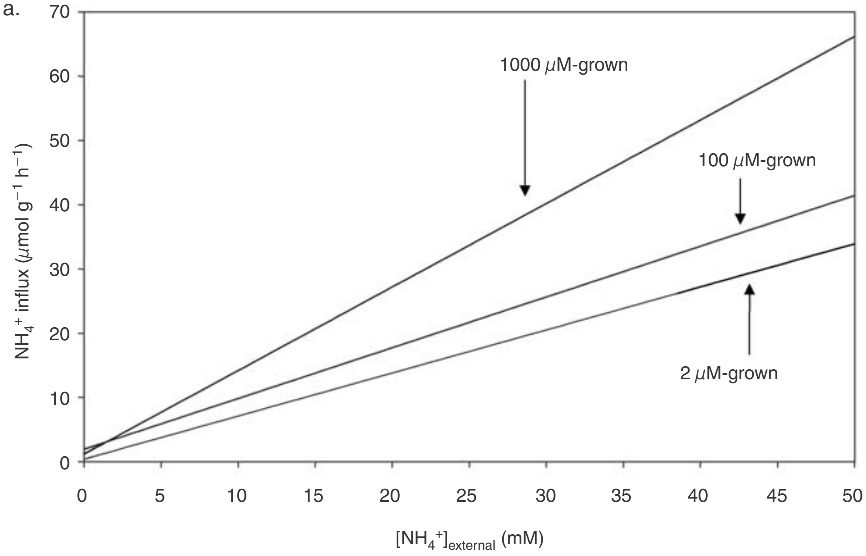
LOW-AFFINITY TRANSPORT SYSTEMS

Plants taking up NO_3^- and NH_4^+ from higher external concentrations (> 1 mM) have high-capacity uptake kinetics very different from those observed in the low-concentration range. Instead of saturating patterns of influx, linear responses to changes in external concentration are typically observed in this "low-affinity transport system" (LATS) concentration range (Figure 4), suggesting the operation of channel-type transport proteins with very high saturation limits (particularly in the case of NH_4^+, the influx of which is energetically passive for most LATS-range soil-NH_4^+ concentrations). In response to changing N availability in this concentration range, substantial differences in flux modulation can be seen between NO_3^- and NH_4^+ transport systems (Figure 4). With NO_3^-, the highest LATS-range fluxes at a given external concentration are found in plants that have had no recent exposure to NO_3^-. Treatment with NO_3^- in this range downregulates influx within a few days, the extent of the downregulation dependent on external $[NO_3^-]$ (Figure 4b). Surprisingly, this situation is reversed with NH_4^+ nutrition, in which the highest fluxes observed are in plants adapted to the highest external $[NH_4^+]$ (Figure 4a; Wang et al., 1993b; Rawat et al., 1999; Min et al., 2000; Cerezo et al., 2001).

Lest low-affinity N transport systems be considered irrelevant in an ecological context, it should be pointed out that soil concentrations of NH_4^+ and NO_3^- can at times reach magnitudes that lie well within the response range of LATS, particularly in agricultural soils but also in natural ecosystems (see Introduction). The lack of downregulation in

FIGURE 4. Linear patterns of nitrogen influx in the low-concentration range, as influenced nitrogen source and supply. **a.** NH_4^+ influx (adapted from Wang et al., 1993b); **b.** NO_3^- influx (adapted from Siddiqi et al., 1990).

a.



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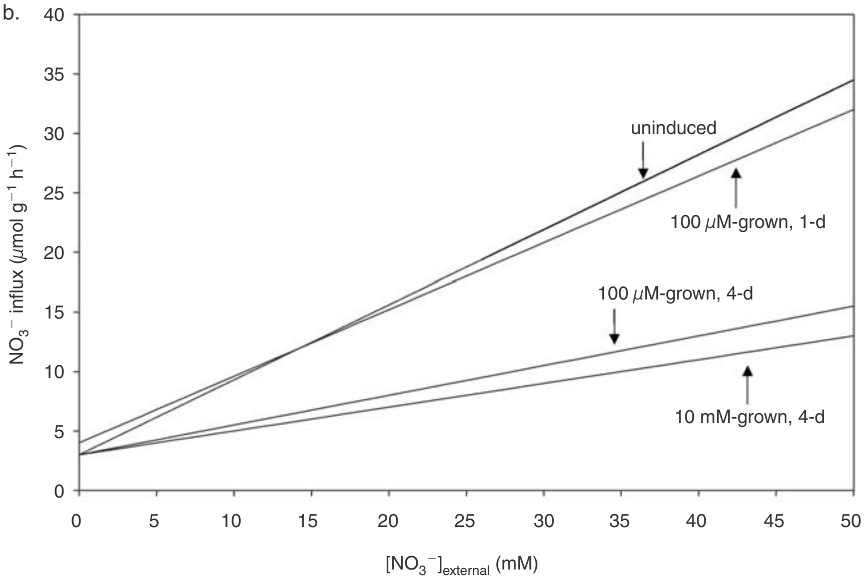

the NH_4^+-LATS range could significantly account for the low threshold for NH_4^+ toxicity in most plants (Britto et al., 2001; Kronzucker et al., 2001; Britto and Kronzucker, 2002; Britto et al., 2002; Kronzucker et al., 2003a). An enhanced flux of NH_4^+ can result in the accumulation of large amounts of NH_4^+ in the plant, both on a tissue basis, and, specifically, in the cytosol of root cells. This accumulation is associated with two effects that have been linked to NH_4^+ toxicity in plants. First, tissue (and cytosolic, in some cases) concentrations of nutritionally important cations such as K^+, Ca^{2+}, and Mg^{2+} drop significantly with NH_4^+ excess (Magalhaes and Wilcox, 1983; Holldampf and Barker, 1993; Speer and Kaiser, 1994; Gloser and Gloser, 2000; Kronzucker et al., 2003a), resulting in pathological conditions with symptoms similar to those seen under deprivation of these nutrients. Second, increased NH_4^+ influx is strongly associated with an even greater increase in NH_4^+ efflux (with efflux approaching 100% of influx). Efflux transporters appear to function in the release of excessive amounts of this ion from root cells, in a manner similar to that of antibiotic-resistant bacteria pumping lethal substances out of the cell (Britto et al., 2001). Because of the inwardly-negative electrical polarization of the plant plasma membrane, the efflux component of NH_4^+ transport is often energy-demanding, despite the high cytosolic accumulation of NH_4^+ observed under conditions of NH_4^+ toxicity. Given the very substantial magnitude of NH_4^+ efflux (~50 μmol gfw^{-1} h^{-1}), the energy requirement could account for much, if not all, of the large (40%) increase in respiratory oxygen consumption observed with NH_4^+-sensitive barley plants under these conditions (Kronzucker et al., 2001). Interestingly, NH_4^+-tolerant rice plants show no such respiratory increase, and display neither an uphill electrochemical gradient for NH_4^+ efflux across the plasma membrane, nor NH_4^+ fluxes of the magnitude seen in barley (Britto et al., 2001).

CONCLUSION

While the above discussions focus on details of cellular mechanisms, it is important to consider that such mechanisms, and their modulations in response to both limited and excessive supply of nitrogen, have profound influences on plant growth and yield. For this reason, the events and biochemical agents causing up- and downregulation of N fluxes, and the energetic costs of excess NH_4^+ fluxes, have important implications both for ecological processes and for the human food supply. Thus, their effects need to be considered by those who would seek to improve plant N acquisition either by conventional breeding or biotechnological means, particularly under conditions in which N is not limiting (Britto and Kronzucker, 2004; cf. Britto and Kronzucker, 2001b). Raising the capacity of plant roots to absorb N from soil might alternatively be achieved by providing the plant with mixed sources of nitrogen, which can maximize the total plant capture of N, simultaneously increasing plant yield and diminishing eutrophication (Kronzucker et al., 1999a). Ultimately, the challenge lies in the extension of physiological findings to practical applications in agricultural and natural ecosystems.

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