Nitrogen regulation of transpiration controls mass-flow acquisition of nutrients

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Abstract

Transpiration may enhance mass-flow of nutrients to roots, especially in low-nutrient soils or where the root system is not extensively developed. Previous work suggested that nitrogen (N) may regulate mass-flow of nutrients. Experiments were conducted to determine whether N regulates water fluxes, and whether this regulation has a functional role in controlling the mass-flow of nutrients to roots. Phaseolus vulgaris were grown in troughs designed to create an N availability gradient by restricting roots from intercepting a slow-release N source, which was placed at one of six distances behind a 25 μm mesh from which nutrients could move by diffusion or mass-flow (termed ‘mass-flow’ treatment). Control plants had the N source supplied directly to their root zone so that N was available through interception, mass-flow, and diffusion (termed ‘interception’ treatment). ‘Mass-flow’ plants closest to the N source exhibited 2.9-fold higher transpiration (E), 2.6-fold higher stomatal conductance (gₛ), 1.2-fold higher intercellular [CO₂] (Cᵢ), and 3.4-fold lower water use efficiency than ‘interception’ plants, despite comparable values of photosynthetic rate (A), E, gₛ, and Cᵢ first increased and then decreased with increasing distance from the N source to values even lower than those of ‘interception’ plants. ‘Mass-flow’ plants accumulated phosphorus and potassium, and had maximum concentrations at 10 mm from the N source. Overall, N availability regulated transpiration-driven mass-flow of nutrients from substrate zones that were inaccessible to roots. Thus when water is available, mass-flow may partially substitute for root density in providing access to nutrients without incurring the costs of root extension, although the efficacy of mass-flow also depends on soil nutrient retention and hydraulic properties.

Key words: Interception, phosphate, potassium, urea, water flux, water use efficiency.

Introduction

Terrestrial plants transpire 32 trillion tonnes of water vapour annually (Hetherington and Woodward, 2003). Although this is commonly viewed as a by-product of photosynthetic CO₂ uptake (e.g. Cowan and Troughton, 1971; Monteith, 1988; Kramer and Boyer, 1995), large variations in the rate at which water is traded for each CO₂ mole assimilated [i.e. water use efficiency (WUE); Hack et al., 2006] as well as evidence for substantial night-time transpiration in photosynthetically inactive C₃ and C₄ plants (Caird et al., 2007; Kupper et al., 2012) suggest that transpiration plays an important functional role in plants. Apart from facilitating leaf cooling (Parkhurst and Loucks, 1972; Nobel, 1999) and root to shoot solute transport (Tanner and Beevers, 1990, 2001), transpiration also powers the movement of water and dissolved nutrients to root surfaces by mass-flow (Barber, 1995; Tinker and Nye, 2000; Cramer et al., 2008; Cramer and Hawkins, 2009), reducing rhizosphere nutrient depletion resulting from active nutrient uptake (Scholz et al., 2007; Kupper et al., 2012).

Although mass-flow plays no direct role in uptake across the plasma membrane, the increased rhizosphere solute

Abbreviations: A, photosynthetic rate, Cᵢ, atmospheric [CO₂], Cₛ, intercellular [CO₂], E, transpiration rate, gₛ, stomatal conductance, PAR, photosynthetically active radiation, WUE, water use efficiency.

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concentrations may enhance membrane nutrient transport (Cernusak et al., 2011). Thus, transpirational water fluxes appear to play a fundamental role in nutrient acquisition, explaining their up-regulation in plants grown in low-nutrient soils (Wilkinson et al., 2007; Cramer et al., 2008; Kupper et al., 2012) and their down-regulation in plants grown under supraoptimal nutrient supply (Wilkinson et al., 2007). High transpirational water fluxes may be especially important in the acquisition of mobile nutrients or in zones where roots are sparsely distributed (Scholz et al., 2007; Cramer et al., 2008; Kupper et al., 2012). While mathematical models have been used to estimate the spatial extent of nutrient depletion around the rhizosphere (e.g. Rengel, 1993; Syring and Claassen, 1995), the magnitude of the distance over which mass-flow is effective remains unknown. Since knowledge of the spatial scale over which mass-flow operates is highly relevant to our understanding of nutrient acquisition from the soil (e.g. the merits of producing smaller, denser versus larger, sparser root networks), there is a clear need for further work.

Although half a century has passed since water fluxes were first suggested to be important for nutrient uptake (Barber, 1962), the role of nutrients in regulating water fluxes remains poorly understood (Raven, 2008). Several authors have suggested a critical signalling role for xylem nitrogen concentration ([N]) in the regulation of water fluxes (Wilkinson et al., 2007; Gloser et al., 2007; Gorska et al., 2008; Cramer et al., 2009), but this idea lacks substantial empirical support (Gorska et al., 2008). Cramer et al. (2008) observed elevated water fluxes in Ehrharta calycina (Poaceae) in response to restricted nutrient access; their results suggest a key regulatory role for N. Subsequently, Cramer et al. (2009) proposed a model of N regulation in which NO$_3^-$ modulates root hydraulic conductance through its control of aquaporins, and foliar nitric oxide (NO) modulates stomatal conductance ($g_s$), alongside the regulatory effects of pH and phytohormones. The interaction of these processes is expected to generate a biphasic response of $g_s$ to [N] (Wilkinson et al., 2007) in which decreasing N availability stimulates $g_s$ until some threshold value is reached, beyond which it once again decreases, probably due to compromised growth. Existing models have emphasized the role of NO$_3^-$ in regulating water fluxes (Wilkinson et al., 2007; Cramer et al., 2008, 2009; Kupper et al., 2012), neglecting the potential regulatory effects of NH$_4^+$. Given the importance of ammonical fertilizers (i.e. including urea) in agriculture (Miller and Cramer, 2004), however, understanding the regulatory effects of ammonical fertilizers on water flux is important.

Water use efficiency (WUE) has been found to be more strongly positively correlated with the foliar ratio of N to phosphorus (N:P) than with foliar [N] (Cernusak et al., 2011), identifying foliar N:P as a more likely modulator of WUE than foliar [N]. However, Garrish et al. (2010) found that plant water fluxes at a given vapour pressure deficit varied as a function of foliar [N]. The internal to ambient CO$_2$ mole fractions, $C_i$/$C_a$, and δ$^{13}$C also indicated that water fluxes varied as a function of N availability, but not as a function of P availability (Cernusak et al., 2007; Garrish et al., 2010), suggesting that excess N in the cytosol (measured as foliar N:P) may modulate water fluxes.

Phaseolus vulgaris was used here to determine whether N, supplied as urea, regulates water fluxes and consequent nutrient delivery to plants and whether such fluxes display biphasic responses or monotonic decline with distance from the N source. The plants were grown in PVC troughs in which a mesh screen prevented their roots from accessing the N source directly, though they had free access to all other nutrients. The N fertilizer was placed at varying distances from the screen, thus creating a gradient of N accessibility, while a control treatment comprised plants having free access to all nutrients including N. It was hypothesized that plants lacking direct access to N should show higher water fluxes than control plants and that water flux should increase with distance from the N source to an optimum value, beyond which it should decline due to N deficiency.

Materials and methods

Plant culture

Forty-two PVC 6 litre troughs (Price and Sons Inc., South Africa) were divided into two sections, one for growing plants (4 litres) and another for nutrient supply (2 litres), using PVC plates with a 30 cm$^2$ window covered with a nylon mesh (Nytal 25 µm; Draht-Center, Stuttgart, Germany). The mesh screen restricted roots to one compartment while allowing free transfer of solutes between the compartments (Fig. 1). The plant compartment and the nutrient compartment were filled with 4 kg and 2 kg, respectively, of thoroughly rinsed acid-washed sand (pH ~7, grade 30/10, Consol Minerals, Cape Town, South Africa). Two Phaseolus vulgaris cv. Starke 2000 plants (Starke Ayres, Cape Town, South Africa) were planted on one side of the partitioning plate, 5 cm from the plate. In the nutrient compartment a 6.5 cm$^2$ core was excavated in wet sand using a 9 mm diameter cork borer at 0, 9, 18, 27, 36, and 45 mm from the mesh to allow addition of fertilizers, thus creating a gradient of N availability. The N source was Multicote 4* urea–N (42-0-0 N:P:K, Haifa Group, South Africa), a slow-release granular fertilizer encapsulated in a multilayer polymeric coating. A 5 g aliquot of Multicote 4* was placed in the sand cores and they were covered with sand. The use of slow-release urea fertilizer avoids rapid volatilization of N. An independent chemical assay of Multicote 4* indicated a 50% (w/w) [N] and a δ$^{15}$N value of –0.84‰. Because roots could not penetrate the fertilizer compartment, nutrient acquisition was only possible through diffusion and mass-flow of solutes to the roots, and these were consequently termed ‘mass-flow’ plants. Roots of control plants could intercept nutrients, and were consequently referred to as ‘interception’ plants, although both mass-flow and diffusion must also contribute to nutrient mobility in this treatment.

All plants were cultured in a glasshouse at the University of Cape Town for 64 d. Water was supplied sparingly (200 ml d$^{-1}$) using spray bottles to prevent leaching of the sand. The sand was maintained at water contents of between 0.15 and 0.2 g H$_2$O g$^{-1}$ dry weight (DW) sand, which was separately estimated as gravimetric moisture from six additional pots for each treatment. The plant compartment was supplied twice weekly with 200 ml of N-free Long Ashton nutrient solution (Hewitt, 1952) containing 2.4 mM PO$_4^{3-}$, 2 mM K, 4 mM Ca, 1.5 mM Mg, 3.5 mM SO$_4^{2-}$, 0.1 mM FeEDTA, 0.02 mM Mn, 0.14 mM H$_2$BO$_3$, 4.2 mM Na, 4 mM Cl, 0.003 mM Cu, 0.0002 mM Mo, and 0.002 mM Zn. Plants were exposed to uniform growing conditions by regularly rearranging the positions of the troughs within the glasshouse every second day. The greenhouse received an average light intensity of 1660 μmol m$^{-2}$ s$^{-1}$, and daytime relative
humidity inside the greenhouse was ~40% (Power et al., 2010), while the temperatures were kept below 25 °C (day) and above 15 °C (night). After 62 d, the plants were transferred to a growth chamber and left to acclimatize for 48 h prior to gas exchange measurements. The growth chambers were equipped with 14 × 400 W HQI-T metal halide lights (Osram Powerstar, Osram, Cape Town, South Africa), 28 × 400 W NAV-T sodium lights (Osram Violox), and 24 × 150 W, 230 V incandescent (Sicca, Osram, Cape Town) lamps providing a light intensity of 1000–1200 μmol m⁻² s⁻¹ with 16 h light and 8 h dark and day/night temperatures of 25 °C/20 °C, with mean day/night relative humidity of ~65%.

Gas exchange analysis
Gas exchange measurements were performed on the third fully expanded leaf of each plant. All plants were watered before the gas exchange measurements. Photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), and intercellular [CO₂] (Ci) were determined using a Licor 6400-02B cuvette connected to a portable gas exchange system (LICOR6400, Li-Cor, Inc., Lincoln, NE, USA). Gas exchange characteristics were measured after equilibration in the cuvette (~5 min) at a saturating photosynthetically active radiation (PAR) level of 1500 μmol quanta m⁻² s⁻¹ (determined from preliminary light response curves) with 400 μl l⁻¹ CO₂ and a flow rate of 500 μmol s⁻¹. Leaf temperature was maintained at 25 °C and relative humidity was ~65% during the measurements. WUE was calculated as A/gs.

Biomass measurements
Shoot and root biomass was measured at the end of the 64 d growth period, at their early reproductive stage. There were no apparent differences in the developmental stages of plants, apart from variations in plant sizes. The root systems were carefully excavated onto 2 mm sieves and the sand removed under running water. Above-ground biomass was separated from root material and dried at 70 °C for 48 h in a forced draught oven and weighed. Since nodules were absent from all plants, only total root biomass was measured. The shoot biomass of each plant was milled in a Wiley mill using a 0.5 mm mesh (Arthur H. Thomas Co., Philadelphia, CA, USA). The milled material was analysed for tissue nutrient concentrations and used for mass spectrometry.

Foliar elemental and isotope analysis
Foliar nutrient concentrations were determined by ashing the milled leaf material at 480 °C for 8 h before dissolving 1:1 (v/v) with HCl (Kalra, 1998). Assessment of the element concentrations in solution was performed using inductively coupled plasma atomic emission spectrometry (Varian Vista MPX, Mulgrave, Australia). To verify the N source used by plants, foliar [%N] and 15N/14N isotope ratios (expressed as δ¹⁵N) were determined using mass spectrometry. Atmospheric N₂ fixation is expected to give a δ¹⁵N signature closest to the natural abundance values of almost zero, whilst urea-N should show an enriched δ¹⁵N signature due to losses of N through volatilization (Högberg and Johannisson, 1993). Based on variations in foliar δ¹⁵N, N sources used by plants may be distinguishable (e.g. Vitousek et al., 1989; Robinson, 2001; Cernusak et al., 2009). Between 1.9 mg and 2.0 mg of ground leaf sample was weighed into a 5 mm × 9 mm tin capsule (Santis Analytical AG, Teufen, Switzerland). The tin capsules were then combusted in a Thermo Flash EA 1112 series elemental analyser coupled to a Delta Plus XP isotope ratio mass spectrometer via a Thermo Finnigan Conflo III control unit (Thermo Electron Corporation, Milan, Italy). International Atomic Energy Authority standards were used to determine the values.

Data analysis
One-way analyses of variance (ANOVA) and post-hoc Tukey’s HSD tests were performed using Statistica (version 10 Statsoft Inc., Tulsa, OK, USA) to evaluate differences in total biomass, shoot/root ratios, water flux, and foliar nutrient content between the fertilizer treatments. Linear models relating total biomass, E, gs, WUE, C₅, A, and foliar elemental concentrations to distance from the N source were generated in R (R Development Core Team, 2006). In each instance, model optimality was determined using Akaike information criterion (AIC) scores (Crawley, 2005). Analyses of covariance (ANCOVA) were used in comparing the slopes of A versus gs.
Results

Plant biomass response to N accessibility

The biomass of ‘mass-flow’ *P. vulgaris* plants was negatively correlated with distance from the N source (Fig. 2A), indicating that growth was limited by N availability. When the N source was adjacent to the plants (i.e. plants 0 mm from N source), the biomass of ‘mass-flow’ plants was statistically indistinguishable from that of ‘interception’ plants, as were their shoot:root ratios. Despite evidence of increased N limitation with distance from the N source, shoot:root ratios of mass-flow plants did not change significantly with distance from the N source (Fig. 2B).

Gas exchange response to N accessibility

Although *g*ₚ and *A* were positively correlated in both ‘mass-flow’ and ‘interception’ plants, the latter showed a steeper slope (Fig. 3), indicating that changes in *g*ₚ and *A* were approximately half as responsive in ‘mass-flow’ as in ‘interception’ plants (ANOVA interaction term: *t* = –2.495; *P* = 0.014). ‘Mass-flow’ plants had a wider range of *g*ₚ values (0.1–1.1 μmol m⁻² s⁻¹) than the ‘interception’ plants (<0.4 μmol m⁻² s⁻¹), suggesting a greater plasticity of response of stomatal conductance in ‘mass-flow’ plants.

*A*, *g*ₚ, *E*, and *Cᵢ* of the ‘mass-flow’ plants all declined with increasing distance from the N source, attaining the highest values over a range of distance from 0 mm to 20 mm (Figs 4, 5).

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**Fig. 2.** (A) Biomass (g dry weight per plant) and (B) shoot:root ratios of *Phaseolus vulgaris* plants accessing a slow-release urea fertilizer either dibbled around the roots (‘interception’, open circles) or placed at six distances from the mesh barrier (‘mass-flow’, filled circles). Each symbol and bar represents a mean ±SE (*n* = 6). The regression equation relates total biomass to distance from the N source.

**Fig. 3.** Correlation of stomatal conductance (*g*ₚ) with photosynthetic rate (*A*) in *Phaseolus vulgaris* accessing a slow-release urea fertilizer either dibbled around the roots (‘interception’, open circles) or placed at six distances from the mesh barrier (‘mass-flow’, filled circles). Each symbol and bar represents a mean ±SE (*n* = 6). The regression equation, coefficient of determination (*R²*), and probability of significance (*P*) are shown on the panel. (This figure is available in colour at *JXB* online.)

**Fig. 4.** Variation of the photosynthetic rate (*A*) with distance from the N source in *Phaseolus vulgaris* accessing a slow-release fertilizer either by ‘interception’ (open circles) or by ‘mass-flow’ (filled circles). Each circle and bar represents a mean ±SE (*n* = 6). Means with different letters showed significant differences after a one-way ANOVA with post-hoc Tukey’s HSD. The regression equation, coefficient of determination (*R²*), and probability of significance (*P*) are shown on the graph.
While ‘mass-flow’ plants adjacent to the N source (0 mm) had similar A to that of ‘interception’ plants, the former had much higher E (2.9-fold), gₛ (2.6-fold), and Cᵢ (1.2-fold), and lower WUE (3.4-fold). E and gₛ of ‘mass-flow’ plants were statistically indistinguishable from those of ‘interception’ plants between 27 mm and 45 mm from the N source. The changes in A and E resulted in increased WUE with distance from the N source.

**Foliar nutrient responses to N accessibility**

Foliar [N] had an asymmetric biphasic relationship with increasing distance from the N source, with a peak at ~10 mm (Fig. 6A). Foliar N contents were negatively correlated with distance from the N source (Fig. 6B), corroborating the importance of N in limiting growth. No nodules were observed, and the positive δ¹³C values ranging from 5‰ to 15‰ (mean 11‰, n=42), suggest use of a more enriched N source following volatilization of NH₃ from urea-N (e.g. Högberg, 1990; Högberg and Johannisson, 1993).

Foliar [P] and [K] increased with distance from the N source (Fig. 6C, E), reaching their maxima at peak foliar [N] (~10 mm from the N source), and total P and K contents declined as the distance from the N source increased (Fig. 6D, F). To determine whether water fluxes are modulated by foliar [N] or excess N (i.e. N:P), the foliar [N] and N:P ratios were correlated to δ¹³C values (Fig. 7). Plant δ¹³C was used as it provides a time-integrated estimate of intercellular to ambient CO₂ mole fractions (Cᵢ/Cₐ) (Farquhar et al., 1982; Brugnoli and Farquhar, 2000), which are a long-term proxy of WUE. δ¹³C was more strongly correlated with N:P (R²=0.53; P = 0.001) than with [N] (R²=0.29; P=0.03; Fig. 7). The low δ¹³C values also indicated no water stress in these plants, as may be expected, since the sand was kept close to field capacity.

**Discussion**

The present study indicates that N availability partially regulates transpiration, and that transpiration modulates the acquisition of other nutrients. Two lines of evidence support the interpretation that N availability varied with distance from the N source. First, in the absence of nodulation, the supplied N fertilizer constituted the only source of plant-accessible N. The high foliar δ¹³N values indicate that the plants utilized the urea-N from which NH₃ had been lost through volatilization, resulting in higher δ¹³N values than that of the plants supplied urea (e.g. Högberg, 1990; Högberg and Johannisson, 1993), suggesting that the slow-release fertilizer was the main source of N. Secondly, plant biomass and tissue [N] declined with distance from the N source.

![Fig. 5. Relationship between distance from N source and (A) transpiration (E), (B) stomatal conductance (gₛ), (C) internal CO₂ concentration (Cᵢ), and (D) water use efficiency (WUE) in Phaseolus vulgaris accessing a slow-release N-fertilizer either by ‘interception’ (open circles) or by ‘mass-flow’ (filled circles). Each circle and bar represents a mean (n=6) ±SE. Means with different letters showed significant differences (at significance value P) after a one-way ANOVA with post-hoc Tukey’s HSD. The equations for the fitted lines, coefficients of determination (R²), and significance values (P) are indicated in each panel.](https://academic.oup.com/jxb/article-abstract/65/1/159/429577)
indicating N limitations. Although the $[\text{NH}_4^+]$ derived from the supplied urea was probably higher than that of $[\text{NO}_3^-]$ in the sand, both $\text{NH}_4^+$ and $\text{NO}_3^-$ were likely to be present in the sand as a result of hydrolysis and nitrification of urea-N (Sahrawat, 1980). Thus it is not possible to differentiate the effects of the different N forms from these data. The plants were not limited by water and, therefore, variation in transpiration was not a consequence of differences in the available water. This lack of water limitation may facilitate regulation of transpiration by N, resulting in greater water flux when N availability is restricted, but not deficient. Thus, the notion of transpiration as a passive wicking of water from soils by vascular plants when stomata open for CO$_2$ uptake (e.g. Nobel, 1999) is questionable and needs to incorporate the regulatory role played by N.

To verify that N regulates transpiration, a common agricultural N fertilizer (urea) was placed at varying distances from the plants, as opposed to varying access to a complex fertilizer containing a suite of nutrients, such as used by Cramer et al. (2008). The hypothesis that N regulates transpiration was supported by the higher $E$, $g_s$, and $C_i$ values and lower WUE in ‘mass-flow’ than ‘interception’ plants at 0 mm from the N source. This was associated with differential responsiveness of $A$ to $g_s$ in ‘interception’ versus ‘mass-flow’ plants. Consistent with changes in WUE, the slope of $A/g_s$ for ‘mass-flow’ plants was half that of ‘interception’ plants and there

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**Fig. 6.** Relationship between distance from the N source and (A) foliar [N], (B) foliar N content, (C) foliar [P], (D) foliar P content, (E) foliar [K], and (F) foliar K content in *Phaseolus vulgaris* accessing a slow-release fertilizer either by ‘interception’ (open circles) or by ‘mass-flow’ (filled circles). Each circle and bar represents a mean ($n=6$) ±SE. Significant differences among distances (one-way ANOVA with post-hoc Tukey’s HSD). The regression equation, coefficient of determination ($R^2$), and probability of significance ($P$) are shown on the graph.
was a greater range of \( g_s \) in ‘mass-flow’ plants challenged with limited N availability. This indicates that \( A \) was not strongly limited by \( g_s \), as was also apparent from the higher \( C_i \) values in the ‘mass-flow’ plants <36 mm from the N source than in the ‘interception’ plants. Instead, \( A \) is likely to be limited by demand for photosynthate that is determined by growth rates (e.g. McCormick et al., 2008). Nutritionally induced elevation of \( E \) and \( g_s \) is consistent with a role for transpiration in increasing water flow through soil, thereby compensating partly for reduced availability of N in the rhizosphere. Indeed, low WUE has been observed in a wide range of plants grown with limited nutrients (e.g. Raven et al., 2004).

Since ‘interception’ plants had more direct access to the N source, the lower \( E \) of these plants may be linked to supraoptimal [N], as predicted by Wilkinson et al. (2007) (Fig. 8). However, the limitation in access to N imposed on the ‘mass-flow’ plants within 20 mm of the N source resulted in strong up-regulation of \( E \). The almost monotonic decline in \( E \) beyond 20 mm suggests the down-regulation of \( E \) evoked by the increasing limitation in N availability, as has been previously observed (e.g. Radin and Parker, 1979; Radin and Ackerson, 1981). The present ‘interception’ and ‘mass-flow’ data seem to match parts of the biphasic trajectory proposed by Wilkinson et al. (2007) (Fig. 8). The decline in total biomass with increasing distance from the N source also implies decreasing mass-flow delivery of N. This is consistent with the fact that water flux density at distance \( (d) \) from the root axis must be proportional to \( 1/d^2 \) (Tinker and Nye, 2000).

Despite the gradual N limitation of biomass accumulation with distance from the N source, shoot:root ratios did not vary significantly, suggesting that these plants adjusted their transpiration more than allocation to root biomass for N uptake. Such physiological adjustment of water flux may precede the well-established changes in carbon allocation for adjustment of shoot:root ratios (e.g. Forde, 2002) and accompanying changes in root architecture in response to nutrient supply limitations (Wiersum, 1958; Hackett, 1968; Forde and Lorenzo, 2001).

The higher transpiration rates of ‘mass-flow’ plants were associated with higher foliar [P] and [K], these reaching maximum concentrations when the distance from the N source was 10 mm and transpiration was high. Beyond this distance, foliar [P] and [K] remained constant, presumably because of reduced P and K demand as N availability limited biomass production, possibly resulting in feedback suppression of P and K uptake (Marschner and Cakmak, 1986; Valizadeh et al., 2002; Hammond and White, 2008; Tsay et al., 2011). The stronger relationship between \( \delta^{13}C \) and N:P ratios than

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**Fig. 7.** Relationship of (A) foliar [N] and (B) foliar N:P with \( \delta^{13}C \) in Phaseolus vulgaris accessing a slow-release fertilizer sequestered from six distances through a mesh-lined substrate compartment. The regression equation, coefficient of determination (\( R^2 \)), and the significance (\( *P < 0.05; **P < 0.01 \)) of the slope are shown on the panel. (This figure is available in colour at JXB online.)

**Fig. 8.** A schematic representation of the effect of decreasing N availability on leaf stomatal conductance (\( g_s \)) and transpiration rate (\( E \)) modified after Wilkinson et al. (2007). Excess [N] availability in the ‘interception’ treatment is indicated to result in low \( E \) and \( g_s \), which increase as N becomes more limiting to compensate for N availability, and then decrease when N is extremely limiting (e.g. 45 mm from the N source). The dotted line shows the position of the nylon mesh relative to the N source. (This figure is available in colour at JXB online.)
with foliar [N] may suggest that it is small excesses of N in the cytosol (possibly inorganic N), rather than the overall tissue [N], that is key in the N regulation of transpiration and consequently mass-flow of other nutrients. While accumulation of inorganic N may result in osmotic influences (e.g. Raven, 1985), it is not known whether tissue N represented inorganic or assimilated N. The correlation of N:P ratios with E in tropical trees and lianas (Cernusak et al., 2009b, 2011) supports the idea that excess N modulates transpiration. Furthermore, despite a significant decreasing trend of foliar [N] with distance, there were no significant differences in foliar [N] (Fig. 6A), possibly indicating that it is N flux that is important in biochemical regulation of gs and WUE, as suggested previously (Cramer et al., 2009). The N acquired by roots as NH$_4^+$ is mostly assimilated into amino acids (Miller and Cramer, 2004), and may not alter root hydraulic conductance or the expression of root aquaporins (Guo et al., 2007). Unlike NH$_4^+$, root NO$_3^-$ increases aquaporin-mediated root hydraulic conductivity (Carvajal et al., 1996; Clarkson et al., 2000; Gloser et al., 2007; Gorska et al., 2008) and, when in excess of the capacity of root nitrate reductase, is taken to the leaves where it is reduced to NO (Cramer et al., 2009) or it can alter xylem sap pH (Mengel et al., 1994; Mühling and Lauchli, 2001), resulting in increased sensitivity of guard cells to abscisic acid (ABA), which elicits stomatal closure (Wilkinson et al., 2004, 2007; Jia and Davies, 2007).

The experimental design used here provided an opportunity to evaluate the spatial scale over which mass-flow is effective in sand. Mass-flow acquisition diminished in effectiveness with distance, with significant reductions in biomass when N was >36 mm from the roots. Nevertheless, even at these distances, the plants managed to acquire sufficient N for growth. Whilst sand is experimentally simpler, soils come with a complex cocktail of nutrients and bind different nutrients and nutrient forms to variable extents. This effective mass-flow distance may also vary with soil porosity and texture (Horn et al., 1994), soil moisture (Clarkson, 1981; Gahoonia et al., 1994), and the flux of water to the root. Thus, clay soils with smaller pores, lower hydraulic conductivities (Child and Collis-George, 1950), and greater binding capacity for nutrients may reduce the effective distances for nutrient mass-flow. The limited spatial efficacy of mass-flow and its interactions with soil moisture availability and soil texture are potentially important for understanding the evolutionary ‘choices’ plants make in root system architecture and biomass allocation.

The spatial effectiveness of mass-flow for acquisition of N may have important implications for carbon allocation. The extent to which plants rely on mass-flow may allow a physiological trade-off between the investment in root architecture and the maintenance of water flux. This trade-off may be particularly complex when nutrients and moisture are differently spatially or temporally localized in the soil (López-Bucio et al., 2003; Ho et al., 2005). For instance, in moisture-limited soils, plants would be expected to invest in root biomass for accessing soil nutrients and moisture. In highly permeable soils with abundant moisture, however, ‘mass-flow’ acquisition may complement investment in a costly root system. Whilst mobile elements such as N are known to be acquired through mass-flow (Barber, 1995), acquisition of immobile nutrients such as P are thought to depend largely on root architectural modifications for uptake (e.g. mycorrhizae and cluster roots; Lambers et al., 2006). There is, however, evidence that P acquisition also benefits from mass-flow (Cernusak et al., 2011). This may be particularly the case for more mobile organic P or in soils with low binding capacity for P. Overall, the recognition that N partially regulates transpiration and thus mass-flow of N and possibly other nutrients is important. In a warming global climate where water supplies are dwindling (Wilkinson and Hartung, 2009), strategic N fertilization may provide an opportunity for moderating plant water demands.

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