

## RESEARCH PAPER

# Dynamic changes in root hydraulic properties in response to nitrate availability

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## Abstract

Changes in root hydraulic resistance in response to alterations in nitrate supply were explored in detail as a potential mechanism that allows plants to respond rapidly to changes in their environment. Sunflower (*Helianthus annuus* cv. Holiday) plants grown hydroponically with limited nitrate availability ( $200 \mu\text{mol l}^{-1}$ ) served as our model system. Experimental plants were 6–9-weeks-old with total dry mass of 2–4 g. Root pressurization of intact plants and detached root systems was used to elucidate the temporal dynamics of root hydraulic properties in sunflower plants following changes in external nitrate availability. The response was rapid, with a 20% decrease in hydraulic resistance occurring within the first hour after the addition of 5 mM nitrate and the magnitude of the effect was dependent on nitrate concentration. The change in root hydraulic resistance was largely reversible, although the temporal dynamics of the response to nitrate addition versus nitrate withdrawal was not symmetric (a gradual decrease in resistance versus its fast increase), raising the possibility that the underlying mechanisms may also differ. Evidence is presented that the observed changes in root hydraulic properties require the assimilation of nitrate by root cells. The hydraulic resistance of roots, previously stimulated by the addition of nitrate, increased more than in control plants in low nitrate under anoxia and that suggests a key role of aquaporin activity in this response. It is proposed that a rapid decrease in root hydraulic resistance in the presence of increased nitrate availability is an important trait that could

enhance a plant's ability to compete for nitrate in the soil.

Key words: Aquaporins, *Helianthus annuus*, nitrate reductase, nitrogen, root hydraulic resistance, sunflower.

## Introduction

Nitrogen supply is highly variable in both time and space and is often a limiting factor for plant growth (Chapin, 1980; Marschner, 1995). An important source of nitrogen for plants is nitrate released from soil organic matter during mineralization (Attiwill and Adams, 1993; Jackson and Caldwell, 1993). Nitrate availability in soils is thought to fluctuate rapidly due to variation in rates of mineralization and the high mobility of nitrate ions in soil (Jackson and Caldwell, 1993; Robinson, 1994; Jackson and Caldwell, 1996). To cope with this heterogeneity, plants respond both developmentally and physiologically to local increases in nitrate availability. Developmental responses include root proliferation (Hodge *et al.*, 1999) and changes in specific root length (Robinson and Rorison, 1983), while physiological responses include up-regulation of nitrate transporters and enzymes involved in nitrate assimilation (Krapp *et al.*, 2002). Because physiological responses can occur much more quickly than responses that require the production of new roots, they allow roots to respond rapidly to short-term changes in the root environment.

The supply of mineral nutrients, especially nitrate, is known to influence rates of transpiration and leaf expansion. Physiological studies beginning in the 1980s suggested that this was mediated, at least in part, by an

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effect of nitrate availability on root hydraulic properties (Fiscus *et al.*, 1983; Radin and Boyer, 1982; Radin and Eidenbock, 1984). In general, root hydraulic resistance increased when nitrate availability was low and decreased when nitrate supply was high (Carvajal *et al.*, 1996; Clarkson *et al.*, 2000). These changes, however, were observed over a period of several days, or at least several hours, and usually preceded the response of stomata or any changes in growth (for review see Clarkson *et al.*, 2000). Whether sudden changes in nitrate availability can lead to rapid changes in root hydraulic properties, possibly through the modification of the number or activity of plasma membrane aquaporins, is not known (Carvajal *et al.*, 1996; Henzler *et al.*, 1999). In this paper, the temporal dynamics with which root hydraulic properties respond to alterations in external nitrate availability are examined and the basic mechanisms connecting nitrate availability with changes in root hydraulic properties are discussed.

## Materials and methods

### Cultivation of plants

Sunflower (*Helianthus annuus* cv. Holiday) plants were grown hydroponically in modified Hoagland's solution with 2.0 mM or 0.2 mM nitrate as the sole source of nitrogen, resulting in high (HN) and low nitrate (LN) treatments. The HN nutrient solution contained 603  $\mu\text{M}$   $\text{Ca}(\text{NO}_3)_2$ , 795  $\mu\text{M}$   $\text{KNO}_3$ , 190  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ , 270  $\mu\text{M}$   $\text{MgSO}_4$ , 2  $\mu\text{M}$   $\text{MnSO}_4$ , 0.85  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.15  $\mu\text{M}$   $\text{CuSO}_4$ , 20  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 0.25  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$ , and 40.5  $\mu\text{M}$  FeNa-EDTA, while the composition of the LN nutrient solution was 60.3  $\mu\text{M}$   $\text{Ca}(\text{NO}_3)_2$ , 79.5  $\mu\text{M}$   $\text{KNO}_3$ , 360  $\mu\text{M}$   $\text{K}_2\text{SO}_4$ , 540  $\mu\text{M}$   $\text{CaCl}_2$ , 190  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ , 270  $\mu\text{M}$   $\text{MgSO}_4$ , 2  $\mu\text{M}$   $\text{MnSO}_4$ , 0.85  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.15  $\mu\text{M}$   $\text{CuSO}_4$ , 20  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 0.25  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$ , and 40.5  $\mu\text{M}$  FeNa-EDTA. Approximately 20 plants were grown in 30 l containers in which the well-aerated nutrient solution was renewed once or twice weekly depending on the size of plants. As plants increased in size, the number per container decreased due to several plants per day being removed for measurements. Natural (greenhouse) illumination was supplemented with metal halide lamps, providing a minimum intensity of 450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR and a constant photoperiod of 16 h. Mean day/night temperatures were 25/16 °C. All plants were grown in the HN solution for 4 weeks, prior to the initiation of the LN treatment. Measurements took place 6–9 weeks after germination, at which time the plants had 6–10 leaves and a total dry mass 2–4 g.

### Determination of root hydraulic resistance

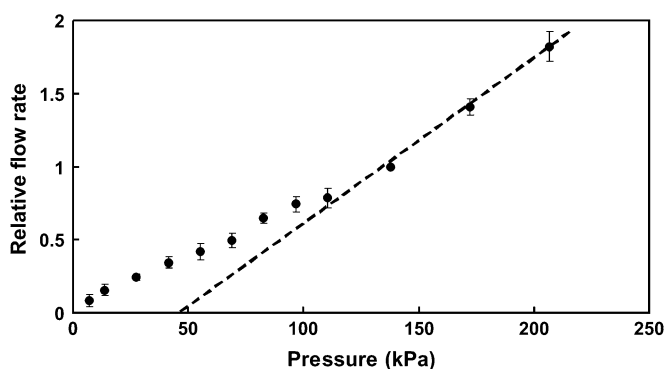
Two methods were used to quantify the effect of nutrient availability on root hydraulic properties: a modified root pressure chamber described by Stirzaker and Passioura (1996) was used to determine changes in hydraulic resistance *in vivo*, while a standard hydraulic apparatus was used to quantify water flux through excised root systems.

The root pressure chamber technique allowed us to monitor instantaneous changes in the hydraulic resistance of intact, transpiring plants. Resistance was calculated as the pressure gradient from the root chamber to the shoot divided by the transpiration rate. An active feedback system controlled the pressure applied to roots such that the pressure in the shoot xylem was held constant (at

atmospheric pressure). Pressure in the shoot xylem was monitored after removing the lamina of a leaf located in the middle of the stem and attaching a pressure transducer (PX26 Omega Engineering Inc. Stamford, CT, USA). The transducer was connected to the petiole using a short piece of thick-wall tubing that was sealed on the petiole using Parafilm (Pechinez Plastic Packaging, Menasha, WI, USA).

Plants were sealed in the root pressure chamber using a flexible dental impression material (Regular type Exaflex, GC America Inc., Alsip, IL, USA) to form a gasket around the base of the stem. Within the pressure chamber, the container containing the roots+ nutrient solution of desired composition was continuously aerated using a battery-operated pump. Access ports allowed us to amend or exchange the hydroponic solution while maintaining the pressure in the root chamber. The gas composition supplied to the root chamber was controlled so as to keep the partial pressure of oxygen equal to that of the air. Transpiration rates were determined from changes in the weight of the pressure chamber, which was placed on an electronic balance ( $\pm 0.01$  g; Model 3808, Sartorius, Germany). Changes in mass due to changes in gas concentration within the root pressure chamber were taken into account when calculating transpiration rate.

A hydraulic apparatus, in which the pressure gradient driving xylem flow was created by compressed air, was used to quantify changes in the hydraulic properties of excised root systems in response to nitrate availability and anoxia. Flow-through detached root systems, immersed in a well-aerated and stirred nutrient solution, was driven by a pressure difference of 175 kPa. Under this delivery pressure the hydraulic component of the driving force dominates (Fig. 1), allowing changes in root hydraulic properties to be quantified independent of any changes in solute accumulation rates (root pressure). Flow rates were measured by directing the outflow from the root systems to an analytical balance ( $\pm 0.00001$  g Model 3200BX, Sartorius, Germany) using thick-walled tygon tubing. Pressure in the system was continuously monitored using a pressure transducer (PX01K1-050GV, Omega, Stamford, CT, USA) and recorded by a computer. The nutrient solutions used in these measurements were identical to those used in cultivation, except for the addition of 10 mM 2-(*N*-morpholino) ethanesulphonic acid (MES) set to pH 6.1 by KOH. The dry mass of root systems used for all measurements ranged from 0.3–0.5 g.



**Fig. 1.** Relationship between the externally applied pressure and resulting flow rate of xylem sap through detached sunflower root systems. Each point represents the mean of six independent replicates, error bars represent 2 SE. At pressures above 100 kPa the pressure:flow relationship was linear, indicating that the osmotic component of the driving force (i.e. flow due to root pressure) was negligible. All further measurements on detached root systems were conducted using an externally supplied pressure of 175 kPa.

### Experimental treatments

Changes in nitrate concentration were carried out by adding sodium nitrate (stock solution) to the root medium or exchanging a HN solution for a LN one. Anoxia was elicited by switching the aeration system intake to a nitrogen tank. Inhibition of nitrate reductase was induced by adding 0.15 mM sodium tungstate (Deng *et al.*, 1989; Barthes *et al.*, 1996) to the LN nutrient solution 20 h prior to any measurements. This treatment was shown to reduce NR activity by more than 50% compared with control plants (Barthes *et al.*, 1996).

Sunflower, like many plants (Clarkson *et al.*, 2000; Uehlein and Kaldenhoff, 2006), exhibits significant diurnal changes in root hydraulic permeability under control (constant) conditions. To account for this natural diurnal variation, experimental treatments were imposed at the same time of day and the experimental data compared against control plants monitored over the same time period. Data describing the response of hydraulic properties of root systems were calculated as the mean of flow rates measured on three to four different root systems normalized to the flow rate at the time when nitrate was applied.

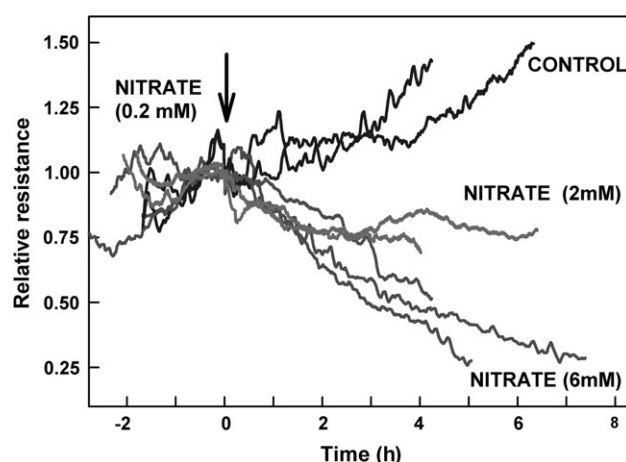
### Nitrate content

Nitrate content of roots was determined for oven-dried tissue. Roots were finely ground, extracted in hot demineralized water, and measured colorimetrically (Cataldo *et al.* 1975).

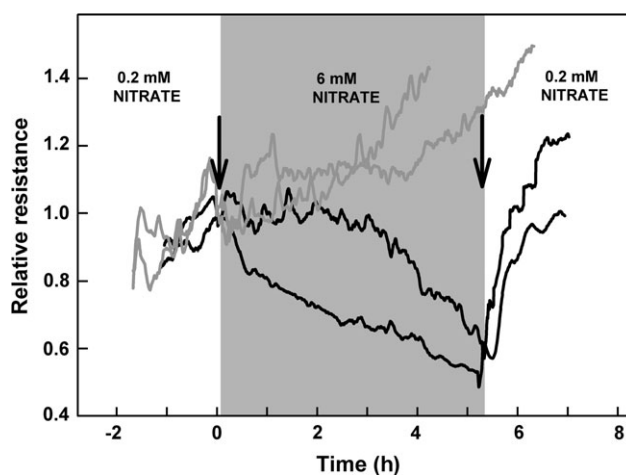
## Results

Addition of nitrate to plants grown in low nitrate (LN) conditions resulted in a fast decrease in root hydraulic resistance: about 20% in the first hour, with the resistance continuing to fall over a period of several hours (Fig. 2). The initial response to the addition of 2 mM and 6 mM of nitrate was similar, however, the effect elicited by the higher nitrate level persisted for a longer period of time, eventually leading to much lower root hydraulic resistance. The resistance of the control plants (no addition of nitrate) increased throughout the experiment, reflecting an underlying diurnal trend of increasing resistance during the morning into mid-afternoon (data not shown). Interestingly, the response of LN plants to a subsequent nitrate withdrawal from 6 mM to 0.2 mM concentration was much faster than their response to nitrate addition. Furthermore, hydraulic resistance following this decrease in nitrate levels did not return to control values suggesting that some of the hydraulic modifications induced by exposure to high nitrate remained in place (Fig. 3). Nitrate addition did not induce any significant changes in transpiration rate of intact plants (data not shown), nor did plants with severed roots respond to nitrate addition suggesting that the entire response occurs within the roots.

Dramatic changes in root hydraulic properties in response to nitrate addition or its subsequent withdrawal were limited to LN-grown plants. Plants grown in high nitrate (HN) conditions (2 mM) were not responsive either to further increases in nitrate levels (6 mM) or to reduced nitrate availability (0.2 mM). This lack of response was presumably due to high  $\text{NO}_3^-$  content in HN plants (47 mg



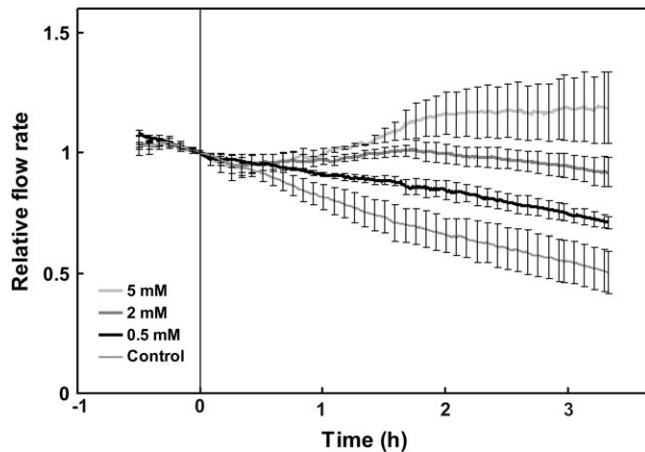
**Fig. 2.** Changes in the relative hydraulic resistance of LN-grown intact sunflower plants in response to changes in nitrate concentration. Hydraulic resistance was measured using the root pressure chamber technique; values are scaled relative to the value at the time of when the experimental treatments were imposed. Concentrated stock solution was added to the hydroponic solution (at time=0) so that the concentration of nitrate supplied to the roots increased from 0.2 mM to 2 mM or 6 mM. Separate runs of plants with no addition of nitrate served as control.



**Fig. 3.** Hydraulic resistance in response to both increases and decreases in nitrate levels (arrows) for two sunflower plants (black lines). Hydraulic resistance decreased following an increase in nitrate concentrations from 0.2 mM to 6 mM and then recovered quickly upon return to a 0.2 mM nitrate solution. Hydraulic resistance was measured *in vivo* using the root pressure chamber technique; values are scaled relative to the value at the time of when the first experimental treatment was imposed. To aid comparison, data from two control plants (grey lines) are reprinted from Fig. 2.

nitrate  $\text{g}^{-1}$  of dry root matter) as opposed to 5.5  $\text{mg g}^{-1}$  in roots of LN plants. This difference might reflect the storage capacity of the vacuoles and the potential for stored nitrate to influence root hydraulic properties.

Flow rate through the roots of decapitated LN plants was related to the nitrate concentration in the root solution (Figs 2, 4). Increasing nitrate concentrations (0.5 mM to 5 mM) resulted in higher flow rates, corresponding to lower root hydraulic resistances.



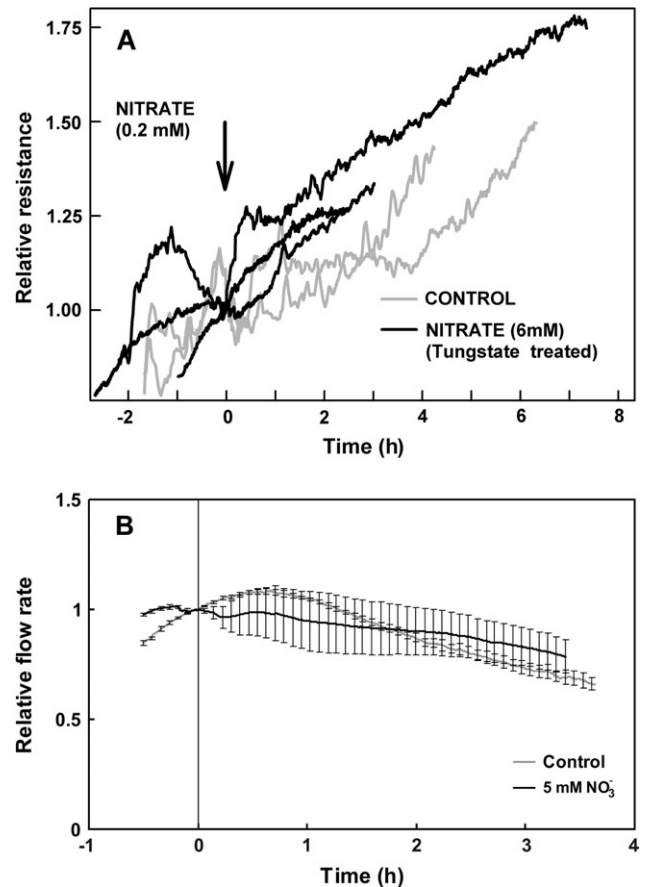
**Fig. 4.** Relative flow rate of water through detached sunflower roots in response to increases in nitrate concentration. Each point represents the mean of three independent measurements per treatment; error bars represent 2 SE. An externally applied hydrostatic pressure of 175 kPa was used to generate the measured flow. Data were normalized relative to the flow rate at the time of nitrate addition (at time zero) for each measurement.

Treatment with sodium tungstate eliminated the effect of nitrate additions on the root hydraulic properties of LN-grown plants both *in vivo* (Fig. 5A) and with detached root systems (Fig. 5B). Reductions in oxygen levels resulted in a significant increase in root hydraulic resistance (Fig. 6; seen as drop in flow rate). The inhibitory effect of anoxia was much greater in root systems previously exposed to 5 mM nitrate than in control plants (Fig. 6).

## Discussion

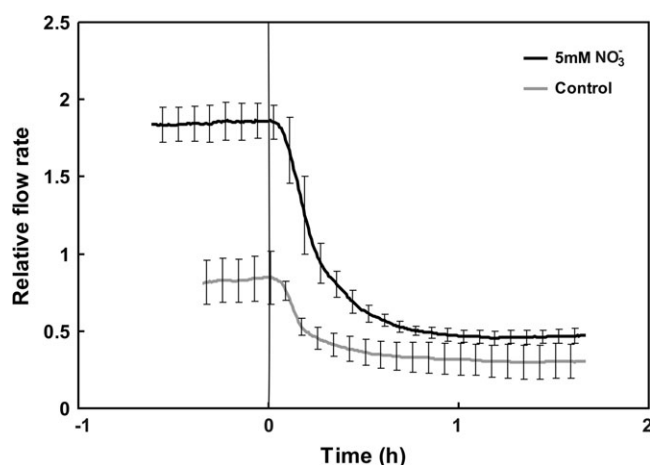
The ability to monitor, essentially continuously, the hydraulic properties of intact, transpiring plants allowed the interaction between nitrate supply and root properties to be probed in considerable detail. The results indicate shorter response times to changes in external nitrate availability than previously reported (Radin and Matthews, 1989; Clarkson *et al.*, 2000). For example, experiments with *Lotus japonicus* showed that significant changes in root hydraulic properties requires, at a minimum, several hours following nitrate re-supply (Clarkson *et al.*, 2000; Prosser *et al.*, 2006). These results suggest that roots become more permeable to water within 20–30 min after the application of nitrate and that this increase in permeability continues over time with the maximum enhancement being dependent on nitrate concentration (Fig. 2). Such a fast response requires not only rapid signal transduction but also an equally rapid mechanism leading to changes in root hydraulic properties.

Mean hydraulic resistance calculated for excised roots of LN plants before the start of the experimental treatments was  $0.0013 \pm 0.0002 \text{ MPa g}^{-1} \text{ FW h}^{-1} \mu\text{l}^{-1}$  (mean  $\pm$  SE, that equals hydraulic conductivity  $840 \pm 108 \mu\text{l h}^{-1} \text{ g}^{-1}$



**Fig. 5.** Response of root hydraulic properties of plants previously exposed for 20 h to sodium tungstate, an inhibitor of nitrate reductase, to increases in nitrate concentration supplied to the roots. (A) Changes in the relative hydraulic resistance of intact sunflower plants in response to changes in nitrate concentration as measured by the root pressurization technique. Root hydraulic resistance of tungstate-treated plants was unaffected by an increase of nitrate concentration from 0.2 mM to 6 mM (addition indicated by arrow). Values are scaled relative to the value at the time of nitrate addition (arrow); to aid comparison, the time-course of two control plants are reprinted from Fig. 2. (B) Relative flow rate of water through detached roots of sunflower plants in response to an external (175 kPa) pressure gradient. Data were normalized relative to the flow rate measured at the time of nitrate addition (time zero). Shown are means of three plants; error bars represent 2 SE.

$\text{FW MPa}^{-1}$ ), similar to values previously reported for root systems of sunflowers grown in hydroponics (Quintero *et al.*, 1999). Because the addition of nitrate changes the osmotic properties of the solution surrounding the roots, it is worth asking to what extent the changes reported here could be the direct result of osmotic effects. Increased osmotic pressure of the solution, as well as the potential build-up of unstirred layers in the root cortex, would slow the flux of water. This suggests that the magnitude of changes that are presented here may be underestimated. On the other hand, rapid uptake of nitrate and its transport to xylem could build up an osmotic gradient into the xylem, stimulating water flux across the root cortex. Nitrate concentration in the xylem of sunflower plants



**Fig. 6.** Relative flow rate of water through detached roots of sunflower plants when challenged with anoxia. One set of plants received 5 mM nitrate several hours before the anoxic treatment, while the second group of plants served as control. An externally applied hydrostatic pressure of 175 kPa was used to generate the measured flow. Data were normalized relative to the flow rate at the time of nitrate application. Each line represents the mean of three plants; error bars represent 2 SE.

can vary by an order of magnitude in the millimolar range, depending on the external nitrate supply (Nikolic and Romheld, 2003). This study's measurements of changes in osmolality of xylem sap after nitrate addition revealed that the osmotic potential of xylem sap decreased from 0.02 MPa to 0.015 MPa within the first 30 min after addition and remained constant over the next 120 min. This suggests that in these experiments, accumulation of nitrate and other ions in the root xylem did not contribute to the observed increase in water flow.

Similar to intact plants, the hydraulic resistance of root systems from decapitated plants also decreased in response to increased nitrate supply (Fig. 4). Furthermore, although the decrease in hydraulic resistance after the addition of nitrate to LN plants was rapid, the increase in resistance after subsequent nitrate withdrawal was even faster (Fig. 3). Differences in the dynamics of the response to nitrate addition versus nitrate withdrawal suggest that the mechanisms underlying these changes in root hydraulic properties may differ. In particular, the response to nitrate withdrawal was so abrupt that it may indicate the closure of a water pathway through the root, whereas the steady increases in permeability with greater nitrate supply suggest a process by which the water transport capacity of the root is enhanced. Similar dynamics after nitrate withdrawal and re-supply were reported for roots of *Lotus japonicus* (Prosser *et al.*, 2006).

Nitrate assimilation represents a potentially important step in the signal transduction chain (Forde and Clarkson, 1999; Prosser *et al.*, 2006). LN-grown sunflower plants exposed for 20 h to sodium tungstate no longer altered their hydraulic properties when supplied with nitrate. This suggests that it is not nitrate itself but the downstream

products of its assimilation that are required to induce hydraulic changes in the root. Results of Barthes *et al.* (1996) indicate that urea, a source of  $\text{NH}_4^+$ , can reverse the negative effect of tungstate treatment. The rate of xylem exudation in detopped maize seedlings after tungstate treatment matched the rate of control plants when urea was the sole nitrogen source. These results also suggest that a product of nitrate assimilation is involved in the signal transduction pathway responsible for changes in root hydraulic properties (Barthes *et al.*, 1996).

Nitrate uptake and assimilation involves a net consumption of protons, raising the possibility of a direct feedback between nitrate assimilation and the regulation of aquaporins. Despite the existence of biochemical and biophysical mechanisms for pH homeostasis (Marschner, 1995), Espen *et al.* (2004) showed that blocking nitrate reductase can lead to a measurable change in cytosolic pH. Decreases in cytosolic pH have also been shown reversibly to alter root hydraulic properties due to the protonation of a tyrosine residue on the cytosolic side of majority PIPs, resulting in a dramatic increase of root hydraulic resistance (Tournaire-Roux *et al.*, 2003). This gating mechanism raises the possibility that changes in cytosolic pH due to nitrate assimilation could be involved in triggering nitrate-induced changes in the permeability of roots to water.

Aquaporins are responsible for a major part of the permeability of root cell membranes to water (Javot and Maurel, 2002). A number of studies have demonstrated that changes in aquaporin expression are correlated with changes in root hydraulic properties (Steudle and Henzler, 1995; Maurel, 1997; Henzler *et al.*, 1999; Martre *et al.*, 2002; Uehlein and Kaldenhoff, 2006). Anoxia was applied as a test for the engagement of aquaporins in the examined response (Tournaire-Roux *et al.*, 2003). Although anoxia is not a selective blocker of aquaporins, it causes decreased activity of plasma membrane  $\text{H}^+$ -ATPases and, therefore, rapid decreases in cytoplasmic pH. The increase in resistance (presented here as a decrease in the measured flow rate) was greater for plants resupplied with nitrate, presumably because more aquaporins were present (Fig. 6). Anoxia never entirely blocked water transport through roots, suggesting the presence of an apoplastic pathway (Steudle and Peterson, 1998) or the existence of intrinsic (i.e. non-aquaporin-mediated) membrane permeability to water.

The proposed mechanism of fast and reliable changes of root hydraulic properties to nitrate availability may play a critical role in the plant's overall strategy for acquiring resources from a highly dynamic source. Nitrate concentrations in soil solution frequently fluctuate within days or even hours due to environmental factors affecting mineralization rates and bacterial activity in the rhizosphere (Attiwill and Adams, 1993; Jackson and Caldwell, 1993). It is proposed that the ability to increase water uptake by

roots in nitrate-rich soil domains, while at the same time reducing water uptake from nitrate-poor regions represents an important mechanism by which plants overcome their sessile life form, dynamically utilizing available resources without long-term developmental modifications. An increase in water uptake rates will cause highly mobile nitrate ions to be brought via convection flow to the root surface, which significantly contributes to greater nitrate uptake (Buysse *et al.*, 1996). Hence, a rapid decrease in root hydraulic resistance in the presence of increased nitrate availability could enhance a plant's ability to compete for nitrate in the soil and thus would be an important trait for non-nitrogen-fixing plants. It is worth noting that our experiments examined only plants grown in hydroponics. The root systems of hydroponically grown plants typically differ in their morphology (longer, thinner roots with less branching and greater water content) and, due to their less robust structure, generally have greater permeability for water (Steudle and Peterson, 1998) than roots grown in soil. Therefore, the magnitude and dynamics of root response to nitrate availability may differ from what occurs in natural conditions.

This paper lays the groundwork for more detailed explorations of the underlying mechanism(s) of in the interaction between root hydraulic properties and external nitrate availability. Future research should examine the role of changes in cytoplasmic pH upon nitrate uptake and assimilation and investigate the relationship between external nitrate availability and expression of aquaporins in root cells. Of equal importance will be studies on the physiological significance of this interaction, emphasizing how local changes in root physiology enhance a global strategy for acquiring nitrogen from the highly dynamic soil environment.

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## References

- Attwill PM, Adams MA. 1993. Nutrient cycling in forests. *New Phytologist* **124**, 561–582.
- Barthes L, Deleens E, Bousser A, Hoarau J, Prioul JL. 1996. Xylem exudation is related to the nitrate assimilation pathway in detopped maize seedlings: use of nitrate reductase and glutamine synthetase inhibitors as tools. *Journal of Experimental Botany* **47**, 485–495.
- Buysse J, Smolders E, Merckx R. 1996. Modelling the uptake of nitrate by a growing plant with an adjustable root nitrate uptake capacity. I. Model description. *Plant and Soil* **181**, 19–23.
- Carvajal M, Cooke DT, Clarkson DT. 1996. Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function. *Planta* **199**, 372–381.
- Cataldo DA, Haroon M, Schrader LE, Youngs VL. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis* **6**, 71–80.
- Chapin FS. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**, 233–260.
- Clarkson DT, Carvajal M, Henzler T, Waterhouse RN, Smyth AJ, Cooke DT, Steudle E. 2000. Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. *Journal of Experimental Botany* **51**, 61–70.
- Deng MD, Moureaux T, Caboche M. 1989. Tungstate, a molybdate analog inactivating nitrate reductase, deregulates the expression of the nitrate reductase structural gene. *Plant Physiology* **91**, 304–309.
- Espen L, Nocito FF, Cocucci M. 2004. Effect of NO<sub>3</sub><sup>-</sup> transport and reduction on intracellular pH: an *in vivo* NMR study in maize roots. *Journal of Experimental Botany* **55**, 2053–2061.
- Fiscus EL, Klute A, Kaufmann MR. 1983. An interpretation of some whole plant water transport phenomena. *Plant Physiology* **71**, 810–817.
- Forde BG, Clarkson DT. 1999. Nitrate and ammonium nutrition of plants: physiological and molecular perspectives. *Advances in Botanical Research Incorporating Advances in Plant Pathology* **30**, 1–90.
- Henzler T, Waterhouse RN, Smyth AJ, Carvajal M, Cooke DT, Schaffner AR, Steudle E, Clarkson DT. 1999. Diurnal variations in hydraulic conductivity and root pressure can be correlated with the expression of putative aquaporins in the roots of *Lotus japonicus*. *Planta* **210**, 50–60.
- Hodge A, Robinson D, Griffiths BS, Fitter AH. 1999. Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. *Plant, Cell and Environment* **22**, 811–820.
- Jackson RB, Caldwell MM. 1993. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. *Ecology* **74**, 612–614.
- Jackson RB, Caldwell MM. 1996. Integrating resource heterogeneity and plant plasticity: modelling nitrate and phosphate uptake in a patchy soil environment. *Journal of Ecology* **84**, 891–903.
- Javot H, Maurel C. 2002. The role of aquaporins in root water uptake. *Annals of Botany* **90**, 301–313.
- Krapp A, Ferrario-Mery S, Touraine B. 2002. Nitrogen and signaling. In: Foyer C, Noctor G, eds. *Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism*. Dordrecht: Kluwer Academic Publishers, 205–225.
- Marschner H. 1995. *Mineral nutrition of higher plants*. Cambridge: Academic Press.
- Martre P, Morillon R, Barrieu F, North GB, Nobel PS, Chrispeels MJ. 2002. Plasma membrane aquaporins play a significant role during recovery from water deficit. *Plant Physiology* **130**, 2101–2110.
- Maurel C. 1997. Aquaporins and water permeability of plant membranes. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 399–429.
- Nikolic M, Romheld V. 2003. Nitrate does not result in iron inactivation in the apoplast of sunflower leaves. *Plant Physiology* **132**, 1303–1314.
- Prosser IM, Massonneau A, Smyth AJ, Waterhouse RN, Forde BG, Clarkson DT. 2006. Nitrate assimilation in the forage legume *Lotus japonicus* L. *Planta* **223**, 821–834.
- Quintero JM, Fournier JM, Benlloch M. 1999. Water transport in sunflower root systems: effects of ABA, Ca<sup>2+</sup> status and HgCl<sub>2</sub>. *Journal of Experimental Botany* **50**, 1607–1612.

- Radin JW, Boyer JS.** 1982. Control of leaf expansion by nitrogen nutrition in sunflower plants: role of hydraulic conductivity and turgor. *Plant Physiology* **69**, 771–775.
- Radin JW, Eidenbock MP.** 1984. Hydraulic conductance as a factor limiting leaf expansion of phosphorus-deficient cotton plants. *Plant Physiology* **75**, 372–377.
- Radin JW, Matthews MA.** 1989. Water transport-properties of cortical-cells in roots of nitrogen-deficient and phosphorus-deficient cotton seedlings. *Plant Physiology* **89**, 264–268.
- Robinson D.** 1994. The responses of plants to nonuniform supplies of nutrients. *New Phytologist* **127**, 635–674.
- Robinson D, Rorison IH.** 1983. A comparison of the responses of *Lolium perenne*, *Holcus lanatus* and *Deschampsia flexuosa* to a localized supply of nitrogen. *New Phytologist* **94**, 263–274.
- Stedle E, Henzler T.** 1995. Water channels in plants: do basic concepts of water transport change? *Journal of Experimental Botany* **46**, 1067–1076.
- Stedle E, Peterson CA.** 1998. How does water get through roots? *Journal of Experimental Botany* **49**, 775–788.
- Stirzaker RJ, Passioura JB.** 1996. The water relations of the root-soil interface. *Plant, Cell and Environment* **19**, 201–208.
- Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, Bligny R, Maurel C.** 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* **425**, 393–397.
- Uehlein N, Kaldenhoff R.** 2006. Aquaporins and biological rhythm. *Biological Rhythm Research* **37**, 315–322.