

Patchy nitrate promotes inter-sector flow and ^{15}N allocation in *Ocimum basilicum*: a model and an experiment

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Abstract. Root conductance increases under high nitrate conditions. This plasticity might increase water and nutrient transport between parallel xylem pathways, but restrictions to lateral flow – called sectoriality – are expected to limit this crossover. We simulated the effects of a high nitrate patch on root conductance, water uptake and inter-sector water transport, then empirically tested whether a high nitrate patch affects water uptake and nitrogen distribution (applied ^{15}N as $^{14}\text{NH}_4^{15}\text{NO}_3$ to half the root system) within the crowns of split-root hydroponic basil (*Ocimum basilicum* L.). Simulations showed that at low sectoriality, the proportion of water taken up in a patch scales with the relative change in root resistance and that this fraction decreases with increasing tangential resistance. The effect of sectoriality decreased when a higher background root resistance was assumed. Empirically, water flow through excised basil roots was 1.4 times higher in the high nitrate than the no nitrate solution. In split-root basil, a nitrate patch resulted in a marginally significant increase in the proportion of water taken up from the patch and water uptake patterns significantly predicted the distribution of ^{15}N . Our results suggest that root conductance can mediate nitrogen allocation between sectors, a previously unexplored benefit.

Additional keywords: hydraulic conductivity, nitrogen transport, nitrogen 15 isotope, water uptake, xylem.

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Introduction

Plant responses to nutrient heterogeneity in the soil include proliferation of roots into nutrient patches, changes to the anatomy of newly formed roots and physiological adjustments to increase the uptake rate for a nutrient (Robinson 1994; Hodge 2004, 2005). It has also been noted that at least some species can increase root conductance in response to the presence of high nitrate patches (Gloser *et al.* 2007; Gorska *et al.* 2008). The adaptive value of hydraulic responses to nutrient patches is not known, but researchers have suggested that this increased hydraulic conductance improves competition for nitrate ions, which are highly mobile, so are easily carried by water flux towards the roots (Gloser *et al.* 2007; Gorska *et al.* 2008; Maurel *et al.* 2010). Other benefits may exist as well. If a high nutrient patch locally elevates hydraulic conductance, it could result in a pressure gradient between parallel xylem pathways, increasing movement of water and nutrients between them (Zwieniecki *et al.* 2003). Here, we examine the combined effect of root and stem hydraulics on water uptake by roots and evaluate the hypothesis that these hydraulic changes could benefit the plant by helping to overcome vascular constraints to nitrogen transport within the crown.

The transportation and distribution of resources within the plant is constrained by vascular anatomy (Watson and Casper 1984; Zanne *et al.* 2006a; Glaser *et al.* 2008). Vascular constraints – called sectoriality – affect xylem connections

from roots to leaves as well as phloem connections from leaves to roots and other sinks and species vary in their capacity to transport resources between sectors (Orians *et al.* 2004; Orians *et al.* 2005; Zanne *et al.* 2006b; Ellmore *et al.* 2006). In the case of xylem transport, sap can move between parallel vessels in response to inter-sector hydraulic gradients, primarily at bordered pit fields. These pit fields vary among species in frequency and conductive properties (e.g. Ellmore *et al.* 2006; Hacke *et al.* 2006), resulting in differences in the resistance to flow between sectors, i.e. differences in tangential resistance. Since water transport in the xylem is driven by water potential gradients, inter-sector transport is expected to be minimal under uniform conditions, even for species with high anatomical integration (Zwieniecki *et al.* 2003; Ellmore *et al.* 2006). However, in patchy resource conditions, vascular constraints could limit growth by preventing allocation of resources from a high quality patch (one with relatively high light, nutrients, or water) to the entire plant (Gloser *et al.* 2008) unless there are factors that increase inter-sector transport. In the case of patchy water or light, environmental heterogeneity naturally favours flow between sectors (Orians and Jones 2001; Thorn and Orians 2011). For example, through its direct effect on transpiration rates, patchy light is expected to result in lower water content – and, therefore, water potential – for some leaves relative to others and so increase inter-sector crossover (Thorn and Orians 2011). In contrast, it is unknown whether patchy

nutrients increase inter-sector crossover. Zwieniecki *et al.* (2003) argued that the xylem conductivity changes associated with ion concentration will increase water supply to high nutrient sectors and, thus, facilitate nutrient transport between sectors. Similarly, the increased root conductance observed in some species (Gloser *et al.* 2007) should also generate a hydrostatic gradient that would increase the spread of nutrient rich xylem sap throughout the crown.

In order to examine the interactive effects of root hydraulics and tangential resistance on water uptake and nutrient allocation, we used two sectoriality models to evaluate patterns of water uptake under steady-state conditions. Then, to empirically evaluate how changes to root resistance influence nutrient allocation in an intact plant, we used split-root hydroponic basil (*Ocimum basilicum* L.) to test the effects of patchy nitrate on water uptake and ¹⁵N distribution in whole plants. We hypothesised that changes in the distribution of water uptake in split-root plants would match model predictions and that the distribution of ¹⁵N would be correspondingly more uniform when the ¹⁵N coincided with a high nutrient patch than when ¹⁵N was locally supplied in a uniform nutrient environment (Fig. 1).

Materials and methods

Models

To simulate the effects of patchy nitrate on xylem hydraulics, we use two different Ohm’s Law circuit models to simulate flow of resources within and between sectors (Fig. 2). The first model (Model 1) uses the same framework as Thorn and Orians (2011), directly simulating the fact that flow between sectors is the consequence of numerous connections at different heights up

the stem. To do this, the stem is simplified as two parallel axial pathways and the various routes for tangential flow between sectors are simulated by a large number of resistors ($n = 100$), each with resistance equal to $n \times r_t$, where r_t is the overall tangential resistance (Fig. 2a). The second model (Model 2) sacrifices some of the structural realism of Model 1 in favour of a simpler, less computationally intensive, circuit. Specifically, Model 2 assumes that axial resistance below the branch point is negligible, so that inter-sector pathways can be simulated as a single resistor with resistance r_t (Fig. 2b).

For both models, flow within and between sectors is driven by the root pressure (Ψ_r), which is assumed constant and the leaf

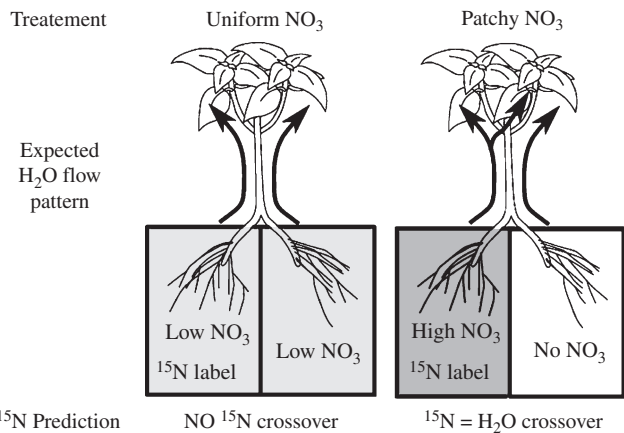


Fig. 1. Diagram of qualitative predictions for how water and nutrient movement will be affected if roots in a nutrient patch have lower resistance than roots in the background nutrient environment.

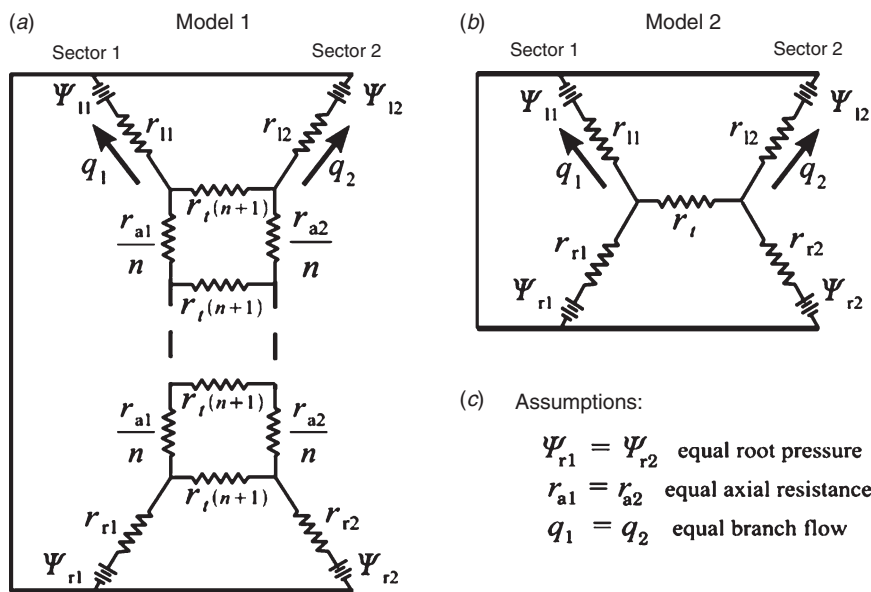


Fig. 2. Circuits for Ohm’s law models for hydraulic sectoriality. (a) Model 1 includes n repeating units in the stem, incorporating both axial (r_a) and tangential (r_t) resistance. (b) Model 2 is a simplified circuit representing the case in which $r_a = 0$. (c) We used the model to simulate water uptake by the two root sectors in response to localised changes in root resistance, under steady-state conditions and otherwise uniform hydraulics.

water potential for each sector ($\Psi_{l,2}$), which varies as a function of the content of water in the leaves of that sector. In particular, the water potential of the leaves of sector i (Ψ_{li}) is the sum of the osmotic (Ψ_{oli}) and pressure (Ψ_{pli}) potentials for those leaves:

$$\Psi_{li} = \Psi_{pli} + \Psi_{oli}. \quad (1)$$

Pressure potential is always positive and increases with water content above the turgor loss point (*sensu* Thornley and Johnson 2000):

$$\Psi_{pli} = \max(0, \varepsilon \times (c_{dw} W_{wli}/W_{li} - 1)), \quad (2)$$

where W_{wli} is the dynamically modelled mass of water in the leaves of a sector, W_{li} is the dry weight of leaves in a sector, ε is the elastic modulus of leaf cell walls and c_{dw} is the ratio of leaf DW to leaf water content at $\Psi_{pli}=0$. Osmotic potentials are always negative, but become more positive with increasing leaf water content:

$$\Psi_{oli} = -K_o \times W_{li}/W_{wli}, \quad (3)$$

where K_o is a parameter for the osmotic properties of the mesophyll cells.

The water content of leaves is dynamically modelled by:

$$dW_{wli}/dt = q_{li} - E_{ti}, \quad (4)$$

where q_{li} is the flux of water into the leaves of sector i calculated by solving our model circuit (Fig. 2a, b) and E_{ti} is the total rate of transpiration from leaves in the sector, assumed for our purposes to scale uniformly with leaf area.

Flows through the circuit used for Model 1 are determined by a large system of equations, which can be easily computed numerically, but for which the analytical solution is extremely complicated. Model 2 provides a more tractable system, with simple analytical solutions that can provide a more intuitive framework for quantifying expected flow. In particular, under steady-state conditions of equal transpiration from the two halves of the crown (Fig. 2c), the proportion of water uptake that is taken up by roots in sector 1 (P_{u1}) is given by the expression:

$$P_{u1} = (2 \times r_{r2} + r_{r1}) / (2 \times (r_{r1} + r_{r2} + r_t)), \quad (5)$$

where r_{r1} and r_{r2} are the root resistances of sectors 1 and 2 respectively.

The same default parameter values were used for both models, based on estimates for basil (Table 1).

Simulations

We used the models to evaluate the potential effect of root resistance changes on water uptake patterns. To simulate the steady-state sap fluxes for plants in asymmetrical nutrient environments, we varied the difference in hydraulic resistance between the two sectors, assuming resistance on the low nutrient side was elevated by a factor of 1.1, 1.25, 1.5, or 2.0. To understand the role of sectoriality in water uptake patterns we varied the tangential resistance from 0.0 to 0.1 MPa day mL⁻¹ a broad range that includes our unpublished measurements of 0.01 MPa day mL⁻¹ for mature basil plants. We also examined the importance of root resistance relative to the rest of the plant by varying the high nutrient root resistance from 0 to 0.01 MPa day mL⁻¹, a range that included published root resistances for herbaceous plants Newman (1973). For all simulations, we have presented output for the steady-state case, with equal flow into the two branches. We focussed on the consequences for the proportion of total water uptake by roots in the nitrate patch, which correlate with changes to water uptake for those roots, assuming that transpiration is unaffected. This variable directly determines the amount of water and, therefore, nutrients, expected to cross to the opposite side of the plant (Fig. 1).

We compared the predictions of the two models, using output from the simulations described above, as well as additional runs of the model with the axial resistance varied between 0.0005 and 0.05 MPa day mL⁻¹.

Cultivation of plants for ¹⁵N experiment

To evaluate the predictions of our model and assess the consequences for nitrate nitrogen allocation, we used basil (*Ocimum basilicum* L. var. sweet basil from Burpee, Warminsler, PA, USA), an ideal species for sectoriality studies because it grows quickly and has opposite leaf growth habit, permitting inter-sector comparison of leaves or branches of the same age.

Seeds were sown in January and February 2007 into moist MetroMix potting soil with water supplied from below. About 1 month after sowing, seedlings were transplanted into individual pots containing 50% sand and 50% zeolite (ZeoPro, Zeoponix, Inc., Boulder, CO). We watered plants every 2 days and added

Table 1. Parameter values used in hydraulic model

Symbol	Description	Default values	Units	Estimate based on
W_{li}	Dry mass of leaves of sector i	1.0	g	Our measurements in basil
ε	Elastic modulus of leaf cell walls	1.0	MPa	Thornley (1996)
c_{dw}	Ratio of leaf dry weight to leaf water content at $\Psi_{pli}=0$	0.15	g biomass mL ⁻¹	Comparing dry and fresh weights for basil leaves
K_o	Osmotic parameter	1.83	MPa	Thorn and Orians (2011)
r_{ri}	Resistance to water uptake by roots of sector i	0.0015	MPa day mL ⁻¹	Typical of herbaceous plants (Newman 1973)
r_a	Resistance to axial flow through stems of sector i	0.01	MPa day mL ⁻¹	Unpublished basil measurements
r_t	Stem tangential resistance	0.01	MPa day mL ⁻¹	Unpublished basil measurements
r_{li}	Resistance to axial flow through leaves of sector i	0.003	MPa day mL ⁻¹	Typical ratios of leaf to axial resistance (Sack <i>et al.</i> 2003)
Ψ_{sri}	Soil-root water potential associated with sector i	0.1	MPa	Typical of herbaceous plants (Taiz and Zeiger 2002)

70 mL of a Hoagland solution (603.0 μM $\text{Ca}(\text{NO}_3)_2$, 795.0 μM KNO_3 , 190.0 μM KH_2PO_4 , 270.0 μM MgSO_4 , 0.09 μM ZnSO_4 , 0.15 μM CuSO_4 , 20.0 μM CuSO_4 , 20.0 μM H_3BO_3 , 0.25 μM Na_2MoO_4 and 40.5 μM FeNa EDTA) twice a week. Throughout cultivation and experiments, plants were grown in a greenhouse at Tufts University, Medford MA, USA. Natural light was supplemented with 400 W sodium halide light to give a total of 16 h of light in each 24 h period. For the root hydraulics experiment, unpruned plants were harvested and roots excised in June 2007 (see below).

For the split-root experiment, we took advantage of the known vascular anatomy of plants in the Lamiaceae, in which each branch or leaf is fed by two vascular traces, which connect to roots in the same orthostichy (Rinne and Langston 1960; Murphy and Watson 1996). Plants were pruned just above the fourth node after fifth-node leaves had emerged. When lateral branches had emerged from the fourth node of decapitated plants, all other leaves and branches were removed from the main axis. On 10 October 2007, pruned plants were transferred to split-root hydroponics containers. We rinsed the roots and carefully split the taproot down the centre, such that each half of the root system was orthostichous to one of the two branches. Previous studies using dye tracers have confirmed that for basil plants in this type of split-root setup, orthostichous roots and branches share direct xylem connections not shared by structures on the opposite side of the plant (A. M. Thorn and C. M. Orians, unpubl. data). Parts of the main root too narrow to split were removed. Each half of the root system was trained into one of a pair of 250 mL uncapped Corning cell culture flasks containing hydroponic solution and stabilised using a stake taped to one side of the flask pair. Hydroponic containers received a 50% dilution of the Hoagland solution described above. Air was supplied at a minimum rate of 30 mL air min^{-1} to each half of the root system via plastic tubing (interior diameter = 1.6 mm). These plastic tubes were in turn attached to a 20 gauge hypodermic needle inserted into a short length of vacuum tubing connected to a pressure pump (Model UN035.1.2 STP, KNF Neuberger Inc., Trenton, NJ, USA). Once a day, deionised water was added to each container to maintain constant solution volume and the volume of water added was recorded as a measure of water uptake. Once every 2 days, just after the addition of the deionised water, the hydroponic solution was fully replaced for all containers.

Split-root experimental treatments were applied on 6 November 2007 and plants were harvested between 9 and 11 November 2007.

Measuring nitrate effects on root hydraulics in basil

To prepare plants for root hydraulic measurements, the base of the root system was wrapped in parafilm and the root system was cut from the rest of the plant underwater, cutting through the parafilm. Water-filled tubing was then pushed over the cut base of the root system and secured in place using a zip tie. The root system was placed in a hydroponic container with a 50% dilution of either the complete (+N) Hoagland solution described above or of a no nitrate (-N) Hoagland solution in which CaCl_2 and K_2SO_4 replaced $\text{Ca}(\text{NO}_3)_2$ and KNO_3 respectively. The hydroponic container was elevated 1 m above the height of an electronic

balance, on which the outflow of water from the root system was weighed at 10 s intervals.

For each of six plants, changes in flow rates were measured both when roots were immersed in +N solution and transferred to -N solution and when roots were immersed in -N solution and transferred to +N solution. A minimum of 60 min acclimation time was permitted for each nutrient environment. Measurements were made in the late morning (0900–1200 hours) for three plants and the mid afternoon (1400–1700 hours) for the other three plants. As has been observed in other species (e.g. Gloser *et al.* 2007), there was clear evidence of diurnal variation in root conductance, with flow rates trending upward during the hours when measurements were taken. To control for this diurnal variation, measurements were recorded both for the transition from +N to -N solution and from -N to +N solution and analysis focussed on short-term changes. For each transition, flow rates were measured immediately before and 10 min after nutrient solutions were changed. After flow measurements, roots were dried in a 70°C oven for a minimum of 3 days and dried roots were weighed.

This protocol does not permit calculation of the absolute hydraulic resistance of roots, but it does show the relative short-term change in resistance with nitrate environment. The flow is driven by a combination of gravitational potential (0.01 MPa for 1 m of elevation) and the osmotic potential of the roots xylem. Although we did not measure the xylem osmolarity, the literature suggests that it will only change modestly with nitrate supply (Gloser *et al.* 2007). To quantify the short-term effects of nitrate addition or removal on root conductance, we calculated the flow rate per unit dry mass for each plant in each of the nutrient environments. We used paired *t*-tests to compare the flow rates before and after nitrate addition and before and after nitrate removal.

Patchy nutrient and ^{15}N treatments

To determine the effects of patchy nitrate supply on water uptake and crown allocation of ^{15}N , 13 split-root hydroponic plants were divided into two treatment groups: a patchy nutrient treatment group ($n=6$) and a uniform nutrient treatment group ($n=7$) (Fig. 1). Starting on 6 November 2007, plants in the patchy nutrient treatment received 50% dilution +N solution in one container, with ^{15}N added as 2.0 to 2.5 mg $^{14}\text{NH}_4^{15}\text{NO}_3$ giving ~8 atom% ^{15}N for nitrate in the +N container. The other container for patchy treatment plants received a 50% dilution of unlabelled Hoagland -N solution. Plants in the uniform nutrient treatment received a 50% dilution of a 50% N Hoagland solution created by mixing equal volumes of the +N and -N solutions. For the uniform nutrient treatments, one container also received the same amount of ^{15}N label that was added for the patchy treatment (giving ~15 atom% ^{15}N for the labelled container, since the overall nitrate concentration is lower in these containers). For both treatments, the side of the plant receiving the ^{15}N label (and high nitrate for the patchy treatment) was randomised. After application of nutrient treatments, nutrients were not replenished before harvest, but water continued to be added to measure water uptake. Plants were harvested between 9 and 11 November 2007. Main roots, lateral roots, leaves and branches for each sector were separated from the main stem and

all plant components were oven dried and weighed. Each tissue type, except for labelled roots, were then ground in a ball mill and analysed for ^{15}N content at the UC Davis Stable Isotope Facility, Davis, CA.

Calculations – estimated water crossover and actual ^{15}N distribution

The proportion of total water uptake crossing from the labelled container to opposite side leaves was estimated as the % of leaf biomass on the opposite side (assumed to equal the % of total transpiration) minus the % of total water taken up in the first day of the treatment by roots in the opposite side container. This value was then divided by the % of water uptake from the labelled container to give the estimated fraction of water from the labelled container that would flow into leaves on the opposite side of the plant. This value was our prediction for the proportion of ^{15}N crossing over to the opposite side of each plant.

We compared this prediction to the actual rate of crossover of ^{15}N label. For all experimental plants, as well as an unlabelled control, the mass % ^{15}N was calculated for nitrogen in roots, stems, branches and leaves. For each sample the mass % ^{15}N was calculated as $(100 \times 15 \times \text{atom}\% \ ^{15}\text{N}) / (100 \times 15 + \text{atom}\% \ ^{15}\text{N} \times (15 - 14))$. Then the mass % ^{15}N label was calculated as the mass % ^{15}N in each sample, minus the mass % ^{15}N in the control. The resulting value was multiplied by the total N content of each tissue (= tissue mass \times sample mg N/sample mass) to give the total mass accumulation of ^{15}N label in each tissue. The percentage of ^{15}N crossing over to the opposite side of the plant was calculated as the total mass of ^{15}N label in opposite-side leaves, divided by the total mass of ^{15}N label in all leaves of that plant.

We had hypothesised that ^{15}N crossover would be proportional to water crossover when there was a net flow of water from the labelled side to the opposite side, but would be unaffected by water crossover when the flow was in the opposite

direction. To test this hypothesis, we divided the plants into two groups: those with a positive estimated water crossover and those with a negative water crossover. For each group, we used linear least-squares regression to test for correlations between the percentage of ^{15}N crossing to the opposite side and the proportion of water crossing to the opposite side.

Results

Model output

Results from both models showed that water uptake distribution should be affected by local changes to root hydraulics and that the change is sensitive to tangential resistance (Fig. 3). The maximum change in water uptake – and, therefore, in inter-sector flow – occurs at zero tangential resistance, with the proportion of water determined by the ratio of root resistances between the two halves of the plant. Thus, when resistance for the low nitrate roots is 2 times that of the high nitrate roots, two-thirds (0.67) of the water is taken up in the high nitrate sector. With a 1.5 times change in root resistance, three-fifths (0.60) of the water is taken up in the high nitrate sector and with a 1.2 times change in root resistance, six-elevenths (0.55) is taken up in the high nitrate sector.

However, the simulated proportion water taken up in the nitrate patch dropped off with increasing tangential resistance. At our estimated tangential resistance for basil and a 2 times change in root resistance, a 56% change in water uptake is expected in the high nitrate patch for Model 1 (boxed area of Fig. 3a), or about a 58% change for Model 2 (boxed area of Fig. 3b). Similar tangential resistance effects are observed with lower changes in root resistance with nutrient environment.

The simulated effects of tangential resistance decreased as root resistance is increased. According to our models, as the root resistance increases the proportion of water taken up on the high nitrate side asymptotically approaches its theoretical maximum (Fig. 4). This result reflects the equation for steady-state water

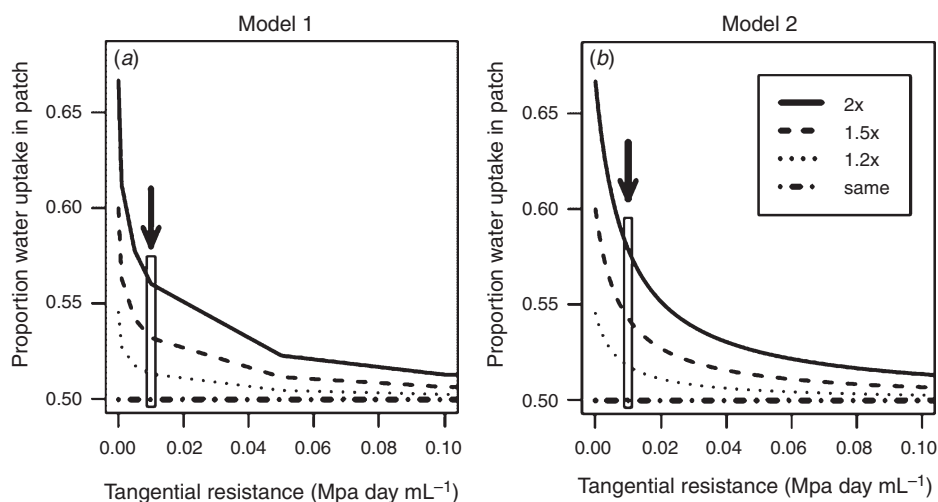


Fig. 3. Simulated patterns of water uptake as a function of the hydraulic resistance to tangential flow, based on (a) Model 1 and (b) Model 2. Hydraulic resistance in roots in low nutrients was either multiplied by a factor of 2, 1.5 or 1.2 times or was unchanged (same) relative to the high nutrient patch. For each model, an arrow and rectangle indicates the outputs for our default tangential resistance, as estimated for basil. Default values were used for all other parameters.

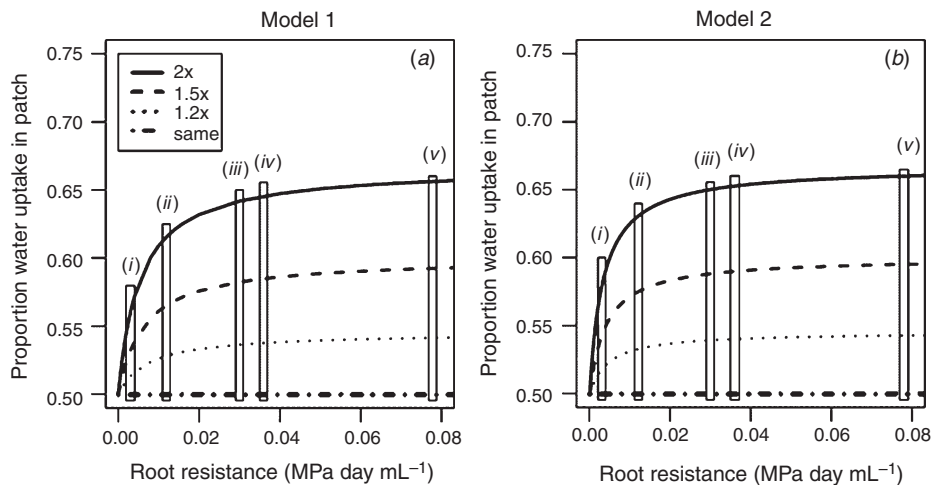


Fig. 4. Simulated patterns of water uptake as a function of the root resistance in high nutrient conditions, based on (a) Model 1 and (b) Model 2. Hydraulic resistance in roots in low nutrients was either multiplied by a factor of 2, 1.5 or 1.2 times or was unchanged (same) relative to the high nutrient patch. Simulated results for the herbaceous species by Newman (1973) are boxed and labelled with lower case Roman numerals: (i) tomato (= 0.003 MPa day mL⁻¹, our default value), (ii) maize (= 0.012 MPa day mL⁻¹), (iii) sunflower (= 0.03 MPa day mL⁻¹), (iv) dwarf bean (= 0.036 MPa day mL⁻¹) and (v) broad bean (= 0.078 MPa day mL⁻¹). These values are based on Newman's (1973) measurement of root conductance per root volume, assuming of 5 cm³ roots per sector. The value for tomato (i) was used as our default root resistance. Default values were used for all other parameters.

uptake distribution according to Model 2: as r_{t1} and r_{t2} get large, the effect of r_t diminishes (Eqn 5). Thus, at the relatively low root resistance estimated for tomato (Newman 1973), the proportion water taken up in the high nitrate patch is substantially below the theoretical maximum and is noticeably higher for Model 2 (Fig. 4b) than for Model 1 (Fig. 4a). At root resistances measured for other herbaceous species (Newman 1973), the effects are closer to their theoretical maximum. In particular, an increase in root resistance from the value for tomato (0.0015 MPa day mL⁻¹) to the value for maize (0.012 MPa day mL⁻¹) or sunflower (0.030 MPa day mL⁻¹) results in a substantial increase in nutrient effects, whereas the higher root resistances observed for dwarf bean (0.036 MPa day mL⁻¹) and broad bean (0.079 MPa day mL⁻¹) are already close to the theoretical maximum for both models (Fig. 4a, b).

It should be noted that although our two models are qualitatively similar, they do not give identical output (Fig. 5). The similarity between the models was greatest when axial resistance was less than or equal to half of the tangential resistance. At higher ratios of axial resistance to tangential resistance, Model 2 predicted a larger proportion of water taken up in the high nutrient patch compared with Model 1 (Fig. 5). At our default values, the ratio was 1.0, resulting in moderate discrepancies between the two models.

Nitrate effects on excised roots

Water flow through excised basil roots that had acclimated >1 h in -N solution was significantly lower than the same roots acclimated >1 h in +N solution. This change in flow rates was much faster when +N solution was replaced with -N solution than when -N was replaced with +N, as indicated by a significant (paired *t*-test, $t = -2.81$, d.f. = 4, $P < 0.05$) change within 10 min

for nitrate removal, but not over the time scale for nitrate addition (paired *t*-test, $t = 1.06$, d.f. = 4, $P = 0.35$). The average ratio of flow rates between the +N and -N treatments was 1.4 ± 0.3 (mean \pm s.d.) (see supplementary data available as an Accessory Publication to this paper).

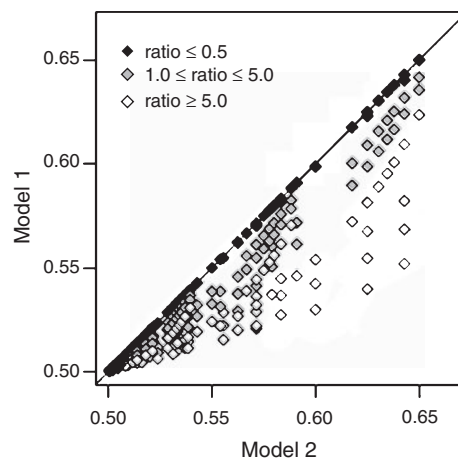


Fig. 5. Relationship between outputs from the two models analysed in this paper using the same parameters. The scatter of points here represents results when tangential resistance between 0.0005 and 0.1 MPa day mL⁻¹, when low root resistance is varied between 0 and 0.01 MPa day mL⁻¹, when the ratio between high and low root resistances is varied between 1.1 and 2.0 and when axial resistances varied between 0.0 and 0.05 MPa day mL⁻¹. Otherwise, model defaults are used. Shading indicates the ratio of axial resistance to tangential resistance, with ratios up to 0.5 shown in black, ratios from 1.0 to 5.0 shown in grey and ratios above 5.0 shown in white.

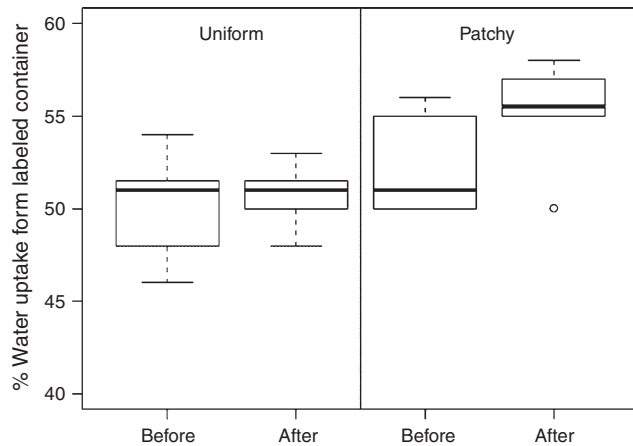


Fig. 6. Average percentage water uptake from the labelled container for plants with patchy ($n=6$) or uniform ($n=7$) nitrate supply, before and after beginning of nutrient treatments and labelling. No significant change was observed for the uniform treatment (paired t -test, $t=-0.57$; d.f. = 6; $P=0.59$). For the patchy nutrient treatment, a marginally significant increase was observed (paired t -test, $t=-2.16$; d.f. = 5; $P=0.08$).

Patchy nutrient and ^{15}N experiment

Application of the patchy nitrate treatment resulted in a slight and marginally significant trend of increased proportion of water uptake from the high nitrate (labelled) side of the root system (paired t -test, $t=2.16$; d.f. = 5; $P=0.08$) whereas no change in water uptake was observed for the uniform nutrient treatment (paired t -test, $t=0.57$; d.f. = 5; $P=0.59$) (Fig. 6).

There was no significant treatment effect on ^{15}N distribution (t -test, $t=-0.20$; d.f. = 10.5; $P=0.85$), but as predicted by the model, the pattern of ^{15}N distribution in the leaves (as expressed by the % ^{15}N above background that was found in the leaves of the

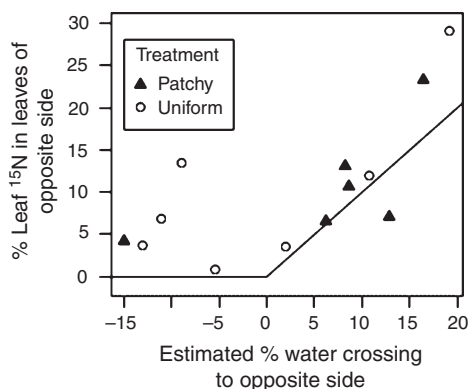


Fig. 7. Percentage of ^{15}N label accumulation in opposite branch leaves as a function of the estimated percentage water crossing over to the opposite side in the first day after nutrient treatments and labelling were applied. Estimated percentage of water crossing to the opposite side is calculated as (% leaf area on opposite side - % water uptake on opposite side)/(% water uptake from labelled side). The curve is the predicted distribution if ^{15}N label is passively carried by water movement (= % water crossover for water crossover $>0\%$, = 0% for water crossover $<0\%$). Closed triangles indicate plants receiving the patchy nitrate treatment (with all nitrate supplied to the labelled side of the plant). Open circles indicate plants receiving the uniform nitrate treatment.

unlabelled side of the plant) was positively related to the estimated percentage when the estimated percentage water crossover was positive ($R^2=0.77$; $P<0.01$) and unrelated when estimated percentage water crossover was negative ($R^2<0.01$; $P=0.97$) (Fig. 7). The ^{15}N distribution for individual plants varied as predicted from water uptake patterns, but with higher values for the calculated % ^{15}N crossover than what would be predicted based on estimated water crossover.

Discussion

Taken together, our modelling and empirical work demonstrate that xylem sectoriality can limit the effect of dynamically varied root resistance on water uptake patterns and support the hypothesis that these changes in water uptake directly influence the internal distribution of nitrogen taken up in a nitrate patch.

Outputs from both of our models support the expectation that patterns of water uptake following a local change in root resistance should be constrained by stem tangential resistance in basil. However, the simulated effect of sectoriality also depends on the magnitude of the root resistance relative to the tangential resistance: when root resistance is higher, a small relative change produces a larger change in water uptake. Model 2 – which is much simpler and, therefore, less computationally intensive than Model 1 – provide similar outputs to Model 1, especially when the ratio of axial to tangential resistance is low. Under these conditions, Model 2 can be useful for general predictions about sectorised water transport. Eqn 5 provides an easy means of calculating expected patterns of water uptake, although it should be emphasised that this equation assumes symmetry of biomass distribution. When circumstances are less equal, such as when only a small portion of the root system is in the nutrient patch, these models would have to be modified to account for differences between the two sectors.

Results from the root conductance experiment suggest that, on average, the root conductance is 40% higher in a high nitrate environment than a no nitrate environment, i.e. that the resistance is 40% higher in the no nitrate environment. The simulations suggest that this relative change in root resistance should result in between 52 and 53% of water uptake in the nitrate patch when 50% of the root system receives nitrate. In actuality, the average proportion of water taken up in the nutrient patch was 56%. This difference is unsurprising given the difference in time scales between the two studies, 1 hour for the root conductance experiment vs several days for the patchy nutrient experiment, but could also be explained if the background root resistance in basil is higher than Newman's (1973) value for root resistance in tomato and more similar to his measurement for sunflower or bean (Newman 1973). Dynamic changes to stem sectoriality due to xylem hydrogel properties could also contribute to increased crossover not accounted for by our models (Zwieniecki *et al.* 2001).

We hypothesised that changes to root resistance would result in nitrogen crossover rates proportional to the resulting changes in the water uptake distribution. Our results support this hypothesis. We were able to detect a marginally significant change in water uptake by comparing water uptake before and

after the nutrient treatments were applied. Since before and after comparisons could not be made for nitrogen allocation, we would not expect a significant treatment effect on ^{15}N distribution given these results and indeed no treatment effect was observed. Also as expected, the observed patterns of ^{15}N distribution were significantly correlated with our predictions based on water uptake patterns. This strongly suggests that any short-term adjustments to nitrate allocation in basil are mediated by xylem fluxes rather than, e.g. by activity of transfer cells (Pate and Jeschke 1995). These changes to xylem fluxes most likely result from the aggregated effects of root conductivity changes (Gloser *et al.* 2007) and stem xylem conductance changes due to hydrogel responses to ion concentrations (Zwieniecki *et al.* 2001).

The confirmation that nitrate from a patch follows xylem fluxes suggests a previously unexamined benefit of physiological changes to root conductance in response to nutrient heterogeneity. Previous studies have emphasised the role of these adjustments in increasing water flow through the soil towards roots in nutrient patches, thereby drawing mobile soil ions towards those roots and increasing whole plant competitiveness (Gloser *et al.* 2007; Gorska *et al.* 2008; Maurel *et al.* 2010). We present the view that there could also be benefits for nutrient allocation within the plant. Although the model outputs presented here suggest only a modest increase in nutrient crossover due root hydraulic changes, larger effects are predicted by a less symmetrical circuit, e.g. Thorn and Orians (2011), suggesting a larger effect of root hydraulics on nutrient movement when only a small portion of the root system experiences high nitrate conditions. Greater benefits are also expected when a patchy nutrient environment is combined with a patchy light environment (Thorn and Orians 2011) or localised defoliation (Orians and Jones 2001). If, for example, most of the transpiration occurs in a portion of the crown in a canopy gap, then changes in root conductance will ensure that a higher proportion of xylem sap delivered to those leaves contains high nitrate content, maximising photosynthesis and growth potential in the light patch. Conversely, the importance of increased movement of nitrate towards the roots through the soil may be of greater importance for plants in direct competition with one another. Future studies should evaluate the relative selective benefit of these factors through ecologically realistic competition experiments, e.g. Hodge *et al.* (1999).

In conclusion, we have shown that basil, like sunflower (Gloser *et al.* 2007), tomato and cucumber (Gorska *et al.* 2008), adjusts its root hydraulic properties in response to its nitrate environment. We confirm that there is also an elevated proportion of water taken up by roots in a nitrate patch for split-root hydroponic basil. In addition, we have shown that the distribution of ^{15}N label applied in the nitrate patch can be explained by water uptake patterns. These observations indicate that the short-term crown distribution of nitrate nitrogen is determined by xylem fluxes. Thus, our research suggests an adaptive reason for nutrient-dependent changes in root hydraulics that has not been previously explored. Further research is needed to determine the relative role of root hydraulics and xylem hydrogel properties in influencing these fluxes. Other fruitful avenues for future research should include closer examination of the mechanisms for uptake changes and

competition experiments to establish the fitness benefit of root hydraulic changes.

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