

# **Functional role of plant water fluxes in nutrient acquisition**

Ignatious Matimati

October 2013

Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

in the

Department of Botany

UNIVERSITY OF CAPE TOWN

Supervisors:

M.D. Cramer and G.A. Verboom

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

To Vimby, Mikayla and Victor for your love, support and patience

## **Declarations**

I Ignatious Matimati hereby: (a) grant the University of Cape Town free license to reproduce the above thesis in whole or in part, for the purpose of research; (b) declare that: i) the above thesis is my own unaided work, both in conception and execution, and that apart from the normal guidance of my supervisor, I have received no assistance apart from that stated below; ii) neither the substance nor any part of the above thesis has been submitted in the past, or is being, or is to be submitted for a degree at this University or at any other University, except as stated below; I am now presenting the thesis for examination for the Degree of PhD.

Signed by candidate

Signature Removed

Ignatious Matimati, October 2013.

## **Acknowledgements**

I sincerely thank everyone who made this thesis possible. This would not have been possible without the tremendous support, constant engagement, guidance and enthusiasm of my dedicated supervisors, Michael Cramer and Tony Verboom. Thank you for your patience as you engaged with all my challenges. I sincerely appreciate the effort to ensure that I had all the necessary equipment and adequate funding. I am grateful for your advice and insight and the constructive criticism and the effort you put in reviewing this thesis.

I have been fortunate to share an office with Simon Power, Justin Nyaga and Taryn Morris. I greatly appreciate their willingness to engage in discussions over some of my challenges. I greatly appreciate their field and lab support. I had the opportunity to interact with members of the Plant Ecophysiology Journal Club of the Biological Sciences Department, Samson Chimphango, Edmund February, Adam West, Justin Nyaga, Simon Power, Tarryn Morris, Mark Maistry, Rob Skelton and Jack Viljoen.

I acknowledge the technical support from Edward Chirwa, especially the burdensome task of rinsing sand for plant culture. Ian Newton is sincerely thanked for running all foliar samples on the mass spectrometer for stable isotopes. I also thank Shamiela Davids for procuring all the resources I used for stable water isotopes. Special thanks to Desmond Barnes, Tamara Nozewu and Sandy Smuts for procurement of my lab consumables and for providing all the administrative support that I needed during my studies.

This study was made possible with the generous funding from the NRF Grantholder's bursary, through Michael Cramer, the Oppenheimer Memorial Trust (OMT 19170) and from the Department of Biological Sciences (UCT). I sincerely thank Laurence Dworkin for granting us permission to use Bloemfontein farm for the hydraulic redistribution study.

Finally, I thank my family in Zimbabwe, my parents and siblings and in South Africa Vimbai, Mikayla and Victor for their moral support, love and patience. Vimbai, I greatly

appreciate your patience and your love during my study. To DP Dan and Ria Zimuwandeyi,  
thank you for your encouragement.

## Abstract

Transpiration is inevitable during photosynthesis; however, it may also function to cool leaves, transport nutrients and drive nutrient acquisition via mass-flow. In addition to transpiration, plants water fluxes occur through hydraulic redistribution (HR). I hypothesized that an important function of plant water fluxes is to drive mass-flow nutrient acquisition, with flux rates positively correlated with nutrient limitation but not deficiency. To test whether nutrient availability regulates mass-flow, *Phaseolus vulgaris* was grown with N placed at one of six distances behind a root-impenetrable mesh whilst control plants intercepted the N-source. In plants forced to acquire N through mass-flow transpiration rates were 2.9-fold higher and P and K accumulation was greater compared to control plants. The contribution of nocturnal transpiration and HR to nutrient acquisition was assessed by supplying *Aspalathus linearis* (N-fixer) with no fertilizer or Na<sup>15</sup>NO<sub>3</sub> and CaP/FePO<sub>4</sub> either above or below-ground with varying rates of below-ground irrigation. <sup>2</sup>H<sub>2</sub>O was used to trace HR. HR by *A. linearis* accounted for the bulk of surface soil moisture at dawn and responded positively to surface fertilization. In contrast, plants supplied below-ground fertilizer exhibited both HR and nocturnal transpiration with increased <sup>15</sup>N and P acquisition. Finally, to establish whether clay fraction moderates mass-flow P availability, *Triticum aestivum* was grown with 0, 1, 5 or 10% (w/w) clay combined with either Ca-P, Fe-P or inositol-P. Transpiration and nutrient accumulation were monitored. Plants acquired P through mass-flow and diffusion. The acquisition of N and P increased from 0 to 5% clay (w/w) due to enhanced moisture retention, but further additions (10%) reduced P-availability (Inositol-P > Fe-P > Ca-P).

Overall, this thesis explored and confirmed the relatively novel idea that nocturnal and diurnal transpiration by plants are not merely the consequence of stomatal opening for CO<sub>2</sub> acquisition. Rather nocturnal and diurnal transpiration are regulated by nutritional

requirements and serves as a driving force for nutrient transport to roots. Likewise, hydraulic redistribution serves to draw water from deep and wet soil layers to the upper layers, which serves as a means to enable uptake of nutrients from the rich, but often dry, upper soil. Plants may thus be opportunistic in their water uptake, taking it up when it is available in order to improve the acquisition of nutrients through mass-flow delivery. Plants in low nutrient substrates elevated their water fluxes for mass-flow nutrient acquisition. Consequently, plants growing in mesic climates with low clay soils are likely to display greater dependence on mass-flow nutrient acquisition. This might vary between  $C_3$  and  $C_4$  plants, which differ in  $WUE$ . Plants may also increase mass-flow nutrient acquisition during inter-specific competition thus reducing investment in root proliferation for nutrient interception. Plants growing in elevated atmospheric  $[CO_2]$  with suppressed transpiration could show limited mass-flow nutrient acquisition.

## Abbreviations and Symbols

ABA	abscisic acid
AIC	akaike information criterion
ANCOVA	analysis of covariance
ANOVA	analysis of variance
Ca-P	calcium phosphate
CEC	cation exchange capacity
CFR	Cape Floristic Region
$C_i/C_a$	intercellular to ambient CO <sub>2</sub> mole fractions
$C_i$	intercellular CO <sub>2</sub> mole fractions
CTMP	Cape Town Meteoric Precipitation
$d$	distance of root from nutrient source
$D_e$	diffusion coefficient
dH <sub>2</sub> O	distilled water
DM	dry mass
$E$	transpiration
$E_{night}$	night-time transpiration
Fe-P	iron phosphate
FW	fresh weight
GCFR	Greater Cape Floristic Region
$g_s$	stomatal conductance
HR	hydraulic redistribution
HSD	highest significant difference
IH-P	inositol hexa- <i>kis</i> -phosphate
[K]	potassium concentration
LSD	least significant difference
MAP	mean annual precipitation
[N]	nitrogen concentration
OCBILS	old, climatically buffered, infertile landscapes
[P]	phosphorous concentration
PAR	photosynthetically active radiation
Pi	inorganic phosphate
PVC	polyvinyl chloride
SWBP	South West Botanical Province
$t$	time
TDM	total dry mass
VPD	vapour pressure deficit
V-SMOW	Vienna Standard Mean Ocean Water
$WUE$	photosynthetic water use efficiency
$\theta$	rate of soil water loss
$\omega_s$	saturated soil mass
$\rho_t$	soil mass after some time ( $t$ ) of drying
$\phi_m$	final mass of soil after oven-drying until it reached constant mass

# Contents

<b>1 General Introduction .....</b>	<b>1</b>
<b>1.2 Hypothesis .....</b>	<b>8</b>
<b>1.3 References .....</b>	<b>9</b>
<b>2 Nitrogen regulation of transpiration controls mass-flow acquisition of nutrients .....</b>	<b>17</b>
<b>2.1 Abstract.....</b>	<b>17</b>
<b>2.2 Introduction .....</b>	<b>18</b>
<b>2.3 Materials and Methods .....</b>	<b>20</b>
2.3.1 <i>Plant culture</i> .....	20
2.3.2 <i>Gas exchange analysis</i> .....	23
2.3.3 <i>Biomass measurements</i> .....	23
2.3.4 <i>Foliar elemental and isotope analysis</i> .....	24
2.3.5 <i>Data analysis</i> .....	24
<b>2.4 Results .....</b>	<b>25</b>
2.4.1 <i>Plant biomass response to N accessibility</i> .....	25
2.4.2 <i>Gas exchange response to N accessibility</i> .....	25
2.4.3 <i>Foliar nutrient responses to N accessibility</i> .....	30
<b>2.5 Discussion .....</b>	<b>33</b>

<b>2.6 References .....</b>	<b>39</b>
<b>3 Do hydraulic redistribution and nocturnal transpiration facilitate nutrient acquisition in <i>Aspalathus linearis</i>? .....</b>	<b>48</b>
<b>3.1 Abstract.....</b>	<b>48</b>
<b>3.2 Introduction .....</b>	<b>49</b>
<b>3.3 Materials and Methods .....</b>	<b>52</b>
3.3.1 Site description and experimental set-up .....	52
3.3.2 Foliar and soil sampling before and after supply of <sup>2</sup> H-enriched water .....	54
3.3.3 Foliar nutritional analysis .....	55
3.3.4 δ <sup>2</sup> H analysis of soil, stem and irrigation water.....	55
3.3.5 Statistical analysis.....	57
<b>3.4 Results .....</b>	<b>57</b>
3.4.1 Water-related measures .....	57
3.4.2 Foliar nutrients .....	65
<b>3.5 Discussion.....</b>	<b>69</b>
<b>3.6 Conclusion.....</b>	<b>73</b>
<b>3.7 References .....</b>	<b>73</b>
<b>4 Clay in soil substrates modulates the acquisition of different soil phosphates.....</b>	<b>83</b>

<b>4.1 Abstract</b> .....	<b>83</b>
<b>4.2 Introduction</b> .....	<b>84</b>
<b>4.3 Materials and Methods</b> .....	<b>88</b>
4.3.1 <i>Soil characteristics</i> .....	88
4.3.2 <i>Sampling and P extraction from Greater Cape Floristic Region soils</i> .....	88
4.3.3 <i>Plant material and experimental culture</i> .....	89
4.3.4 <i>Gas exchange measurements</i> .....	92
4.3.5 <i>Biomass measurements and foliar elemental analyses</i> .....	92
4.3.6 <i>Data analysis</i> .....	93
<b>4.4 Results</b> .....	<b>93</b>
4.4.1 <i>Substrate water and P retention</i> .....	93
4.4.2 <i>Biomass response to clay and P form</i> .....	96
4.4.3 <i>Gas exchange response to clay and P form</i> .....	96
4.4.4 <i>Tissue nutrient responses to clay and P form</i> .....	99
<b>4.5 Discussion</b> .....	<b>102</b>
<b>4.6 References</b> .....	<b>107</b>
<b>5 Synthesis</b> .....	<b>117</b>
<b>5.1 References</b> .....	<b>130</b>

# 1 General Introduction

Terrestrial plants transpire *ca.* 32 trillion tonnes of water vapour annually representing 29 % of annual terrestrial precipitation (Hetherington and Woodward, 2003). Furthermore, their roots hydraulically redistribute from 0.04 to 3.23 mm H<sub>2</sub>O d<sup>-1</sup>, (expressed on a land area basis) from wetter to drier soil zones (Neumann and Cardon, 2012). Transpiration is by far the largest atmospheric water flux from the terrestrial surfaces of Earth's continents, representing *ca.* 90 % of total terrestrial evapotranspiration (Jasechko et al. 2013). Thus, plants incur large-scale transpirational losses (Rutter, 1972; Sutcliffe, 1974), retaining less than 5% of the water absorbed by their roots for cell expansion and plant growth (McElron et al., 2013). Considering that water is vital to plant growth and productivity, and is a key climatic factor determining the distribution of vegetation (e.g. Stephenson, 1990), this raises questions over why plants transpire so much of it. Transpirational fluxes may function in facilitating evaporative leaf cooling (e.g. Nobel, 1999), driving root to shoot xylem transport and delivering nutrients to the root surfaces *via* mass-flow (Cramer et al., 2008; Cramer et al., 2009; Christman et al., 2009). Of these three functions, mass-flow acquisition is a frequently neglected function of transpiration (Cramer et al., 2009). Yet, understanding the delivery of dissolved soil nutrients to the root surface is a pressing issue in terrestrial ecosystems where nutrients are limiting and water resources are inadequate since plants lose large quantities of water through their roots and leaves.

Plants are sessile and therefore rely mostly on the combination of root 'interception' of nutrients and fluxes of water and nutrients delivered to their root surfaces by diffusion and transpirational mass-flow (Barber, 1995; Tinker and Nye 2000; McDonald et al. 2002). Root 'interception' is the positioning of roots close to nutrient sources to facilitate nutrient diffusion and mass-flow (Tinker and Nye, 2000). Thus, nutrient 'interception' increases with proliferation of roots in the soil (Kage, 1997). Although 'interception' is important for

acquiring less mobile nutrients such as P (e.g. Lambers et al., 2006), root proliferations incur significant carbon costs representing both structural and metabolic costs (Lynch et al., 1991; Lynch and Brown, 2001). Thus, root proliferation consumes a large fraction of whole-plant carbon budgets, particularly straining the plant when under nutrient stress (Eissenstat, 1997), which reduces the efficacy of nutrient acquisition *via* root interception. Root associations with mycorrhizal fungi can increase the effective ‘interception’ of immobile nutrients by extending the volume of accessible soil (Smith et al. 2011). However, mycorrhizal associations often lead to increased root respiration as a consequence of high maintenance and growth respiration of the fungal tissue (Nielsen et al. 1998; Graham and Eissenstat, 1998). Thus, such associations may be costly in terms of carbon, particularly in nutrient-poor ecosystems. For example, most plant families native to extremely P-limited soils of particular regions relied more on root proliferations (e.g. cluster roots) than mycorrhiza for acquiring the less mobile P (Lambers et al. 2006).

For diffusive fluxes, nutrient transport to root surfaces is driven by a concentration gradient that develops around the roots due to active root uptake (Nye, 1966). Diffusion is a passive process whereby nutrients follow an often-tortuous network of water-filled pores in the soil (Tinker and Nye, 2000). The contribution of diffusive fluxes to root nutrient acquisition therefore varies with the diffusion coefficient of the ionic form of the nutrient and the water present in the soil. Although the diffusion coefficients of  $\text{NO}_3^-$  ( $1.9 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ ) and  $\text{NH}_4^+$  ( $1.96 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ ) are similar in water, they differ substantially in effective soil diffusion coefficients between  $\text{NO}_3^-$  ( $3.26 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ ) and  $\text{NH}_4^+$  ( $2.70 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ ) (Miller and Cramer, 2005). The difference is related to soil diffusion coefficients being additionally determined by ion size and charge, viscosity of water, temperature, soil moisture, route of diffusion (tortuosity) and the soil buffer capacity (Miller and Cramer, 2004). On the contrary, mass-flow acquisition of nutrients relies on transpirational fluxes to draw soil water

containing nutrients towards the root surface (Barber, 1995). Transpirational fluxes increase nutrient concentration at root surfaces, which reduces the nutrient depletion zone that develops in the rhizosphere as a consequence of active nutrient uptake (Scholze et al., 2007). When transpirational fluxes deliver fewer nutrients than those demanded by roots, diffusive fluxes may also contribute to the nutrient supply.

Mobility of nutrients in the soil in response to diffusive and transpirational fluxes is determined by the amount and nature of nutrients in the soil and their interaction with nutrients adsorbed by the soil particles (Barber, 1995). Soil particles are broadly divided into sand, silt and clay. Sand and silt have a similar mineralogy. Although clay develops mostly from the weathering of sand and silt, it has a high surface area per gram (Cihacek and Bremner, 1979). Variation in clay is one of the factors that affect diffusion coefficients in soils (Lambers et al., 1998), this in turn influencing the bioavailability of nutrients, particularly in natural ecosystems where soil solution concentrations are much lower than in managed agricultural systems. The recovery of applied nutrients such as P by crop plants can be very low, because in the soil more than 80% of the P becomes immobile and unavailable for plant uptake as a result of adsorption to clay and metal sesquioxides, precipitation, or conversion to the organic form (Holford, 1997).

Root studies are still lagging well behind other studies on aboveground plant parts because of the technical difficulties of studying roots in their natural environment, the soil (Silberbush, 2013), particularly the mass-flow acquisition of nutrients by roots (e.g. Cramer et al., 2009). The limited research on mass-flow acquisition may possibly be related to studies that have reported insignificant contributions of transpirational fluxes in delivering soil nutrients. According to Howard and Donovan (2007), Tanner and Beevers (2001) is commonly cited as contrary evidence for mass-flow acquisition, yet it dealt only with the effects of transpiration on long-distance N transport within the xylem, not with mass-flow

delivery to roots. Mechanistic models have been used to predict nutrient acquisition by roots (e.g. Barber and Cushman 1981; Silberbush, 2013). Such mechanistic models, however, often predicted nutrient acquisition by mass-flow, particularly P, to be inconsequential (e.g. Barber, 1995). However, mass-flow provided the bulk of the nutrients (79% N, 5% P, 18% K, 73% Ca, 88% Mg and 95% S) delivered to the root surfaces of *Zea mays* growing in a fertile loam soil, the remainder being delivered through root interception and diffusive fluxes by the roots (Barber 1995). Current plant nutrition models still fail to predict accurately the actual uptake of poorly mobile nutrients such as P because they hardly take rhizosphere processes into account (Hinsinger et al., 2011; Schnepf et al. 2012). The Barber-Cushman model, for example, employs the simplification of ignoring root hairs, mycorrhiza, and acidification of the rhizosphere by root exudates (Schenk, and Barber, 1980, Silberbush and Barber, 1984). Despite this, these components may be important; for example root hairs are the most active parts of the system in terms of water and nutrient acquisition (McCully 1999).

Over the past *ca.* 400 million years, plants have evolved a diversity of leaf structures and attributes which have enabled them to persist in virtually all terrestrial ecosystems (Boyce and Knoll, 2002; Boyce, 2005), including features that may be interpreted as promoting the reported high water fluxes. Nicotra et al. (2008) showed that highly dissected leaf shapes, for example, did not correlate with VPD, which was inconsistent with the function of transpiration in leaf cooling, but possibly for promoting water delivery to leaf tissues. Evidence exists that plants also adjust their venation in order to deliver more water per given carbon investment (McKown et al., 2010). Presence of aquaporins in mesophyll cells of several species may be construed as features that promote water fluxes (Cochard et al., 2007). Transpiration may thus serve a key functional role in nutrient acquisition, besides leaf cooling and promoting xylem transport, particularly given the enormous transpirational losses, and the occurrence of night-time transpiration in C<sub>3</sub> and C<sub>4</sub> plants when they are not actively fixing

CO<sub>2</sub> (Raven, 2008). Plants may be opportunistic in their water uptake, taking it up when it is available in order to improve the acquisition of nutrients through mass-flow delivery.

The roles of transpiration in evaporative leaf cooling and xylem transport of solutes may not adequately explain the transpirational fluxes observed in many plants, suggesting nutrient acquisition as a key function of transpiration. Although transpiration functions in evaporative leaf cooling (Nobel, 1999), for example, some alpine plants in frosty habitats show unexpectedly high rates of transpiration, given the low VPDs they experience (Mooney et al., 1965, Smith and Geller, 1979). Also, nocturnal transpiration occurs in C<sub>3</sub> and C<sub>4</sub> species (Daley and Phillips, 2006; Dawson et al., 2007, Phillips et al., 2010) when conditions are cooler and there is no significant CO<sub>2</sub> fixation. Further, midday closure of stomata is common in many plants growing under natural conditions (Tenhunen et al., 1980), when transpirational cooling is expected to be highest. Despite a lack of experimental evidence, high transpirational fluxes have generally been suggested as vital in driving long-distance transport of inorganic nutrients in the xylem of higher plants (e.g. Campbell et al., 1999). This suggested function in xylem transport of nutrients has not, however, been supported in *Helianthus annuus* (Tanner and Beevers, 2001) and *Zea mays* (Tanner and Beevers, 1990). Since evaporative leaf cooling and long distance transport of solutes fail to account for the enormous transpirational fluxes observed in many species, an alternative key function of high water fluxes may be the delivery of nutrients to the root surface through mass-flow.

There is evidence consistent with the function of transpiration in powering mass-flow in the soil (Barber 1995; Tinker and Nye 2000). If transpiration-driven mass-flow is an essential strategy for nutrient acquisition, then soil nutrient availability may at least partially control plant water fluxes. Previous studies indicated an association between plant water fluxes and nutrient availability (Cramer et al., 2008, Kupper et al., 2012, Raven et al., 2004, Radin and Mathews, 1988, Radin 1990, Gorska et al, 2008, Cernusak et al., 2011), whereby

nutrient availability partially regulated water fluxes in plants. A typical example is where *Ehrharta calycina* forced to obtain nutrients through mass-flow and diffusion (i.e. not interception) plants transpired 60% more and had 40% more tissue P, Ca and Na than plants that acquired nutrients through a combination of interception, diffusion and mass-flow (Cramer et al., 2008). In addition, Gorska et al. (2008) reported rapid increases in water uptake by *Solanum lycopersicum* and *Cucumis sativus* roots that had been exposed to  $\text{NO}_3^-$ -rich soil patches, such that water and  $\text{NO}_3^-$  were preferentially absorbed from these patches. These responses to  $\text{NO}_3^-$  were very rapid (ca. 1.8 s; Gorska et al., 2008), identifying them as a potential functional trait, which would allow plants growing in heterogeneous soil environments to control mass-flow nutrient acquisition. This association between transpiration and nutrient fluxes has important implications in nutrient acquisition from larger soil volumes, or where roots are poorly developed to access limiting nutrients. I expect plant water fluxes to be up- or down-regulated by the concentration of nutrients absorbed in their leaf tissues (e.g. Wilkinson et al., 2007; Cramer et al., 2009).

Although mass-flow delivery of nutrients to the root surfaces has been recognised for at least half a century (e.g. Barber, 1962), its regulation by nutrients is still unclear (Raven, 2008). The control of water flux through plants has been regulated through N-flux-linked signalling mechanisms (e.g. Wilkinson et al. 2007; Clarkson et al. 2000; Desikan et al. 2002; Gloser et al. 2007) that operate in both roots and shoots. Increasing rhizosphere  $[\text{NO}_3^-]$  above deficiency has been shown to rapidly increase aquaporin-mediated root hydraulic conductivity (Carvajal et al. 1996; Clarkson et al. 2000; Gloser et al. 2007; Gorska et al. 2008). The  $[\text{NO}_3^-]$  of the xylem sap has a biphasic effect on stomatal conductance ( $g_s$ ) increasing with  $\text{NO}_3^-$  supply from deficiency to a maximum  $[\text{NO}_3^-]$  (Radin and Parker, 1979; Radin and Ackerson, 1981; Wilkinson et al., 2007), beyond which  $[\text{NO}_3^-]$  evokes a concentration-dependant closure of stomata (Wilkinson et al., 2007) possibly mediated by cytosolic pH and/or phytohormones

concentrations (e.g. ABA; Wilkinson et al, 1998) and NO signalling (e.g. Neill et al., 2008). Such signalling effects become ineffective when N deficiency is prolonged, resulting in decreased water flux (Radin and Parker, 1979), possibly due to N starvation.

Unlike  $\text{NO}_3^-$ , very little is known about the role of  $\text{NH}_4^+$  in modulating plant water fluxes, apart from the mechanism proposed by Goodger and Schachtman (2010). Guo et al. (2007) suggested that  $\text{NH}_4^+$  nutrition does not alter root hydraulic conductance or increase the expression of aquaporins in the root. A question arises as to how plants depending on  $\text{NH}_4^+$  regulate their water fluxes for potential nutrient acquisition by mass-flow.  $\text{NH}_4^+$  absorbed by roots is transported as electro-neutral amino acids such as glutamine (Miller and Cramer, 2004; Goodger and Schachtman, 2010). The transportation of  $\text{NH}_4^+$  as amino-acids may partly explain the very low xylem  $[\text{NH}_4^+]$ , but high concentrations of glutamine when plants are grown on  $\text{NH}_4^+$  only (Gollan, 1992). As a consequence, the pH of the cytosol does not become alkaline, and the flux of protons is at its highest, and the low levels of abscisic acid (ABA) are insufficient to reduce stomatal conductance (Goodger and Schachtman, 2010).

Besides transpirational fluxes, plants incur substantial root-mediated fluxes of water from wetter to drier soil zones, known as hydraulic redistribution (HR). HR is a commonly observed process, documented for over 60 plant species (Jackson et al. 2000, Armas et al., 2010; 2012), which is powered by differences in water potentials between wet and dry soils, using roots as conduits (e.g. Burgess and Bleby, 2006). HR supplies *ca.* 2–80 % (mean of 15%,  $n = 25$ ) of the water consumed by daily transpiration (Neumann and Cardon, 2012). The daily contributions of HR, when considered during summer-drought periods, may improve nutrient acquisition when sustained. Theoretically, hydraulic redistribution could act to enhance litter decomposition, nutrient availability and nutrient uptake by plants (Ryel, 2004; Armas et al., 2012). However, empirical evidence for the nutritional role of HR is scarce, and enhanced nutrient acquisition following HR has not been universally supported with literature

(e.g. online resource 1 of Armas et al., 2012). Soil nutrients exist in the rhizosphere in bound forms or in solution, and must be transported towards the root surfaces for their absorption. This movement may occur through transpiration-powered mass-flow or diffusion (Nye and Marriot, 1969). The flux of HR water from wetter to drier soil may consequently power the mass-flow of water and dissolved nutrients towards the root surfaces for active uptake in the wetter soil. Such HR fluxes appear important in environments where rainfall is strongly seasonal (e.g. Brooks et al., 2002). Mediterranean-type climates are a classic example of such environments because during the seasonal summer-drought period, roots may hydraulically lift deep soil moisture to drier shallow soils, which are often richer in strongly-recycled nutrients from plant litter (Jobbágy and Jackson, 2004).

## **1.2 Hypothesis**

Although transpirational water fluxes are inevitable and hydraulic redistribution is common, plants in nutrient-poor soils may elevate these water fluxes to power mass-flow, exhibiting profligate use of water when it is available. Consequently, the amount of soil nutrients acquired may regulate plant water flux. My overall hypothesis is that transpirational fluxes and hydraulic redistribution of water are important in powering movement of soil nutrients to the root surfaces and that foliar [N] or flux signals the modulation of water flux, for regulating mass-flow nutrient acquisition. Similarly, below-ground water fluxes as a result of hydraulic redistribution may have a functional role in delivering both deep and surface soil nutrients to roots. Based on this, I hypothesized that the distance of soil N from roots, regulates plant water fluxes and consequent nutrient delivery to plants and that such fluxes may display biphasic responses (increase followed by a decrease) with distance from the N source (Chapter 2). Secondly, I hypothesized that hydraulic redistribution functions in nutrient acquisition in two ways; i) driving water fluxes from deep to shallow soil, thus enabling

acquisition of nutrients from shallow soil; ii) driving mass-flow of nutrients in deep soil towards the roots, with a concurrent release of water in the topsoil. In addition nocturnal transpiration may act as a competitive sink for water, limiting HR, but also contributing to mass-flow at night from deep wet soils (Chapter 3). Finally, soil edaphic conditions, particularly clay, may modulate the acquisition of nutrients differently from soils, especially P, which binds easily to clay particles. I therefore hypothesised that; i) Fe-P, Ca-P and IH-P have different mobilities in substrates of varying clay, because of their different rates of dissolution in the soil solution and that ii) the availability of P must be determined by both soil moisture retention and transpirational fluxes driving P mass-flow; and iii) mobility of P should be greatest in low-clay substrates, although these substrates also dry out quickly, thus reducing the opportunity for P delivery (Chapter 4).

### **1.3 References**

- Armas C. et al., 2010. Hydraulic lift and tolerance to salinity of semiarid species: consequences for species interactions. *Oecologia*, 162, 11-21.
- Armas C, Kim JH, Bleby TM, Jackson RB. 2012. The effect of hydraulic lift on organic matter decomposition, soil nitrogen cycling, and nitrogen acquisition by a grass species. *Oecologia* 168, 11-22.
- Barber SA. 1962. A diffusion and mass-flow concept of soil nutrient availability. *Soil Science* 93, 39-49.
- Barber SA, Cushman JH. 1981. Nitrogen uptake model for agronomic crops. In IK Iskander, ed, *Modeling Waste Water Renovation-Land Treatment*. Wiley-Interscience, New York.
- Barber S. 1995. *Soil nutrient bioavailability: a mechanistic approach*. 2<sup>nd</sup> Edition, John Wiley and Sons, p89-99

- Bieleski RL. 1973. Phosphate pools, phosphate transport, and phosphate availability. Annual Review in Plant Physiology 24, 225–252.
- Burgess SSO, Bleby TM. 2006. Redistribution of soil water by lateral roots mediated by stem tissues. J Exp Bot 57: 3283–3291.
- Boyce CK, Knoll, AH. 2002. Evolution of developmental potential and the multiple independent origins of leaves in Paleozoic vascular plants. Paleobiology 28,70–100.
- Boyce CK. 2005. Patterns of segregation and convergence in the evolution of fern and seed plant leaf morphologies. Paleobiology 31, 117-140.
- Campbell NA, Reece JB, Mitchell LG. 1999. Biology, Addison Wesley Longman, Reading, MA, 5th Ed., p. 703.
- Eissenstat DM. 1997. Trade-offs in root form and function. In: Jackson LE. (ed.) Ecology in Agriculture. Academic Press, San Diego. pp.173-199.
- Cernusak LA, Winter K, Turner BL. 2011. Transpiration modulates phosphorus acquisition in tropical tree seedlings. Tree Physiology 31, 878-885.
- 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.
- Guo S, Kaldenhoff R, Uehlein N, Sattelmacher B, Brueck H. 2007a. Relationship between water and nitrogen uptake in nitrate- and ammonium-supplied *Phaseolus vulgaris* L. plants. Journal of Plant Nutrition and Soil Science 170, 73–80.
- Gollan T, Schurr U, Schulze ED. 1992. Stomatal response to drying soil in relation to changes in xylem sap composition of *Helianthus annuus*. I. The concentration of cations, anions, amino acids in, and pH of, the xylem sap. Plant Cell and Environment 15, 551–559.

- Christman MA, Donovan LA, Richards JH. 2009. Magnitude of nighttime transpiration does not affect plant growth or nutrition in well-watered *Arabidopsis*. *Physiologia Plantarum*, 136, 264-273.
- Cihacek LJ, Bremner JM. 1979. A simplified ethylene glycol monoethyl ether procedure for assessment of surface area. *Soil Science Society of America Journal* 43, 821-822.
- 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.
- Cochard H, Casella E, Mencuccini M. 2007. Xylem vulnerability to cavitation varies among poplar and willow clones and correlates with yield. *Tree Physiology* 27, 1761–1767.
- Cramer MD, Hoffmann V, Verboom GA. 2008. Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist* 179, 1048–1057.
- Cramer MD, Hawkins H-J, Verboom GA. 2009. The importance of nutritional regulation of plant water flux. *Oecologia* 161, 15-24.
- Daley MJ, Phillips NG. 2006. Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. *Tree Physiol.* 26, 411-419.
- Dawson T, Burgess S, Tu K, Oliveira R, Stantiago L, Fisher J, Simonin K, Ambrose A. 2007. Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiol.* 27, 561–576.
- Desikan R, Griffiths R, Hancock J, Neill S. 2002. A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 99, 16314–16318.

- Gloser V, Zwieniecki MA, Orians C, Holbrook NM. 2007. Dynamic changes in root hydraulic properties in response to nitrate availability. *Journal of Experimental Botany* 58, 2409–2415.
- Goodger JQ, Schachtman DP. 2010. Nitrogen source influences root to shoot signaling under drought. In: *Abiotic stress adaptation in plants*. Springer Netherlands, pp. 165-173.
- Gorska A, Ye Q, Holbrook NM, Zwieniecki MA. 2008. Nitrate control of root hydraulic properties in plants: translating local information to whole plant response. *Plant Physiology* 148, 1159–1167.
- Graham JH, Eissenstat DM. 1998. Field evidence for the carbon cost of citrus mycorrhizas. *New Phytologist* 140, 103-110.
- Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. *Nature*, 424, 901–908.
- Holford ICR. 1997. Soil phosphorus: its measurement and its uptake by plants. *Australian Journal of Soil Research* 35, 227–239.
- Howard AR, Donovan LA. 2007. Helianthus night-time conductance and transpiration respond to soil water but not nutrient availability. *Plant Physiol* 143, 145–155.
- Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237, 173–195.
- Jackson RB, Sperry JS, Dawson TE. 2000. Root water uptake and transport: using physiological processes in global predictions. *Trends in Plant Science* 5, 482–488.
- Jasechko S, Sharp ZD, Gibson JJ, Birks SJ, Yi Y, Fawcett PJ. 2013. Terrestrial water fluxes dominated by transpiration. *Nature* 496, 347-350.
- Kage H. 1997. Is low rooting density of faba beans a cause of high residual nitrate content of soil at harvest? *Plant Soil* 190, 47–60

- Kupper P, Rohula G, Saksing L, Sellin A, Lõhmus K, Ostonen I, Helmisaari H-S, Sõber A. 2012. Does soil nutrient availability influence night-time water flux of aspen saplings? *Environmental and Experimental Botany* 82, 37–42.
- Lambers H, Chapin FS, Pons TL. 1998. *Plant physiological ecology*, 2nd edn. Springer, New York, p 255-310.
- Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* 98, 693-713.
- Lynch J, Lauchli A, Epstein E. 1991. Vegetative growth of the common bean in response to P nutrition. *Crop Science* 31, 380-387.
- Lynch JP, Brown KM. 2001. Topsoil foraging - an architectural adaptation of plants to low phosphorus availability. *Plant and Soil*, 237, 225-237.
- McDonald EP, Erickson JE, Kruger EL. 2002. Can decreased transpiration limit plant nitrogen acquisition in elevated CO<sub>2</sub>? *Functional Plant Biology* 29, 1115–1120.
- McKown AD, Cochard H, Sack L. 2010. Decoding leaf hydraulics with a spatially explicit model: principles of venation architecture and implications for its evolution. *The American Naturalist* 175, 447–460.
- McElrone A J, Choat B, Gambetta GA, Brodersen CR. 2013. Water uptake and transport in vascular plants. *Nature Education Knowledge* 4(5), 6.
- Miller AJ, Cramer MD. 2004. Root nitrogen acquisition and assimilation. *Plant Soil* 274,1–36
- Mooney HA, Hillier RD, Billings WD. 1965. Transpiration Rates of Alpine Plants in the Sierra Nevada of California. *American Midland Naturalist* 74, 374-386.
- Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I. 2008. Nitric oxide, stomatal closure, and abiotic stress. *Journal of Experimental Botany* 59,165–176.

- Nielsen KL, Bouma TJ, Lynch JP, Eissenstat DM. 1998. Effects of phosphorus availability and vesicular-arbuscular mycorrhizas on the carbon budget of common bean (*Phaseolus vulgaris*). *New Phytologist* 139, 647-656.
- Neumann RB, Cardon ZG. 2012. The magnitude of hydraulic redistribution by plant roots: a review and synthesis of empirical and modelling studies. *New Phytologist* 194, 337–352.
- Nicotra AB, Cosgrove MJ, Cowling A, Schlichting CD, Jones CS. 2008. Leaf shape linked to photosynthetic rates and temperature optima in South African pelargonium species. *Oecologia* 154, 625–63.
- Nobel PS. 1999. *Physicochemical and environmental plant physiology*. Academic Press, San Diego
- Nye PH, Marriott FHC. 1969. A theoretical study of the distribution of substances around roots resulting from simultaneous diffusion and mass-flow. *Plant and Soil* 30, 459-472.
- Nye PH. 1966. The effect of nutrient intensity and buffering power of a soil, and the absorbing power, size and root hairs, on nutrient absorption by diffusion. *Plant and Soil* 25, 81-105.
- Phillips NG. et al., 2010. Inter- and intra-specific variation in nocturnal water transport in *Eucalyptus*. *Tree Physiology* 30, 586-596.
- Radin JW, Parker LL. 1979. Water relations of cotton plants under nitrogen deficiency. I. Dependence upon leaf structure. *Plant Physiology* 64, 495–498.
- Radin JW, Ackerson RC. 1981. Water relations of cotton plants under nitrogen deficiency. III. Stomatal conductance, photosynthesis, and abscisic acid accumulation during drought. *Plant Physiology* 67, 115–119.

- Raven JA, Handley LL, Wollenweber B. 2004. Plant nutrition and water use efficiency. In: Bacon MA. (ed.) Water use efficiency in plant biology. Oxford: Blackwell Publishing Ltd, 171–197.
- Radin JW, Matthews MA. 1988. Water transport properties of cortical cells in roots of nitrogen and phosphorus-deficient cotton seedlings. *Plant Physiology* 89, 264–268.
- Radin JW. 1990. Responses of transpiration and hydraulic conductance to root temperature in nitrogen and phosphorus-deficient cotton seedlings. *Plant Physiology* 92, 855–857.
- Raven JA. 2008. Transpiration: how many functions? *New Phytologist* 179, 905-907.
- Rutter AJ. 1972. *Transpiration*. Oxford University, London.
- Ryel RJ. et al., 2004. Water conservation in *Artemisia tridentata* through redistribution of precipitation. *Oecologia* 141, 335-45.
- Smith SE, Jakobsen I, Grønlund M, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology* 156, 1050-1057.
- Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F. 2007. Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. *Tree Physiology* 27, 551–559.
- Schenk MK, Barber SA. 1980. Potassium and phosphorus uptake by corn field grown genotypes as influenced by root characteristics. *Plant Soil* 54, 65–76.
- Schnepf A, Leitner D, Klepsch S. 2012. Modelling p uptake by a growing and exuding root system. *Vadose Zone Journal* 11(3). doi:10.2136/vzj2012.0001
- Silberbush M, Barber SA. 1984. Phosphorus and potassium uptake of field-grown soybean cultivars predicted by a simulation model. *Soil Science Society of America Journal* 48, 592–596.

- Sutcliffe J. 1974. *Plants and Water*. Studies in Biology, London: Arnold. 14, 29-30.
- Smith WK, Geller GN. 1979. Plant transpiration at high elevations: Theory, field measurements, and comparisons with desert plants. *Oecologia* 41, 109-122.
- Silberbush M. 2013. Root Study: Why Is It behind Other Plant Studies? *American Journal of Plant Sciences* 4, 198-203.
- Stephenson NL. 1990. Climatic control of vegetation distribution: the role of the water balance. *American Naturalist* 135, 649-670.
- Tinker PB, Nye PH. 2000. *Solute movement in the rhizosphere*. Oxford University Press, New York
- Tanner W, Beevers H. 1990. Does transpiration have an essential function in long-distance ion transport in plants? *Plant Cell Environ.* 13, 745–750.
- Tenhunen JD, Lange OL, Braun M, Meyer A, Losch R, Pereira JS. 1980. Midday stomatal closure in *Arbutus unedo* leaves in a natural macchia and under simulated habitat conditions in an environmental chamber. *Oecologia* 47, 365-367.
- Tanner W, Beevers H. 2001. Transpiration, a prerequisite for long-distance transport of minerals in plants? *PNAS*, 98, 3-7.
- Wilkinson S, Corlett JE, Oger L, Davies WJ. 1998. Effects of xylem pH on transpiration from wild-type and *flacca* tomato leaves: a vital role for abscisic acid in preventing excessive water loss even from well-watered plants. *Plant Physiology* 117, 703–709.
- Wilkinson S, Bacon MA, Davies WJ. 2007. Nitrate signalling to stomata and growing leaves: interactions with soil drying, ABA and xylem sap pH in maize. *Journal of Experimental Botany* 58, 1705–1716.

## 2 Nitrogen regulation of transpiration controls mass-flow acquisition of nutrients

### 2.1 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 N may regulate mass-flow of nutrients. I determined whether N regulates water fluxes, and whether this regulation has a functional role in controlling the mass-flow of nutrients to roots. I grew *Phaseolus vulgaris* 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- $\mu\text{m}$  mesh from which nutrients could move by diffusion or mass-flow (dubbed ‘mass-flow/diffusion’ treatment). Control plants had the N-source supplied directly to their root zone so that N was available through interception, mass-flow and diffusion (dubbed ‘interception’ treatment). ‘Mass-flow/diffusion’ plants closest to the N-source exhibited 2.9-fold higher transpiration ( $E$ ), 2.6-fold higher stomatal conductance ( $g_s$ ), 1.2-fold higher intercellular  $[\text{CO}_2]$  ( $C_i$ ) and 3.4-fold lower  $WUE$  than ‘interception’ plants, despite comparable values of photosynthetic rate ( $A$ ).  $E$ ,  $g_s$  and  $C_i$  first increased and then decreased with increasing distance from the N-source to values even lower than those of ‘interception’ plants. ‘Mass-flow/diffusion’ plants accumulated P and K, and had maximum concentrations at 10 mm from 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.

## 2.2 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 CO<sub>2</sub> 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 CO<sub>2</sub> mole assimilated (i.e. water use efficiency; Hack et al., 2006) as well as evidence for substantial night-time transpiration in photosynthetically-inactive C<sub>3</sub> and C<sub>4</sub> 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 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 supra-optimal 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. Syring and Claassen, 1995; Rengel, 1993), 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 acquisition (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 [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  $\text{NO}_3^-$  modulates root hydraulic conductance through its control of aquaporins (Henzler et al., 1999), and foliar 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  $\text{NO}_3^-$  in regulating water fluxes (Wilkinson et al., 2007; Cramer et al., 2008, 2009; Kupper et al., 2012), neglecting the potential regulatory effects of  $\text{NH}_4^+$ . Given the importance of nitrogenous fertilizers (including urea) in agriculture (Miller and Cramer, 2004), however, understanding the different regulatory effects of varying forms of N on water flux, and the feed back of water flux on nutrient acquisition is important.

Water use efficiency has been found to be more strongly positively correlated with foliar N:P than with foliar [N] (Cernusak et al., 2011), identifying foliar N:P as a more likely modulator of water use efficiency 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<sub>2</sub> mole fractions,  $C_i / C_a$ , and  $\delta^{13}\text{C}$  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.

I used *Phaseolus vulgaris* 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-fertiliser was placed at varying distances from the screen, thus creating a gradient of N-accessibility, whilst a control treatment comprised plants having free access to all nutrients including N. I 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.

## 2.3 Materials and Methods

### 2.3.1 Plant culture

Forty-two PVC 6-L troughs (Price and Sons Inc., South Africa) were each divided into two sections, one for growing plants (4 L) and another for nutrient supply (2 L), using PVC plates with a 30 cm<sup>2</sup> window covered with a nylon mesh (Nytal 25  $\mu\text{m}$ ; Draht-Center, Stuttgart, Germany). The mesh screen restricted roots to one compartment while allowing free-transfer of solutes between the compartments (Fig. 2.1). I filled the plant compartment and the nutrient compartment with 4 and 2 kg, respectively, of thoroughly rinsed acid-washed sand (ca. pH 7, grade 30/10, Consol Minerals, Cape Town, South Africa). Two *Phaseolus vulgaris* cv. *Star 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<sup>3</sup> 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 multi-layer polymeric coating. I placed 5 g of Multicote 4\* in the sand cores and covered them with sand. The use of slow release urea fertilizer avoids rapid volatilisation of N. To verify the N concentration in Multicote 4\*, the fertilizer was milled and [N] and <sup>15</sup>N/<sup>14</sup>N isotope ratios determined using mass spectrometry by Archaeometry Lab, University of Cape Town. The fertilizer had [N] and <sup>15</sup>N/<sup>14</sup>N isotope ratios of 50% and -0.84‰ respectively.



Fig. 2.1 Experimental set-up showing a modified trough containing plants and nutrient placement positions highlighted by blue circles. A white PVC plate, which has a 25 micron nylon mesh window (30 cm<sup>2</sup>), divided the plant and nutrient compartments of the trough. The picture was taken at harvest, showing 2 senescing older leaves.

An independent chemical assay of Multicote 4\* by ashing milled fertilizer at 480°C for 8 h before dissolving with a 1:1 (v/v) of HCl (Kalra, 1998) indicated a 50-0-0 N:P:K (w/w) (analysed by BemLab, De Beers Rd, Somerset West, South Africa). Because roots could not penetrate the fertiliser compartment, nutrient acquisition was only possible through diffusion and mass-flow of solutes to the roots and these were consequently termed ‘mass-flow/diffusion’ 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. To ensure an even soil moisture distribution while minimising leaching irrigation water was supplied sparingly (200 ml d<sup>-1</sup>) to the sand substrate using spray bottles. The sand was maintained at water contents of between 0.15 and 0.2 g H<sub>2</sub>O g<sup>-1</sup> 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<sub>4</sub><sup>3-</sup>, 2 mM K, 4 mM Ca, 1.5 mM Mg, 3.5 mM SO<sub>4</sub><sup>2-</sup>, 0.1 mM FeEDTA, 0.02 mM Mn, 0.14 mM H<sub>3</sub>BO<sub>3</sub>, 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<sup>-2</sup> s<sup>-1</sup> and daytime RH inside the greenhouse was *ca.* 40 % (Power et al., 2010), whilst 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 acclimatise for 48 h prior to gas exchange measurements. The growth chambers were equipped with 14 x 400W- HQI-T metal halide lights (Osram Powerstar, Osram, Cape Town, South Africa), 28 400W- NAV-T sodium lights (Osram Violox) and 24 x 150 W, 230V incandescent (Sicca, Osram, Cape Town) lamps providing a light intensity of

1000 - 1200  $\mu\text{mol PPF D m}^{-2} \text{ s}^{-1}$  with 16 h light and 8 h dark and day/night temperatures of 25°C/20°C, with mean day/night RH *ca.* 65 %.

### 2.3.2 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 ( $g_s$ ), transpiration rate ( $E$ ) and intercellular  $[\text{CO}_2]$  ( $C_i$ ) 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 (*ca.* 5 min) at a saturating photosynthetically active radiation (PAR) level of 1500  $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  (determined from preliminary light response curves) with 400  $\mu\text{l L}^{-1} \text{ CO}_2$  and a flow rate of 500  $\mu\text{mol s}^{-1}$ . Leaf temperature was maintained at 25°C and relative humidity was *ca.* 65% during the measurements. Photosynthetic water use efficiency ( $WUE$ ) was calculated as  $A/g_s$ .

### 2.3.3 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 whole root system of each plant was carefully excavated onto 2-mm<sup>2</sup> 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. 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.

#### 2.3.4 Foliar elemental and isotope analysis

Foliar nutrient concentrations were determined by ashing the milled leaf material at 480 °C for 8 h before dissolving with a 1:1 (v/v) of 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  $^{15}\text{N}/^{14}\text{N}$  isotope ratios (expressed as  $\delta^{15}\text{N}$ ) were determined using mass spectrometry. Atmospheric  $\text{N}_2$  fixation is expected to give a  $\delta^{15}\text{N}$  signature closest to the natural abundance values of almost zero whilst urea-N should show enriched  $\delta^{15}\text{N}$  signature due to losses of N through volatilization (Högberg, 1990, Högberg and Johannisson 1993). Based on variations in foliar  $\delta^{15}\text{N}$ , N sources used by plants may be distinguishable (e.g. Vitousek et al., 1989; Robinson, 2001; Cernusak et al., 2009a). Between 1.900 and 2.000 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 analyzer 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.

#### 2.3.5 Data analysis

One-way analyses of variance (ANOVA) and *post-hoc* Tukey's HSD tests were performed using Statistica (version 10 Statsoft Inc., Tulsa, USA) to evaluate differences in total biomass, shoot:root ratios, water flux and foliar nutrient content between the fertiliser treatments. Linear models relating total biomass,  $E$ ,  $g_s$ ,  $WUE$ ,  $C_i$ ,  $A$  and foliar elemental concentrations to distance from 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  $A$  vs.  $g_s$ .

## 2.4 Results

### 2.4.1 Plant biomass response to N accessibility

The biomass of ‘mass-flow/diffusion’ *P. vulgaris* plants was negatively correlated with distance from the N source (Fig. 2.2A), 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/diffusion’ plants was statistically indistinguishable from that of ‘interception’ plants, as were their shoot:root ratios. Total root weight did not vary significantly with distance from N-source. Despite evidence of increased N limitation with distance from the N source, the shoot:root ratios of mass-flow plants did not change significantly with distance from the N source (Fig. 2.2B).

### 2.4.2 Gas exchange response to N accessibility

Although  $g_s$  and  $A$  were positively correlated in both ‘mass-flow/diffusion’ and ‘interception’ plants, the latter showed a steeper slope (Fig. 2.3), indicating that changes in  $g_s$  and  $A$  were approximately half as responsive in ‘mass-flow/diffusion’ as in ‘interception’ plants (ANCOVA interaction term:  $t = -2.495$ ;  $P = 0.014$ ). ‘Mass-flow/diffusion’ plants had a wider range of  $g_s$  values ( $0.1 - 1.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than the ‘interception’ plants ( $< 0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), suggesting greater plasticity of response of stomatal conductance in ‘mass-flow/diffusion’ plants.

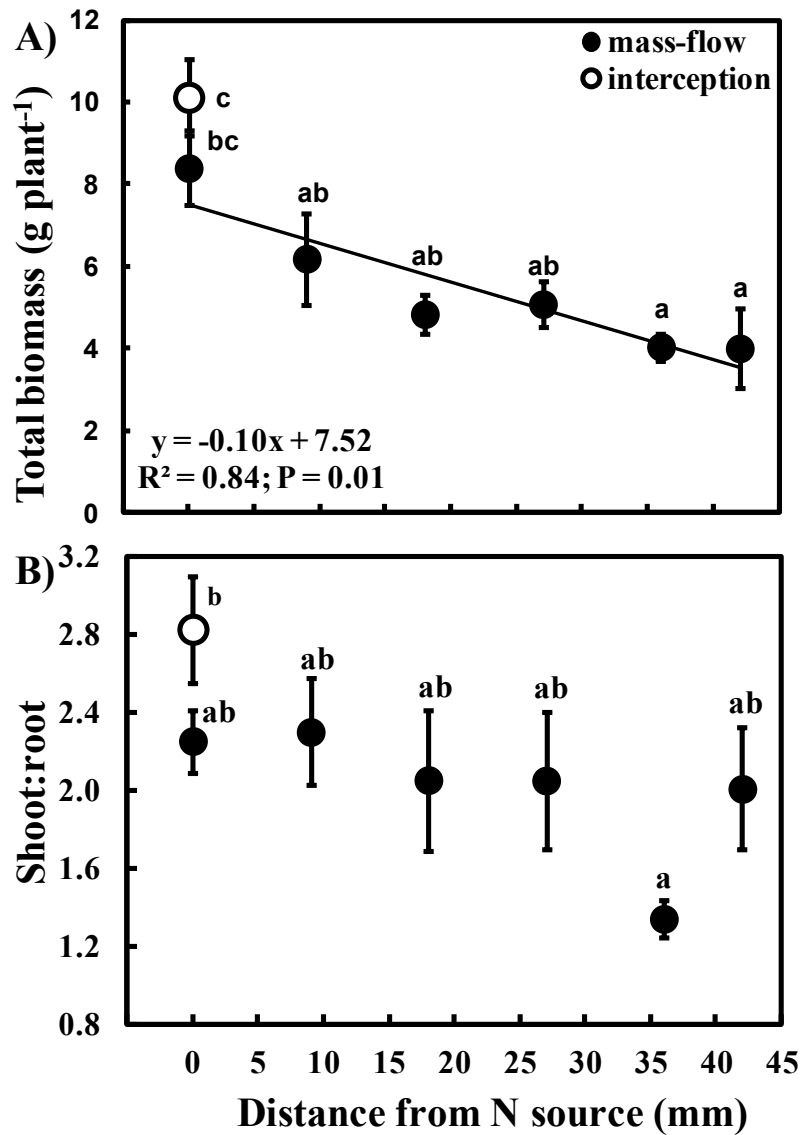


Fig. 2.2. A) Biomass (g dry weight per plant) and B) shoot:root ratios of *Phaseolus vulgaris* plants accessing a slow release urea fertilizer either dibbled around the roots ('interception') or placed at six distances from the mesh barrier ('mass-flow/diffusion'). Each circle and bar represents a mean  $\pm$  SE (n = 6); significantly different means (after a one-way ANOVA with *post-hoc* Tukey's HSD,  $P < 0.001$  for total biomass and  $P < 0.01$  for shoot:root) have different letters. Regression equation relates total biomass to distance from N-source.

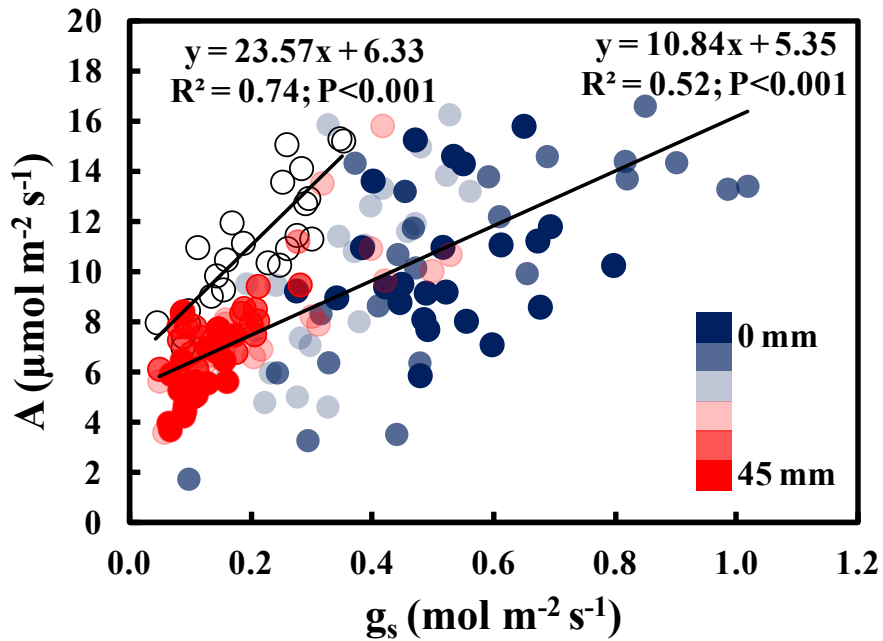


Fig. 2.3. Correlation of stomatal conductance ( $g_s$ ) with photosynthetic rate ( $A$ ) in *Phaseolus vulgaris* accessing a slow release urea fertilizer either dibbled around the roots ('interception', open circles) or placed at six distances from the mesh barrier ('mass-flow/diffusion', coloured circles). Colours indicate the variation in distance from N-source, as shown in the key. The regression equation, coefficient of determination ( $R^2$ ) and probability of significance ( $P$ ) are shown on the panel.

$A$ ,  $g_s$ ,  $E$  and  $C_i$  of the 'mass-flow/diffusion' plants all declined with increasing distance from the N source, attaining the highest values over a range of distance from 0 to 20 mm (Fig. 2.4, 2.5). While 'mass-flow/diffusion' 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),  $g_s$  (2.6-fold),  $C_i$  (1.2-fold) and lower  $WUE$  (3.4-fold).  $E$  and  $g_s$  of 'mass-flow/diffusion' plants were statistically indistinguishable from those of 'interception' plants between 27 and 45 mm from the N source. The changes in  $A$  and  $E$  resulted in increased  $WUE$  with distance from the N source.

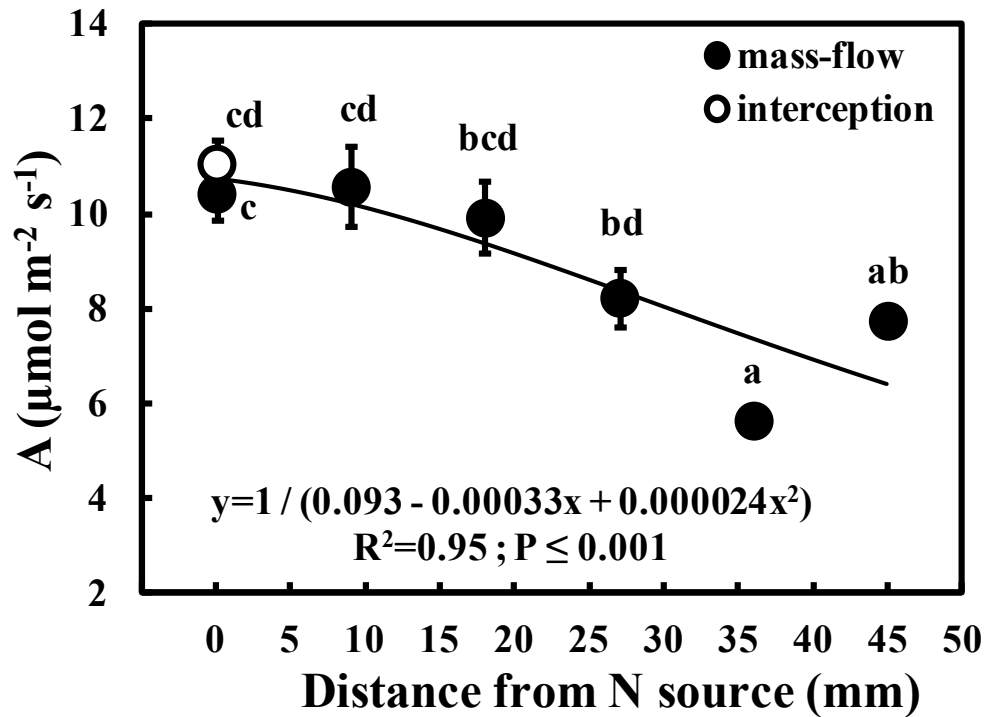


Fig. 2.4. Variation of photosynthetic rate ( $A$ ) with distance from the N-source in *Phaseolus vulgaris* accessing a slow release fertilizer either by 'interception' (open circles) or by 'mass-flow/diffusion' (closed circles). Each circle and bar represents a mean  $\pm$  SE ( $n = 6$ ). Means with different letters showed significant differences after a one-way ANOVA with *post-hoc* Tukey's HSD. The regression equation, coefficient of determination ( $R^2$ ) and probability of significance ( $P$ ) are shown on the graph.

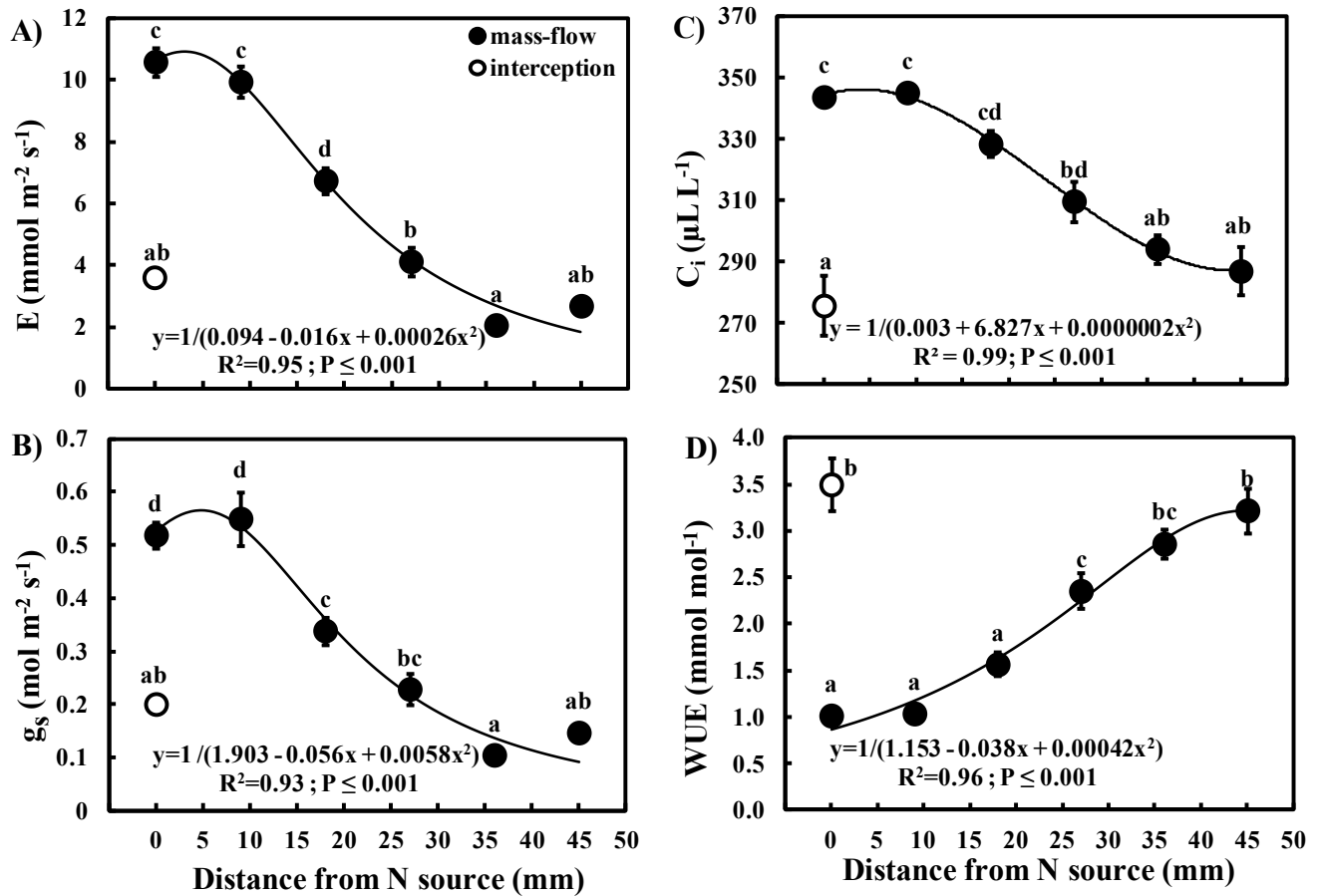


Fig. 2.5. Relationship between distance from N-source and A) transpiration ( $E$ ), B) stomatal conductance ( $g_s$ ), C) internal  $\text{CO}_2$  concentration ( $C_i$ ) and D) photosynthetic water use efficiency ( $WUE$ ) in *Phaseolus vulgaris* accessing a slow release N-fertilizer either by 'interception' (open circles) or by 'mass-flow/diffusion' (closed circles). Each circle and bar represents a mean ( $n = 6$ )  $\pm$  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 ( $R^2$ ) and significance values ( $P$ ) are indicated in each panel.

### 2.4.3 Foliar nutrient responses to N accessibility

Foliar [N] had an asymmetric biphasic relationship with increasing distance from the N source, with a peak at *ca.* 10 mm (Fig. 2.6A). Foliar N contents were negatively correlated with distance from N-source (Fig. 2.6B), corroborating the importance of N in limiting growth. I observed no nodules and the positive  $\delta^{15}\text{N}$  values ranging from 5 – 15 ‰ (mean 11 ‰,  $n = 42$ ), suggests use of a more enriched N source following volatilisation of  $\text{NH}_3$  from urea-N (e.g. Högberg, 1990, Högberg and Johannisson, 1993).

Foliar [P] and [K] increased compared to 0-distance but stayed rather constant thereafter (Fig. 2.6C and E), and total P and K contents declined as distance from N-source increased (Fig. 2.6D and 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  $\delta^{13}\text{C}$  values (Fig. 2.7). Plant  $\delta^{13}\text{C}$  was used as it provides a time integrated estimate of intercellular to ambient  $\text{CO}_2$  mole fractions ( $C_i/C_a$ ) (Farquhar et al., 1982; Brugnoli and Farquhar, 2000), which are a long-term proxy of *WUE*.  $\delta^{13}\text{C}$  was more strongly correlated with N:P ( $R^2 = 0.53$ ;  $P < 0.001$ ) than with [N] ( $R^2 = 0.29$ ;  $P = 0.03$ ; Fig. 2.7). The low  $\delta^{13}\text{C}$  values also indicated no water stress in these plants, as might be expected, since the sand was kept close to field capacity.

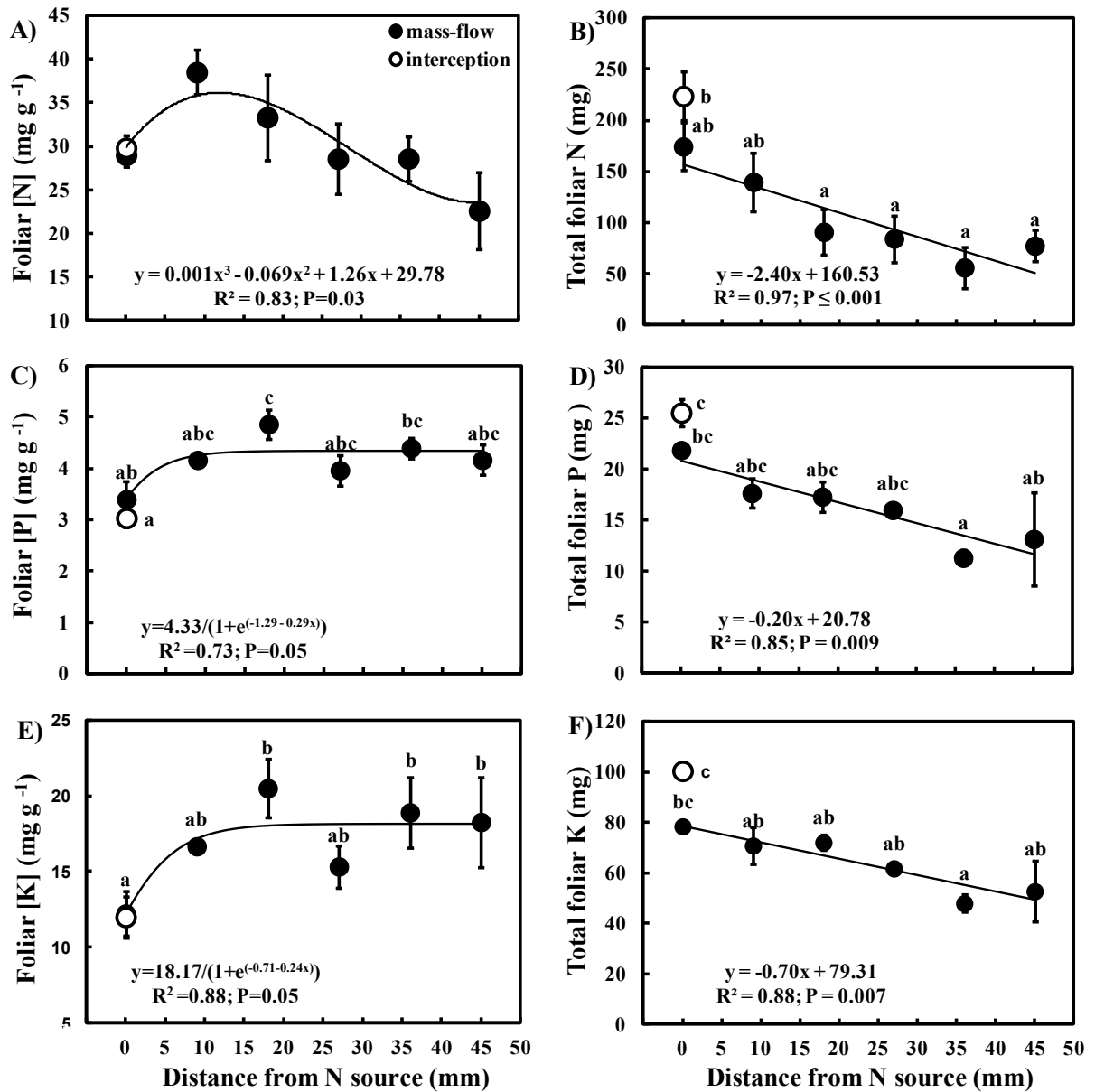


Fig. 2.6. Relationship between distance from N-source and A) foliar [N], B) foliar N content, C) foliar [P], D) foliar P content, E) foliar [K] and F) foliar K content in *Phaseolus vulgaris* accessing a slow release fertilizer either by ‘interception’ (open circles) or by ‘mass-flow/diffusion’ (closed circles). Each circle and bar represents a mean ( $n = 6$ )  $\pm$  SE. Significant differences amongst 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.

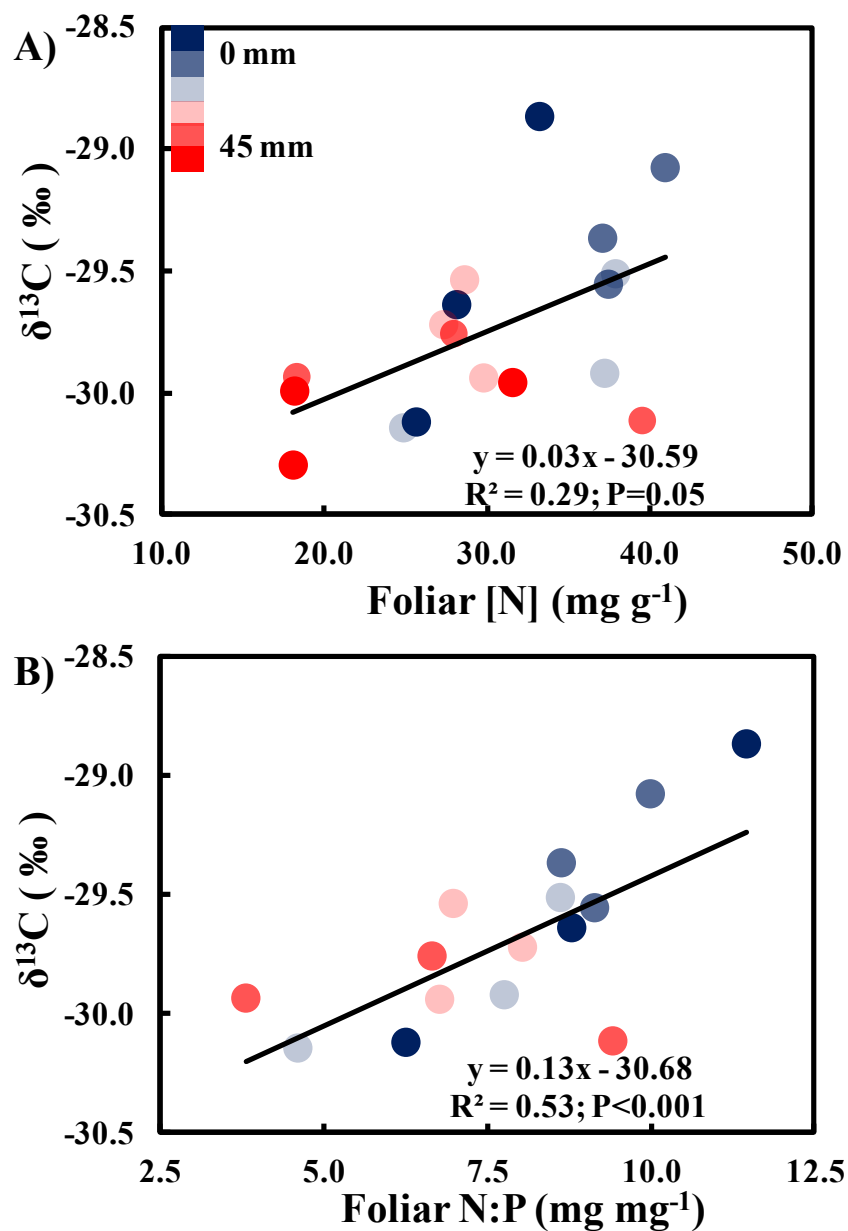


Fig. 2.7. Relationship of A) foliar [N] and B) foliar N:P with  $\delta^{13}\text{C}$  in *Phaseolus vulgaris* accessing a slow release fertilizer sequestered from six distances through a mesh-lined substrate compartment. Colours show the variation in distance from N-source as shown on the key. The regression equation, coefficient of determination ( $R^2$ ) and the significance ( $* = P < 0.05$ ;  $***P < 0.001$ ) of the slope are shown on the panel.

## 2.5 Discussion

This 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  $\delta^{15}\text{N}$  values indicate that the plants utilised the urea-N from which  $\text{NH}_3$  had been lost through volatilisation resulting in higher  $\delta^{15}\text{N}$  values than that of the plants supplied urea (e.g. Högberg, 1990; Högberg and Johannisson 1993), suggesting that the slow release fertiliser was the main source of N. Second, plant biomass and tissue [N] declined with distance from the N-source, 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  $\text{CO}_2$  uptake (e.g. Nobel, 1999) is questionable and needs to incorporate the regulatory role played by N.

To verify that N regulates transpiration I placed a common agricultural N-fertilizer (urea) 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). My hypothesis that N regulates transpiration was supported by the higher  $E$ ,  $g_s$  and  $C_i$  values and lower  $WUE$  in ‘mass-flow/diffusion’ than ‘interception’ plants at 0 mm from N-source. This was associated with differential responsiveness of  $A$  to  $g_s$  in ‘interception’ versus ‘mass-

flow/diffusion' plants. Consistent with changes in  $WUE$ , the slope of  $A/g_s$  for 'mass-flow/diffusion' plants was half that of 'interception' plants and there was a greater range of  $g_s$  in 'mass-flow/diffusion' 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/diffusion' 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 supra-optimal [N], as predicted by Wilkinson et al. (2007) (Fig. 2.8). The 'interception' plants had direct access to ca. 2.5 g N plant<sup>-1</sup> from the Multicote 4\*, which was 8-fold higher than the calculated total plant uptake of ca. 0.3 g N plant<sup>-1</sup> (calculated as 30 mg N g<sup>-1</sup> of DM x 10 g DM plant<sup>-1</sup>). Given that leaching was minimal, such supra-optimal [N] potentially suppressed the transpiration rates (Wilkinson et al. 2007) by altering pH within the cytosol and phytohormone levels (ABA and cytokinins), which regulate stomatal conductance (Wilkinson and Davies, 1997; Bacon et al., 1998; Wilkinson et al., 1998, Wilkinson et al., 2007; Cramer et al., 2009). However, the limitation in access to N imposed on the 'mass-flow/diffusion' 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). My 'interception' and 'mass-flow/diffusion' data seem to match parts of the biphasic trajectory proposed by Wilkinson et al. (2007) (Fig. 2.8). 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 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.

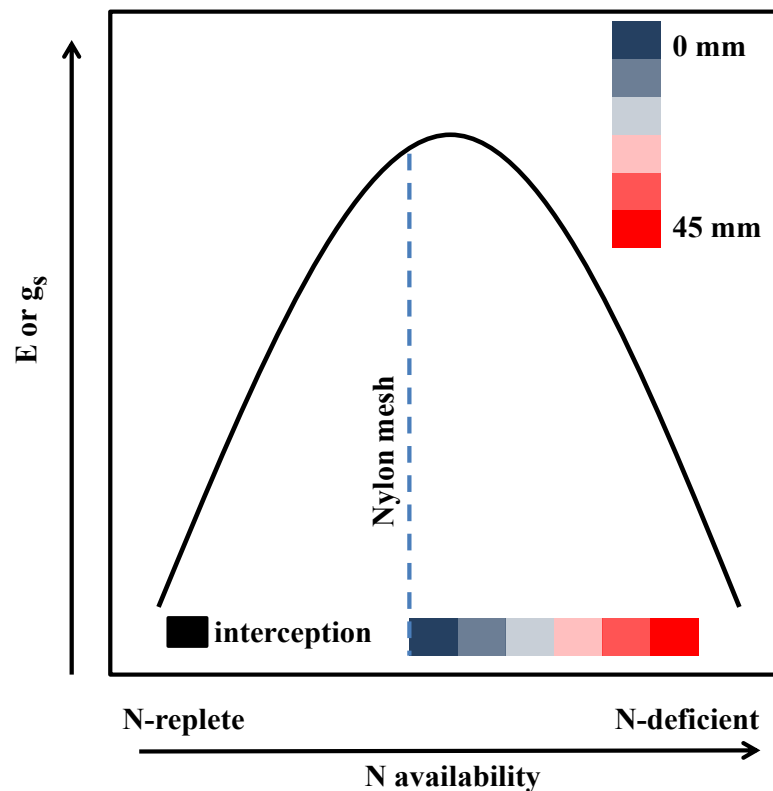


Fig. 2.8. A schematic representation of the effect of decreasing N availability on leaf stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) modified from Wilkinson et al. (2007). Excess [N] availability in the ‘interception’ treatment is indicated to result in low  $E$  and  $g_s$ , which increase as N becomes more limiting to compensate for N availability, and then decrease when N is extremely limiting (e.g. 45 mm from N-source). Colours indicate the variation in distance from N-source as shown on the key. Black square indicates the ‘interception’ treatment. Blue dotted line shows the position of nylon mesh relative to the N source.

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).

As  $\text{NO}_3^-$  concentration at the root surface drops during active uptake (Taylor and Bloom, 1998), a coordinated increase in water fluxes may be necessary to ensure delivery and maintenance of high concentrations at the root surface (Cramer et al., 2008; 2009). Early investigations on cotton (*Gossypium hirsutum*) plants that were exposed to different levels of N availability revealed that exposure of  $\text{NO}_3^-$ -deprived plants to elevated  $\text{NO}_3^-$  levels increased their root hydraulic conductance (Radin 1984, Radin and Ackerson 1981, Radin et al. 1982; Radin and Matthews 1988; Radin 1990). Since then, multiple lines of evidence have emerged linking exogenous and endogenous  $\text{NO}_3^-$  availability to both root and shoot hydraulic properties. For instance,  $\text{NO}_3^-$ -deprived plants up-regulate their water fluxes (Gorska et al. 2008; Cramer et al., 2008; Gloser 2008) for the mass flow of  $\text{NO}_3^-$  toward the root surface thus maintaining high nutrient concentrations at the root surface. Nitrate transporters and proteins involved in its assimilation are up-regulated in response to exogenous  $\text{NO}_3^-$  concentrations, thus increasing its uptake in the event of detecting a  $\text{NO}_3^-$ -rich patch (Forde and Clarkson, 1999; Forde, 2002). When  $\text{NO}_3^-$  levels are high, plants may down-regulate these water fluxes (e.g. Wilkinson et al., 2007; Scholz et al., 2007; Cramer et al., 2008) through multiple pathways that are controlled by  $\text{NO}_3^-$  levels (e.g. Stitt, 1999; Sakakibara et al., 2006; Cramer et al. 2009) and involving the downstream products of  $\text{NO}_3^-$  assimilation (Hoarau et al., 1996; Clarkson et al., 2000).

The higher transpiration rates of ‘mass-flow/diffusion’ plants were associated with higher foliar [P] and [K], these reaching maximum concentrations when the distance from N-source was  $\geq 10$  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 (Tsay et al., 2011; Hammond and White, 2008; Marschner and Cakmak, 1986; Valizadeh et al., 2002). The stronger relationship between  $\delta^{13}\text{C}$  and N:P ratios than 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) we do not know 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. 2.6A), possibly indicating that it is N-flux that is important in biochemical regulation of  $g_s$  and WUE, as suggested previously (Cramer et al., 2009). The N acquired by roots as  $\text{NH}_4^+$  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  $\text{NH}_4^+$ , root  $\text{NO}_3^-$  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, it 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 ABA, which elicit stomatal closure (Wilkinson et al., 2004; Wilkinson et al., 2007; Jia and Davies, 2007).

Leaf-level water-use efficiency generally increases in response to increasing leaf N concentration in  $\text{C}_3$  plants (Wong, 1979; Toft et al., 1989; Duursma and Marshall, 2006). This may be due to the down-regulation of transpiration because the plant has sufficient N (Cramer et al., 2008). Alternatively, this may be because more leaf [N] is usually associated with more

photosynthetic capacity, which allows for a greater photosynthetic rate at a given rate of water loss (Cernusak et al., 2007). The possible reason why water use efficiency correlated better with N:P ratio than with [N] alone is that P may not function in this second role (Cernusak et al., 2007; Garish et al., 2010). Phosphorus can therefore potentially act loosely as a tracer for mass flow, all else being equal, and N:P ratios are related to the ratios of photosynthesis to transpiration, i.e. water use efficiency.

My experimental design 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 more than 36 mm from the roots. Nevertheless, even at these distances the plants managed to acquire sufficient N for growth. The distance of N-source effectively generated different concentrations of N supply that were growth limiting, which was analogous to growing the plants at a range of different growth limiting N concentrations. Whilst N-limitation of growth was observed in sand, this N gradient may not necessarily be shown by plants in hydroponics where nutrients are well mixed and easily accessed by the roots. 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 (Chapter 4). 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 localised 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 like N are known to be acquired through mass-flow (Barber, 1995), acquisition of immobile nutrients like P are thought to largely depend on root architectural modifications for uptake (e.g. mycorrhiza 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, in which water supplies are dwindling (Wilkinson and Hartung, 2009), strategic N-fertilization may provide an opportunity for moderating plant water demands.

## 2.6 References

- Barber SA. 1962. A diffusion and mass-flow concept of soil nutrient availability. *Soil Science* 93, 39–49.
- Barber SA. 1995. *Soil nutrient bioavailability: a mechanistic approach*, 2nd edn. New York: Wiley.

- Brugnoli E, Farquhar GD. 2000. Photosynthetic fractionation of carbon isotopes. In: Leegood RC, Sharkey TC, von Caemmerer S. (eds.) *Photosynthesis: physiology and metabolism*. Dordrecht, The Netherlands: Kluwer, 399-434.
- Caird MA, Richards JH, Donovan LA. 2007. Nighttime stomatal conductance and transpiration in C<sub>3</sub> and C<sub>4</sub> plants. *Plant Physiology* 143, 4–10.
- 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.
- Cernusak LA, Winter K, Aranda J, Turner BL, Marshall JD. 2007. Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *Journal of Experimental Botany* 58, 3549-3566.
- Cernusak LA, Winter K, Turner BL. 2009a. Plant  $\delta^{15}\text{N}$  Correlates with the transpiration efficiency of nitrogen acquisition in tropical trees. *Plant Physiology* 151, 1667-1676.
- Cernusak LA, Winter K, Turner BL. 2009b. Leaf nitrogen to phosphorus ratios of tropical trees: experimental assessment of physiological and environmental controls. *New Phytologist* 185, 770–779.
- Cernusak LA, Winter K, Turner BL. 2011. Transpiration modulates phosphorus acquisition in tropical tree seedlings. *Tree Physiology* 31, 878-885.
- Childs EC, Collis-George N. 1950. The permeability of porous materials. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences* 201, 392–405.
- 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.
- Clarkson DT. 1981. Nutrient interception and transport by root systems. In: Johnson CB. (ed.) *Physiological factors limiting plant productivity*. London: Butterworths, 307–314.

- Cowan IR, Troughton JH. 1971. The relative role of stomata in transpiration and assimilation. *Planta* 97, 325-336.
- Cramer MD, Hawkins H-J, Verboom GA. 2009. The importance of nutritional regulation of plant water flux. *Journal of Experimental Botany* 161, 15-24.
- Cramer MD, Hawkins HJ. 2009. A physiological mechanism for the formation of root casts. *Palaeogeography, Palaeoclimatology, Palaeoecology* 274, 125-133.
- Cramer MD, Hoffmann V, Verboom GA. 2008. Nutrient availability moderates transpiration in *Erharta calycina*. *New Phytologist* 179, 1048–1057.
- Crawley MJ. 2005. *Statistics: an introduction to using R*. Sussex: John Wiley and Sons Ltd, West Sussex.
- Duursma RA, Marshall JD 2006. Vertical canopy gradients in  $\delta^{13}\text{C}$  correspond with leaf nitrogen content in a mixed-species conifer forest. *Trees* 20,496-506.
- Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* 9, 121-37.
- Forde BG, Clarkson DT. 1999. Nitrate and ammonium nutrition of plants: physiological and molecular perspectives. *Advances in Botanical Research* 30, 1–90.
- Forde BG, Lorenzo H. 2001. The nutritional control of root development. *Plant Soil* 232, 51-68.
- Forde BG. 2000. Nitrate transporters in plants: structure, function and regulation. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1465, 219-235.
- Forde BG. 2002. Local and long-range signalling pathways regulating plant responses to nitrate. *Annual Review of Plant Biology* 53, 203-224.
- Gahoonia TS, Raza S, Nielsen NE. 1994. Phosphorus depletion in the rhizosphere as influenced by soil moisture. *Plant Soil* 159, 231–218.

- Garrish V, Cernusak LA, Winter K, Turner BL. 2010. Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*. *Journal of Experimental Botany* 61, 3735-3748.
- Gloser V, Zwieniecki MA, Oriens C, Holbrook NM. 2007. Dynamic changes in root hydraulic properties in response to nitrate availability. *Journal of Experimental Botany* 58, 2409–2415.
- Gorska A, Ye Q, Holbrook NM, Zwieniecki MA. 2008. Nitrate control of root hydraulic properties in plants: translating local information to whole plant response. *Plant Physiology* 148, 1159–1167.
- Guo S, KaldenhoVR, Uehlein N, Sattelmacher B, Brueck H. 2007. Relationship between water and nitrogen uptake in nitrate- and ammonium-supplied *Phaseolus vulgaris* L. plants. *Journal of Plant Nutrition and Soil Science* 170, 73–80.
- Hack JJ, Caron JM, Yeager SG, Oleson KW, Holland MM, Truesdale JE, Rasch PJ. 2006. Simulation of the global hydrological cycle in the CCSM Community Atmosphere Model Version 3 (CAM3): mean features. *Journal of Climate* 19, 2199–2221.
- Hackett C. 1968. A study of the root system of barley. I Effects of nutrition on two varieties. *New Phytologist* 67, 287-299.
- Hammond JP, White PJ. 2008. Sucrose transport in the phloem: integrating root responses to phosphorus starvation. *Journal of Experimental Botany* 59, 93–109.
- Henzler T, Waterhouse RN, Smyth AJ, Carvajal M, Cooke DT, Schäffner 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.
- Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424, 901–908.

- Hewitt EJ. 1952. *Sand and water culture methods used in the study of plant nutrition*. Technical communication No. 22. Farnham Royal, UK: Commonwealth Agricultural Bureau.
- Ho MD, Rosas JC, Brown KM, Lynch JP. 2005. Root architectural tradeoffs for water and phosphorus acquisition. *Functional Plant Biology* 32, 737–748.
- Hoarau J, Barthes L, Bousser A, Deleens E, Prioul JL. 1996. Effect of nitrate on water transfer across roots of nitrogen pre-starved maize seedlings. *Planta* 200, 405–415.
- Högberg P, Johannisson C. 1993.  $^{15}\text{N}$  abundance of forests is correlated with losses of nitrogen. *Plant and Soil* 157, 147-150.
- Högberg P. 1990. Forests losing large quantities of nitrogen have elevated  $^{15}\text{N}$  ratios. *Oecologia* 84, 229-231.
- Horn R, Taubner H, Wuttke M, Baumgartl T. 1994. Soil physical properties related to soil structure. *Soil Tillage Research* 30, 187-216.
- Jia W, Davies WJ. 2007. Modification of leaf apoplastic pH in relation to stomatal sensitivity to root-sourced abscisic acid signals. *Plant Physiology* 143, 68-77.
- Kalra YP. 1998. *Handbook of standard methods of plant analysis*. Boca Raton, FL, USA: CRC Press.
- Kramer PJ, Boyer JS. 1995. Stomata and gas exchange. In: Kramer PJ, Boyer JS. (eds.) *Water relations of Plants*. San Diego: Academic Press, 257-282.
- Kupper P, Rohula G, Saksing L, Sellin A, Löhmus K, Ostonen I, Helmisaari H-S, Söber A. 2012. Does soil nutrient availability influence night-time water flux of aspen saplings? *Environmental and Experimental Botany* 82, 37–42.
- Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* 98, 693-713.

- López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L. 2003. The role of nutrient availability in regulating root architecture. *Current Opinions in Plant Biology* 6, 280–287.
- Marschner H, Cakmak I. 1986. Mechanism of phosphorus-induced zinc deficiency in cotton. II. Evidence for impaired shoot control of phosphorus uptake and translocation under zinc deficiency. *Physiologia Plantarum* 68, 491–496.
- McCormick AJ, Cramer MD, Watt DA. 2006. Sink strength regulates photosynthesis in sugarcane. *New Phytologist* 171, 759–770.
- Mengel K, Planker R, Hoffmann B. 1994. Relationship between leaf apoplast pH and iron chlorosis of sunflower (*Helianthus annuus* L.). *Journal of Plant Nutrition* 17, 1053–1065.
- Miller AJ, Cramer MD. 2004. Root nitrogen acquisition and assimilation. *Plant and Soil* 274, 1–36.
- Monteith JL. 1988. Does transpiration limit the growth of vegetation or vice versa? *Journal of Hydrology* 100, 57–68.
- Mühling KH, Lauchli A. 2001. Influence of chemical form and concentration of nitrogen on apoplastic pH of leaves. *Journal of Plant Nutrition* 25, 399–411.
- Nobel PS. 1999. *Physicochemical and environmental plant physiology*. 2nd edn. Academic Press, San Diego, California, USA.
- Olivera, M., Tejera, N., Iribarne, C., Ocaña, A. and Lluch, C. (2004), Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris*): effect of phosphorus. *Physiologia Plantarum* 121, 498–505.
- Parkhurst DF, Loucks OL. 1972. Optimal leaf size in relation to environment. *Journal of Ecology* 60, 505–537.
- Pereira PAA, Bliss FA. 1987. Nitrogen fixation and plant growth of common bean (*Phaseolus vulgaris* L.) at different levels of phosphorus availability. *Plant and Soil*. 104, 79–84.

- Power SC, Cramer MD, Verboom GA, Chimphango SB. 2010. Does phosphate acquisition constrain legume persistence in the fynbos of the Cape Floristic Region? *Plant and Soil* 334, 33-46.
- R Development Core Team, 2006. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Radin JW, Ackerson RC. 1981. Water relations of cotton plants under nitrogen deficiency. III. Stomatal conductance, photosynthesis and abscisic acid accumulation during drought. *Plant Physiology* 67,115-119.
- Radin JW, Ackerson RC. 1981. Water relations of cotton plants under nitrogen deficiency: III. Stomatal conductance, photosynthesis, and abscisic acid accumulation during drought. *Plant Physiology* 67, 115-119.
- Radin JW, Matthews MA. 1988. Water transport properties of cortical cells in roots of nitrogen and phosphorus-deficient cotton seedlings. *Plant Physiology* 89, 264–268.
- Radin JW, Parker LL, Guinn G. 1982. Water relations of cotton plants under nitrogen deficiency. V. Environmental control of abscisic acid accumulation and stomatal sensitivity to abscisic acid. *Plant Physiology* 70, 1066-1070.
- Radin JW. 1984. Stomatal responses to water stress and to abscisic acid in phosphorus-deficient cotton plants. *Plant Physiology* 76,392-394.
- Radin JW. 1990. Responses of transpiration and hydraulic conductance to root temperature in nitrogen and phosphorus-deficient cotton seedlings. *Plant Physiology* 92, 855–857.
- Raven JA, Handley LL, Wollenweber B. 2004. Plant nutrition and water use efficiency. In: Bacon MA. (ed.) *Water use efficiency in plant biology*. Oxford: Blackwell Publishing Ltd, 171–197.

- Raven JA. 1985. Regulation of pH and generation of osmolarity in vascular plants: a cost-benefit analysis in relation to efficiency of use of energy, nitrogen and water. *New Phytologist* 101, 25-77.
- Raven JA. 2008. Transpiration: how many functions? *New Phytologist* 179, 905-907.
- Rengel Z. 1993 Mechanistic simulation models of nutrient uptake: a review. *Plant and Soil* 152, 161–173.
- Robinson D. 2001.  $\delta^{15}\text{N}$  as an integrator of the nitrogen cycle. *Trends in Ecology and Evolution* 16, 153–162.
- Sahrawat KL. 1980. Control of urea hydrolysis and nitrification in soil by chemicals - prospects and problems. *Plant and Soil* 57, 335-352.
- Sakakibara H, Takei K, Hirose N. 2006. Interactions between nitrogen and cytokinin in the regulation of metabolism and development. *Trends in Plant Science* 11, 440–448.
- Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F. 2007. Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. *Tree Physiology* 27, 551–559.
- Stitt M. 1999. Nitrate regulation of metabolism and growth. *Current Opinion in Plant Biology* 2, 178–186.
- Syring KM, Claassen N. 1995. Estimation of the influx and the radius of the depletion zone developing around a root during nutrient uptake. *Plant and Soil* 175, 115-123.
- Tanner W, Beevers H. 1990. Does transpiration have an essential function in long-distance ion transport in plants? *Plant, Cell and Environment* 13, 745–750.
- Tanner W, Beevers H. 2001. Transpiration, a prerequisite for long-distance transport of minerals in plants? *Proceedings of the National Academy of Sciences, USA* 98, 9443–9447.

- Taylor A, Bloom A. 1998. Ammonium, nitrate, and proton fluxes along the maize root. *Plant Cell and Environment* 21, 1255–1263.
- Tinker PB, Nye PH. 2000. *Solute movement in the rhizosphere*. New York: Oxford University Press.
- Toft NL, Anderson JE, Nowak RS. 1989. Water-use efficiency and carbon isotope composition of plants in a cold desert environment. *Oecologia* 80,11-18.
- Tsay YF, Ho CH, Chen HY, Lin SH. 2011. Integration of nitrogen and potassium signalling. *Annual Review of Plant Biology* 62, 207-226.
- Valizadeh GR, Rengel Z, Rate AW. 2002. Role of phosphorus fertiliser banding and the ratio of nitrate to ammonium on the uptake of phosphorus and wheat growth: a glasshouse study. *Australian Journal of Experimental Agriculture* 42, 1095-1102.
- Vitousek PM, Shearer G, Kohl DH. 1989. Foliar  $^{15}\text{N}$  natural abundance in Hawaiian rainforest: Patterns and possible mechanisms. *Oecologia* 78, 383–388.
- Wiersum LK. 1958. Density of root branching as affected by substrate and separate ions. *Acta Botanica Neerlandica* 7, 174-190.
- Wilkinson S, Bacon MA, Davies WJ. 2007. Nitrate signalling to stomata and growing leaves: interactions with soil drying, ABA and xylem sap pH in maize. *Journal of Experimental Botany* 58, 1705–1716.
- Wilkinson S, Hartung W. 2009. Food production: reducing water consumption by manipulating long-distance chemical signalling in plants. *Journal of Experimental Botany* 60, 1885-1891.
- Wilkinson S. 2004. Water use efficiency and chemical signalling. In: Bacon MA. (ed.) *Water use efficiency in plant biology*. Oxford, UK: Blackwell Publishing Ltd, pp 75–112.
- Wong SC. 1979. Elevated atmospheric partial pressure of  $\text{CO}_2$  and plant growth. *Oecologia* 44:68-74

### **3 Do hydraulic redistribution and nocturnal transpiration facilitate nutrient acquisition in *Aspalathus linearis*?**

#### **3.1 Abstract**

The significance of soil water redistribution by roots and nocturnal transpiration for nutrient acquisition were assessed for deep-rooted three-year old leguminous *Aspalathus linearis* shrubs of the Cape Floristic Region (South Africa). I hypothesised that hydraulic redistribution and nocturnal transpiration facilitate nutrient acquisition by releasing moisture in shallow soil to enable acquisition of shallow-soil nutrients during the summer drought periods and by driving water fluxes from deep to shallow soil powering mass-flow nutrient acquisition, respectively. *A. linearis* was supplied with sub-surface (1 m deep) irrigation rates of 0, 2 or 4 L d<sup>-1</sup> plant<sup>-1</sup>. Some plants were unfertilized, whilst others were surface- or deep-fertilized (1 m depth) with Na<sup>15</sup>NO<sub>3</sub> and CaP/FePO<sub>4</sub>. I also supplied deuterium oxide (<sup>2</sup>H<sub>2</sub>O) at 1 m depth at dusk and measured its predawn redistribution to shallow soil and plant stems. Hydraulic redistribution of deep water was substantial across all treatments, accounting for 34% to 72% of surface-soil predawn moisture. Fourteen days after fertilization the surface-fertilized plants exhibited increased hydraulic redistribution and increased <sup>15</sup>N and P acquisition with higher rates of deep-irrigation. Deep-fertilization also increased hydraulic redistribution to surface soils, although these plants additionally accumulated <sup>2</sup>H<sub>2</sub>O in their stem tissue over-night, probably due to nocturnal transpiration. Plants engaged in nocturnal transpiration also increased <sup>15</sup>N and P acquisition from deep fertilizer sources. Thus both nocturnal transpiration and hydraulic redistribution increased acquisition of shallow soil N and P, possibly through a combination of increased nutrient availability and mobility.

## 3.2 Introduction

The root systems of many plants explore large volumes of soils, as a consequence encountering soil patches that differ greatly with respect to moisture and nutrients (Jackson and Caldwell, 1993; Prieto et al., 2012). Under these circumstances, roots may serve as conduits for the movement of water from wetter to drier regions of the soil profile, a phenomenon known as ‘hydraulic redistribution’ (hereafter, HR; Burgess et al., 1998) that has been documented in more than 60 plant species, including trees, shrubs and grasses (Prieto et al., 2012, Neumann and Cardon, 2012). Although HR is especially prevalent in species from seasonally droughted environments, it has also been reported in tropical forest (Jackson et al. 2000) and crop taxa (Pang et al., 2013; Sekiya et al., 2011; Sekiya and Yano, 2004; Allen et al., 2004). Despite being common, the ecological and physiological benefits of HR remain unclear (Ryel, 2004).

HR is powered by water potential gradients that exist between the roots and soil (e.g. Burgess and Bleby, 2006; Hawkins et al., 2009). Thus the magnitude and direction of HR is controlled by the location and relative strengths of sources of water and sinks for water, which are in turn influenced by a network of competing water potential gradients in the soil-plant-atmosphere continuum (Nadezhdina et al., 2010; Prieto et al., 2012). High nocturnal transpiration, for example, lowers leaf water potentials, thereby forcing water to flow towards the canopy and reducing HR towards the drier soil (Hutline et al., 2004; Howard et al., 2009; Prieto et al., 2010). At night in the absence of strong nocturnal transpiration when stomatal conductance is low, water is moved by roots from wetter to drier soil patches to equilibrate the existing gradients in soil and plant water potentials (Prieto et al., 2012). Thus, HR may be modulated by conditions that alter plant and soil water potentials such as soil moisture and evaporative demand (Scholz et al., 2002). Water deep in the soil profile, for instance, provides

the conditions for upward HR to shallow soil layers (Ludwig et al., 2003; Kurz-Besson et al., 2006; Muñoz et al., 2008; Burgess et al. 2000; Ryel et al. 2002)

HR of water from deep to shallow soil may enable access to nutrients (e.g. N and P) that accumulate in topsoil due to plant litterfall (Jobbagy and Jackson, 2001; 2004). Indeed, HR has been found to enhance uptake of some nutrients; e.g. N (Matzner and Richards, 1996; Dawson, 1997; Leffler et al., 2004, Armas, et al., 2012; Shen et al., 2011), Zn (Nambiar, 1976) and  $\text{PO}_4^{3-}$  (Wang et al., 2009). Other studies, however, have reported no HR associated uptake of  $\text{NO}_3^-$  (Snyder et al., 2008),  $\text{PO}_4^{3-}$  (Rose et al., 2008; Wang et al., 2009) or Li (a tracer for K; Hawkins et al., 2009; Rose et al., 2008). Such inconsistencies have largely been attributed to lack of suitable control treatments, limited experimental time scales, or differences in the form of supplied nutrients (Armas et al., 2012). Moreover, the prevailing environmental conditions during these experiments also affect the magnitudes and the direction of HR fluxes (Prieto et al., 2012) resulting in downward, upward or lateral fluxes of water (e.g. Burgess et al., 1998; Hutline et al., 2003; Smart et al., 2005) and inconsistent effects on nutrient acquisition.

There are several mechanisms through which HR may function to promote nutrient acquisition: 1) through prolonging the life span of fine roots and maintaining root–soil contact in dry soils by redistributing water into the soil occupied by these roots (Dawson, 1993; Caldwell et al., 1998; Bauerle et al., 2008); 2) through maintaining active microbial floras, including mycorrhizas (Querejeta et al., 2003; Warren et al., 2008; Lehto and Zwiazek, 2011); 3) through increasing mineralisation and/or nutrient acquisition from the soil that is wetted by HR (e.g. Prieto et al., 2012; Armas et al., 2012); and 4) through facilitating root acquisition of nutrients in fertile, but dry soil patches through daily cycles of soil wetting and drying which improves nutrient mobility (Aanderud and Richards, 2009; Cardon and Gage, 2006; Matzner and Richards, 1996; Leffler et al., 2004; Shen et al., 2011). Nutrient mobility in soils wetted

by HR may be enhanced by both improved diffusion of nutrients (Barber 1995) and mass-flow of nutrients (Cramer et al. 2009).

Nutrients may in turn moderate water potential gradients through physiological controls on water flux or through determining the growth of plants. For example, root conductance changes with exposure to  $\text{NO}_3^-$  (e.g. Gloser et al., 2007). Acquisition of  $\text{NO}_3^-$  also modulates both day (Clarkson et al., 2000; Gorska et al., 2008; Cramer et al., 2008; 2009) and nocturnal transpiration (Kupper et al., 2012), possibly altering plant water potentials and consequently the magnitude of HR. Whilst HR is suggested to be a passive physical process that occurs even through the roots of fully senesced plants (e.g. Leffler et al., 2005), I envisage the nutritional control of transpiration to modulate HR, particularly where nutrients are leached to wetter deep soil layers. Increased transpiration suppresses HR; possibly because leaves become stronger sinks forcing water inflow from the soil through the stems towards the leaves, rather than releasing it back to the soil (Howard, 2009; Prieto et al., 2012). Modulation of HR may also occur secondarily through the  $\text{NO}_3^-$  regulation of transpiration (Wilkinson et al., 2007; Cramer et al., 2009), which may alter water potential gradients and consequently HR. To my knowledge, there has been no report of nutrients modulating the magnitude or direction of HR.

In this study, I used *Aspalathus linearis* (Burm. F) Dahlg. (Fabaceae) shrubs to test the hypothesis that HR promotes nutrient acquisition by i) driving water fluxes from deep to shallow soil enabling acquisition of nutrients from shallow soil; and ii) driving mass-flow of nutrients in deep soil towards roots with concurrent release of water in the topsoil. I also examined evidence that nocturnal transpiration acts as a competitive sink for water with HR enabling mass-flow at night from deep wet soils. To create a range of soil water potentials, three deep-soil irrigation levels were supplied to *A. linearis* plants in the field and a fertilizer containing  $^{15}\text{N}$  and P supplemented either in surface or deep soils.

### 3.3 Materials and Methods

#### 3.3.1 Site description and experimental set-up

The study site was on the farm Bloemfontein (South Africa; 31°44'04.96"S; 19°08'18.77"E) where *Aspalathus linearis* is cultivated 'organically' for commercial *rooibos* tea production. The *ca.* 1.5 m tall shrub is native to the western portion of the Cape Floristic Region (CFR), South Africa, and forms cluster roots, arbuscular mycorrhizal associations and also rhizobial nodules that fix N<sub>2</sub> (Hawkins et al., 2011). *Aspalathus linearis* has a tap root extending to 2 m or more in depth (Morton, 1983), and often reaching the ground water, which is why the plant can survive periods of drought (Gérard, 2010). My observations of root distribution down a soil profile of 1.63 m depth showed *ca.* 75% of roots to be distributed in the surface soil (*ca.* 30 cm). The vegetation is arid-fynbos (Moll et al., 1984) and the site receives 135 mm year<sup>-1</sup> of rain and has a Mediterranean-type climate, the lowest monthly rainfall falling in January and the highest (43 mm per month) in June, with a negligible amount of rain falling between mid-November and mid-March (Fig. 3.1). The mean annual temperature for the nearby town of Nieuwoudtville is 19°C. To monitor soil volumetric moisture at the beginning of the study, four 5-TE moisture sensors (Decagon Devices Inc., Pullman, USA) were buried at 0.2, 0.4, 0.6 and 0.8 m depths at 0.3 m distance from the plant and interfaced with EM50 data loggers (Decagon Devices Inc., Pullman, USA) that logged at 2 h intervals. The 5-TE moisture sensor is designed to measure the water content, electrical conductivity, and temperature of a soil based on a 5-point dielectric calibration that provides dielectric permittivity measurements. Three-year old *A. linearis* (n = 72) growing in three rows 4 m apart were tagged, leaving single "guard" rows of non-experimental plants between the tagged rows. To supplement groundwater, holes (70 mm diameter) were augered to 1.2 m depth at 0.25 m from the stem of each plant and 1.2 m long PVC access tubes (60 mm Ø) inserted.

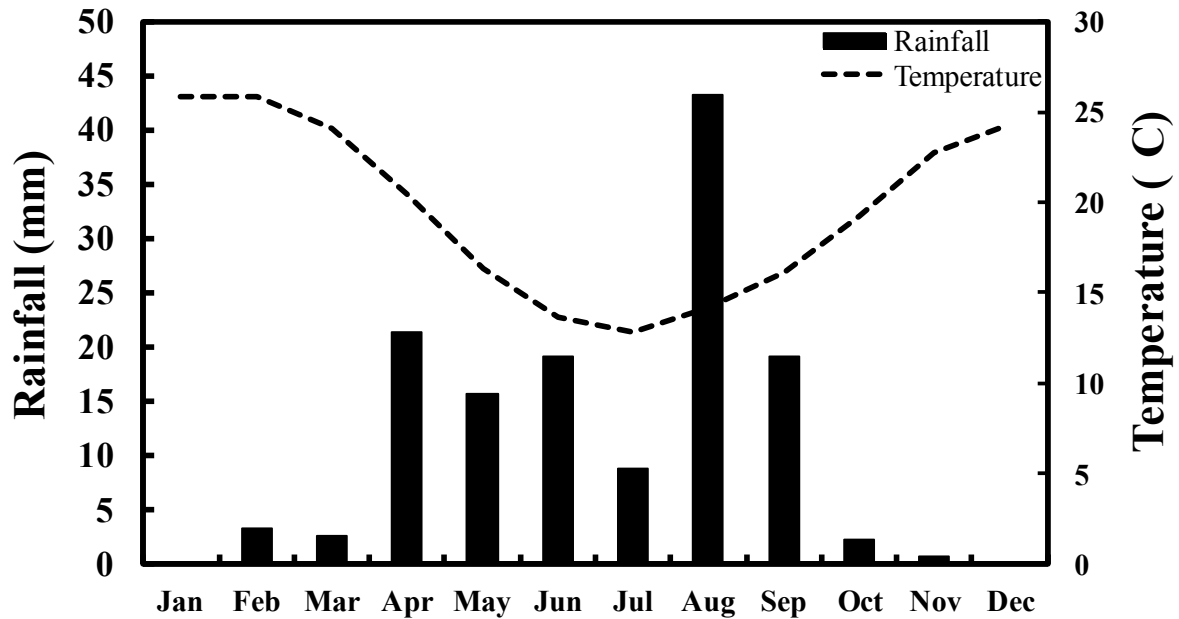


Fig. 3.1. Monthly rainfall and average temperatures at Bloemfontein Farm, Niewoudtville during the period May 2010 to April 2011. There was no rain between the 8<sup>th</sup> Nov 2010, when fertilizer treatments were supplied and the 26<sup>th</sup> Feb 2011(at 110 d post-fertilization). Rainfall on the 27<sup>th</sup> and 28<sup>th</sup> of February 2011 was 0.6 mm and 2.6 mm, respectively, at 111-112 d post-fertilization.

After allowing the roots to adjust to the soil disturbance for 14 d, an automated drip irrigation system was set up to supply the deep soil layers with 0 L d<sup>-1</sup>, 2 L d<sup>-1</sup> or 4 L d<sup>-1</sup> (per plant) through the access tubes, dripped from 18H00-19H00. Nutrients were then supplied as a solid fertilizer, coated onto dried sand. I made the fertilizer from 65 kg of medium-grained commercial sand (grade 30/10, Consol Minerals, South Africa) mixed with 30 mg kg<sup>-1</sup> of 96% Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, 70 mg kg<sup>-1</sup> FePO<sub>4</sub>·2H<sub>2</sub>O, 600 mg kg<sup>-1</sup> of 99% NaNO<sub>3</sub>, 5.12 mg kg<sup>-1</sup> of 98 atom % Na<sup>15</sup>NO<sub>3</sub> (Sigma-Aldrich, St. Louis, MO, USA) and 12 L distilled water. The slurry was mixed in a cement mixer for 1 h and then air-dried in a temperature controlled greenhouse (25°C). The fertilizer had a δ<sup>15</sup>N value of 200 ± 1.6 ‰ (n = 4).

Eight tagged plants in each row were left unfertilized, the remainder being supplied with nutrients either in shallow (n = 8) or deep (n = 8) soil layers. To avoid cross-contamination between treatments, gaps of 6 m with two rows of untreated plants separated the treatments along each row. For the deep fertilization treatment, 0.8 kg of fertilizer-sand mix was poured through the PVC access pipes. The deep-fertilizer mix was released from the 1.2 m PVC pipes inserted into the augered holes by gently pulling the pipes 0.2 m above the soil surface. For shallow-soil fertilization, the litter layer was removed and 0.8 kg of fertilizer-sand mix poured in a circle of radius 0.3 m in surface-soil to 0.1 m depth. The stand of *A. linearis* was maintained by fortnightly weeding.

### 3.3.2 Foliar and soil sampling before and after supply of $^2\text{H}$ -enriched water

To determine the initial foliar elemental contents, samples (*ca.* 50 g FW) of young apical shoots (*ca.* 0.2 m long) were cut from all experimental plants prior to fertilization (n = 10). At 14 and 125 d post-fertilization, sets of other 50 g samples of foliage were similarly cut from each experimental plant. All foliar samples were oven dried at 70°C for 48 h and 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 elemental concentrations as described below.

To determine HR, I used  $^2\text{H}$ -enriched water (e.g. Dawson et al., 2002) prepared from 2 mL aliquots of 99.9 atom %  $^2\text{H}_2\text{O}$  (Sigma-Aldrich, St. Louis, MO, USA) diluted in 20 L of  $\text{H}_2\text{O}$ . The  $\delta^2\text{H}$  value after dilution was 496‰ (see methods below). Initial stem and soil  $\delta^2\text{H}$  values were determined at 14 d post-fertilization on stem samples (*ca.* 10 mm diameter X 60 mm long, n = 5) of suberized wood collected at dusk (18H00) from six plants of each treatment, while soil cores (400 g) were augered directly under the canopy of each plant to 0.1 m depth, away from fertilized patches. Following this dusk sampling, I supplied 1 L of the diluted  $^2\text{H}_2\text{O}$  through each PVC-access pipe at 18H15. A second set of stem and soil samples

were collected predawn (06H00). Four samples of irrigation water were also collected for  $\delta^2\text{H}$  analysis. To avoid fractionation, the stem, soil and irrigation water samples were collected directly into airtight borosilicate tubes (Kimax–Kimble, Vineland, USA), sealed with Parafilm M (Sigma-Aldrich) and transported in cooler boxes with dry ice. All samples were kept in a cold room ( $4^\circ\text{C}$ ) 3 d prior to analysis. Gravimetric moisture of shallow soil was measured both at 14 d and 125 d by weighing sub-samples of shallow soil before oven-drying them at  $70^\circ\text{C}$  for *ca.* 48 h before re-weighing them.

### *3.3.3 Foliar nutritional analysis*

Foliar P was extracted by ashing pulverized leaf material at  $480^\circ\text{C}$  for 8 h before dissolving in 1:1 (v/v) of HCl (Kalra, 1998). Elemental concentrations of P were then determined using inductively coupled plasma atomic emission spectrometry (Varian Vista MPX, Mulgrave, Australia). Foliar [N], [ $^{15}\text{N}$ ] and [ $^{13}\text{C}$ ] ratios were determined using mass spectrometry. Between 1.900 and 2.000 mg of ground leaf sample was weighed into a  $5 \times 9$  mm tin capsule (Santis Analytical AG, Teufen, Switzerland). The tin capsules were then combusted in a Thermo Flash EA 1112 series elemental analyzer 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.

### *3.3.4 $\delta^2\text{H}$ analysis of soil, stem and irrigation water*

Water was extracted from the soil and stem samples using the cryogenic vacuum distillation method of West et al. (2006). After extraction, the water samples were analysed for  $\delta^2\text{H}$  values using closed tube Zn reduction method (Coleman et al. 1982). Between 100-105 mg of Zn was loaded into oven-dried 6-mm flame-sealed break-seal vials before connecting them to

an H<sub>2</sub> sample preparation line developed after Coleman et al. (1982). The break-seal vials loaded with Zn were heated at 450°C for 5 min with an HE2300 Metabo heat gun (Nürtingen, Germany), whilst evacuating them to 10<sup>-4</sup> torr. The vials were then loaded with 2 µL aliquots of water samples using Hirschmann micro-capillary pipettes (Eberstadt, Germany) and quickly returned to the vacuum line and frozen (*ca.* -200°C) with liquid-N<sub>2</sub> for 5 min. After evacuating impurities from the frozen samples to 10<sup>-4</sup> torr, the vials were flame-sealed with the oxyacetylene torch. The loaded vials were then combusted in a furnace at 450°C for 1 h to release H<sub>2</sub> gas. The H<sub>2</sub> gas was analysed with an isotopic ratio mass spectrometer (Finnigan Mat 252, Bremen, Germany). Internal standards (CTMP and DML ice) were run to calibrate the measurements relative to standard Mean Ocean Water (V-SMOW) and to correct for drift in the reference gas. The enrichment ( $\Delta^2\text{H}$  values) of shallow soil and stems was calculated as the difference in  $\delta^2\text{H}$  values of dusk and predawn samples across the soil moisture and nutrient treatments.

The contributions of groundwater, <sup>2</sup>H<sub>2</sub>O-tracer, irrigation and dusk shallow-soil water to the predawn shallow-soil water were estimated using the Isosource isotope mixing model version 1.3.1 available at <http://www.epa.gov/wed/pages/models.htm>. In this model, all possible combinations of each source contribution (0–100%) are examined in small increments (e.g. 1%). Combinations that sum to the observed mixture isotopic signatures within a small tolerance (e.g. 0.1‰) are considered to be feasible solutions, from which the frequency and range of potential source contributions can be determined (Phillips et al., 2005). I used predawn shallow-soil  $\delta^2\text{H}$  values as the “mixture” isotopic signatures, with source water having  $\delta^2\text{H}$  values of 496‰ for <sup>2</sup>H<sub>2</sub>O, -48‰ for irrigation water, -65‰ for groundwater and the measured shallow-soil dusk  $\delta^2\text{H}$  values. I used an increment of 1% and a tolerance of  $\pm 0.1\%$  for estimating the proportions contributed by source waters to the predawn shallow-soil water.

### 3.3.5 Statistical analysis

A 3x3 factorial ANOVA was used to test the effect of nutrient location, and irrigation levels using Statistica 10 (Statsoft Inc., Tulsa, OK, USA). For comparing variables at 14 d with those at 125 d post fertilization, a repeated measures ANOVA was used. A post-hoc test using Fisher's LSD test was used to identify significantly different means.

## 3.4 Results

### 3.4.1 Water-related measures

No rain fell at the study site for 110 d post-fertilization (Fig. 3.1). Although, 3.2 mm fell on the 27<sup>th</sup> and 28<sup>th</sup> of February 2011, this did not override the treatment differences in gravitational water content of shallow soil measured 125 d post-fertilization (see below). The volumetric water content at the beginning of the study was lower in the shallow soil than in the deeper soil (Fig. 3.2), suggesting the existence of a water potential gradient that could drive HR. Dam-water that was used for deep irrigation for 14 d prior to the experimental treatments had a  $\delta^2\text{H}$  value of  $-48 \pm 1.8\text{‰}$  ( $n = 4$ ) and the  $\delta^2\text{H}$  value for rain was *ca.*  $-75.1\text{‰}$ , which is the volume-weighted average for the year (e.g. Jaeschke et al. 2008). The shallow-soil water at dusk, before the application of  $^2\text{H}_2\text{O}$ , had different  $\delta^2\text{H}$  values for non-irrigated and irrigated treatments, the differences varying in response to fertilization (Fig. 3.3). For unfertilized plants, the dusk  $\delta^2\text{H}$  values were higher with irrigation at  $4 \text{ L plant}^{-1} \text{ d}^{-1}$ , whilst for surface-fertilized plants the values became more negative with increased irrigation. The irrigated deep-fertilized plants had more positive  $\delta^2\text{H}$  values than the unirrigated plants. After supply of  $^2\text{H}_2\text{O}$  at dusk, the  $\delta^2\text{H}$  values of shallow-soil water at predawn were enriched to varying levels, expressed as  $\Delta^2\text{H}$ , calculated by subtracting predawn from dusk  $\delta^2\text{H}$  values (see Fig. 3.4 A).

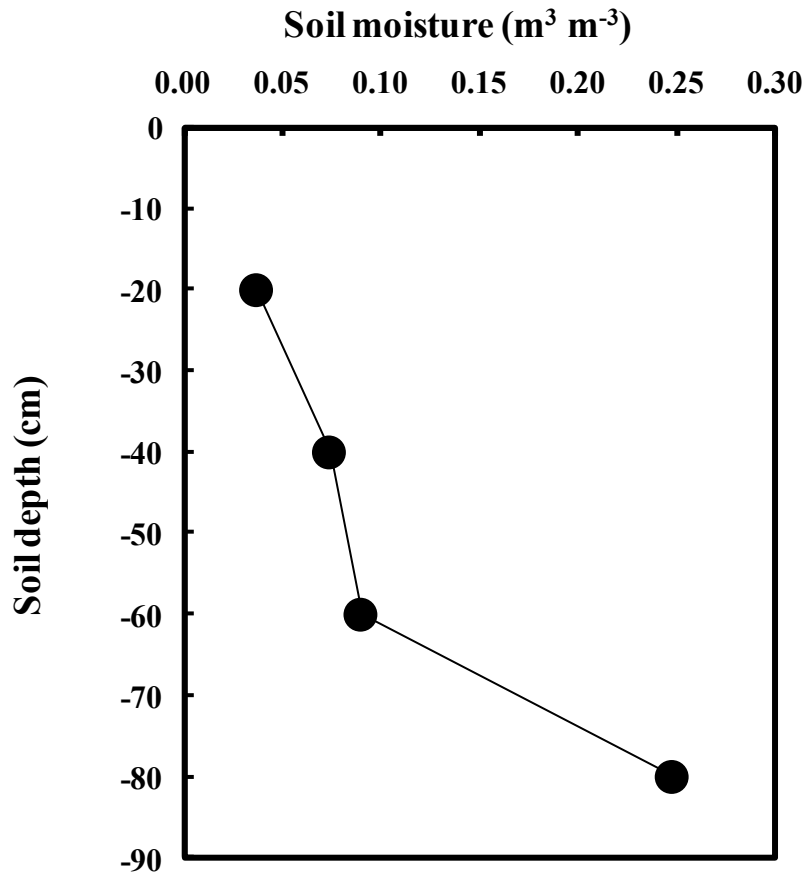


Fig. 3.2. Volumetric soil moisture at varying soil depth as measured using EchoTE sensors at the beginning of the study. Soil moisture was measured at 2-h intervals using 5-TE moisture sensors buried at 0.2, 0.4, 0.6 and 0.8 m depths and 0.3 m from *Aspalathus linearis* stems. The values from each depth are averaged for the first day before treatment application ( $n = 12$ ). Sensors were interfaced with EM50 data loggers (Decagon Devices Inc., Pullman, USA).

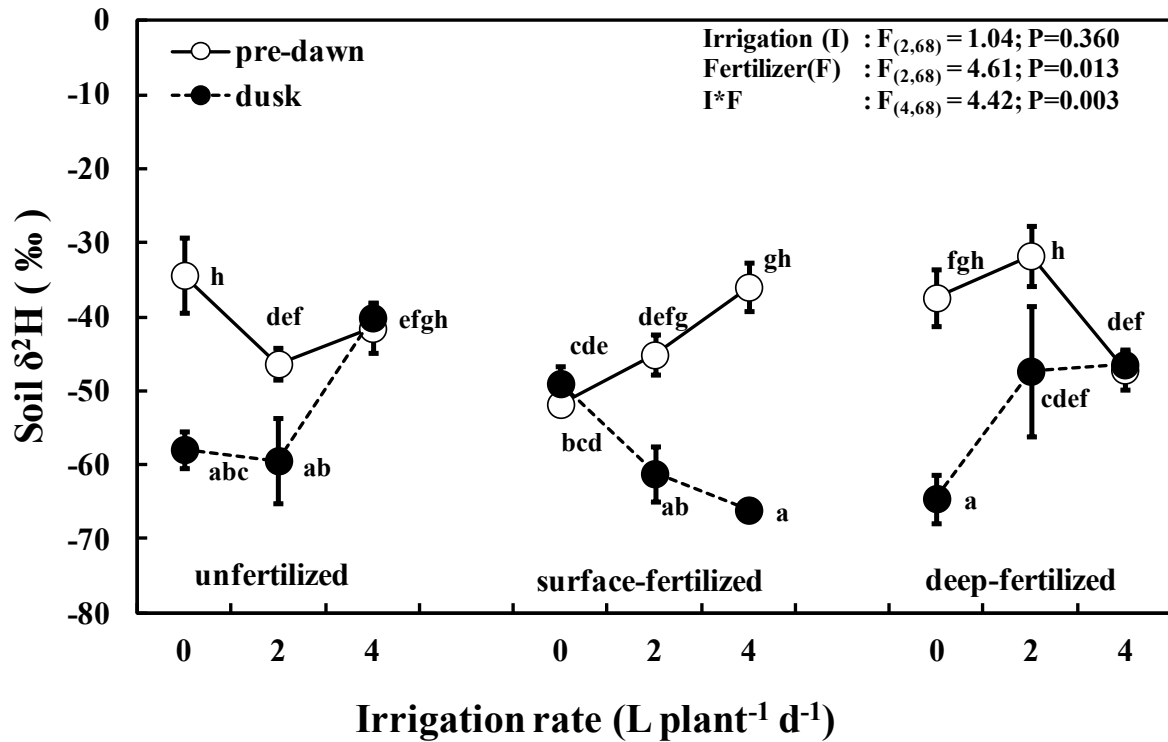


Fig. 3.3. Hydrogen isotope ( $\delta^2\text{H}$ ) values of surface-soil of unfertilized, surface-fertilized or the deep-fertilized *Aspalathus linearis*, receiving sub-surface (1 m) irrigation at 0, 2 or 4  $\text{L plant}^{-1} \text{d}^{-1}$  at dusk and predawn. Symbols and error bars represent means  $\pm$  SE. Different letters indicate significantly different means ( $P < 0.05$ ) as determined using a three way ANOVA with *post-hoc* Fischer's LSD tests. Rain had  $\delta^2\text{H} = -75\text{‰}$ , groundwater  $-65\text{‰}$ , irrigation water from an open earth dam had  $\delta^2\text{H} = -48\text{‰}$ ; deuterium oxide had  $\delta^2\text{H} = 496\text{‰}$ .

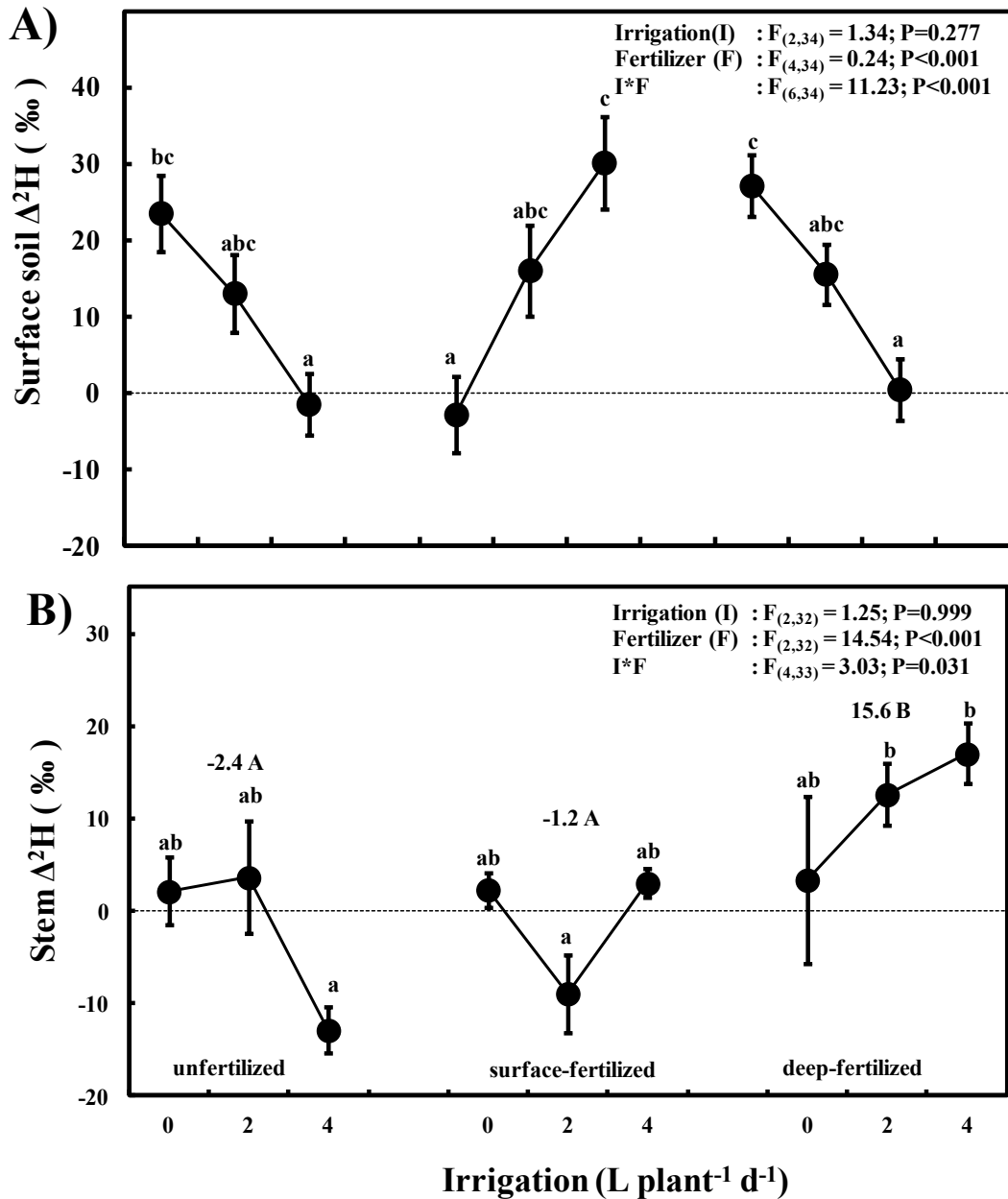


Fig. 3.4. Enrichment ( $\Delta^2\text{H}$ ) values of A) surface-soil and B) stems of unfertilized, surface-fertilized or the deep-fertilized *Aspalathus linearis*, receiving sub-surface (1 m) irrigation at 0, 2 or 4  $\text{L plant}^{-1} \text{d}^{-1}$ .  $\Delta^2\text{H}$  was calculated by subtracting dusk  $\delta^2\text{H}$  values prior to supply of  $^2\text{H}_2\text{O}$  from predawn values 14 d post-fertilization. Symbols and error bars represent means  $\pm$  SE. Different letters indicate significantly different means ( $P < 0.05$ ) as determined using two-way ANOVA with *post-hoc* Fischer's LSD tests. The dotted horizontal line represents zero enrichment. Values above lines in panel B) show the average stem nocturnal  $\Delta^2\text{H}$  for plants supplied with different fertilizer treatments.

The unfertilized and the deep-fertilized plants had higher shallow-soil  $\Delta^2\text{H}$  at lower rates of irrigation. In contrast, the  $\Delta^2\text{H}$  of shallow-soil under surface-fertilized plants increased with irrigation (Fig. 3.4A). Unlike soil  $\Delta^2\text{H}$ , stem predawn  $\Delta^2\text{H}$  was not significantly altered by variation in irrigation, but was increased by deep fertilization when compared to unfertilized or surface-fertilized plants (Fig. 3.4B).

The estimated proportional contribution of  $^2\text{H}_2\text{O}$  to predawn surface-soil moisture ranged between 2% and 7% (Table 3.1). This contribution followed the trends in shallow-soil  $\Delta^2\text{H}$  values (Fig. 3.4A). Although the proportion of tracer lifted to surface-soil by HR was relatively small, the estimated HR of deep water (sum of groundwater, irrigation water and tracer) was large, accounting for between 34% and 72% of predawn surface-soil moisture (Table 3.1). In unfertilized and surface-fertilized plants, HR of deep water increased with irrigation, whereas with deep fertilization it changed rather little. The deep water contributed a smaller proportion of the shallow-soil water beneath the unirrigated, unfertilized plants, than under unirrigated plants receiving either surface- or deep-fertilization.

Across the fertilization treatments, the gravimetric soil moisture of shallow-soil at 14 d was increased by deep irrigation (Fig. 3.5A). In contrast, at 125 d post-fertilization the surface-soil moisture was reduced by deep irrigation (Fig. 3.5B). There was no interaction between irrigation and fertilizer treatments with regard to soil moisture content. Overall, surface-soil moisture was higher at 125 d than at 14 d post-fertilization ( $F_{(1,100)} = 134.06$ ;  $P < 0.001$ ), possibly because of the rain that fell a few days before sample collection. Deep irrigation generally resulted in more negative foliar  $\delta^{13}\text{C}$  (Fig. 3.6A, B). Plant  $\delta^{13}\text{C}$  was used to provide a time-integrated estimate of intercellular to ambient  $\text{CO}_2$  mole fractions ( $C_i/C_a$ ), which are a long-term proxy for  $WUE$  (Farquhar et al., 1982; Brugnoli and Farquhar, 2000). There was no significant effect of fertilizer treatment on  $\delta^{13}\text{C}$  values at either 14 d or 125 d post-fertilization.

Table 3.1: Estimated proportions of water sources present in shallow-soil at predawn, calculated using the Isosource isotope mixing model version 1.3.1 (e.g. Phillips and Gregg, 2003; Phillips et al., 2005). The values for each treatment indicate the proportions of predawn surface soil water derived from the pre-existing (i.e. dusk) surface soil water, irrigation water, ground water and isotopically enriched water. Values presented are means and (lower, upper) quartiles in parentheses.

Fertiliser	Irrigation	Surface-soil	<sup>2</sup> H <sub>2</sub> O tracer	Irrigation	Ground H <sub>2</sub> O	Total deep-H <sub>2</sub> O
	L plant <sup>-1</sup> d <sup>-1</sup>	Dusk δ <sup>2</sup> H %	δ <sup>2</sup> H = 496‰ %	δ <sup>2</sup> H = -48‰ %	δ <sup>2</sup> H = -65‰ %	%
Unfertilized	0	67 (53, 83)	6 (5, 6)	0	28 (12, 44)	34
	2	33 (15, 49)	3(3, 5)	31(11, 48)	32(14, 49)	66
	4	34 (15, 54)	3(2, 4)	31(12, 47)	32 (12, 49)	66
Surface-fertilized	0	45 (25,55)	2 (1, 3)	0	53 (34, 65)	55
	2	31(14, 44)	3 (3, 4)	33 (13, 53)	32 (14, 46)	68
	4	31(12, 45)	5 (5, 6)	34 (16, 52)	30 (12, 45)	69
Deep-fertilized	0	29 (16, 42)	7(6, 7)	0	65 (51, 77)	72
	2	29 (12, 49)	5 (4, 6)	31(14, 49)	36 (14, 53)	72
	4	35 (16, 49)	3(1, 3)	28 (12, 47)	35 (6, 45)	66

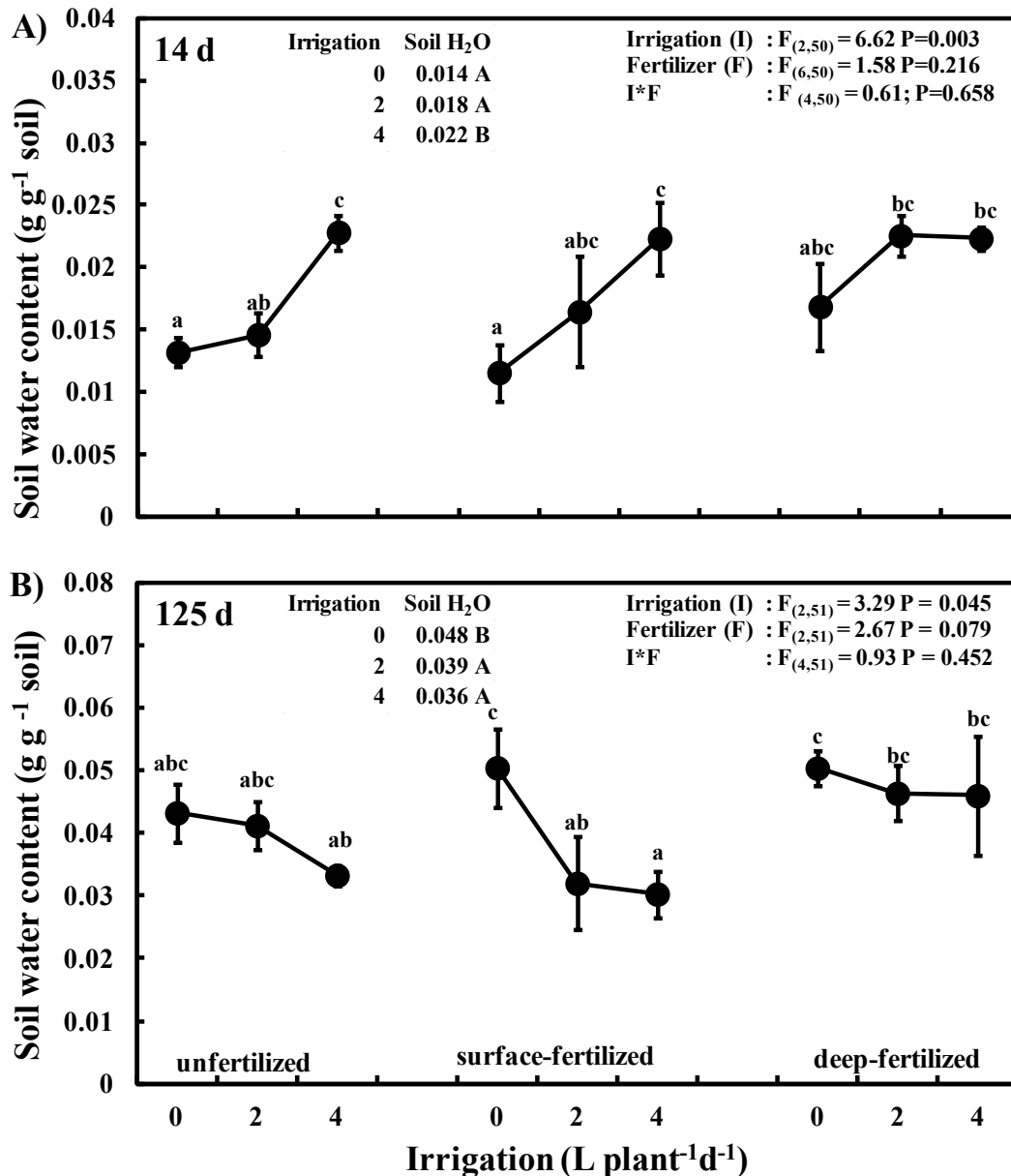


Fig. 3.5. Surface-soil gravimetric water content at dusk of unfertilized, surface-fertilized and deep-fertilized (1 m depth) *Aspalathus linearis* receiving sub-surface irrigation at 0, 2 or 4 L plant<sup>-1</sup> d<sup>-1</sup> at 1 m depth after A) 14 d and B) 125 d post-fertilization. Symbols and error bars are means  $\pm$  SE. Different letters indicate significantly different means ( $P < 0.05$ ) as determined using two-way ANOVA with *post-hoc* Fischer's LSD tests. In the absence of significant irrigation  $\times$  fertilizer interactions, inserted tables show the average soil H<sub>2</sub>O concentrations across irrigation levels.

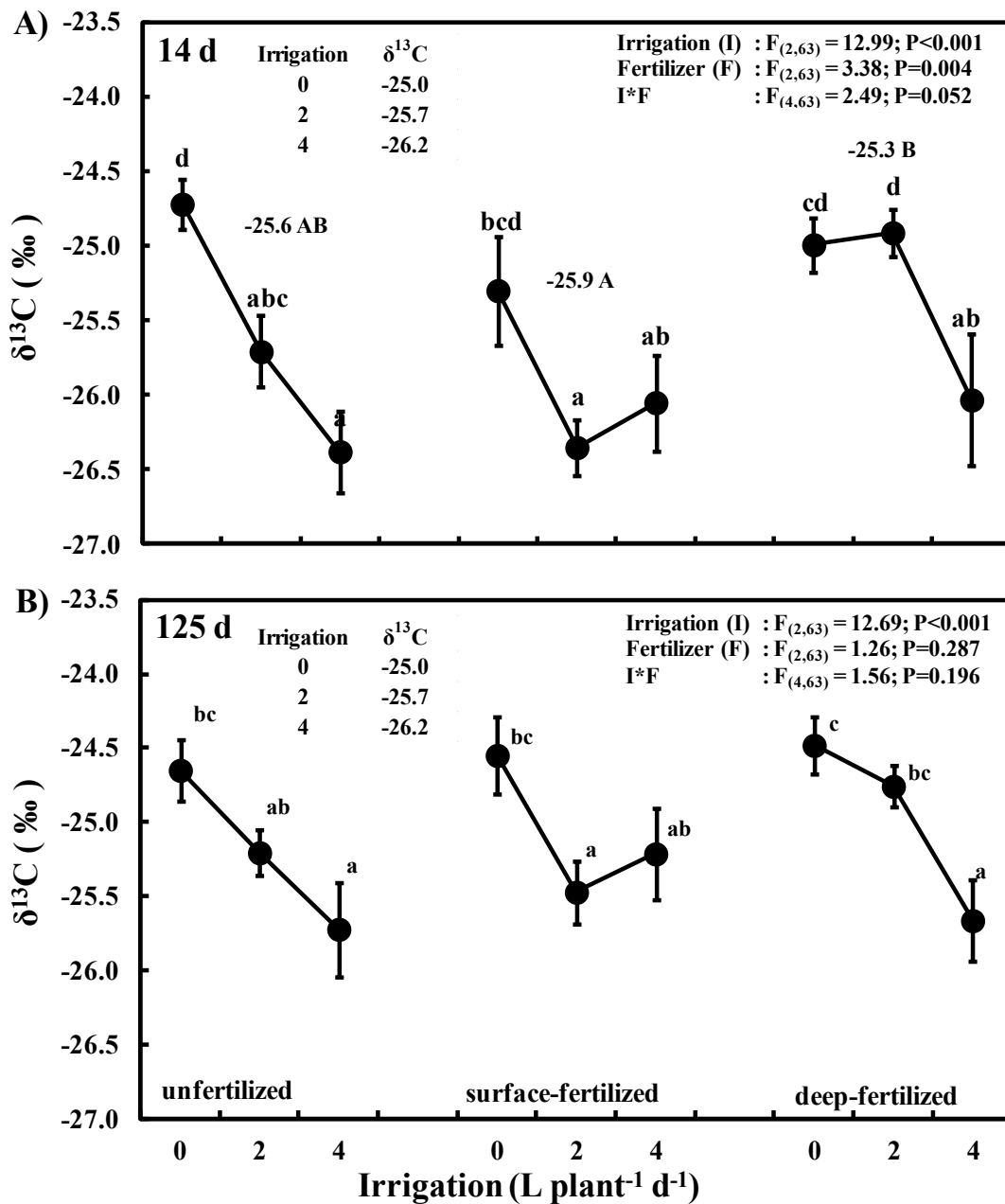


Fig. 3.6. Foliar  $\delta^{13}\text{C}$  of unfertilized, surface-fertilized or deep-fertilized (1 m depth) *Aspalathus linearis* plants receiving sub-surface irrigation at 0, 2 or 4  $\text{L plant}^{-1}\text{d}^{-1}$  at 1 m depth after A) 14 d and B) 125 d post-fertilization. Symbols and error bars represent means  $\pm$  SE. Different letters indicate significantly different means ( $P < 0.05$ ) as determined using two-way ANOVA with *post-hoc* Fischer's LSD tests. In the absence of significant irrigation x fertilizer interactions, inserted tables show the average foliar  $\delta^{13}\text{C}$  across irrigation levels. Values above lines in panel A) show the average foliar  $\delta^{13}\text{C}$  for plants supplied with different fertilizer treatments

### 3.4.2 Foliar nutrients

Foliar [N] 14 d post-fertilization (Fig. 3.7A) increased with higher rates of irrigation, but there was no significant effect of fertilization on foliar [N]. At 125 d post-fertilization, however, there were no significant differences in foliar [N] (Fig. 3.7B). Unlike foliar [N] which originates from soil sources, nodules and the supplied fertilizer, the changes in foliar  $\delta^{15}\text{N}$  values predominantly reflect the uptake of  $^{15}\text{N}$ -labelled fertilizer. At both 14 d and 125 d post-fertilization (Fig. 3.8A, B),  $\delta^{15}\text{N}$  values were higher in both deep-fertilized and surface-fertilized plants than in the unfertilized plants, indicating uptake of the supplied  $^{15}\text{N}$  label. At 14 d post-fertilization, plants supplied with fertilization took up more  $^{15}\text{N}$  when irrigated, except in the case of those receiving surface-fertilization and  $2 \text{ L plant}^{-1} \text{ d}^{-1}$ . At 125 d post-fertilization, all fertilized plants had incorporated large amounts of  $^{15}\text{N}$ , especially the unirrigated surface-fertilized plants. In contrast to these surface-fertilized plants,  $\delta^{15}\text{N}$  increased with irrigation of the deep-fertilized plants.

Overall, foliar [P] 14 d post-fertilization increased with deep irrigation in response to the added fertilizer with similar increases in foliar [P] with both surface- and deep-fertilization (Fig. 3.9A). At 125 d post-fertilization, however, foliar [P] did not vary with irrigation or fertilization (Fig. 9B). However, the overall foliar [P] of  $291 \pm 9 \text{ mg kg}^{-1}$  measured at 125 d was significantly ( $F_{(1,54)} = 82.53$ ;  $P < 0.001$ ) lower than  $465 \pm 21 \text{ mg kg}^{-1}$  measured at 14 d post-fertilization.

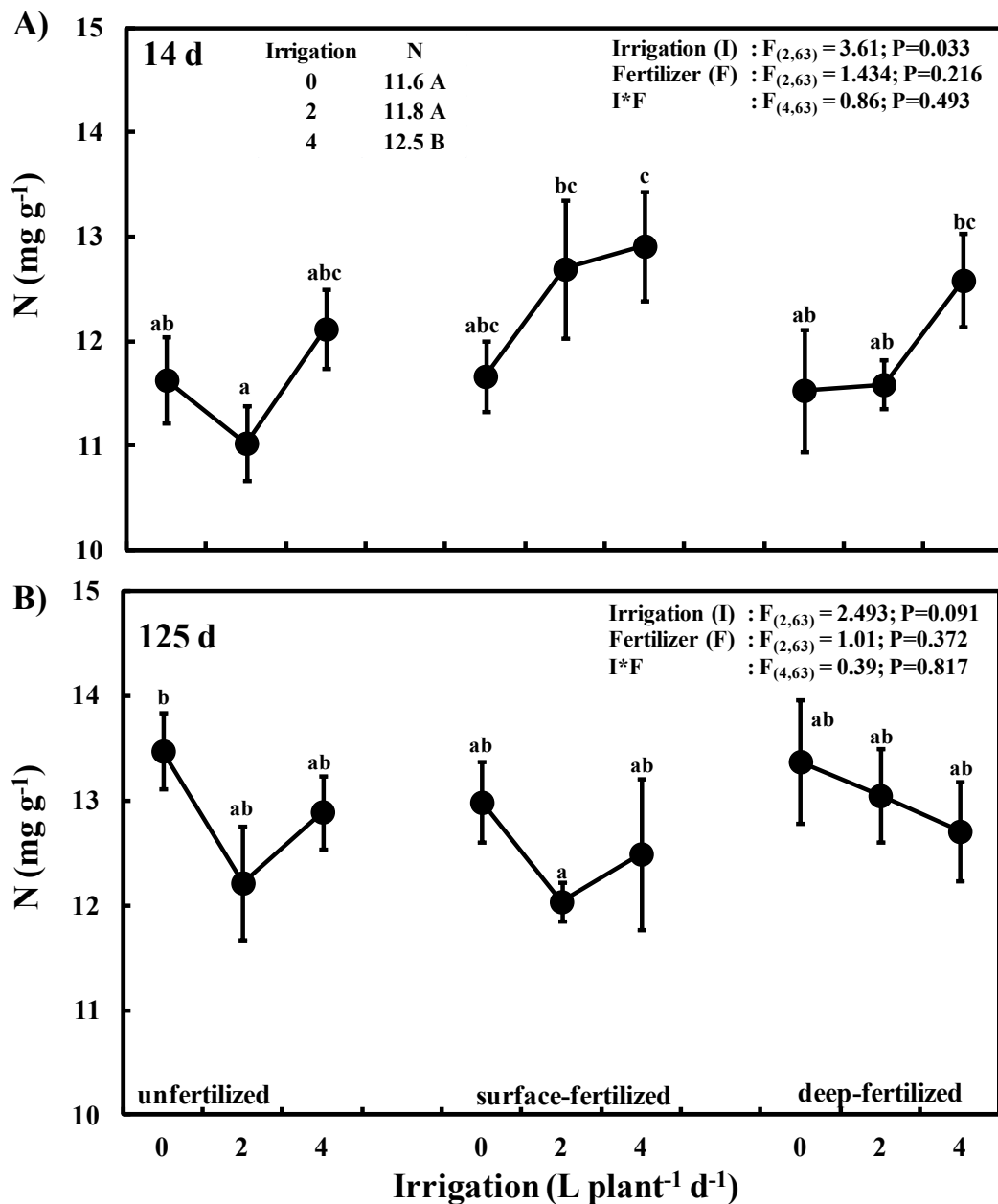


Fig. 3.7. Foliar N of unfertilized, surface-fertilized or deep-fertilized (1 m depth) *Aspalathus linearis* plants receiving sub-surface irrigation at 0, 2 or 4 L plant<sup>-1</sup> d<sup>-1</sup> at 1 m depth after A) 14 d and B) 125 d post-fertilization. Symbols and error bars represent means  $\pm$  SE. Different letters indicate significantly different means ( $P < 0.05$ ) as determined using two-way ANOVA with *post-hoc* Fischer's LSD tests. In the absence of significant irrigation x fertilizer interactions, inserted table shows the average foliar [N] across irrigation levels.

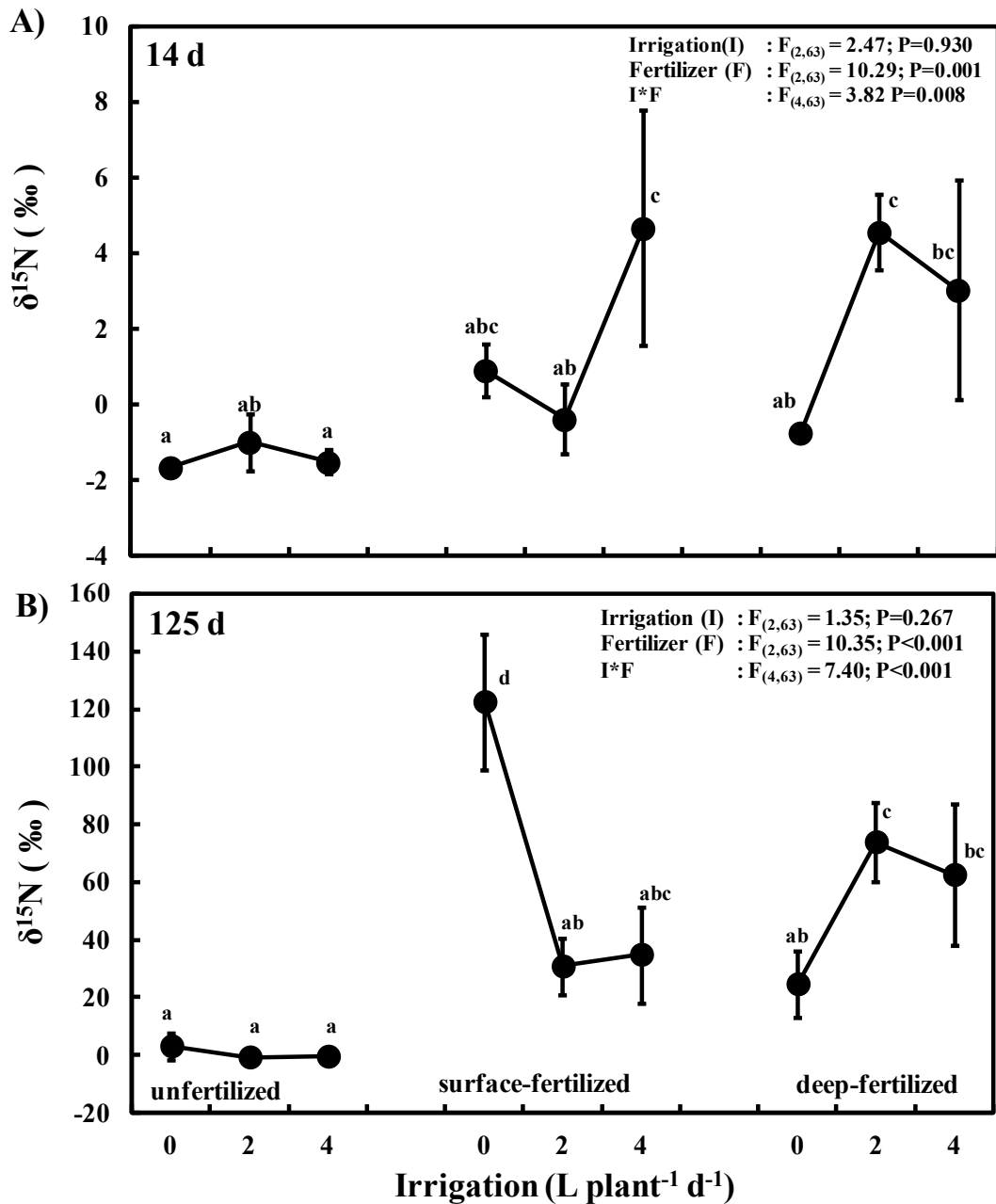


Fig. 3.8. Foliar  $\delta^{15}\text{N}$  of unfertilized, surface-fertilized or deep-fertilized (1 m depth) *Aspalathus linearis* plants receiving sub-surface irrigation at 0, 2 or 4  $\text{L plant}^{-1} \text{d}^{-1}$  at 1 m depth after A) 14 d and B) 125 d post-fertilization. Each symbol and bar represents a mean  $\pm$  SE. Different letters indicate significantly different means ( $P < 0.05$ ) as determined using two-way ANOVA with *post-hoc* Fischer's LSD tests after log transformation to normalise the values. Values above lines in the panels show the average foliar  $\delta^{15}\text{N}$  for plants supplied with different fertilizer treatments.

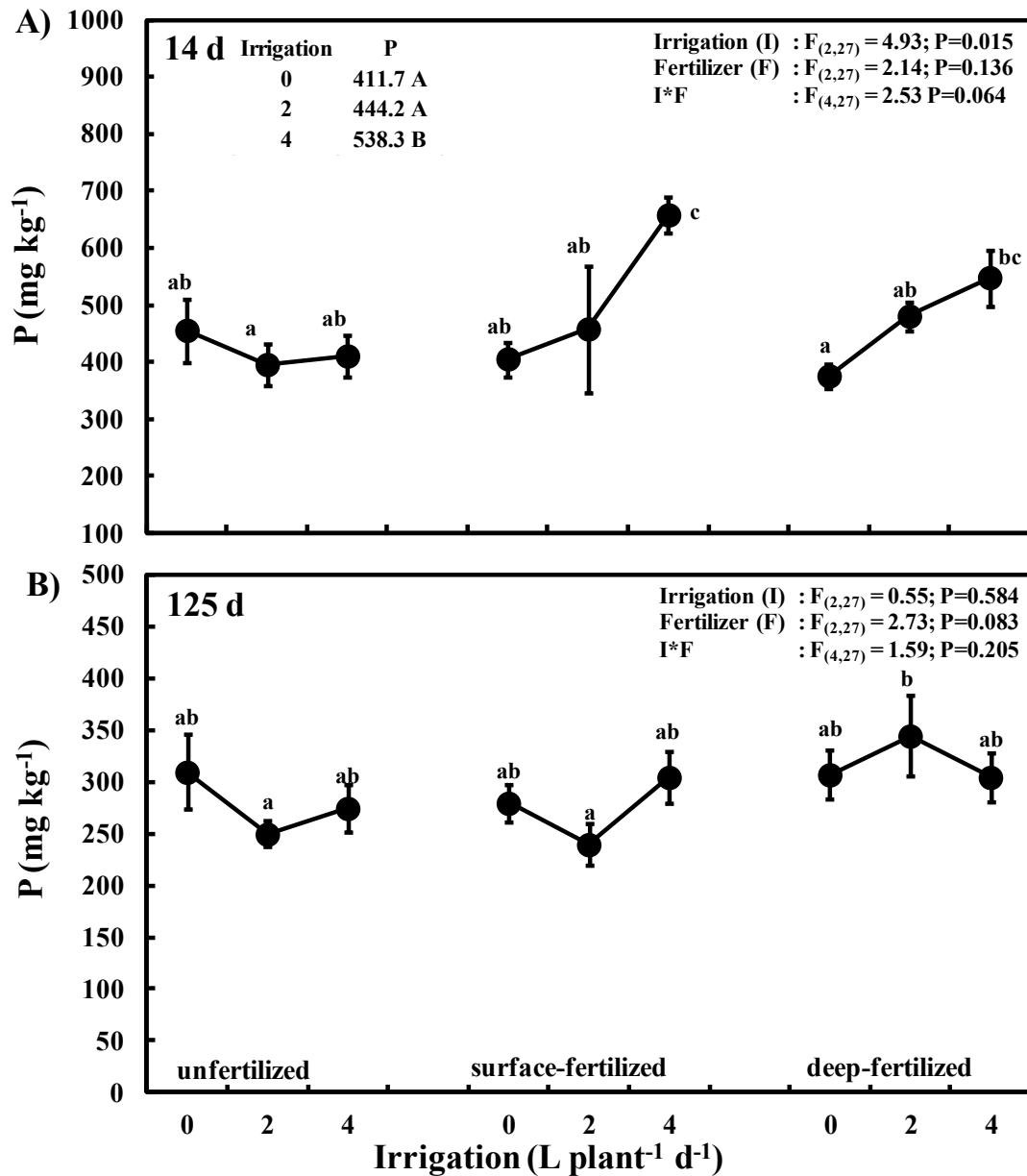


Fig. 3.9. Foliar P of unfertilized, surface-fertilized or deep-fertilized (1 m depth) *Aspalathus linearis* plants receiving sub-surface irrigation at 0, 2 or 4 L plant<sup>-1</sup>d<sup>-1</sup> at 1 m depth after A) 14 d and B) 125 d post-fertilization. Each circle and bar represents a mean  $\pm$  SE. In the absence of significant irrigation  $\times$  fertilizer interactions, inserted tables show the average foliar P across irrigation levels. Different letters indicate significantly different means ( $P < 0.05$ ) as determined using two-way ANOVA with *post-hoc* Fischer's LSD tests. Inserted table shows foliar P across irrigation levels.

### 3.5 Discussion

I used  $^2\text{H}_2\text{O}$  as a tracer for HR (e.g. Dawson 1993; Brooks et al., 2002) and found evidence for the occurrence of HR and nocturnal transpiration in the arid fynbos legume, *A. linearis*. Since  $^2\text{H}_2\text{O}$  uptake responded to irrigation and fertilization treatments, it is unlikely that capillary flow through the sands explains the overnight redistribution of  $^2\text{H}_2\text{O}$ , as also concluded previously in a similar edaphic context (Hawkins et al., 2009).

The differences in surface-soil  $\delta^2\text{H}$  values at dusk 14 d post-fertilization were caused by a combination of differences in HR and fractionation of residual shallow-soil water exposed to daytime evaporative losses. Soil water may be variably bound to soil particles (Sun et al., 2009; Lund, 1959; Tyree, 2003), particularly clay (Kovda, 1983; Yao Xianliang and Cheng Yun-sheng, 1983) protecting it from evaporative fractionation. The dusk  $\delta^2\text{H}$  values are therefore a product of a combination of HR during the preceding night, the utilization of surface-soil water by plants during the day and the evaporative fractionation of residual soil water not bound to particles. Unlike surface-soil natural abundance  $\delta^2\text{H}$  values and gravimetric moisture which both may accumulate over time, the redistributed  $^2\text{H}_2\text{O}$ -tracer indicates the short-term (< 12 h) magnitude and direction of HR and was used to estimate the contribution of HR to surface-soil moisture. I estimated that nocturnal HR contributed the bulk of surface-soil water in deep-irrigated plants and that HR was increased in response to both surface- and deep fertilization of unirrigated treatments.

Decreased surface-soil  $\Delta^2\text{H}$  in unfertilized plants with increased rates of deep irrigation may be due to a combination of dilution of the supplied label by unlabelled irrigation water, suppression of HR by increased rates of deep irrigation and plant uptake of surface water. The estimation of total redistribution of deep water and the increase in shallow-soil moisture with deep irrigation, however, indicates that irrigation did increase HR. Furthermore, the increased deep irrigation led to more negative  $\delta^{13}\text{C}$  values, also suggesting

increased daytime transpiration and decreased *WUE*. Plant uptake of surface-soil moisture, mostly from HR, may also have been increased from subsequent growth in response to deep irrigation. Indeed, the stem length increment ( $\text{cm d}^{-1}$ ) over 14 d for unfertilized plants was  $0.10 \pm 0.09$ ,  $0.19 \pm 0.1$  and  $0.49 \pm 0.20$  for the 0, 2 and 4 L  $\text{plant}^{-1} \text{d}^{-1}$  irrigation rates, respectively. This increased growth due to the greater availability of water may contribute to utilisation of shallow-soil moisture. Thus deep irrigation increased HR, but probably also increased the utilisation of surface-soil moisture due to increased growth of the plants.

Supply of fertilizers to surface and deep soil increased HR of deep water in unirrigated plants. The estimated proportion of surface-soil water lifted overnight by HR by deep-irrigated plants was, however, not increased by surface- or deep-fertilization. Thus HR was increased primarily by deep irrigation, and nutrition only increased HR in the absence of additional irrigation water. The results also suggest the existence of a trade-off between engagement of HR and nocturnal transpiration. Irrigated plants supplied with deep fertilization engaged in both HR and nocturnal transpiration. In contrast, there was no evidence for uptake of  $^2\text{H}_2\text{O}$ -labelled irrigation water through nocturnal transpiration in unfertilized or surface-fertilized plants, either directly from deep soil or indirectly from surface soil. During the following diurnal period, however, uptake of this water is likely to have occurred (not measured). In situations where nutrients are most abundant in surface soils it may be strategic to favour HR and recharge of surface moisture, which would be compromised by simultaneous nocturnal transpiration of surface-soil moisture. This trade-off between HR and nocturnal transpiration has been reported previously (Richards and Caldwell, 1987; Bauerle et al., 2008; Howard et al., 2009; Prieto et al., 2010).

During the summer drought period the increased HR of surface-fertilized plants 14 d post-fertilization was associated with increased acquisition of shallow-soil nutrients. This was partially evident from higher  $\delta^{15}\text{N}$  values and tissue [P]. However, fertilization can potentially lower nutrient acquisition through symbiotic relationships. Inhibition of mycorrhizal

symbioses, for instance, can possibly reduce the capacity of the roots to acquire water and nutrients as the fungus directly enhances root uptake or indirectly by modifying transpiration rates and the composition of rhizosphere microflora (Marshner and Dell, 1994; Auge 2001). Although, effects of fertilization on the amount of symbiotic N fixation were not assessed, the  $^{15}\text{N}$  data showed that new growth sampled was driven by the added fertilizers. Because of the generally low P in CFR soils (e.g. Witkowski and Mitchells, 1987), the lower foliar P at 125 d post fertilization compared to 14 d possibly indicates the exhaustion of the relatively small amount of fertilizer-P supplied to the rhizosphere. Alternatively, chemical change in the forms of P and fixation by the soil minerals may partly explain the decrease of P at 125 d relative to 14 d. Increased acquisition of these inorganic fertilizer forms is independent of mineralisation and must reflect access either through increased mobility of the nutrients in the soil or increased root interception of nutrients. Increased nutrient mobility due to higher soil moisture includes both diffusive and mass-flow mobility. I speculate that the striking accumulation at 125 d of  $\delta^{15}\text{N}$  in the foliar tissue of unirrigated shallow-fertilized plants results from the slower growth of these plants. In the longer-term (i.e. 125 d), increased growth due to irrigation may have diluted  $^{15}\text{N}$ -enrichment in shallow-fertilized plants, complicating interpretation of the influence of HR on  $^{15}\text{N}$  tracer data.

There was also evidence that the deep-fertilized plants increased their uptake of  $^{15}\text{N}$  and P in response to irrigation. As with surface-fertilized plants, this could be due to increased nutrient mobility or root growth. Since I interpret the stem  $\Delta^2\text{H}$  as indicating greater nocturnal water flux in these plants and also found evidence of greater HR, I suggest that nutrient uptake may have been increased by a combination of increased mass-flow driven by nocturnal water flux and HR induced uptake of nutrients from surface soils. Although diffusion and mass-flow of N is well known, P mobility has been considered limited in most soils (e.g. Bielecki, 1973, Lambers et al., 1998) owing to low diffusion coefficients (Barber and Olson, 1968; Clarkson, 1981). P-mobility may, however, be relatively high in low P-binding soils.

Examples of such soils are the low-clay, highly weathered and leached soils of the CFR (Witkowski and Mitchell, 1987; Rebelo, 1996; Rebelo et al., 2006; Goldblatt, 1997) to which *A. linearis* is native. Plant water flux has also been linked to P acquisition in tropical species (Cernusak et al., 2011). Thus plants supplied with deep fertilization apparently increased both nocturnal transpiration and HR, although the combination of these two processes was only manifested with irrigation.

If HR, diurnal and nocturnal transpiration have functional roles in nutrient acquisition through mass-flow, it is likely that these physiological activities will be regulated by nutrient availability. Evidence is accruing for nutritional regulation of diurnal transpiration (Clarkson et al., 2000; Gorska et al., 2008) and this has been linked to mass-flow acquisition of nutrients (Cramer et al., 2008; 2009). The induction of HR by fertilizers in the shallow soil also indicates that this process is inducible by nutrition. This is at odds with the view that HR is a passive process, dependent only on the water potential gradient within the soil. Instead I suggest a role for nutritional regulation of HR, possibly functioning through aquaporin regulation of water uptake from the soil or release to the soil (e.g. Carvajal et al., 1996; Hoarau et al., 1996; Clarkson et al., 2000) or indirectly through interactions with nocturnal transpiration (Prieto et al., 2010; Howard et al., 2009). Since the placement of fertilizer in the soil modulated stem  $\Delta^2\text{H}$  I conclude that nocturnal transpiration is regulated by nutrient availability, as also reported by Kupper et al. (2012). The fertilizer supplied included  $\text{NO}_3^-$ , which is known to partially regulate stomatal conductance (Wilkinson, et al., 2007; Gloser et al., 2007; Gorska et al., 2008; Garish et al., 2010). Further work is required to ascertain the physiological mechanism through which nutrients regulate HR and nocturnal transpiration and how these processes may interact.

Variation in the functional significance of HR and nocturnal transpiration in acquiring nutrients situated in shallow versus deep soil profiles accords with the conclusion of Prieto et al. (2012) that it is the selective placement of roots in nutrient-rich patches and deeper moist

soils that most strongly determine nutrient capture and plant performance during summer drought. Many plants employ mixed strategies, in which the development of deep roots is prioritized to ensure access to deep water and leached nutrients (Lynch, 2012), but with some shallow root development being maintained to enable the acquisition of shallow-soil nutrients (Lynch and Brown, 2001; Lynch, 2011; Richardson et al., 2011), possibly with participation of HR.

### 3.6 Conclusion

I conclude that *A. linearis* utilised transpiration and HR to increase acquisition of both shallow and deep soil nutrients. I suggest that both diurnal and nocturnal transpiration are particularly important for promoting mass-flow of nutrients by these plants growing in nutrient poor sands. HR responded to both surface fertilization and deep fertilization, indicating that this process may have dual functionality in increasing uptake of deep nutrients by driving water fluxes at night, and also by mobilizing surface nutrients. The fact that both nocturnal transpiration and HR responded to fertilizer placement indicates that these processes are partially regulated by nutrient availability.

### 3.7 References

Aanderud ZT, Richards JH. 2009. Hydraulic redistribution may stimulate decomposition.

Biogeochemistry 95,323–333.

Allen SC, Jose S, Nair PKR, Brecke BJ, Nkedi-kizza P, Ramsey CL. 2004. Safety-net role of tree roots: evidence from a pecan (*Carya illinoensis* K. Koch) – cotton (*Gossypium hirsutum* L.) alley cropping system in the southern United States. Forest Ecol Manage 192, 395-407.

- Armas C, Kim JH, Bleby TM, Jackson RB. 2012. The effect of hydraulic lift on organic matter decomposition, soil nitrogen cycling, and nitrogen acquisition by a grass species. *Oecologia* 168, 11-22.
- Augé RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11, 3-42.
- Barber SA, Olson RA. 1968. Fertilizer use on corn. In: Nelson LB, McVickar MH, Munson RD, Seatz LF, Tisdale SL, White WC (eds) *Changing Patterns in Fertilizer Use*, Soil Sci. Soc. of Amer., Inc., Madison, Wis. pp. 163-188.
- Bauerle TL, Richards JH, Smart DR, Eissenstat DM. 2008. Importance of internal hydraulic redistribution for prolonging the lifespan of roots in drying soil. *Plant Cell Environ.* 31, 177–186.
- Bialeski RL. 1973. Phosphate pools, phosphate transport, and phosphate availability. *Ann. Rev. Plant Physiol* 24, 225 – 252.
- Burgess SSO, Bleby TM. 2006. Redistribution of soil water by lateral roots mediated by stem tissues. *Journal of Experimental Botany* 57, 3283–3291.
- Burgess SSO, Adams MA, Bleby TM. 2000. Measurement of sap flow in roots of woody plants: a commentary. *Tree Physiology* 20, 909–913.
- Burgess SSO, Adams MA, Turner NC, Ong CK. 1998. The redistribution of soil water by tree root systems. *Oecologia* 115, 306–311.
- Brooks JR, Meinzer FC, Coulombe R. 2002. Hydraulic redistribution of soil water during summer drought in two contrasting Pacific Northwest coniferous forests. *Tree Physiology* 22, 1107–1117.
- Cabrera-Bosquet L, Molero G, Nogue S, Araus JL. 2009. Water and nitrogen conditions affect the relationships of  $\Delta^{13}\text{C}$  and  $\Delta^{18}\text{O}$  to gas exchange and growth in durum wheat. *Journal of Experimental Botany* 60, 1633-1644.

- Caldwell MM, Dawson TE, Richards JH. 1998. Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia* 113,151-161.
- Cardon ZG, Gage, DJ. 2006. Resource exchange in the rhizosphere: molecular tools and the microbial perspective. *Annual Review in Ecology Evolution and Systematics* 37, 459-488.
- 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.
- Cernusak LA, Winter K, Turner BL. 2011. Transpiration modulates phosphorus acquisition in tropical tree seedlings. *Tree Physiology* 31, 878-885.
- Cernusak LA, Winter K, Aranda J, Turner BL, Marshall JD. 2007. Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *Journal of Experimental Botany* 58, 3549-3566.
- Clarkson DT. 1981. Nutrient interception and transport by root systems. In: Johnson, C.B. (ed.) *Physiological factors limiting plant productivity*. Butterworths, London, pp 307–314.
- 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* 342, 61-70.
- Cocks MP, Stock WD. 2001. Field patterns of nodulation in fifteen *Aspalathus* species and their ecological role in the fynbos vegetation of southern Africa. *Basic and Applied Ecology* 125, 115-125.
- Coleman ML, Shepherd TJ, Durham JJ, Rouse JE, Moore GR. 1982. Reduction of water with zinc for hydrogen isotope analysis. *Analytical Chemistry* 54, 993–995.
- Cramer MD, Hawkins H-J, Verboom AG. 2009. The importance of nutritional regulation of plant water flux. *Oecologia* 161,15–24.

- Dawson TE. 1993. Hydraulic lift and water use by plants: implications for water balance, performance and plant-plant interactions. *Oecologia* 95, 565–574.
- Dawson TE. 1997. Water loss from tree influences soil water nutrient status and plant performance. In: Flores EH, Lynch JP, Eissenstat D (eds) *Radical biology: advances and perspectives on the function of plant roots*, vol 18. American Society of Plant Physiologists, Rockville, pp 235–250.
- Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP. 2002. Stable isotopes in plant ecology. *Annu Rev Ecol Syst* 33: 507–559.
- Espeleta JF, West JB, Donovan LA. 2004. Species-specific patterns of hydraulic lift in co-occurring adult trees and grasses in a sandhill community. *Oecologia* 138:341–349.
- Farquhar GD, O’Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust J Plant Physiology* 9, 121-137.
- Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review Plant Physiology* 40, 503–537.
- Garrish V, Cernusak LA, Winter K, Turner BL. 2010. Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*. *Journal of Experimental Botany* 61, 3735-3748.
- Goldblatt P. 1997. Floristic diversity in the Cape Flora of South Africa. *Biodivers Conserv* 6, 359-377.
- Gloser V, Zwieniecki MA, Orians C, Holbrook NM. 2007. Dynamic changes in root hydraulic properties in response to nitrate availability. *Journal of Experimental Botany* 58, 2409–2415.
- Gorska A, Ye Q, Holbrook NM, Zwieniecki MA. 2008. Nitrate control of root hydraulic properties in plants: translating local information to whole plant response. *Plant Physiology* 148, 1159-1167.

- Hawkins H-J, Malgas R, Biénabe E. 2011. Ecotypes of wild rooibos (*Aspalathus linearis* (Burm . F) Dahlg., Fabaceae ) are ecologically distinct. South African Journal of Botany 77, 360-370.
- Hawkins H-J, Hettasch H, West AG, Cramer MD. 2009. Hydraulic redistribution by Protea “Sylvia ” (Proteaceae) facilitates soil water replenishment and water acquisition by an understorey grass and shrub. Functional Plant Biology 36,752-760.
- Hoarau J, Barthes L, Bousser A, Deleens E, Prioul JL. 1996. Effect of nitrate on water transfer across roots of nitrogen pre-starved maize seedlings. Planta 200, 405–415.
- Howard AR, Van Iersel MW, Richards JH, Donovan LA. 2009. Nocturnal transpiration can decrease hydraulic redistribution. Plant Cell Environ 32, 1060-1070.
- Hultine KR, Cable WL, Burgess SSO, Williams DG. 2003. Hydraulic redistribution by deep roots of a Chihuahuan Desert phreatophyte. Tree Physiol 23,353–360.
- Hultine KR, Scott RL, Cable WL, Goodrich DC, Williams DG. 2004. Hydraulic redistribution by a dominant, warm-desert phreatophyte: seasonal patterns and response to precipitation pulses. Functional Ecology 18, 530–538.
- Jackson RB, Sperry JS, Dawson TE. 2000. Root water uptake and transport: using physiological processes in global predictions. Trends in Plant Science 5, 482–488.
- Jackson RB, Caldwell MM. 1993. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. Ecology 74, 612–614.
- Jaeschke JB, Scholl MA, Cozzarelli IM, Masoner JR, Christenson S, Qi H. 2011. Stable-isotope ratios of hydrogen and oxygen in precipitation at Norman, Oklahoma, 1996–2008: U.S. Geological Survey Scientific Investigations Report, 2011–5262, 22 p
- Jobbágy EG, Jackson RB 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10, 423-436.
- Jobbágy EG, Jackson RB. 2004. The uplift of soil nutrients by plants: biogeochemical consequences across scales. Ecology 85, 2380-2389.

- Kalra YP. 1998. *Handbook of standard methods of plant analysis*. Boca Raton, Florida, USA: CRC Press.
- Keitel C, Adams MA, Holst T, Matzarakis A, Mayer H, Rennenberg H, Geßler A. 2003. Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). *Plant Cell and Environment* 26, 1157-1168.
- Kovda BA. 1983. *Agrology principle*. Science Press, B.A. Science Press, Beijing. pp.17.
- Kupper P, Rohula G, Saksing L, Sellin A, Lõhmus K, Ostonen I, Helmisaari H-S, Sõber A. 2012. Does soil nutrient availability influence nocturnal water flux of aspen saplings? *Environmental and Experimental Botany* 82, 37–42.
- Kurz-Besson C, Otieno D, Lobo do Vale R, Siegwolf R, Schmidt M, Herd A, Nogueira C, David TS, David JS, Tenhunen J, Pereira JS, Chaves M. 2006. Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance. *Plant and Soil* 282, 361-378.
- Lambers H, Chapin III FS, Pons TL, 1998. *Plant physiological ecology*. Springer- Verlag, New York., USA. pp239-298.
- Laustroer B. 2011. Environmental drivers of population structure of wild Rooibos (*Aspalathus linearis*). BSc Thesis, University of Hamburg, Germany. pp44.
- Lehto T, Zwiazek JJ. 2011. Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza* 21, 71-90.
- Leffler AJ, Ivans CY, Ryel RJ, Caldwell MM. 2004. Gas exchange and growth responses of the desert shrubs *Artemisia tridentata* and *Chrysothamnus nauseosus* to shallow- vs. deep-soil water in a glasshouse experiment. *Environmental and Experimental Botany* 51, 9–19.
- Leffler AJ, Peek MS, Ryel RJ, Ivans CY, Caldwell MM. 2005. Hydraulic redistribution through the root systems of senesced plants. *Ecology* 86, 633–642.

- Ludwig F, Dawson TE, Kroon H, Berendse F, Prins HHT. 2003. Hydraulic lift in *Acacia tortilis* trees on an East African savanna. *Oecologia* 134, 293–300.
- Ludwig F, Dawson TE, Prins HHT, Berendse F, de Kroon H. 2004. Below-ground competition between trees and grasses may overwhelm the facilitative effects of hydraulic lift. *Ecology Letters* 7, 623–631.
- Lund ZF. 1959. Available water-holding capacity of alluvial soils in Louisiana. *Soil Science Society of America Journal* 23, 1-3.
- Lynch JP, Brown KM. 2001. Topsoil foraging – an architectural adaptation of plants to low phosphorus availability. *Plant Soil* 237, 225-237.
- Lynch JP 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiology* 156, 1041-1049.
- Marschner H, Dell B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* 159, 89-102
- Matzner SL, Richards JH. 1996. Sagebrush (*Artemisia tridentata* Nutt.) roots maintain nutrient uptake capacity under water stress. *Journal of Experimental Botany* 47, 1045-1056.
- Moll EJ, Campbell BM, Cowling RM, Bossi L, Jarman ML, Boucher C. 1984. Description of major vegetation categories in and adjacent to the Fynbos biome. National Scientific Programmes Unit: CSIR, SANSP Report 83, Mar 1984, pp 35
- Muñoz MR, Squeo F, León, MF, Tracol Y, Gutiérrez JR. 2008. Hydraulic lift in three shrub species from the Chilean coastal desert. *Journal of Arid Environments* 72, 624-632.
- Nadezhdina N, David TS, David JS, Ferreira MA, Dohnal M, Tesa M, Gartner K, Leitgeb E, Nadezhdin V, Cermak J et al. 2010. Trees never rest: the multiple facets of hydraulic redistribution. *Ecohydrology* 3, 431–444.
- Nambiar EKS. 1976. Uptake of Zn<sup>65</sup> from dry soil by plants. *Plant Soil* 44, 267-271.

- Neumann RB, Cardon ZG. 2012. The magnitude of hydraulic redistribution by plant roots: a review and synthesis of empirical and modelling studies. *New Phytologist*, 194, 337–352.
- Osório J, Osório LM, Chaves MM, Pereira JS. 1998. Effects of water deficits on  $^{13}\text{C}$  discrimination and transpiration efficiency of *Eucalyptus globulus* clones. *Aust J Plant Physiol* 25, 645–653.
- Pang J, Wang Y, Lambers H, Tibbett M, Siddique KH, Ryan MH. 2013. Commensalism in an agroecosystem: hydraulic redistribution by deep-rooted legumes improves survival of a droughted shallow-rooted legume companion. *Physiologia Plantarum*. doi: 10.1111/ppl.12020.
- Prieto I, Kikvidze Z, Pugnaire FI. 2010. Hydraulic lift: soil processes and transpiration in the Mediterranean leguminous shrub *Retama sphaerocarpa* (L.) Boiss. *Plant and Soil* 329, 447–456.
- Prieto I, Armas C, Pugnaire FI. 2012. Water release through plant roots: new insights into its consequences at the plant and ecosystem level. *New Phytologist* 193, 830–841.
- Querejeta JJ, Egerton-Warburton LM, Allen MF. 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia* 134, 55–64.
- Rebello AG, Boucher C, Helme N, Mucina L, Rutherford MC. 2006. Fynbos biome. In: Mucina L, Rutherford MC. (eds). *The vegetation of South Africa, Lesotho and Swaziland*. *Strelitzia* 19, 53-219.
- Rebello AG. 1996. Fynbos biome. In: Low AB, Rebello AG. (eds.) *Vegetation of South Africa, Lesotho and Swaziland*. Department of Environmental Affairs and Tourism, South Africa, pp. 62–74.
- Rewald B, Ephrath JE, Rachmilevitch S. 2011. A root is a root is a root? Water uptake rates of Citrus root orders. *Plant Cell and Environment* 34,33-42. Richards JH, Caldwell

- MM. 1987. Hydraulic lift: substantial nocturnal water transport between soil layers by *Artemisia tridentata* roots. *Oecologia* 73, 486–489.
- Richardson AE, Lynch JP, Ryan PR, et al. 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil* 349, 121-156.
- Rose TJ, Rengel Z, Ma Q. et al (2008). Hydraulic lift by canola plants aids P and K uptake from dry topsoil. *Australian Journal of Agricultural Research* 59, 38–45.
- Ryel RJ, Caldwell MM, Yoder CK, Or D, Leffler AJ. 2002. Hydraulic redistribution in a stand of *Artemisia tridentata*: evaluation of benefits to transpiration assessed with a simulation model. *Oecologia* 130, 173–184.
- Ryel RJ. 2004. Hydraulic redistribution. In: Esser K, Lüttge U, Beyschlag W(eds.) *Prog Bot* 65, 413-435.
- Ryel RJ, Leffler AJ, Peek MS, Ivans CY, Caldwell MM. 2004. Water conservation in *Artemisia tridentata* through redistribution of precipitation. *Oecologia* 141,335-45.
- Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC. 2002. Hydraulic redistribution of soil water by neotropical savanna trees. *Tree Physiology* 22, 603–612. Sekiya N, Yano K. 2004. Do pigeon pea and sesbania supply groundwater to intercropped maize through hydraulic lift?- Hydrogen stable isotope investigation of xylem waters. *Field Crops Res* 86, 167-173.
- Sekiya N, Araki H, Yano K. 2011. Applying hydraulic lift in an agroecosystem: forage plants with shoots removed supply water to neighbouring vegetable crops. *Plant and Soil* 341, 39-50.
- Shen Y, Zhang Y, Li S. 2011. Nutrient effects on diurnal variation and magnitude of hydraulic lift in winter wheat. *Agricultural Water Management* 98, 1589 -1594.
- Smart DR, Carlisle E, Goebel M, Nuñez BA. 2005. Transverse hydraulic redistribution by a grapevine. *Plant Cell and Environ* 28, 157–166.

- Snyder KA, James JJ, Richards JH, Donovan LA. 2008. Does hydraulic lift or nighttime transpiration facilitate nitrogen acquisition? *Plant and Soil* 306, 159–166.
- Tyree MT. 2003. Matric potential. *Encyclopaedia of Water Science*, 615-617.
- Wang X, Tang C, Guppy CN, Sale PWG. 2009. The role of hydraulic lift and subsoil P placement in P uptake of cotton (*Gossypium hirsutum* L.). *Plant and Soil* 325, 263-275.
- Warren J M, Meinzer FC, Brooks JR, Domec JC, Coulombe R. 2007. Hydraulic redistribution of soil water in two old-growth coniferous forests: Quantifying patterns and controls. *New Phytologist* 173, 753-765.
- West AG, Patrickson SJ, Ehleringer JR. 2006. Water extraction times for plant and soil materials used in stable isotope analysis. *Rapid Communications in Mass Spectrometry* 20, 1317–1321.
- Wilkinson S, Bacon MA, Davies WJ. 2007. Nitrate signalling to stomata and growing leaves: interactions with soil drying, ABA and xylem sap pH in maize. *Journal of Experimental Botany* 58, 1705–1716.
- Witkowski ETF, Mitchell DT. 1987. Variations in soil phosphorus in the fynbos biome. *S African Journal of Ecology* 75, 1159–1171.
- Xianliang Y, Yunsheng C. 1983. *Soil Physics*, Agriculture Press, Beijing. pp.1–14.

## 4 Clay in soil substrates modulates the acquisition of different soil phosphates

### 4.1 Abstract

*Triticum aestivum* was used to test whether increasing kaolinite clay fractions (0, 1, 5 and 10% w/w) reduce P acquisition *via* mass-flow and diffusion, due to increased adsorption by clay and whether Ca-P, Fe-P and inositol-P have different rates of mobility in soils of varying clay %. I grew plants in modified pots that restricted their roots to acquiring P by mass-flow and diffusion from behind a 25 $\mu$ m nylon mesh. Although plants acquired P by mass-flow and diffusion from behind the mesh, stomatal conductance and  $\delta^{13}\text{C}$  were not modulated by P. Variation in total biomass, shoot:root ratios, foliar [P] and [N] with clay increases indicated a compromise between enhanced N and P acquisition driven by improved moisture retention *versus* decreased nutrient acquisition with increased clay, possibly due to P and water sorption. Acquisition of N and P increased with clay increases (0 - 5% clay) due to enhanced moisture availability, however, further clay increases (10% clay) led to increased complexation of  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  with clay, reduced moisture availability as soil matric potentials increased, increased path length (tortuous routes) for nutrients in less porous substrates that are rich in clay and possibly reduced root elongation in 'strong' soils. Overall, inositol-P supplied the most P whilst Ca-P supplied the least, with values for Fe-P ranging between the two. Clay proportions in soils modulate the mass-flow mobility of  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$ .

## 4.2 Introduction

Soil phosphate (P) availability is a major constraint on plant productivity in most terrestrial ecosystems (Treseder and Vitousek, 2001, Vance *et al.*, 2003, Vitousek *et al.*, 2010, Cramer, 2010). Phosphates in bulk soil exist as organic and inorganic P forms, which differ in their behaviour and fate in different soils (Hansen *et al.*, 2004; Turner *et al.*, 2007). Globally, between 20 and 80% of soil P exists as organic forms, of which phytic acid (inositol hexaphosphate, hereafter abbreviated IH-P) is a major component (Richardson, 1994). Inorganic P forms *ca.* 170 different mineral P complexes (Holford, 1997), of which Fe-P and Ca-P are commonly the most abundant, particularly in some of the extremely P-impooverished Mediterranean ecosystems (Groom and Lamont, 2010). The scarcity of total P and its reduced availability in the soil are major nutritional challenges, particularly in the Cape Floristic Region (CFR) of South Africa and the South West Botanical Province (SWBP) of Australia (Witkowski and Mitchels, 1987; Lambers *et al.*, 2006; Power *et al.*, 2011). Although total P may be high in soils, it often adsorbs strongly to clay and Fe/Al oxides (e.g. Devau *et al.*, 2010) and is often present in unavailable forms or may be available outside the rhizosphere. Availability of P in soils can also be reduced through leaching, either of dissolved or colloidal P, particularly in areas with coarse textured soils and high rainfalls (Siemens *et al.*, 2008). Plant roots are viewed as active ‘miners’ of unavailable soil P (Lambers *et al.*, 2003) through symbiotic associations with mycorrhizal fungi (Fransson *et al.*, 2003; Solaiman and Abbott, 2003), root-hair formation (Bates and Lynch, 1996) and other morphological responses (De Groot *et al.*, 2003; He *et al.*, 2003), and chemical and physical modifications to the substrate (Jones *et al.*, 2003).

Root proliferation brings soil nutrients into contact with root surfaces, which is particularly important for the acquisition of nutrient ions that are only able to move small distances within the soil, whether due to physical isolation by the soil structure or decreased

mobility (Jungk et al., 2002; Chapman et al., 2012). Root proliferation is also a vital adaptive trait enabling roots to access poorly mobile nutrient ions, such as  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  (Hodge et al., 1999; Robinson et al., 1999). For example, the less mobile  $\text{NH}_4^+$  induces lateral root initiation while  $\text{NO}_3^-$  regulates root proliferation (Lima et al., 2010). An increase in root density facilitates the exploration of a bigger soil volume, which increases the chances of nutrients being in contact (interception) with root surfaces (Kage, 1997) where the nutrients can be actively absorbed.

Soil nutrients can be accessed through root 'interception' or they can be delivered to root surfaces by diffusion or by transpiration-driven mass-flow (Barber 1995; Tinker and Nye 2000; McDonald et al. 2002; Cramer et al. 2008; Cramer and Hawkins 2009; Raven, 2008; Cramer et al. 2009). Active absorption creates depletion zones around the roots, which drive the diffusion of nutrients toward the root surface (Nye, 1966a; Nye and Tinker, 1977; Tinker and Nye, 2000; Barber 1995). Since P readily adsorbs to clay and metal oxides in soils (e.g. Livingstone and Boykin, 1962; Frink, 1969; Lambers and Shane, 2007), its mobility in different soils depend on the buffering capacity of the soil in relation to P, which can be expressed as an effective diffusion coefficient (Clarkson et al., 1981). In soils, the effective diffusion coefficient ( $D_e$ ) of P is very low, *ca.*  $10^{-10}$  -  $3.3 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$  (Clarkson, 1981), although this depends strongly on the soil [P], volumetric moisture content and soil texture (So and Nye, 1989, Barber, 1995). This explains the fact that P is considered an immobile nutrient in soil (e.g. Shen et al., 2011). Regarding mass-flow acquisition, transpiration can play a role in modulating P uptake by delivering nutrients to the root surfaces through a flux of solutes into root cells (Cramer *et al.* 2008, Cramer and Hawkins 2009, Cramer *et al.* 2009). Both diffusion and mass-flow fluxes of nutrients co-occur in soils and depend on the available soil moisture; however, diffusion is driven by a nutrient gradient due to active uptake whilst transpiration powers water fluxes towards the root surfaces, which may deliver P to the root surfaces (Cramer *et al.*, 2008; Cernusak *et al.*, 2011).

Transpiration-induced mass-flow of the soil solution is considered to play only a small role in supplying P to root surfaces (Bielecki 1973; Barber, 1995). Roots mostly absorb P as inorganic P (Pi), which is typically low in most soils and is generally considered to be highly immobile (Bielecki, 1973; Barber, 1995). Consequently, mass-flow of the soil solution is generally assumed to play only a small function in powering P to root surfaces (Bielecki 1973). For example, Lambers et al. (1998) estimate that mass-flow typically delivers as little as 1–5 % of a plant's P demand. The delivery of P by mass-flow may, however, be amplified when plants increase their water fluxes (e.g. Cramer *et al.* 2008, Kupper *et al.*, 2012) and when the concentration of nutrients in the bulk soil is high (Scholz *et al.*, 2007). Moreover, mass-flow may deliver significant proportions of P in low-clay soils with a low buffering power. Buffering power is a value that reflects the soil's ability to maintain nutrient intensity as the nutrient is depleted (Nye, 1966b).

While acquisition of P has often been associated with increased root proliferations, diffusion (e.g. Clarkson 1981, Lambers *et al.*, 1998) and mycorrhizal associations (e.g. Bolan, 1991; Smith *et al.*, 2011), there is empirical evidence suggesting effective P acquisition through transpiration-driven mass-flow. Indeed, some tropical species increased their acquisition of P through increased transpirational fluxes (Cernusak *et al.*, 2011), suggesting effective delivery of P *via* mass-flow. Leaf [P] significantly increased with increasing transpirational fluxes in *Ficus insipida* (Cernusak et al., 2007, 2010, Garrish et al., 2010), which is consistent with the role of transpiration in delivering soil P. Despite the general notion that P is immobile in soils (Lambers and Shane, 2007), increased transpiration by nutrient-constrained *Ehrharta calycina* grown in sand led to 40% higher foliar P acquisition *via* mass-flow delivery (Cramer et al., 2008). Thus, although P binds in high clay soils, it may be readily accessible through transpirational mass-flow in low-clay soils because of the reduced P-binding sites.

At a given soil pH, the availability of Ca-P, Fe-P and inositol phosphates (IH-P) may vary because of differences in their rates of dissolution, which are dictated by the size of their mineral particles (Shen et al., 2011; Pierzynski et al., 2005; Oelkers and Valsami-Jones, 2008) and the adsorption of P to clay and metal oxides. Fe-P has an increasing solubility with increasing pH, whilst Ca-P has a decreasing solubility with increasing pH, except for pH > 8 (Hinsinger, 2001). However, pH has a relatively minor influence on the amount of organic phosphate in soil, although some forms of organic phosphate (e.g. IH-P, DNA) accumulate preferentially under strongly acidic (pH < 4) conditions (Turner and Blackwell, 2013). There is limited empirical evidence, however, for the mobility of these soil P forms. Since Fe-P forms a two-times stronger ionic complex than Ca-P at 25°C (Sillen and Martell, 1964), its P should be less available than when Ca-P is supplied. Although data on the ionic strengths is scarce, I expect IH-P to form weaker complexes with clay and metals than Fe-P due to its larger molecule with a presumably weaker charge. Besides forming complexes with P, clay is effective in retaining moisture due to the strong adhesion (high matric potential) of water to clay particles (Lund, 1959; Tyree, 2003) and may possibly impede root elongation due to high matric potential, thus forming ‘strong’ soils (Bengough and Mullins, 1991; Chapman et al., 2012). The consequent retention of moisture by clay may also deprive plants of water that would otherwise have been accessible for mass-flow nutrient acquisition.

To determine the effectiveness of transpirational and diffusive fluxes in delivering P from Ca-P, Fe-P and IH-P in soils of varying sand: kaolinite proportions, I grew *Triticum aestivum* L. (Poaceae) in modified pots that restricted the roots to acquiring P by mass-flow and diffusion from behind a nylon mesh. I hypothesised that (i) increasing the proportion of clay in the soil (0, 1, 5 and 10% w/w) reduces P acquisition *via* mass-flow and diffusion due to increased adsorption of water and P by clay, and ii) Fe-P, Ca-P and IH-P have different rates of mobility in substrates of varying clay owing to their different rates of dissolution in

the soil. Thus, I expect mobility of P to be fastest in low-clay substrates, although these substrates also dry out faster, reducing the opportunity for P delivery.

## 4.3 Materials and Methods

### 4.3.1 Soil characteristics

To determine the rate of water loss from the soils, *ca.* 10-g samples varying in clay were thoroughly wetted to field capacity. Wet soil samples were weighed in crucibles and transferred to a constant temperature room (25°C) and subsequently reweighed after 12 h and every 6 h thereafter until no further weight loss was recorded. The rate of water loss after 12 h ( $\theta$ ) was expressed as  $\theta = (\omega_s - \rho_t)/12 \cdot \phi_m$ ; where  $\omega_s$  is saturated soil mass,  $\rho_t$  is soil mass after time ( $t = 12$ ) from saturation,  $\phi_m$  is the final mass of soil after oven-drying until it reached constant mass.

To determine the adsorption of Ca-P, Fe-P and IH-P by soil samples of varying clay, 5 g samples were supplied 10 ml of 100  $\mu$ M of P from one of the P forms dH<sub>2</sub>O and left to mix for 48 h at 110 rpm and 25°C on a Labcon continuous rotary shaker (Wirsam Scientific, Cape Town, South Africa). Supernatants were obtained by pipetting 5 ml of the soil solution and then centrifuging it at 10 000 rpm for 20 min. Available PO<sub>4</sub><sup>3-</sup> was determined on 180  $\mu$ l aliquots of the supernatants using the malachite green oxalate method (Motomizu et al., 1983). A 1500 Multiskan spectrum plate reader (Thermo Electron Corporation, Vantaa, Finland) was used to determine sample absorbencies at 650 nm.

### 4.3.2 Sampling and P extraction from Greater Cape Floristic Region soils

To determine the effect of clay on P availability in soils of Greater Cape Floristic Region, replicate ( $n = 5$ ) shallow-soil samples were collected from the top 30 cm and characterized at nine sites along an aridity gradient of 443 km on the winter-rainfall west coast of South Africa

from Springbok in the north to Heksrivier in the south (co-ordinates in Table 1). Soils were oven dried at 80°C and sieved (2-mm mesh). Soil pH was determined by shaking a 2 g soil sample in 20 ml 1 M KCl at 180 rpm for 60 min, centrifuging at 10 000 g for 10 min and measuring the supernatant pH. Total soil P was determined by digesting 1 g soil in a 3.5 mL triacid mixture (10 HNO<sub>3</sub>:1H<sub>2</sub>SO<sub>4</sub>:1HClO<sub>4</sub>) at 150°C for 90 min, diluting to 25 ml and analysing using molybdate green method (Diatloff and Rengel, 2001). Available P was determined from extracts of 6.6 g soil in Bray II solution (Bray and Kurtz, 1945) before filtering and analysing using ICP-AES (Varian Vista MPX, Melbourne, Australia). A soil column was constructed from a 10 mL syringe with the plunger removed where the bottom was plugged with glass wool over a disc of Whatman No. 1 filter paper. Soil was filled to the 10 mL mark, weighed and water added to field capacity and reweighed. The soil column was inserted through a hole in the lid of a 50 ml centrifuge tube, placed in the tube and centrifuged at 150 g for 2 min. The solution collected in the centrifuge tube was taken as the mass-flow displaceable P, which was determined colorimetrically using the molybdate-green method (Diatloff and Rengel 2001).

#### *4.3.3 Plant material and experimental culture*

Sixty 2.5-L pots were furnished with a central compartment to which plant roots did not have access but allowed free-flow of solutes between the compartments (Fig 4.1). The compartment was made by cutting a hole (5 cm diameter) into the base of each pot into which a 0.2 L PVC pipe (5 cm diameter, 10 cm long, 0.25 cm wall thickness) was glued. To allow free movement of solutes between the compartments, the pipe was drilled on its entire surface with 0.5 cm diameter holes in a 2 cm lattice and covered on the outer surface with strips of nylon mesh (Nytal 25 µm; Draht-Center, Stuttgart, Germany). The mesh restricted the root system from penetrating the central nutrient compartment, but not mycorrhiza.

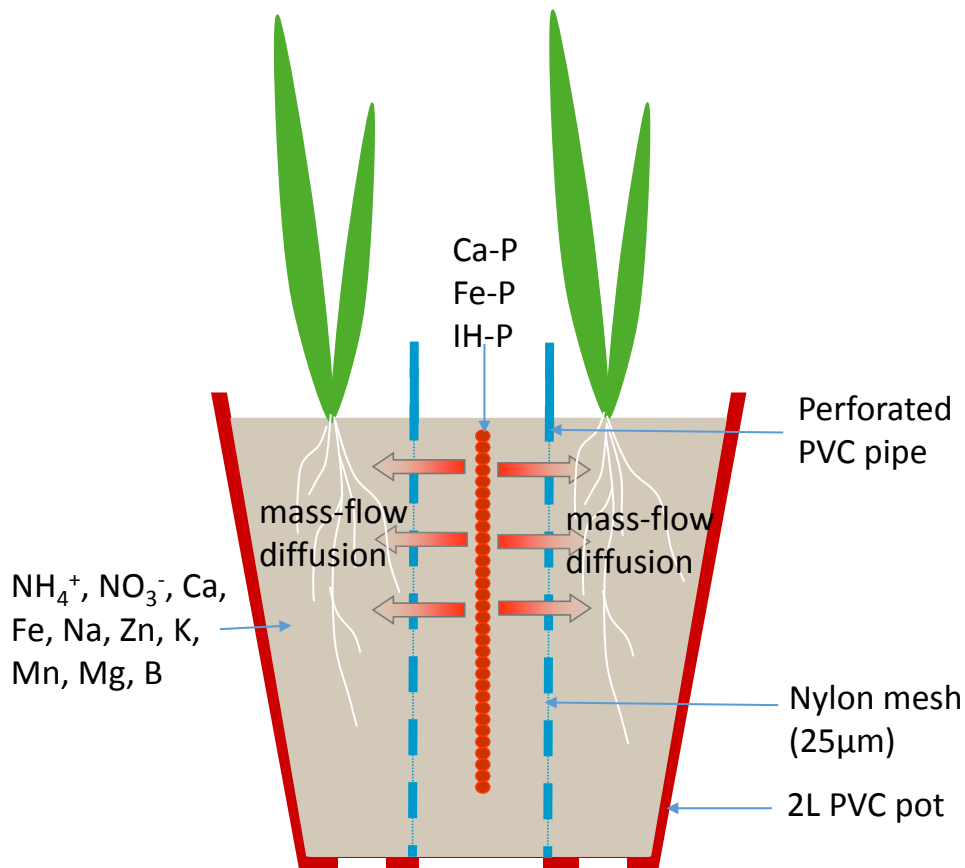


Fig. 4.1. A schematic description of the pot experiment, with one of 0, 1, 5 or 10 % (w/w) kaolinite clay:sand. Plants were supplied with one of Ca-P, Fe-P or IH-P fertilizers in a compartment not directly accessible by the roots (fertiliser sequestered behind a 25 µm mesh), but from which P could move by diffusion and mass-flow, dubbed ‘mass-flow/diffusion’. Other nutrients ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , K, Ca, Fe, Mn, Mg, Zn, B, Na) were supplied twice weekly, outside central compartment, with 200 ml of P-free modified Long Ashton solution (Hewitt, 1952).

To minimize mycorrhizal symbioses I used rinsed acid-washed sand. I also scored the infection of roots by mycorrhiza (see below). Four soil substrates of varying clay were made by mixing 0, 1, 5 and 10 % (w/w) of Kaolin (Sigma-Aldrich, St. Louis, Mo, USA) with the acid- and then dH<sub>2</sub>O-washed sand (*ca.* pH 7). Kaolin is a bolus hydrated aluminium silicate [ $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ ], which is a commercial form of natural kaolinite clay. Pots were filled with

ca. 3.5 kg of sand, with the central compartment filled with one of the four sand:clay substrates. To supply the different P treatments, a cylindrical hole (9-mm diameter) was made in the central compartment using a cork borer. One of the three P forms was supplied to each core at  $5 \mu\text{g P g}^{-1}$  substrate. The P fertilizers used were prepared using one of  $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$  and inositol-phytate ( $\text{C}_6\text{H}_{18}\text{O}_{24}\text{P}_6 \cdot x\text{Na}^+ \cdot y\text{H}_2\text{O}$ ; Sigma-Aldrich, St. Louis, Mo, USA) mixed with sand. Clay substrates and P forms were applied in a 4 x 3 factorial arrangement replicated five times.

Each pot had two imbibed seeds of *T. aestivum* L. planted on opposite sides at a depth of 0.01 m. All pots were kept in a glasshouse and watered with 200 ml  $\text{dH}_2\text{O d}^{-1}$  five times in a week and fertilized twice weekly with 200 ml of P-free modified Long Ashton solution (Hewitt, 1952) containing (mM) 4 N, 2 K, 4 Ca, 1.5 Mg, 3.5  $\text{SO}_4$ , 0.1 FeEDTA, 0.02 Mn, 0.14  $\text{H}_3\text{BO}_3$ , 4.2 Na, 4 Cl, 0.003 Cu, 0.0002 Mo and 0.002 Zn. Soil water holding capacities and available P were determined separately as described below.

To ensure uniformity of the growing conditions the pots were grouped in replicates on movable trolleys and both the pots and replicates were randomly reshuffled from their positions every second day. The greenhouse received an average midday light intensity of  $1660 \mu\text{mol m}^{-2} \text{s}^{-1}$  and daytime RH of ca. 40 %, whilst the temperatures were kept below  $25^\circ\text{C}$  (day) and above  $15^\circ\text{C}$  (night). After 40 d, the plants were transferred to a growth chamber for measurements and left to acclimatise for 48 h prior to gas exchange measurements. The growth chambers were equipped with 14 x 400W- HQI-T metal halide lights (Osram Powerstar, Osram, Cape Town, South Africa), 28 400W- NAV-T sodium lights (Osram Violox) and 24 x 150 W, 230V incandescent (Sicca, Osram, Cape Town) lamps providing a light intensity of  $1000 - 1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  with 16h light and 8h dark and day/night temperatures of  $25^\circ\text{C}/20^\circ\text{C}$ , with mean day/night RH ca. 65 %.

#### 4.3.4 Gas exchange measurements

Gas exchange measurements were performed on the third fully expanded leaf of each plant. All plants were watered before the gas exchange measurements. Stomatal conductance ( $g_s$ ) was determined using a Licor 6400-02B cuvette connected to a portable gas exchange system (LICOR6400, Li-Cor, Inc., Lincoln, NE, USA) after equilibration in the cuvette (*ca.* 5 min) at a saturating photosynthetically active radiation (PAR) level of  $1500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  (determined from preliminary light response curves) with  $400 \mu\text{l L}^{-1} \text{CO}_2$  and a flow rate of  $500 \mu\text{mol s}^{-1}$ . Leaf temperature was maintained at  $25^\circ\text{C}$  and relative humidity was *ca.* 65% during the measurements.

#### 4.3.5 Biomass measurements and foliar elemental analyses

Roots, shoot and ear biomasses were estimated at the end of the 60 d period. Pots were gently excavated onto  $2 \text{ mm}^2$  sieves and the soil removed under running water. To assess mycorrhizal infection, fine roots were sampled (0.1 g FW) and cleared in hot 10% KOH, acidified in 10 % HCl and stained with 0.05 Trypan blue in lactophenol, destained in lactophenol, mounted on slides and observed at 150X magnification under a dissecting microscope (Phillips and Hayman, 1970). Shoots were separated from roots and dried at  $70^\circ\text{C}$  for 48 h in a forced draught oven and weighed. Samples of shoots, ears and roots of each plant were milled separately 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.

Foliar concentrations of nutrients were determined by ashing milled leaf material at  $480^\circ\text{C}$  for 8 h before dissolving them with a 1:1 (v/v) of HCl (Kalra, 1998). Assessment of the element concentrations in solution was performed using inductively coupled plasma atomic emission spectrometry (Varian Vista MPX, Mulgrave, Australia). Foliar N and  $\delta^{13}\text{C}$  were determined using mass spectrometry. Between 1.900 and 2.000 mg of milled samples was

weighed into a 5×9 mm tin capsule (Santis Analytical AG, Teufen, Switzerland). The tin capsules were then combusted in a Thermo Flash EA 1112 series elemental analyzer 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.

#### *4.3.6 Data analysis*

Data on plant water fluxes, biomass and foliar nutrients were analysed through two-way analyses of variance (ANOVA) using Statistica (version 10 Statsoft Inc., Tulsa, USA). Data were log transformed where values ranged over several orders of magnitude. Analysis of covariance (ANCOVA) was used to compare the relationship between availability of P from different forms with increased clay proportions in R (R-Core Development Team, 2008).

## **4.4 Results**

### *4.4.1 Substrate water and P retention*

Water retention of sand-clay mixtures against evaporation increased as the proportion of clay (clay fraction) in the soil increased (Fig. 4.2A). Increased clay fraction also reduced the amount of water available for uptake by plants (Fig. 4.2B). Thus, sand-clay mixtures with higher clay showed both greater water retention and reduced water availability. Increased clay fraction resulted in reduced available [P] regardless of the P form supplied (Ca-P, Fe-P and IH-P, all supplied at the same concentration), the form of this relationship being similar across the three P forms (ANCOVA interaction term  $F = 1.520$   $P = 0.292$ ) (Fig. 4.3A). A similar trend of declining available [P] with increased clay was shown by nine soils of varying clay fraction, sampled from across the Greater Cape Floristic Region (Supplementary Table 1; Fig. 4.3B).

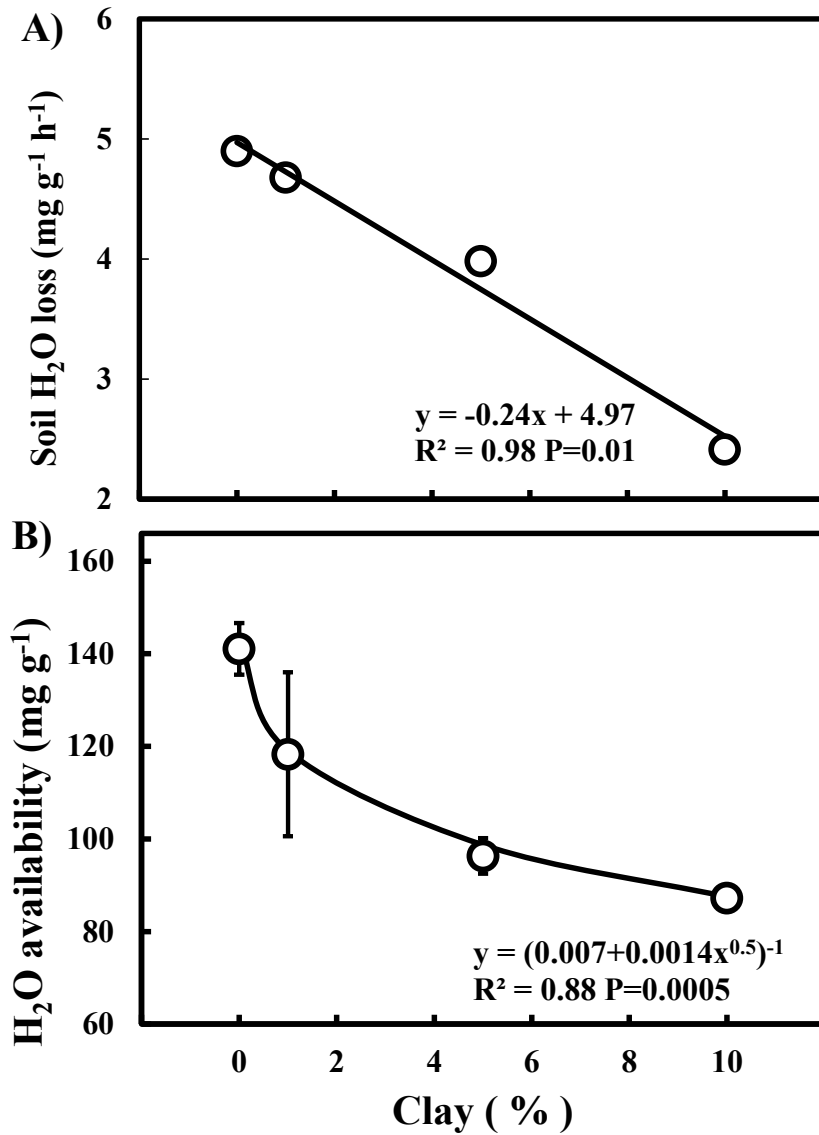


Fig. 4.2. Relationship between a) rate of soil water loss (mg g<sup>-1</sup> h<sup>-1</sup>) and b) plant-extractable water (mg g<sup>-1</sup>) from substrates of varying clay fractions (% w/w) following their saturation with water to field capacity. Symbols and bars denote mean ± SE (n = 4). Lines were estimated with the equation shown, with coefficient of determination (R<sup>2</sup>) and P-value indicated.

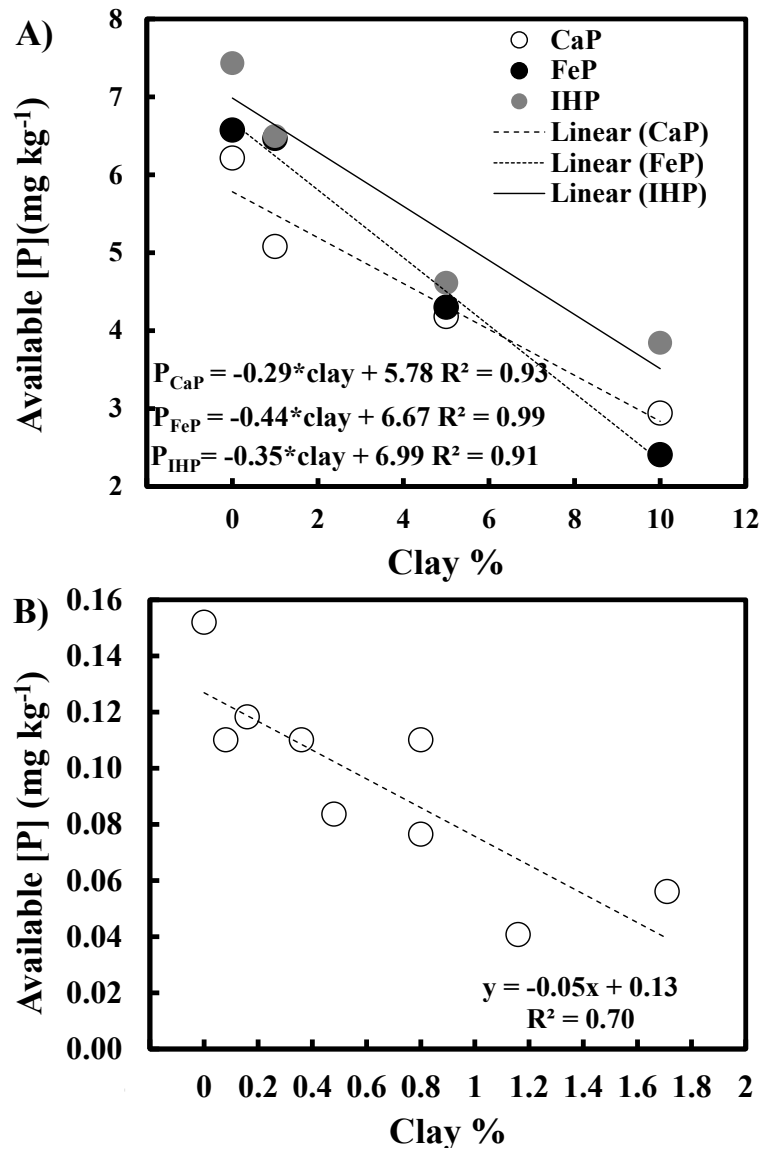


Fig. 4.3. a) Variation in extractable [P] from substrate solution after P was supplied as Ca-P, Fe-P and IH-P to 5-g soil samples that varied in their clay proportions (0, 1, 5 and 10 % w/w). Lines, equations and coefficient of determination ( $R^2$ ) display regressions relating clay % to substrate extractable [P] ( $n = 4$ ). b) Variations in available P displaced from nine soils of varying clay content by water supplied at field capacity of the soil. Soils were collected along an aridity gradient in winter rainfall region of South Africa (which includes the Fynbos biome and adjacent Succulent Karoo). See supplementary Table 1 for sites where soils were collected. Line shows a linear relationship of clay % to available [P] with coefficient of determination ( $R^2$ ) indicated.

#### 4.4.2 Biomass response to clay and P form

Mycorrhizal colonisation did not vary with either P form or clay fraction (P form:  $F_{(2,48)} = 0.867$ ,  $P = 0.724$ ; Clay:  $F_{(3,48)} = 0.028$ ,  $P = 0.994$ ; Clay\*P form:  $F_{(6,48)} = 0.205$ ,  $P = 0.973$ ). Mycorrhizal infection rates were consistently low ( $2.0 \pm 1.8\%$ ), especially when compared with the 40-fold higher ( $85 \pm 2.5$ ) infection rates previously reported for *T. aestivum* grown under similar culture conditions but inoculated with *Glomus mosseae* (BEG 107) (Hawkins and George, 2001). Since mycorrhizal infection rates were very low, when detected, the infected plants were also included in the data analysis. Because soils were not inoculated with mycorrhizae, the low infection rates must be due to natural contamination. The low infection rates suggest that mycorrhizae played a limited role in P acquisition from the central compartment. Direct interception of P by the roots of *T. aestivum* is precluded by the failure of roots to penetrate the central compartment, this being surrounded by a 25  $\mu\text{m}$  nylon mesh.

Clay fraction, but not P form, had a significant effect on plant biomass, with plants grown in 1% and/or 5% clay generally being larger than those grown in 0% and 10% clay (Fig. 4.4A). At least in plants supplied with Ca-P and Fe-P, shoot:root ratio showed an opposite trend, being lowest in plants grown in 1% and/or 5% clay. The high shoot:root ratios shown by plants grown on 0% and 10% clay was due to decreased root dry mass (data not shown).

#### 4.4.3 Gas exchange response to clay and P form

Foliar  $\delta^{13}\text{C}$  varied with clay fraction but not with P form (Fig. 4.5A), being consistently higher in plants grown in 10% clay than in plants grown in 0%, 1% or 5% clay. While this implies increased water use efficiency of plants grown on 10% clay, stomatal conductance ( $g_s$ ) was found not to vary with clay fraction (Fig. 4.5B). Plants supplied with Ca-P and IH-P did, however, show higher  $g_s$  overall, than those supplied with Fe-P.

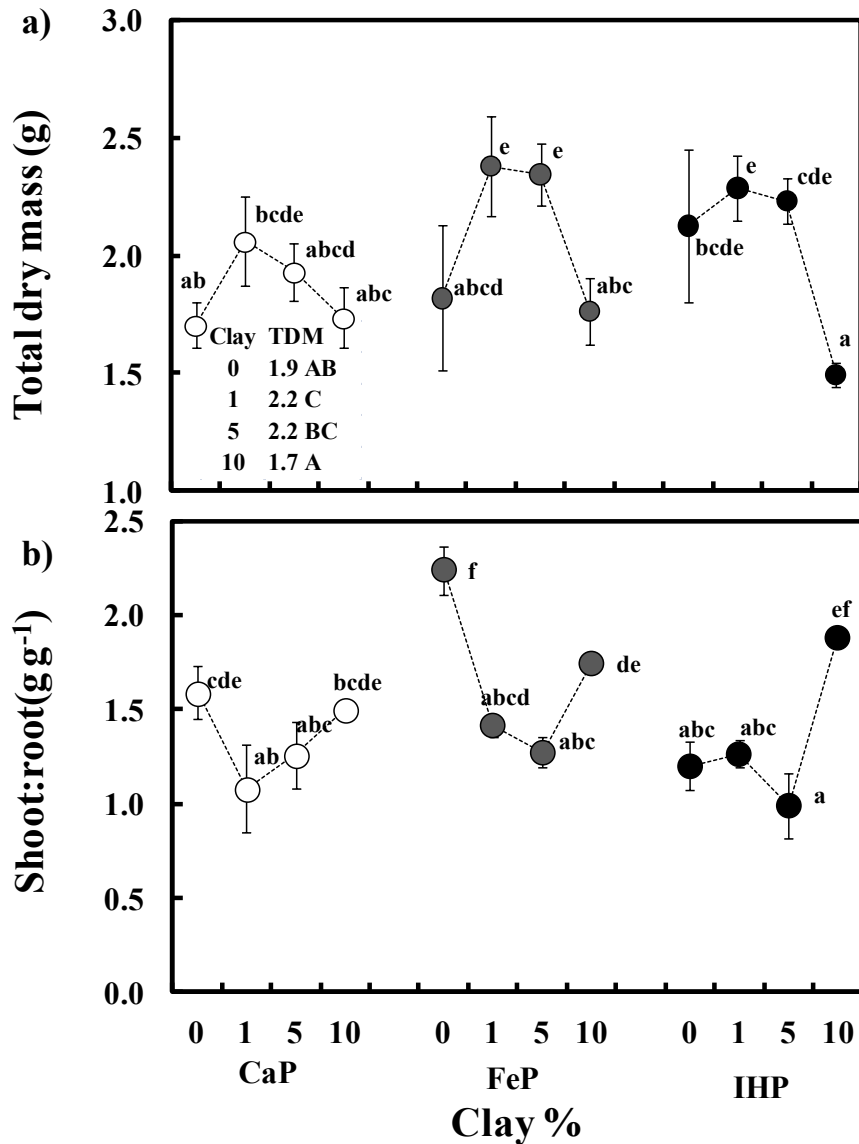


Fig. 4.4. a) Total dry mass (TDM) and b) shoot:root ratios of *Triticum aestivum* plants grown in soils of varying clay (% w/w) and acquiring Ca-P, Fe-P or IH-P from 0.025m behind a 25 $\mu$ m mesh. Symbols and bars represent means  $\pm$  SE (n = 5). ANCOVA for total dry mass yielded Clay:  $F_{(3,48)} = 6.54$   $P < 0.001$ ; P form:  $F_{(2,48)} = 1.64$   $P = 0.204$  and Clay\*P form:  $F_{(6,48)} = 0.90$   $P = 0.500$ . ANCOVA for shoot:root yielded Clay :  $F_{(3,48)} = 10.57$   $P < 0.001$ , P-form :  $F_{(2,48)} = 6.30$   $P = 0.004$  and Clay\*P form :  $F_{(6,48)} = 3.43$   $P = 0.007$ . Means with different letters are significantly different after post-hoc test (Fischer LSD) for mean separation. In the absence of significant clay\*P form, inserted tables and values show TDM across clay levels.

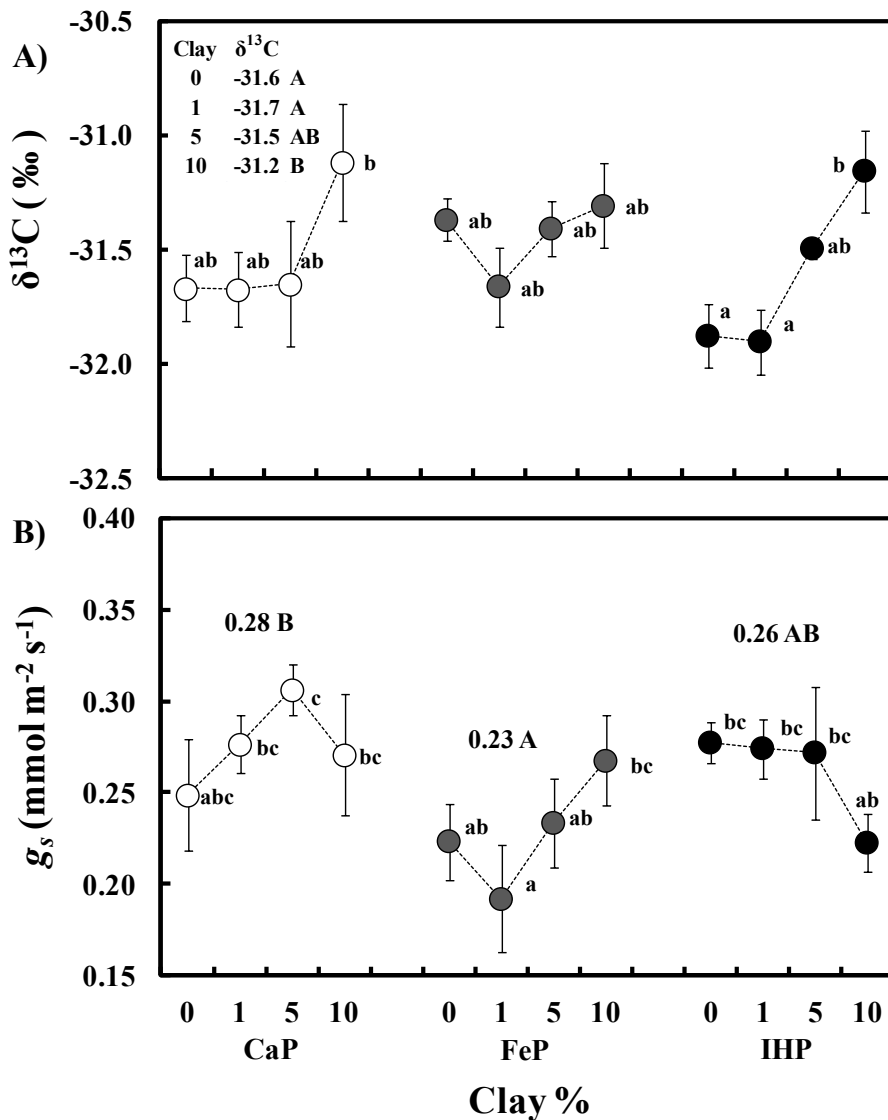


Fig. 4.5. Variation in a)  $\delta^{13}\text{C}$  and b) stomatal conductance ( $g_s$ ) of *T. aestivum* plants grown in soils of varying clay (% w/w) and acquiring Ca-P, Fe-P or IH-P from 0.025m behind a 25 $\mu\text{m}$  mesh. Each symbol and bar represents mean  $\pm$  SE (n = 6). ANCOVA for  $\delta^{13}\text{C}$  Clay:  $F_{(3,24)} = 3.6$   $P = 0.029$ ; P form :  $F_{(2,24)} = 0.6$   $P = 0.551$  and Clay\*P form :  $F_{(6,24)} = 0.5$   $P = 0.767$ . ANCOVA for  $g_s$  Clay :  $F_{(3,48)} = 0.57$   $P = 0.641$ , P form :  $F_{(2,48)} = 3.96$   $P = 0.026$  and Clay\*P form :  $F_{(6,48)} = 1.63$   $P = 0.159$ . Different letters denote means with significant differences after a post-hoc test (Fischer LSD). In the absence of significant clay\*P form, inserted tables and values show [ $\delta^{13}\text{C}$ ] across clay levels and [ $g_s$ ] across P forms.

#### 4.4.4 Tissue nutrient responses to clay and P form

Both clay fraction and P form exerted significant effects on foliar [N], the interaction term being non-significant (Fig. 4.6A). Overall, foliar [N] was highest in plants grown in 5% clay, being significantly lower in plants grown in 0% and 10% clay. Since plants grown in 1% and 5% clay also had the highest biomass, these treatments were also associated with the highest total N contents (Fig. 4.7A). Plants supplied with the inorganic Fe-P had higher foliar [N] than those supplied with organic IH-P, with values of those supplied Ca-P ranging across those of Fe-P and IH-P treatments. The relationship displayed between N content and clay proportion (Fig. 4.7A) was consistent with that of increased clay fraction with biomass, displaying increased N content with increased clay to 5% and a decline at 10% clay. Unlike foliar [N], the total N did not differ overall for plants supplied Ca-P, Fe-P and IH-P.

Foliar [P] varied with clay fraction but not with P form (Fig. 4.6B), with [P] having higher values with increased clay fraction, attaining the highest values at 5%. Foliar [P] of plants grown in 10% clay were, however, not different from those grown in 5%. Both clay fraction and P form exerted significant effects on foliar P content, the interaction term being non-significant (Fig. 4.7B). Overall, P content increased as clay fraction increased. Phosphorus content was lowest for plants supplied Ca-P and highest for those supplied IH-P, with values of plants supplied with Fe-P between those of Ca-P and IH-P treatments. Foliar [K] (Fig. 4.6C) and K content (data not shown), did not differ with clay fraction or P form. Both clay fraction and P form had a significant effects on foliar N:P ratio, the interaction term being non-significant (Fig. 4.7C). The foliar N:P declined as clay increased within the range 0-10% clay, displaying a contrasting trend to that of total foliar P. The N:P ratios of plants varied in the order  $FeP \geq CaP \geq IH-P$ , with significant differences shown between plants supplied with IH-P and Fe-P.

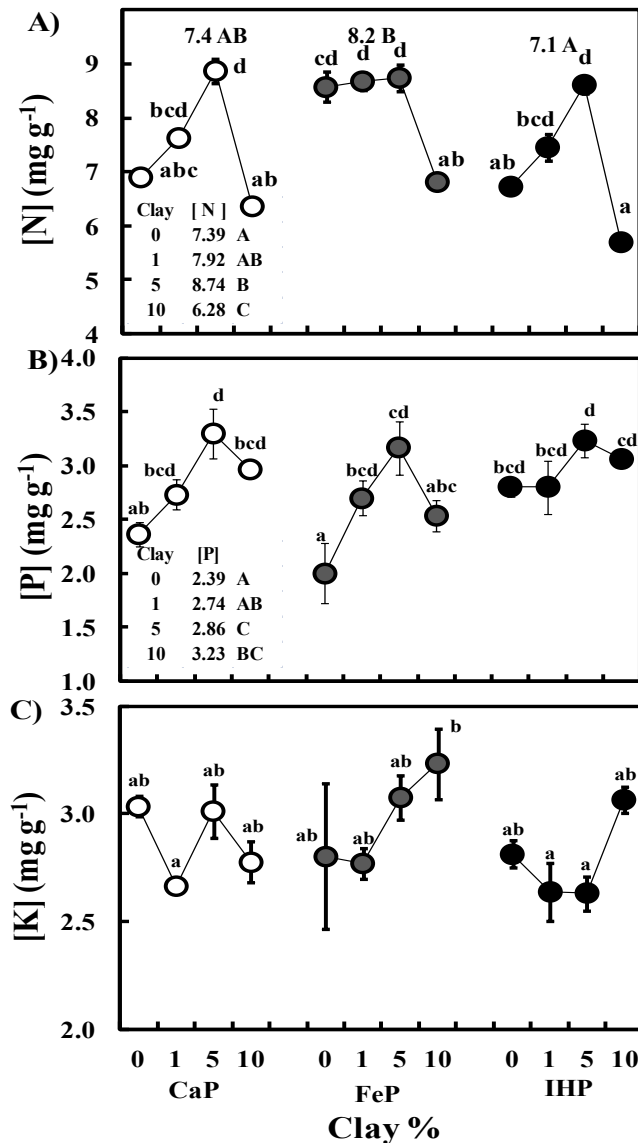


Fig. 4.6. Variation in a) foliar [N], b) foliar [P] and c) foliar [K] of *Triticum aestivum* L. grown in soils of varying clay (% w/w) and acquiring Ca-P, Fe-P or IH-P from 0.025m behind a 25 $\mu$ m mesh. Each symbol and bar represents mean  $\pm$  SE. ANCOVA for [N] Clay:  $F_{(3,48)} = 9.42$   $P < 0.001$ ; P form :  $F_{(2,48)} = 3.71$   $P = 0.039$  and Clay\*P form :  $F_{(6,48)} = 0.58$   $P = 0.743$ . ANCOVA for [P] Clay :  $F_{(3,24)} = 6.973$   $P = 0.002$ , P form :  $F_{(2,24)} = 2.778$   $P = 0.082$  and Clay\*P form :  $F_{(6,24)} = 0.661$   $P = 0.681$ . ANCOVA for [K] Clay :  $F_{(3,24)} = 1.92$   $P < 0.153$ , P form :  $F_{(2,24)} = 1.13$   $P = 0.341$  and Clay\*P form :  $F_{(6,24)} = 1.11$   $P = 0.386$ . Different letters denote different means after a posthoc test (Fischer LSD). In the absence of significant clay\*P form, inserted tables and values show [N] and [P] across clay levels and values above lines show [N] across different P forms.

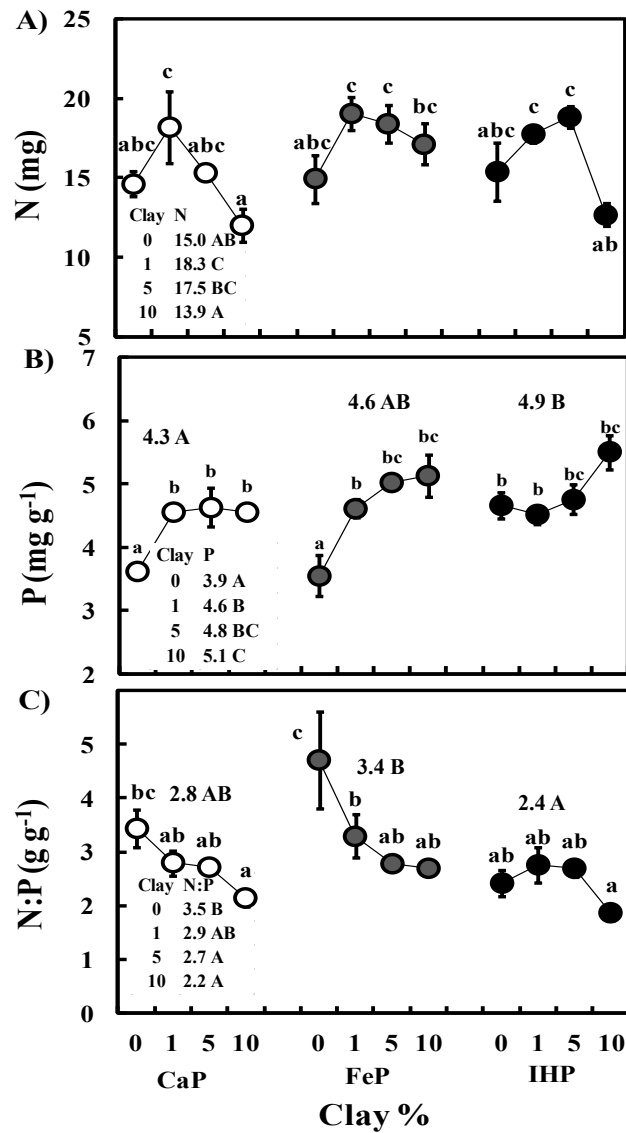


Fig 4.7. Variation in a) total N, b) total P and c) foliar N:P of plants grown in soils of varying clay (% w/w) and acquiring Ca-P, Fe-P or IH-P from 0.025m behind a 25 $\mu$ m mesh. Each symbol and bar represents mean  $\pm$  SE (n = 6). ANCOVA for N Clay:  $F_{(3,48)} = 5.118$   $P = 0.007$ ; P form :  $F_{(2,48)} = 2.26$   $P = 0.130$  and Clay\*P form :  $F_{(6,48)} = 0.857$   $P = 0.540$ . ANCOVA for P Clay :  $F_{(3,24)} = 9.02$   $P < 0.001$ , P form :  $F_{(2,24)} = 3.52$   $P = 0.046$  and Clay\*P form :  $F_{(6,24)} = 1.67$   $P = 0.171$ . ANCOVA for N:P Clay :  $F_{(3,24)} = 4.33$   $P = 0.014$ , P form :  $F_{(2,24)} = 4.56$   $P = 0.021$  and Clay\*P form :  $F_{(6,24)} = 1.16$   $P = 0.359$ . Different letters denote different means after a post-hoc test (Fischer LSD). In the absence of significant clay\*P form, inserted tables show N and P across clay levels and values above the lines show P and N:P across P forms.

To assess whether  $g_s$  was modulated by foliar [N] or excess [N] in the cytosol (as measured by foliar N:P), I compared the trends in  $g_s$  to those of foliar N:P. Variation in foliar N:P was not related to  $g_s$  across any P-forms Ca-P ( $r = 0.20$ ;  $P = 0.609$ ), Fe-P ( $r = 0.24$   $P = 0.444$ ) and IH-P ( $r = 0.20$   $P = 0.531$ ), suggesting that transpiration was not regulated by P.

## 4.5 Discussion

Given the experimental set up used, *T. aestivum* plants must have acquired their P mostly by mass-flow. In the absence of direct root interception, limited mycorrhizal infection and diffusion are the only other processes that could deliver soil P to the roots. Over such long distances (>25 mm) diffusion would be unlikely to account for the observed levels of P acquisition owing to the slow soil diffusion rate of P ( $D_e = 10^{-8}$  to  $10^{-10}$   $\text{cm}^2 \text{s}^{-1}$ ; Barber, 1985). Using the equation for linear diffusion distance ( $\Delta r = 2D_e t$ )<sup>0.5</sup> ( $t = \text{time}$ , Nielsen, 2002), the mobility of P by diffusion can be estimated from  $D_e$  to range from  $\Delta r = 0.04$  to  $0.4$   $\text{mm d}^{-1}$ , thus taking between 6 d and 63 d to reach the closest roots. The low diffusive mobility of P limits the soil volume from which P is acquired to that in close proximity to the root; e.g. < 10 mm for onion roots (Bagshaw et al. 1972), thus P acquisition potentially occurred through a combination of mass-flow and diffusion as the roots were 25 mm from the P-rich zone. Nutrient acquisition by mycorrhiza must have been limited since root colonisation was negligible and the presence of a nylon mesh barriers possibly reduced hyphal penetration.

Foliar [N], foliar [P], total biomass and shoot:root biomass allocation were all influenced by the clay fraction. The foliar [N], [P] and total biomass attained their highest values for intermediate levels of clay (1% and/or 5%) while shoot:root ratio showed the reverse pattern. At 0% clay, the sand substrate would have had poor water retention reducing the effective amount of soil moisture available to plants (Fig. 4.8). Although higher clay

fractions (10%) would have improved water retention by minimizing evaporative loss (Fig. 4.2), it would also have reduced the effective amount of soil moisture available to plants for nutrient uptake, owing to the strong adhesion of water to clay particles and the high soil matric potential (e.g. Lund, 1959; Tyree, 2003). Thus, foliar [N] and [P] possibly declined or did not change at 10% clay due to i) the high matric potential associated with clay (Chapman et al., 2012; Whalley et al., 2013), ii) the adsorption of  $\text{NH}_4^+$  (Miller and Cramer, 2004) to negatively charged soil particles (Appel and Ma, 2002), and iii) the complexation of  $\text{PO}_4^{3-}$  to metals and binding to clay in the soil (Fontes and Weed, 1996; Shen et al., 2011). The proposed model (Fig 4.7), however, still requires verification at varying water potentials, given that the different clay portions were supplied the same amount of water. In addition, clay reduced the amount of P extracted from nine natural soils with low clay proportion (< 2%), collected from the GCFR, which provided further evidence for  $\text{PO}_4^{3-}$  adsorption. In particular, the  $\text{PO}_4^{3-}$  in bulk soil solution was possibly bound to more clay binding sites and Fe/Al, thereby becoming unavailable to plants (Arai and Sparks, 2007), since my clay source kaolin contained Al. Santner et al. (2012) demonstrated that high  $\text{Al}_2\text{O}_3$  in clay reduced the acquisition of P by *Brassica napus*. My clay form was kaolinite, a hydrated aluminium silicate, with the potential to adsorb P to  $\text{Al}^{3+}$  ions and to clay binding sites. Kaolinite, a 1:1 ( $\text{Si}_2\text{O}_5^{2-}$ :  $\text{Al}(\text{OH})_6^{-3}$ ) clay with a low CECs ca. 5-15 meq/100 g (Velde and Meunier, 2008) may be high in free  $\text{Al}^{3+}$  in low soil pH (below 4.5-5.0), which binds  $\text{PO}_4^{3-}$ , yet very low in free  $\text{Al}^{3+}$  at neutral pH. Adsorption of P occurs on an edge  $-\text{Al}(\text{OH})_2$  and in some amorphous region of the clay surface (Muljadi et al., 1966). It is likely that the mobility of P in 2:1 ( $\text{Si}_2\text{O}_5^{2-}$ :  $\text{Al}(\text{OH})_6^{-3}$ ) clays with higher CEC than kaolinite, such as illite (CEC ca. 25-40 meq/100 g), smectite (90-120 meq/100 g) and vermiculite (100-150 meq/100 g) (Velde and Meunier, 2008) would give different rates of P sorption. Thus, differences in total biomass and in shoot:root biomass ratios between the clay treatments may indicate a compromise between enhanced nutrient acquisition with enhanced moisture retention *versus* decreased

nutrient acquisition with increased clay due to nutrient and water sorption (Fig. 4.8). Greater clay may also result in mechanical resistance to root development by ‘strong’ clay soils (Bengough and Mullins, 1991), possibly accounting for increased shoot:root ratios with high clay proportions.

The lack of differences in foliar [K] between clay treatments suggests that plants had adequate supplies of K. The supplied K is also known to be relatively mobile, as it has a higher diffusion coefficient ( $De$ ) of  $ca. 1 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$  compared to  $ca. 1 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$  for P but lower than of  $\text{NO}_3^-$   $ca. 2.5 \times 10^{-6}$  (Barber, 1995).

Organic P forms are often more mobile in the soil solution, being less strongly complexed to metal oxides than inorganic forms (Condrón et al. 2005, Richardson et al. 2005, Turner 2008). In this context the greater delivery of IH-P than Ca-P is fully consistent with a mass-flow delivery mechanism. At the root surface, the IH-P would then be hydrolyzed to accessible P, since the concentration of phytase enzymes is typically greater in the rhizosphere ( $ca. 10$ -fold) compared with that in bulk soil (Neumann and Römheld, 2002; Jones et al. 2003; Zhang et al., 2010). Contrary to expectation, based on the differential mobilities Ca-P and Fe-P under conditions of slightly low pH (e.g. Pierzynski et al., 2005; Oelkers and Valsami-Jones, 2008), there was no difference in the acquisition of P by *T. aestivum* plants supplied with these P forms.

Although P (e.g. Maurel et al., 2008) or N:P (Chapter 2) could potentially be involved in regulation of water fluxes, I found no evidence for the nutritional regulation of water fluxes by foliar [P] or N:P, neither of these variables being related to  $\delta^{13}\text{C}$  or  $g_s$  across the clay gradient. This is consistent with the findings of Garrish et al. (2007), which demonstrate that P exerted no control over transpirational fluxes in *Ficus insipida*. Instead, the authors suggested a functional role for mass-flow delivery of P to root surfaces of this tropical pioneer tree (Garish et al., 2010; Cernusak et al., 2007).

Mass-flow has often been thought to play a rather limited role in the delivery of P to the rhizosphere (Barber, 1995, Lambers et al., 1998), both because soil [P] is typically considered to be low (*ca.* 1  $\mu\text{M}$ , Bielecki 1973) and because the mobility of P in soil is typically considered to be low. This perception needs re-evaluation since some soils, such as in post-agricultural lands, often accumulate higher [P] between 50 and 200  $\text{mg kg}^{-1}$  (Hawkins, et al., 2008), which may potentially supply adequate P *via* transpiration-driven mass-flow of bulk soil solution. Barber (1995) suggests a negligible contribution of water fluxes towards P acquisition by the root and that the main contribution is due to [P] in the soil solution. Therefore, future studies should possibly measure [P] at root surface using  $^{32}\text{P}$  fertilizers and anti-transpirants to establish the P delivered by mass-flow to the root surfaces compared to the contribution of P concentration in the soil solution. My kaolin:sand substrates and the soils collected from the GCFR (Supplementary Table 1) displayed reduced  $\text{PO}_4^{3-}$  mobility as clay proportions increased. The GCFR soils, however, had high total P, most of which was unavailable (possibly organic P) despite the very low clay proportions of *ca.* < 2%. Under field conditions, extrapolating data from simple experimental systems (e.g. sand cultures, hydroponics) may present interpretational challenges, particularly as soil clay regulates the soil hydraulic properties, the availability and mobility of dissolved nutrient ions. Future models on nutrient mobility in soils should, therefore, focus on developing novel techniques of accounting for nutrient adsorption and variations in soil hydraulic conductivity.

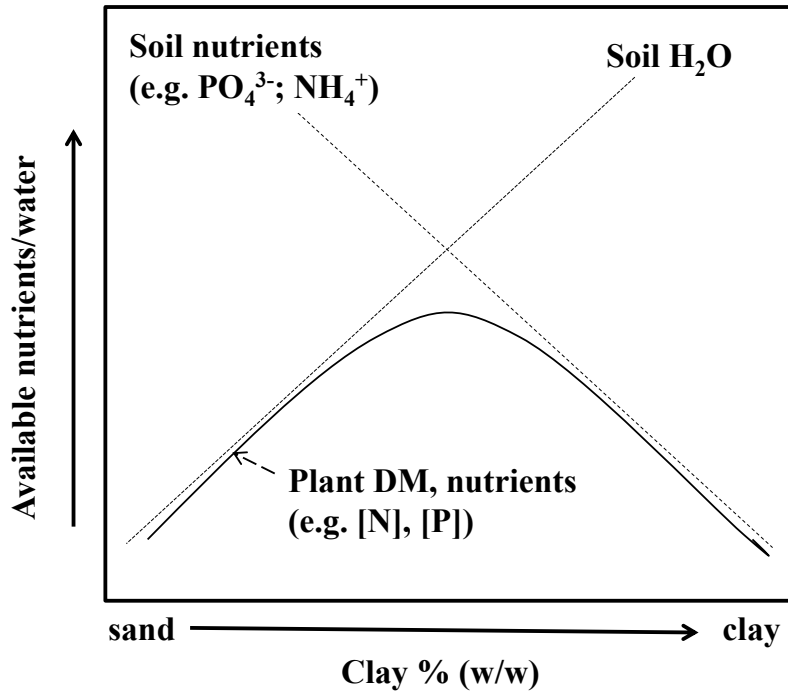


Fig. 4.8. Proposed clay modulation of the availability of soil nutrients, soil moisture, plant biomass and tissue nutrients in *T. aestivum* plants when grown in soils of varying clay proportions. Solid line displays the expected trends in total biomass, [N] and [P] with increased clay. Broken lines represent trends in the availability of nutrients and water as labelled. Increased clay % is expected to result in increased water retention, but decreased nutrients due to adsorption. A trade-off between effects of water and nutrient availability should result in complex responses in plant DM and nutrients with increased clay.

## 4.6 References

- Arai Y, Sparks DL. 2007. Phosphate reaction dynamics in soils and soil minerals: a multiscale approach. *Advances in Agronomy* 94, 135–179.
- Bagshaw R., Vaidyanathan LV, Nye PH. 1972. The supply of nutrient ions by diffusion to plant roots in soil. VI. Effects of onion plant roots on pH and phosphate desorption characteristics in a sandy soil. *Plant and Soil* 37, 627 – 639.
- Barber S. 1995. Soil nutrient bioavailability: a mechanistic approach. 2<sup>nd</sup> Edition, John Wiley and Sons, p89-99
- Bates TR, Lynch JP. 1996. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. *Plant Cell Environ.* 19, 529–538.
- Bengough AG, Mullins CE. 1991. Penetrometer resistance, root penetration resistance and root elongation in 2 sandy loam soils. *Plant and Soil* 131, 59–66.
- Bieleski RL. 1973. Phosphate pools, phosphate transport, and phosphate availability. *Annual Review in Plant Physiology* 24, 225-252.
- Bolan NS. 1991. A critical review on the role of mycorrhizal fungi in uptake of phosphorus by plants. *Plant and Soil* 134, 189-234.
- Bray RH, Kurtz LT. 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Science* 59, 39–45.
- Cernusak LA, Winter K, Aranda J, Turner BL, Marshall JD. 2007. Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *Journal of Experimental Botany* 58, 3549-3566.
- Cernusak LA, Winter K, Turner BL. 2010. Leaf nitrogen to phosphorus ratios of tropical trees: experimental assessment of physiological and environmental controls. *New Phytologist* 185, 770-779.

- Cernusak LA, Winter K, Turner BL. 2011. Transpiration modulates phosphorus acquisition in tropical tree seedlings. *Tree Physiology* 31, 878-885.
- Chapman N, Miller AJ, Lindsey K, Whalley WR. 2012. Roots, water, and nutrient acquisition: let's get physical. *Trends in Plant Science* 17, 701-710.
- Chapman N, Whalley WR, Lindsey K, Miller AJ. 2011. Water supply and not nitrate concentration determines primary root growth in *Arabidopsis*. *Plant, Cell and Environment* 34, 1630-1638.
- Clarkson DT 1981. Nutrient interception and transport by root systems. In: Johnson CB. (ed) *Physiological factors limiting plant productivity*. Butterworths, London, pp 307-314
- Condon LM, Turner BL, Cade-Menun BJ. 2005. Chemistry and dynamics of soil organic phosphorus. In: Sims JT, Sharpley AN, (eds.) *Phosphorus: Agriculture and the Environment*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Inc., Madison, WI, pp 87–121.
- Cramer MD, Hawkins H-J, Verboom GA. 2009. Importance of nutritional regulation of plant water flux. *Oecologia* 161, 15-24. Cramer MD, Hawkins H-J. 2009. A physiological mechanism for the formation of root casts. *Palaeogeography, Palaeoclimatology, Palaeoecology* 274,125-133.
- Cramer MD, Hoffman V, Verboom AG. 2008. Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist* 179, 1048-1057. Cramer MD. 2010. Phosphate as a limiting resource: introduction. *Plant and Soil* 334, 1-10.
- De Groot C, Marcelis L F M, Van den Boogaard R, Lambers H. 2003. Interaction of nitrogen and phosphorus nutrition in determining growth. *Plant Soil* 248, 257–268.
- Devau N, Le Cadre E, Hinsinger P, Gérard F. 2010. A mechanistic model for understanding root-induced chemical changes controlling phosphorus availability. *Annals of Botany* 105, 1183-1197.

- Diatloff E, Rengel Z. 2001. Compilation of simple spectrophotometric techniques for the determination of elements in nutrient solutions. *Journal of Plant Nutrition* 24, 75-86.
- Fontes MPF, Weed SB. 1996. Phosphate adsorption by clays from Brazilian Oxisols: relationships with specific surface area and mineralogy. *Geoderma*, 72, 37-51.
- Fransson A-M, Van Aarle I, Olsson P A and Tyler G 2003. *Plantago lanceolata* L. and *Rumex acetosella* L. differ in their utilisation of soil phosphorus fractions. *Plant Soil* 248, 285–295.
- Frink CR. 1969. Fractionation of phosphorus in lake sediments: analytical evaluation. *Soil Science Society of America Journal* 33, 326-328.
- Garrish V, Cernusak LA, Winter K, Turner BL. 2010. Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*. *Journal of Experimental Botany* 61, 3735-3748.
- Groom PK, Lamont BB. 2010. Phosphorus accumulation in Proteaceae seeds: a synthesis. *Plant and soil* 334, 61-72.
- Hansen JC, Cade-Menun BJ, Strawn DG. 2004. Phosphorus speciation in manure-amended alkaline soils. *Journal of Environmental Quality* 33, 1521-1527
- Hawkins H-J, Hettasch H, Mesjasz-Przybylowicz J, Przybylowicz W, Cramer MD. 2008. Phosphorus toxicity in the Proteaceae: A problem in post-agricultural lands. *Scientia Horticulturae* 117, 357-365.
- He Y, Liao H, Yan X. 2003 Localized supply of phosphorus induces root morphological and architectural changes of rice in split and stratified soil cultures. *Plant Soil* 248, 247–256.
- Hewitt EJ. 1952. Sand and water culture methods used in the study of plant nutrition. Technical communication No. 22. Farnham Royal, UK: Commonwealth Agricultural Bureau.

- Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil*, 237,173 – 195.
- 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.
- Holford ICR. 1997. Soil phosphorus: its measurement and its uptake by plants. *Australian Journal of Soil Research* 35, 227–239.
- Jones DL, Dennis PG, Owen AG, Van Hees PAW. 2003. Organic acid behavior in soils-misconceptions and knowledge gaps. *Plant and Soil* 248, 31–41.
- Jones HG.1992. *Plants and microclimate: a quantitative approach to environmental plant physiology*, 2nd edn. Cambridge University Press, Cambridge.
- Jungk AO, Waisel Y, Eshel A, Kafkafi U. 2002. Dynamics of nutrient movement at the soil-root interface. *Plant roots: The hidden half*, (Ed. 3), 587-616.
- Kage H. 1997. Is low rooting density of faba beans a cause of high residual nitrate content of soil at harvest? *Plant and Soil* 190, 47–60.
- Kalra YP. 1998. *Handbook of standard methods of plant analysis*. Boca Raton, FL, USA: CRC Press.
- Kupper P, Rohula G, Saksing L, Sellin A, Lõhmus K, Ostonen I, Helmisaari H-S, Söber A. 2012. Does soil nutrient availability influence night-time water flux of aspen saplings? *Environmental and Experimental Botany* 82, 37-42.
- Lambers H, Chapin FS III, Pons TL. 1998. *Plant physiological ecology*. 2<sup>nd</sup> Edition, Springer p270-273.
- Lambers H, Cramer MD, Shane MW, Wouterlood M, Poot P, Veneklaas EJ. 2003. Structure and functioning of cluster roots and plant responses to phosphate deficiency. *Plant and Soil* 248, ix – xix.

- Lambers H, Shane MW, 2007. Role of root clusters in phosphorus acquisition and increasing biological diversity in agriculture. In: Spiertz JHJ, Struik PC, HH van Laar. (eds.) *Scale and Complexity in Plant Systems Research: Gene-Plant-Crop Relations* 237-250.
- Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* 98, 693-713
- Lima JE, Kojima S, Takahashi H, von Wirén N. 2010. Ammonium Triggers Lateral Root Branching in Arabidopsis in an ammonium transporter 1;3-Dependent Manner. *Plant Cell* 22, 3621-3633.
- Livingstone DA, Boykin JC. 1962. Vertical distribution of phosphorus in Linsley Pond mud. *Limnology and Oceanography* 7, 57-62.
- Lund ZF. 1959. Available water-holding capacity of alluvial soils in Louisiana. *Soil Science Society of America Journal* 23, 1-3.
- Maurel C, Verdoucq L, Luu D-T, Santoni V. 2008. Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review in Plant Biol* 59, 595–624.
- Miller AJ, Cramer MD. 2004. Root nitrogen acquisition and assimilation. *Plant Soil* 274,1–36
- Motomizu S, Wakimoto T, Toei K. 1983. Spectrophotometric determination of phosphate in river waters with molybdate blue and malachite green. *Analyst* 108, 361–367.
- Muljadi D, Posner AM, Quirk JP. 1966. The mechanism of phosphate adsorption by kaolinite, gibbsite, and pseudoboehmite. *Journal of Soil Science*, 17, 230–237.
- Neumann G, Römheld V. 2002. Root-induced changes in the availability of nutrients in the rhizosphere. In Waisel Y, Eshel A, Kafkafi U. (eds.) *Plant Roots, The Hidden Half*, Ed 3. Marcel Dekker, Inc., New York, pp 617–649.
- Nye PH, Tinker PB. 1977. *Solute movement in the soil-root system*. Oxford: Blackwell Scientific Publishers.

- Nye PH. 1966a. Measurement and mechanism of ion diffusion in soil. 1. Relation between self-diffusion and bulk diffusion. *Journal of Soil Science* 17, 16–23.
- Nye PH. 1966b. The effect of the nutrient intensity and buffering power of a soil, and the absorbing power, size and root hairs of a root, on nutrient absorption by diffusion. *Plant and Soil* XXV, 81-105.
- Oelkers EH, Valsami-Jones E. 2008. Phosphate mineral reactivity and global sustainability. *Elements* 4, 83-87.
- Phillips JM, Hayman DS. 1970. Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhiza for rapid assessment of infection. *Transactions of the British Mycological Society* 55, 158.
- Pierzynski GM, McDowell RW, Sims JT. 2005. Chemistry, cycling, and potential moment of inorganic phosphorus in soils. In Sims JT, Sharpley AN. (eds) *Phosphorus: Agriculture and the Environment*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Inc., Madison, WI, pp 53-86.
- Power SC, Cramer MD, Verboom GA, Chimphango SBM. 2011. Legume seeders of the Cape Floristic Region inhabit more fertile soils than congeneric resprouters -sometimes. *Plant Ecology* 212, 1979-1989.
- R Development Core Team. 2008. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Raven JA. 2008. Transpiration: how many functions? *New Phytologist* 179, 905-907.
- Richardson AE, George TS, Hens M, Simpson RJ. 2005. Utilization of soil organic phosphorus by higher plants. In: Turner BL, Frossard E, Baldwin DS. (eds) *Organic phosphorus in the environment*. CABI, Wallingford, UK, pp 165–184.
- Richardson AE. 1994. Soil microorganisms and phosphorus availability. In: Pankhurst CE, Doube BM, Gupta VVSR, Grace PR (eds) *Management of the soil biota in sustainable farming systems*. CSIRO Publishing, Melbourne, pp 50–62.

- Robinson D, Hodge A, Griffiths BS, Fitter AH. 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proceedings of the Royal Society of London Biological Science* 266, 431–435.
- Santner J, Smolders E, Wenzel WW, Degryse F. 2012. First observation of diffusion-limited plant root phosphorus uptake from nutrient solution. *Plant Cell and Environment* 35, 1558-1566.
- Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F. 2007. Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. *Tree Physiology* 27, 551-559.
- Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, Zhang F. 2011. Phosphorus dynamics: from soil to plant. *Plant Physiology* 156, 997-1005.
- Siemens J, Ilg K, Pagel H, Kaupenjohann M. 2008. Is colloid-facilitated phosphorus leaching triggered by phosphorus accumulation in sandy soils? *Journal of Environmental Quality* 37, 2100 - 2107.
- Sillen GH, Martell AH. 1964 Stability constants of metal-ionic complexes. Special Publication 17, The Chemical Society, London: Burlington House,.
- Smith SE, Jakobsen I, Grønlund M, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology* 156, 1050-1057
- So HB, Nye PH. 1989. The effect of bulk density, water content and soil type on the diffusion of chloride in soil. *Journal of Soil Science* 40, 743-749.
- Solaiman MZ, Abbott LK. 2003. Phosphorus uptake by a community of arbuscular mycorrhizal fungi in Jarrah forest. *Plant Soil* 248, 313–320.

- Tinker PB, Nye PH. 2000. Solute movement in the rhizosphere. New York: Oxford University Press pp 444.
- Treseder KK, Vitousek PM. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian Rain Forests. *Ecology* 82, 946-954.
- Turner BL, Blackwell MSA. 2013. Isolating the influence of pH on the amounts and forms of soil organic phosphorus. *European Journal of Soil Science* 64, 249–259.
- Turner BL, Condron LM, Richardson SJ, Peltzer DA, Allison VJ. 2007. Soil organic phosphorus transformations during pedogenesis. *Ecosystems* 10, 1166-1181.
- Turner BL. 2008. Resource partitioning for soil phosphorus: a hypothesis. *Journal of Ecology* 96, 698–702.
- Tyree MT. 2003. Matric potential. *Encyclopaedia of Water Science*, 615-617.
- Vance CP, Uhde-Stone C, Allan DL. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a non-renewable resource. *New Phytologist*, 157, 423-447.
- Velde B, Meunier A. 2008. The origin of clay minerals in soils and weathered rock. Springer Verlag, Berlin, p33.
- Vitousek PM, Porder S, Houlton BZ, Chadwick OA. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications* 20, 5-15.
- Whalley WR, Ober ES, Jenkins M. 2013. Measurement of the matric potential of soil water in the rhizosphere. *Journal of Experimental Botany*, doi:10.1093/jxb/ert044
- Witkowski ETF, Mitchell DT. 1987. Variations in soil phosphorus in the fynbos biome, South Africa. *Journal of Ecology* 75, 1159-1171.

Zhang F, Shen J, Zhang J, Zuo Y, Li L, Chen X. 2010. Rhizosphere processes and management for improving nutrient use efficiency and crop productivity: implications for China. *Advances in Agronomy* 107, 1–32.

Supplementary Table 1. Mean annual precipitation (MAP), clay proportion, pH and P contents of nine soils collected along an aridity gradient (including the Fynbos biome and the adjacent Succulent Karoo) used for determining the availability of P. Vegetation codes (from Mucina and Rutherford, 2006) are listed for each of the sites. Values for soil represent 5 replicate measurements for each site. For the sake of clarity, standard errors (which are incorporated in Fig 4.2.) are not presented here.

Site	Veg map code	MAP mm	Clay %	Field capacity %	pH	Total P mg kg <sup>-1</sup>	Bray II P mg kg <sup>-1</sup>
29.81°S17.78°E	SKn2	202	0.16	2.86	4.68	87.8	7.8
30.40°S17.93°E	SKn3	355	1.71	2.62	4.34	112.3	15.3
30.71°S18.18°E	SKn1	226	0.8	2.60	4.96	72.2	5.4
32.13°S18.85°E	FFd2	322	0	3.37	4.02	26.6	2.8
32.66°S18.99°E	FFs3	640	0.8	3.30	4.02	38.4	3.6
33.41°S18.58°E	FRg2	551	1.16	2.97	4.16	32.8	2.4
32.97°S19.05°E	FFs5	930	0.48	3.45	3.84	92.8	3.8
33.85°S19.05°E	FFs10	928	0.36	3.94	4.6	80.5	7.0
33.41°S19.44°E	FFs7	639	0.08	4.02	3.98	22.7	2.2

## 5 Synthesis

Following a series of pioneering experiments Woodward (1699) demonstrated that common spearmint plants transpired about 95 – 171 times as much as their total fresh mass gain over 77 d, with a consequent delivery of soil ‘matter’ to roots. Since then, evidence has mounted that nutrients can modulate plant water fluxes (e.g. Raven et al, 2004; Miller and Cramer, 2004; Wilkinson et al., 2007; Cramer et al., 2009; Garish et al., 2010), possibly powering the delivery of soil nutrients via mass-flow to the root surfaces (e.g. Chapter 2; Cramer et al., 2008; Cernusak et al., 2011), where they can be actively taken up. Consequently, mass-flow acquisition of soil nutrients by roots is now widely recognised and is a standard inclusion in most mechanistic models of soil nutrient fluxes (e.g. Nye and Marriott 1969; Hoffland et al. 1990; Rengel 1993; Greenwood and Karpinets 1997). Mass-flow acquisition of nutrients is now a well known process (Silberbush and Barber, 1983; Barber and Silberbush, 1984, Silberbush 2002). Despite the extensive research on plant water fluxes over the centuries, the functional importance of nutritional regulation of these water fluxes in acquiring nutrients via mass-flow has, however, largely remained obscure (Cramer et al., 2009). Although transpirational fluxes through stomatal pores are inevitable during CO<sub>2</sub> exchange with the atmosphere, such water fluxes may also be adaptive, functioning in evaporative leaf cooling (Parkhurst and Loucks, 1972; Nobel, 1999), root to shoot solute transport (Tanner and Beevers, 1990, 2001) and nutrient acquisition (Barber, 1995).

Here I explored the hypothesis that nutrient acquisition is possibly an important function of plant water fluxes, and that the rates of water fluxes should respond positively to nutrient limitation, but not deficiency, in order to facilitate nutrient mass-flow in soil grown plants. This Chapter explores the nutritional regulation of daytime and night-time transpiration as well as hydraulic redistribution for powering mass-flow nutrient acquisition

and the implications these water fluxes may have for plants in competition, in different soils with fluctuating moisture, different climates, for plant physiology, for agriculture and in a changing global climate.

Consistent with the hypothesis (Chapter 1), the present study showed that stomatal conductance and transpiration in *Phaseolus vulgaris* were modulated by the distance ( $d$ ) of the N source from the roots (Chapter 2), as conceptualized in Fig. 5.1. As  $d$  increased, the soil N around the roots was probably diminished (Barber and Cushman, 1981; Silberbush and Barber, 1983; 1984) and plants gradually elevated their water fluxes (i.e.  $E$ ,  $E_{night}$ ,  $g_s$ ) to acquire N up to a maximum accessible  $d$ . Beyond that  $d$ , N possibly remained poorly accessible and plants had to down-regulate their water fluxes to conserve water and as a response to N starvation (Radin et al., 1981; 1982). This biphasic nature of responses in  $g_s$  with N may possibly explain why different responses of transpiration to nutrition have been reported (e.g. Radin and Ackerson 1981; Chapin et al. 1988; Ciompi et al. 1996; Cechin and Fumis 2004). These studies possibly monitored transpiration at deficient *versus* replete soil [N] and subsequently reported on different parts of the biphasic trajectory. Alternatively, the differences may also be a consequence of whole plant water fluxes being determined by the combination of both root and shoot hydraulic conductivities and also differences in the nutrient forms used (e.g.  $\text{NO}_3^-$  vs.  $\text{NH}_4^+$ ; Miller et al., 2004; Cramer et al. 2009) and their availability between experiments, as was shown for  $\text{PO}_4^{3-}$  supplied by IH-P vs. Ca-P (Chapter 4).

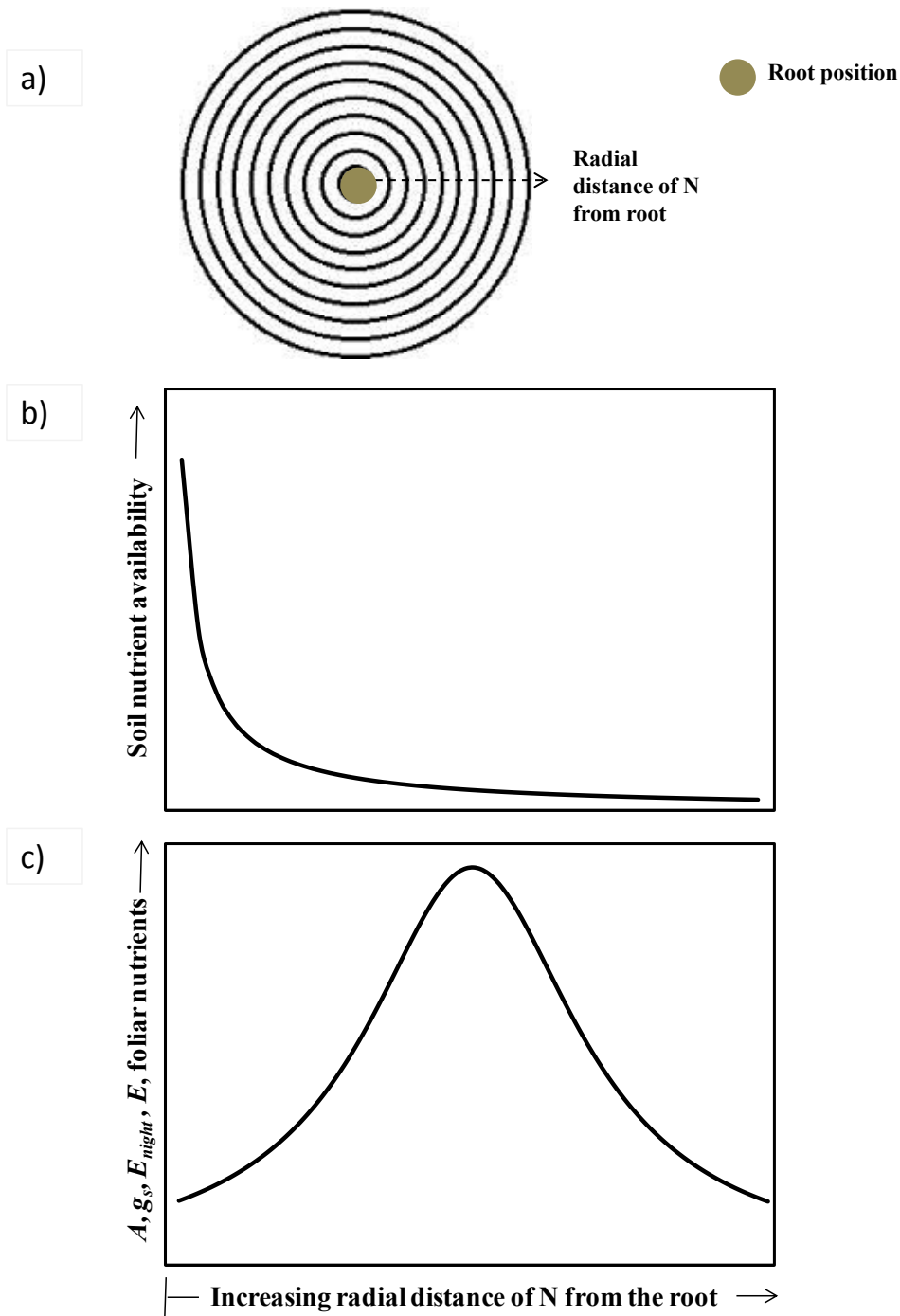


Fig. 5.1 a) Stylistic representation of the radial distance of N from the root, b) Variation of soil nutrient availability with increasing radial distance of N source and c) photosynthesis ( $A$ ), stomatal conductance ( $g_s$ ), night-time transpiration ( $E_{night}$ ), transpiration ( $E$ ) and foliar nutrient concentrations. Arrows show direction of increase in the indicated variables.

Hydraulic redistribution should vary with gradients in soil water potentials at each site, varying with soil texture and conductivity, varying with soil moisture status of different soil zones (Neumann and Cardon 2012), generally increasing with access to ground water, varying with location of soil nutrients in shallow *versus* deep soils (Chapter 3 Fig 5.2A &B) and also varying with transpiration, which can turn on or off the process as recently reviewed (see Neumann and Cardon 2012; Chapter 3). During daytime, the water released to shallow soil by HR must have been taken by *A. linearis* plants (Chapter 3; Fig. 5.2C), delivering nutrients that were present in the shallow soil zone (e.g. Armas and Pugnaire, 2011; Prieto et al., 2012b). Thus, the hydraulic lift of soil water by roots to shallow fertile soils may be an adaptive mechanism for acquiring nutrients, seeing as hydraulic redistribution in *A. linearis* promoted root foraging and nutrient capture in the fertile shallow soil patches (Chapter 3), as has also been reported for *Retama sphaerocarpa* (Prieto et al., 2012b).

Night-time water fluxes in *Aspalathus linearis* growing in deep oligotrophic sands were also elevated when  $^{15}\text{N}$ -labelled  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and irrigation were supplied at depth (Chapter 3), resulting in increased foliar  $\delta^{15}\text{N}$  and P levels, consequently suppressing hydraulic redistribution to drier shallow soils as reported by others (e.g. Prieto et al., 2010). The efficacy of night-time transpiration in delivering nutrients to root surfaces may, however, be reduced in soils with high clay fractions, particularly for  $\text{PO}_4^{3-}$  which adsorbs to clay and metals (e.g. Al and Fe) (Chapter 4). Previous studies have shown that night-time transpiration increases at low availabilities of mineral nutrients (N, P) (Scholz et al., 2007; Kupper et al. 2012) or remains unchanged (Howard and Donovan, 2007; Christman et al., 2009). These conflicting reports could possibly be a consequence of the different nutritional concentrations supplied, with some studies possibly reporting patterns in plants experiencing N-deficiency and others reporting patterns in plants grown under N-replete soil conditions (e.g. Chapter 2; Fig 5.1.). Night-time transpiration may be an adaptive process for delivering nutrients to root surfaces when plants are nutrient limited, but not nutrient deficient.

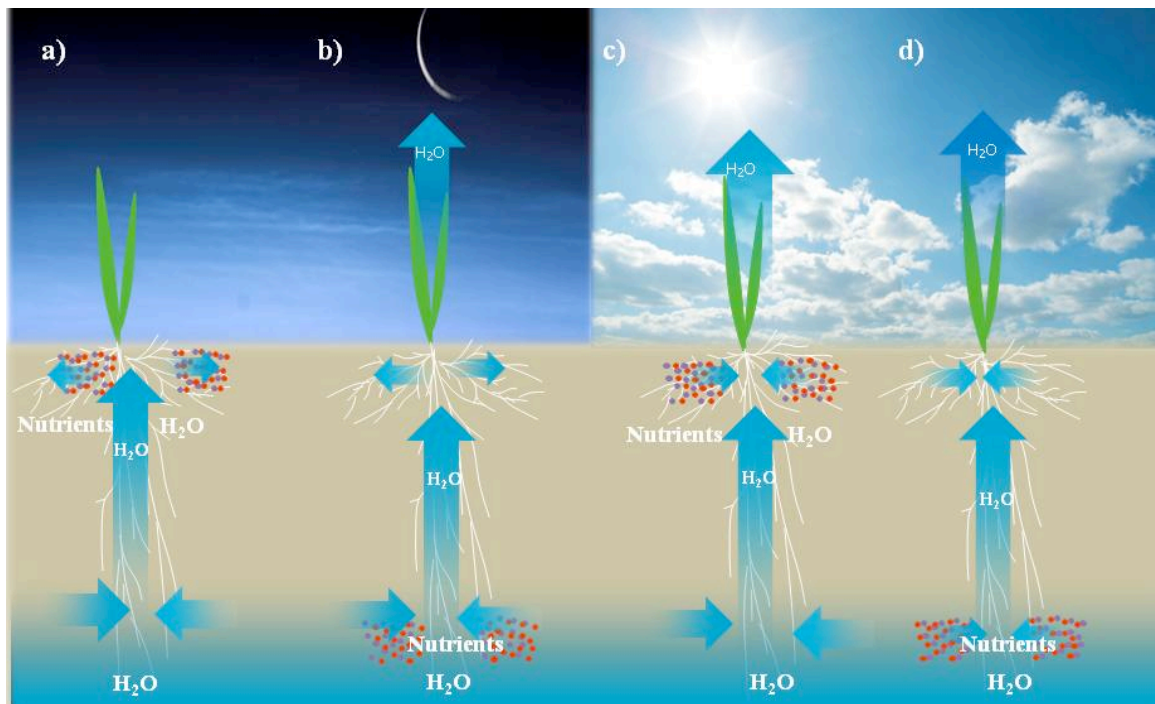


Fig. 5.2 a) Plants were supplied  $^{15}\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  nutrients in shallow water and groundwater increased by irrigation at depth (1m). At night-time a) ground water was hydraulically lifted and released into shallow soil, for acquisition of the shallow soil nutrients, resulting in high tissue  $\delta^{15}\text{N}$  and P. During daytime, the moisture released in shallow soil is reabsorbed resulting in nutrient acquisition (see panel c). When plants were supplied  $^{15}\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and groundwater by irrigation at depth (1 m), the increased their b) night-time transpiration ( $E_{\text{night}}$ ) and d) day-time transpiration ( $E$ ) and moved ground water resulting in mass-flow acquisition of soil nutrients, consequently leading to high tissue  $\delta^{15}\text{N}$  and P. However, hydraulic redistribution was suppressed, presumably by the increased  $E_{\text{night}}$  and  $E$ .

In the face of interspecific competition, trade-offs may exist between mass-flow acquisition of nutrients *versus* investment in root proliferations for nutrient interception. Based on resource-competition theory, species most efficient at acquiring, retaining and using the major limiting resource are considered to be the best competitors (e.g. Tilman, 1982; Fargione and Tilman,

2006). Using this theory,  $R^*$  is defined empirically in monocultures as the concentration of the available resource in the environment, and integrates all the effects a species may have on the resource levels (Fargione and Tilman, 2006). For instance, the traits associated with dominance in strongly N-limited environments may include low tissue N; high root:shoot ratio; long-lived tissue; high nutrient-use efficiency (Grime 1979; Chapin 1980; Vitousek 1982; Tilman 1990; Aerts and Chapin 2000) and the ability to reduce the mineral N concentration to lower levels in monocultures (Tilman 1982, 1990; Tilman and Wedin 1991a; Wedin and Tilman 1993). My data suggest, however, that high water fluxes may contribute to competitive strength in acquiring soil nutrients (e.g. Chapter 2). Thus, plants with high water fluxes (where water is available) should be able to economise on root investments while still acquiring the bulk of their nutrients via mass-flow. Although soil P is least expected to be supplied via mass-flow (e.g. Bielecki, 1983; White et al., 2013), my studies showed that plants growing in relatively low clay soils can still acquire the bulk of their P via mass-flow (Chapter 2, 4). Although water influxes do not result in direct nutrient uptake, mass-flow can concentrate these nutrients at root surfaces where active uptake occurs (Cernusak et al, 2011), thus reducing the distance of the nutrient source from root surfaces (Fig. 5.1. Scholz et al., 2007). Hydraulic redistribution of ground water to shallow nutrient-rich soil (Chapter 3) may possibly benefit shallow-rooted neighbours, with the deep-rooted plant possibly serving as a nurse plant to neighbouring seedlings during the dry season (e.g. Prieto et al., 2012a; Hawkins et al., 2009). Ludwig et al. (2003) found the facilitation by hydraulic redistribution and the competition between trees and grasses to be both co-occurring processes in savannas that varied across sites depending on the groundwater available each year. In this study, when the groundwater was increased through irrigation at depth, *A. linearis* plants increased their hydraulic redistribution to shallow soil, consequently acquiring shallow soil nutrients (Fig 5.2; Chapter 3).

Mass-flow acquisition of nutrients depends on the influx of water into roots, the concentration and the mobility of nutrients in the soil (Barber 1995; Miller and Cramer, 2004; Silberbush, 2013). For most nutrients mobility in the soil limits their uptake by the roots, rather than uptake into the root tissue and transport across a plasmalemma by an appropriate transport system for a given nutrient (Clarkson 1981; Kage 1997). The clay fraction of a soil can be one of the factors modulating nutrient mobility in soils (Chapter 4). In particular,  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  were limited by low mobility (Chapter 4; Marschner 1995, Barber, 1995; Miller and Cramer, 2004) because they were readily bound to clay and metal oxides (e.g. Al, Fe) in the soil (Chapter 4), resulting in a high buffering power with regard to P (Barber, 1995). Further, the rate at which soluble  $\text{PO}_4^{3-}$  is released into the soil solution is generally low (Parfitt, 1979) and may vary depending on the P forms (e.g. Fe-P, Ca-P and IH-P) available in the soil (Chapter 4). Since inorganic forms (Fe-P and Ca-P) undergo dissolution to release  $\text{PO}_4^{2-}$ , which readily forms complexes with metals and clay in soils, organic P forms (e.g. IH-P) that are least adsorbed may be relatively more mobile towards the root surfaces where hydrolysis by phosphatase enzymes exuded by roots may free the P (e.g. Richardson et al., 2005; Chapter 4). Clay fractions are likely to have further imposed soil moisture variations with increased clay fractions and physical limitations on root proliferation (Chapman et al. 2012) and the mass-flow delivery of dissolved  $\text{PO}_4^{3-}$  to the root surface (Chapter 4). In particular, nine soils of varying clay fractions (0 - 2% w/w) from the Greater Cape Floristic Region (GCFR) displayed a decline in available P as clay fractions increased, which supports the reduced mobility of P with higher clay fractions. Although GCFR and the South Western Botanical Province (SWBP) of Australia are typically P-limited biomes, their soils have relatively higher concentrations of organic P forms (Wtkowski and Mitchells, 1987; Lambers et al, 2006), possibly explaining why the cluster-rooted species that exude phosphatase enzymes (e.g. Richardson et al., 2005) thrive in these P-impooverished and low-clay soils (Lambers et al., 2006).

The efficacy of mass-flow nutrient acquisition in delivering soil nutrients to roots is likely to differ with climatic region in response to soil moisture regimes and vapour pressure deficit. Mass-flow in tropical deciduous forest/woodland biomes with transpiration estimated at  $401 \text{ kg H}_2\text{O m}^{-2} \text{ y}^{-1}$  (Ito and Matoko, 2012), may be an effective mechanism for nutrient delivery, particularly in soils with low clay fraction. This may be supported by other recent studies that suggested a role of water fluxes in the acquisition of nutrients by tropical tree and liana seedlings (Cernusak et al., 2007; 2011). Plants growing in tundra and in desert biomes with low transpiration estimated at 14 and 24  $\text{kg H}_2\text{O m}^{-2} \text{ y}^{-1}$  respectively (Ito and Matoko, 2012), are expected to show smaller mass-flow nutrient acquisition. I expect functional ecophysiological plant traits that promote mass-flow nutrient acquisition to be highly represented in old, climatically buffered, infertile landscapes (OCBILS) such as the SWBP of Australia and the GCFR of South Africa (Lambers et al., 2010), which have generally low clay fractions and limited soil nutrients. For example, Yates et al. (2010) suggested that the small leaves characteristic of Fynbos vegetation in the GCFR, which have high boundary layer conductances, were a bifunctional adaptation, promoting heat loss in summer and water loss in during the wet winter months. Thus plants may increase their water fluxes during short-lived periods of high soil moisture, a process that may be particularly be important for mass-flow nutrient acquisition in low-CEC soils. Moreover, my studies have demonstrated mass-flow acquisition of nutrients in relatively sandy soils when amply watered (Chapter 2, 3).

Nutritional regulation of plant water fluxes may occur via N-flux-linked signalling mechanisms (e.g. Wilkinson et al. 1998; 2004; 2007; Clarkson et al. 2000; Desikan et al. 2002; Gloser et al. 2007; Cramer et al., 2009) operating in both roots and shoots. Acquisition of N as  $\text{NO}_3^-$  was possibly the signal that controlled plant water fluxes in my study (Chapter 2), exerting its effects on root hydraulic conductivity and on stomatal conductance as previously reported by others (discussed below). Increasing rhizosphere  $[\text{NO}_3^-]$  above

deficiency is known to rapidly increase aquaporin-mediated root hydraulic conductivity (Carvajal et al. 1996; Clarkson et al. 2000; Gloser et al. 2007; Gorska et al. 2008). The root  $\text{NO}_3^-$ , rather than its reduction/assimilation products can regulate aquaporin expression (Gorska et al. 2008), which is probably an adaptive trait that has functional significance in the mass-flow delivery of  $\text{NO}_3^-$  to the root surface when the roots of N-limited plants are exposed to fertile soil patches (Gorska et al., 2010). Indeed my *P. vulgaris* elevated their water fluxes as distance from the N-source was increased, attaining maximum  $g_s$  at  $d = 10$  mm behind a root barrier (Chapter 2). Excess root uptake of  $\text{NO}_3^-$  beyond the capacity of the nitrate reductase enzyme for reduction, was possibly transported to the leaves for reduction to  $\text{NH}_4^+$ , which produces  $\text{NO}_2^-$  and NO (Desikan et al. 2002; Neill et al. 2008). NO is known to induce stomatal closure (e.g. Neill et al., 2008), which possibly explains why  $g_s$  in *P. vulgaris* began to decline when N-source was beyond  $d = 10$  mm. When the plants are well supplied with  $\text{NO}_3^-$  their xylem/apoplastic pH will generally be more alkaline (Mengel et al., 1994; Mühlhling and Lauchli, 2001; Jia and Davies, 2007) resulting in the accumulation of ABA in the apoplast, which ultimately elicits the closure of stomata (Wilkinson and Davies, 1997). Further, when  $\text{NO}_3^-$  supply is limiting, the transport of cytokinins within the xylem is reduced (Rahayu et al., 2005), which can increase stomatal sensitivity to xylem ABA (Radin et al., 1982; Füsseder et al., 1992), thus possibly explaining why N deficiency also induces sensitivity of tissues to ABA (McDonald and Davies, 1996). Based on the strong correlations between stomatal conductance and the foliar N:P ratios of *P. vulgaris* (Chapter 2), it is likely that excess inorganic [N], and not overall foliar [N], was the signal modulating plant water fluxes. Moreover, P did not regulate transpiration in this study (Chapter 4), but N modulated plant water fluxes as previously discussed (Chapter 2). Although others reported a rapid increase in root hydraulic conductivity following a relief from P deficiency (Radin and Eidenbock 1984; Carvajal et al. 1996), I propose this to be linked to increased aquaporin

expression (e.g. Clarkson et al. 2000), resulting indirectly from the altered N:P ratios and consequently the the excess tissue  $\text{NO}_3^-$ .

The *P. vulgaris* plants that were supplied urea-N, which can be converted to either  $\text{NO}_3^-$  or  $\text{NH}_4^+$  in the soil, modulated their stomatal responses to acquire N (Chapter 2), and the potential role of  $\text{NH}_4^+$  in controlling  $g_s$  could not be ascertained. Contrary to  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  supply may not increase the expression of root aquaporins or alter root hydraulic conductance (Guo et al. 2007a). Roots can readily assimilate  $\text{NH}_4^+$  into amino acids (Miller and Cramer 2004) and  $\text{NH}_4^+$  does not appear to elicit stomatal responses (see Miller and Cramer 2004; Cramer et al., 2009). Nevertheless, it remains unclear how plants supplied  $\text{NH}_4\text{Cl}$  or  $(\text{NH}_4)_2\text{SO}_4$ , which are common fertilizers, modulate their water fluxes. Goodger and Schachtman (2010) proposed a mechanism by which  $\text{NH}_4^+$ -fed plants might control their water fluxes, involving cytosolic pH, amino acids and phytohormone concentrations. However, plants supplied with  $\text{NH}_4^+$  generally display lower water use efficiency (*WUE*) than those acquiring  $\text{NO}_3^-$  (Raven et al. 2004; Guo et al. 2007a, b) and may display wilting and other symptoms of water stress (Cramer and Lewis 1993; Chaillou and Lamaze 2001). My study on *P. vulgaris* showed no apparent symptoms of  $\text{NH}_4^+$ -toxicity. In natural ecosystems high soil  $[\text{NH}_4^+]$  are rare, compared to agricultural soils and plants may thus lack the mechanisms to respond to these variations in soil  $[\text{NH}_4^+]$ .

Although not explored in this study, other nutrients, particularly  $\text{PO}_4^{2-}$ ,  $\text{SO}_4^{2-}$  and  $\text{K}^+$ , have also been implicated in the regulation of plant water fluxes. It has been shown that N-, P- and S-deficiencies result in major reductions of root hydraulic conductivity, which may lead to reduced stomatal conductance (Karmoker et al., 1991; Clarkson et al., 2000). The  $\text{K}^+$  ion is the main osmotic solute in plants (Mengel and Arneke, 1982) and stomatal opening may be prompted by the accumulation of large amounts of  $\text{K}^+$  by guard cells, resulting in water uptake and cell turgor (Benlloch-González, 2010). Stomatal closure is preceded by the release of  $\text{K}^+$  by guard cells, which results in loss of turgor (Kearns and Assmann, 1993). Oddo et al.

(2011) found that *Laurus nobilis* seedlings which had been fertilized with K increased their xylem sap  $[K^+]$  and exhibited a 45% increase in transpiration rate and a 30% increase in plant hydraulic conductance. However, addition of  $K^+$  to deficient *Picea sitchensis* seedlings led to a marked reduction in transpiration (Bradbury and Malcolm, 1977), whilst *P. vulgaris* plants (Chapter 2) showed no correlation between their foliar  $[K^+]$  with  $g_s$  (data not shown). Potassium salts are abundant in guard cells (Outlaw, 1983) and many regulators of stomatal movements, such as ABA, may exert their activity, at least in part, through the modulation of potassium fluxes (MacRobbie, 1981; Blatt, 1990).

My conclusion that N regulates mass-flow acquisition of nutrients has implications for nutrient use efficiency in crop production. However, focusing attention on the most important nutrients, such as nitrogen (N), has in some cases led to nutrient imbalances (Goulding et al. 2008). First, the tissue stoichiometry of nutrients, such as N:P ratios, may indicate nutrient uptake above what is immediately required for growth (e.g. Ågren 1988), this leading to the accumulation of a nutrient in leaf tissues. If  $NO_3^-$  is the excess nutrient, it may alter plant water fluxes, whilst accumulation of, for example,  $PO_4^{3-}$ , Al, Mn have been linked with toxicity. In particular, the Proteaceae are adapted to low soil P levels between 0.8 and 8 mg P  $kg^{-1}$  soil, but are also grown in the relatively P-rich soils often resulting in P toxicity (Hawkins et al., 2008). If the natural foliar stoichiometry of Proteaceae is known, fertilizer application should target to balance this stoichiometry in order to avert P toxicity in areas with high soil P, based on expected tissue nutrient ratios relative to N. A balance in stoichiometry may eliminate the foliar accumulation of nutrients that often leads to toxicity. Some success in breeding and genetic modification of leaf WUE has been reported for wheat (Condon et al. 2004). Given that leaf WUE increases with nutrition (Raven et al. 2004), it is likely that increased N fertilization rates over the past decades (reviewed in Miller and Cramer 2004) have already increased the WUE of crops through decreased dependence on mass-flow. The trade-offs of water flux for nutrient availability, however, means that the

viability of genetic improvement to reduce crop transpiration (e.g. Condon et al. 2004), depends on nutrient availability, since reducing water consumption by the plants may limit productivity, especially in nutrient-limited soils.

As WUE responds to foliar N:P stoichiometry (e.g. Garrish et al, 2010; Chapter 2), it is likely that P loading of the rhizosphere by run-off water and polluted irrigation water may modulate plant water fluxes. Cycles of soil wetting and drying can give flushes of P release from raptured microbial biomass into the rhizosphere and into run-off water (Turner and Haygarth 2001), which increases solubilized soil [P] (Turner et al., 2003). For example, the total amounts of water soluble P in moist soils were small, but increased after drying and rewetting by 185-1 900%, due to direct release of P from the soil microbial biomass (Turner and Haygarth 2001; Turner et al., 2003). Such flushes of P are likely to alter the N:P stoichiometry of the rhizosphere and plants, which potentially reduces excess N in the cytosol, consequently reducing stomatal conductance (see Chapter 2).

As the global climate changes, N regulation of water fluxes raises key questions to resolve in future. The N regulation of water fluxes may have implications for the physiology of C<sub>3</sub> and C<sub>4</sub> plants, which differ in their water use efficiency. The C<sub>4</sub> plants have higher water use efficiency than C<sub>3</sub> plants (e.g. Farquahar et al., 1989), suggesting that they may have greater capacity to modulate transpiration in response to nutrition. Are we likely to find varying responses in persistence of C<sub>3</sub> and C<sub>4</sub> species in low and high nutrient ecosystems if they experience dwindling water resources with global warming? Further physiological research is needed to understand better these functional groups in ecosystems of varying moisture and soil nutrients. Further, it remains to be determined whether the reduced transpirational fluxes predicted in elevated [CO<sub>2</sub>] atmospheres will reduce nutrient acquisition (Conroy and Hocking 1993) as a consequence of mass-flow limitation. A meta-analysis of data from 19 herbaceous and 11 woody species displayed a general decline in the

concentrations of several nutrients (P, K, Ca, S, Mg, Fe, Zn, Mn and Cu) with elevated CO<sub>2</sub> (Loladze 2002), possibly due to a reduction in stomatal aperture rather than stomatal density (Ainsworth and Rogers 2007). Further, Taub and Wang (2008) suggested that the decreased foliar nutrient concentrations with elevated [CO<sub>2</sub>] that have been reported by others may be linked to reduced transpirational fluxes, potentially limiting mass-flow nutrient acquisition, a hypothesis that has not been thoroughly tested. Recently, however, McGrath and Lobell (2013) used metadata gleaned from open-top chambers, growth cabinets and glasshouse experiments and concluded a decline of nutrients with reduced transpiration in elevated [CO<sub>2</sub>] atmospheres.

In conclusion, this thesis explored and confirmed the novel idea that transpiration by plants is not only passive, to fulfil the water requirements of the shoot and opening stomata, but also actively induced by nutritional needs, to serve as driving force for nutrient uptake. Likewise, hydraulic redistribution serves to draw water from deep and wet soil parts to the upper layers, which serves as a means to enable uptake of nutrients from the rich, but often dry, upper soil. Plants may be opportunistic in their water uptake, taking it up when it is available in order to improve the acquisition of nutrients through mass-flow delivery. My perception is that the role of transpiration and other water fluxes in powering nutrient mass-flow has been somewhat neglected in the last decades. This thesis and recent publications (e.g. Cernusak et al., 2011, Kupper et al., 2012; Prieto et al., 2012a; McGrath and Lobell, 2013; Oyewole et al., 2013) have, however, initiated an increased awareness of this important additional function of transpiration that has important implications for understanding plant physiology, ecophysiology and agriculture.

## 5.1 References

- Aerts R, Chapin FS. III 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* 30, 1–67.
- Ågren GI. 1988. The ideal nutrient productivities and nutrient proportions. *Plant, Cell and Environment* 11, 613 - 620.
- Ainsworth EA, Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising [CO<sub>2</sub>]: mechanisms and environmental interactions. *Plant Cell and Environment* 30, 258–270.
- Armas C, Pugnaire FI. 2011. Belowground zone of influence in a tussock grass species. *Acta Oecologica* 37, 284–289.
- Barber S. 1995. *Soil nutrient bioavailability: a mechanistic approach*. 2<sup>nd</sup> Edition, John Wiley and Sons, p89-99.
- Barber SA, Cushman JH. 1981. Nitrogen uptake model for agronomic crops. In: Iskandar IK. (ed.) *Modeling waste water renovation- land treatment*, New York, Wiley Interscience, p. 382-409.
- Barber SA, Silberbush M. 1984. Plant root morphology and nutrient uptake. In: Barber SA, Bouldin DR. (eds.) *Roots, nutrient and water influx and plant growth*. American Society of Agronomy, Madison, WI 49, 43-55.
- Benlloch-González M, Fournier JM, Benlloch M. 2010. K<sup>+</sup> deprivation induces xylem water and K<sup>+</sup> transport in sunflower: evidence for a co-ordinated control. *Journal of Experimental Botany* 61, 157-164.
- Bieleski RL. 1973. Phosphate pools, phosphate transport, and phosphate availability. *Annual Review in Plant Physiology* 24, 225–252.
- Blatt M. 1990. Potassium channel currents in intact stomatal guard cells: rapid enhancement by abscisic acid. *Planta* 180, 445-455.

- Bradbury IK, Malcolm DC. 1977. The Effect of Phosphorus and Potassium on Transpiration, Leaf Diffusive Resistance and Water-Use Efficiency in Sitka Spruce (*Picea sitchensis*) Seedlings. *Journal of Applied Ecology* 14, 631-641.
- 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.
- Cechin I, Fumis TD. 2004. Effect of nitrogen supply on growth and photosynthesis of sunflower plants grown in the greenhouse. *Plant Science* 166, 1379–1385.
- Cernusak LA, Winter K, Aranda J, Turner BL, Marshall JD. 2007. Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *Journal of Experimental Botany* 58, 3549-3566.
- Cernusak LA, Winter K, Turner BL. 2011. Transpiration modulates phosphorus acquisition in tropical tree seedlings. *Tree Physiology* 31, 878-885.
- Chaillou S, Lamaze T. 2001. Ammoniacal nutrition of plants. In: Morot-Gaudry J-F (ed) *Nitrogen assimilation by plants*. Science Publishers, NH, pp 53–69.
- Chapin FS III, Walter CHS, Clarkson DT. 1988. Growth response of barley and tomato to nitrogen stress and its control by abscisic acid, water relations and photosynthesis. *Planta* 173, 352–366.
- Chapin FS. III 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11, 233–260.
- Chapman N, Miller AJ, Lindsey K, Whalley R. 2012. Roots, water, and nutrient acquisition: let's get physical. *Trends in Plant Science* 17, 701-710.
- Christman MA, Donovan LA, Richards JH. 2009. Magnitude of nighttime transpiration does not affect plant growth or nutrition in well-watered *Arabidopsis*. *Physiologia Plantarum*, 136, 264–273.

- Ciampi S, Gentili E, Guidi L, Soldanti GF. 1996. The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters in sunflower. *Plant Science* 118, 177–184.
- 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.
- Clarkson DT. 1981. Nutrient interception and transport by root systems. In: Johnson, C.B. (ed.) *Physiological factors limiting plant productivity*. Butterworths, London, pp 307–314.
- Condon AG, Richards RA, Rebetzke GJ, Farquhar GD. 2004. Breeding for high water-use efficiency. *Journal of Experimental Botany* 55, 2447–2460.
- Conroy J, Hocking P. 1993. Nitrogen nutrition of C<sub>3</sub> plants at elevated atmospheric CO<sub>2</sub> concentrations. *Physiologia Plantarum* 89, 570–576
- Cramer MD, Hoffmann V, Verboom GA. 2008. Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist* 179, 1048–1057.
- Cramer MD, Lewis OAM. 1993. The influence of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> nutrition on the growth of wheat (*Triticum aestivum*) and maize (*Zea mays*) plants. *Annals of Botany* 72, 359–365.
- Cramer MD, Hawkins H-J, Verboom GA. 2009. The importance of nutritional regulation of plant water flux. *Oecologia* 161, 15–24.
- Desikan R, Griffiths R, Hancock J, Neill S. 2002. A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. *Proceedings of National Academy of Science USA* 99, 16314–16318.

- Fargione J, Tilman D. 2006. Plant species traits and capacity for resource reduction predict yield and abundance under competition in nitrogen-limited grassland. *Functional Ecology* 20, 533–540.
- Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Reviews in Plant Physiology* 40, 503–537.
- Fusseder A, Wartinger A, Hartung W, Schulze E-D, Heilmeyer H. 1992. Cytokinins in the xylem sap of desert grown almond (*Prunus dulcis*) trees: daily courses and their possible interactions with abscisic acid and leaf conductance. *New Phytologist* 122, 45-53.
- Garrish V, Cernusak LA, Winter K, Turner BL. 2010. Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*. *Journal of Experimental Botany* 61, 3735-3748.
- Gloser V, Zwieniecki MA, Orians CM, Holbrook NM. 2007. Dynamic changes in root hydraulic properties in response to nitrate availability. *Journal of Experimental Botany* 58, 2409–2415.
- Goulding K, Jarvis S, Whitmore A. 2008. Optimizing nutrient management for farm systems. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363, 667-680.
- Goodger JQD, Schachtman DP. 2010 Nitrogen source influences root to shoot signaling under drought. In: Pareek A, Sopory SK, Bohnert HJ, Govindjee, (eds.) *Abiotic stress adaptation in plants: physiological, molecular and genomic foundation*. Dordrecht: Springer Science, pp 165-173.
- Gorska A, Ye Q, Holbrook NM, Zwieniecki MA. 2008. Nitrate control of root hydraulic properties in plants: translating local information to whole plant response. *Plant Physiol* 148, 1159–1167.

- Gorska A, Lazor JW, Zwieniecka AK, Benway C, Zwieniecki MA. 2010. The capacity for nitrate regulation of root hydraulic properties correlates with species' nitrate uptake rates. *Plant and Soil* 337, 447-455.
- Greenwood DJ, Karpinets TV. 1997. Dynamic model for the effects of K-fertilizer on crop growth, K-uptake and soil-K in arable cropping. 1. Description of the model. *Soil Use Manage* 13,178–183.
- Grime JP. 1979. *Plant strategies and vegetation processes*. Wiley, New York.
- Guo S, Kaldenhoff R, Uehlein N, Sattelmacher B, Brueck H. 2007a. Relationship between water and nitrogen uptake in nitrate- and ammonium-supplied *Phaseolus vulgaris* L. plants. *Journal of Plant Nutrition and Soil Science* 170, 73–80.
- Guo S, Shen O, Brueck H. 2007b. Effects of local nitrogen supply on water uptake of bean plants in a split root system. *Journal of Integrative Plant Biology* 49, 472–480.
- Hawkins H-J, Hettasch H, Mesjasz-Przybylowicz J, Przybylowicz W, Cramer MD. 2008. Phosphorus toxicity in the Proteaceae: A problem in post-agricultural lands. *Scientia Horticulturae* 117, 357–365.
- Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW. 2005. Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. *New Phytologist* 168, 293-303.
- Hoffland E, Bloemhof HS, Leffelaar PA, Findenegg GR, Nelemans JA. 1990. Simulation of nutrient uptake by a growing root system considering increasing root density and inter-root competition. *Plant Soil* 1124, 149–155.
- Howard AR, Donovan LA. 2007. *Helianthus* night-time conductance and transpiration respond to soil water but not nutrient availability. *Plant Physiology* 143,145–155.
- Ito A, Motoko I. 2012. Water-Use Efficiency of the Terrestrial Biosphere: A Model Analysis Focusing on Interactions between the Global Carbon and Water Cycles. *Journal of Hydrometeorology* 13, 681–694.

- Jia W, Davies WJ. 2007. Modification of leaf apoplastic pH in relation to stomatal sensitivity to root-sourced ABA signals. *Plant Physiology* 143, 68-77.
- Kage H. 1997. Is low rooting density of faba beans a cause of high residual nitrate content of soil at harvest? *Plant and Soil* 190, 47–60.
- Karmoker JL, Clarkson DT, Saker L, Rooney JM, Purves JV. 1991. Sulphate deprivation depresses the transport of nitrogen to the xylem and the hydraulic conductivity of barley (*Hordeum vulgare* L.) roots. *Planta* 185, 269-278.
- Kearns EV, Assmann SM. 1993. The guard cell–environment connection. *Plant Physiology* 102, 711-715.
- Kupper P, Rohula G, Saksing L, Sellin A, Löhmus K, Ostonen I, Helmisaari H-S, Söber A. 2012. Does soil nutrient availability influence night-time water flux of aspen saplings? *Environmental and Experimental Botany* 82, 37–42.
- Lambers H, Brundrett MC, Raven JA, Hopper SD. 2010. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 334, 11–31.
- Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* 98, 693–713.
- Loladze I. 2002. Rising atmospheric CO<sub>2</sub> and human nutrition: toward globally imbalanced plant stoichiometry? *Trends in Ecology and Evolution* 17, 457–461.
- Ludwig F, Dawson TE, Kroon H, Berendse F, Prins HHT. 2003. Hydraulic lift in *Acacia tortilis* trees on an East African savanna. *Oecologia* 134, 293–300.
- MacRobbie EAC. 1981. Effects of ABA in ‘isolated’ guard cells of *Commelina communis* L. *Journal of Experimental Botany* 32, 563-572.
- Marschner H. 1995. Mineral nutrition of higher plants (second edition). Academic Press, London.

- McDonald AJS, Davies WJ. 1996. Keeping in touch: responses of the whole plant to deficits in water and nitrogen supply. *Advances in Botanical Research* 22, 229-300.
- McGrath JM, Lobell DB. 2013. Reduction of transpiration and altered nutrient allocation contribute to nutrient decline of crops grown in elevated CO<sub>2</sub> concentrations. *Plant Cell and Environment* 36, 697-705.
- Mengel K, Arneke WW. 1982. Effect of potassium on the water potential the osmotic potential, and cell elongating leaves of *Phaseolus vulgaris*. *Physiologia Plantarum* 54, 402-408.
- Mengel K, Planker R, Hoffmann B. 1994. Relationship between leaf apoplast pH and iron chlorosis of sunflower (*Helianthus annuus* L.). *Journal of Plant Nutrition* 25, 1053–1065.
- Miller AJ, Cramer MD. 2004. Root nitrogen acquisition and assimilation. *Plant Soil* 274,1–36.
- Mühling KH, Lauchli A. 2001. Influence of chemical form and concentration of nitrogen on apoplastic pH of leaves. *Journal of Plant Nutrition* 25, 399–411.
- Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I. 2008. Nitric oxide, stomatal closure, and abiotic stress. *Journal of Experimental Botany* 59, 165–176.
- Neumann RB, Cardon ZG. 2012. The magnitude of hydraulic redistribution by plant roots: a review and synthesis of empirical and modelling studies. *New Phytologist* 194, 337–352.
- Nobel PS. 1999. *Physicochemical and environmental plant physiology*. Academic Press, San Diego.
- Nye PH, Marriot FHC. 1969. A theoretical study of the distribution of substances around roots resulting from simultaneous diffusion and mass-flow. *Plant Soil* 30, 459 – 472.

- Oddo E, Inzerillo S, La Bella F, Grisafi F, Salleo S, Nardini A. 2011. Short-term effects of potassium fertilization on the hydraulic conductance of *Laurus nobilis* L. *Tree Physiology* 31, 131-138.
- Outlaw WH. 1983. Current concepts on the role of potassium in stomatal movements. *Physiologia Plantarum* 59, 302–311.
- Oyewole OA, Inselsbacher E, Näsholm T. 2013. Direct estimation of mass flow and diffusion of nitrogen compounds in solution and soil. *New Phytologist*. doi: 10.1111/nph.12553
- Parfitt R. 1979. The availability of P from phosphate-geothite bridging complexes. Desorption and uptake by ryegrass. *Plant and Soil* 53, 55-65.
- Parkhurst DF, Loucks OL. 1972. Optimal leaf size in relation to environment. *Journal of Ecology* 60, 505–537.
- Prieto I, Armas C, Pugnaire FI. 2012a. Hydraulic lift promotes selective root foraging in nutrient-rich soil patches. *Functional Plant Biology* 39, 804–812.
- Prieto I, Armas C, Pugnaire FI. 2012b. Water release through plant roots: new insights into its consequences at the plant and ecosystem level. *New Phytologist* 193, 830 - 841.
- Prieto I, Kikvidze Z, Pugnaire FI. 2010. Hydraulic lift: soil processes and transpiration in the Mediterranean leguminous shrub *Retama sphaerocarpa* (L.) Boiss. *Plant and Soil* 329, 447–456.
- Prieto I, Padilla FM, Armas C, Pugnaire F. 2011. The role of hydraulic lift on seedling establishment under a nurse plant species in a semi-arid environment. *Perspectives in Plant Ecology, Evolution and Systematics* 13, 181–187.
- Radin JW, Ackerson RC. 1981. Water relations of cotton plants under nitrogen deficiency. III. Stomatal conductance, photosynthesis and abscisic acid accumulation during drought. *Plant Physiology* 67, 115-119.
- 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, Parker LL, Guinn G. 1982. Water relations of cotton plants under nitrogen deficiency. V. Environmental control of abscisic acid accumulation and stomatal sensitivity to abscisic acid. *Plant Physiology* 70, 1066-1070.
- Rahayu YS, Walch-Liu P, Neumann G, Römheld V, von Wiren N, Bangerth F. 2005. Root-derived cytokinins as long-distance signals for formula-induced stimulation of leaf growth. *Journal of Experimental Botany* 56, 1143-1152.
- Raven JA, Handley LL, Wollenweber B. 2004. Plant nutrition and water use efficiency. In: Bacon MS (ed) *Water use efficiency in plant biology*. Blackwell, Oxford.
- Rengel Z. 1993. Mechanistic simulation models of nutrient uptake: a review. *Plant Soil* 152, 161–173.
- Richardson AE, George TS, Hens M, Simpson RJ. 2005. Utilization of soil organic phosphorus by higher plants. In: Turner BL, Frossard E, Baldwin DS. (ed.). *Organic phosphorus in the environment*, Wallingford, Oxfordshire, UK, 165-184.
- Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F. 2007. Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. *Tree Physiology* 27, 551–559.
- Silberbush M, Barber S. 1983. Sensitivity of simulated phosphorus uptake to parameters used by a mechanistic-mathematical model. *Plant and Soil* 74, 93-100.
- Silberbush M, Barber S. 1984. Phosphorus and Potassium Uptake of Field-Grown Soybean Cultivars Predicted by a Simulation Model. *Soil Science Society of America Journal* 48, 592-596.
- Silberbush M. 2002. Simulation of ion uptake from the soil. In: Waisel Y, Eshel A, Kafkafi U. (eds.) *Plant Roots: The Hidden Half*, 3RD Edition, Revised and Expanded, Chapter 37, p. 651-661. Marcel Dekker, New York.
- Silberbush M. 2013. Root Study: Why Is It behind Other Plant Studies? *American Journal of Plant Sciences* 4, 198-203.

- Tanner W, Beevers H. 1990. Does transpiration have an essential function in long-distance ion transport in plants? *Plant Cell and Environment* 13, 745–750.
- Tanner W, Beevers H. 2001. Transpiration, a prerequisite for long-distance transport of minerals in plants? *Proceedings of National Academy of Science USA* 98, 9443 - 9447.
- Taub DR, Wang X. 2008. Why are nitrogen concentrations in plant tissues lower under elevated CO<sub>2</sub>? A critical examination of the hypotheses. *Journal of Integrative Plant Biology* 50, 1365-1374.
- Tilman D, Wedin D. 1991. Dynamics of nitrogen competition between successional grasses. *Ecology* 72, 1038–1049.
- Tilman D. 1982. *Resource Competition and Community Structure*. Princeton University Press, Princeton, NJ, USA.
- Tilman D. 1990. Mechanisms of plant competition for nutrients: the elements of a predictive theory of competition. In: Grace JB, Tilman D. (eds) *Perspectives on Plant Competition*, Academic Press, San Diego, CA, USA. pp. 117–141.
- Tinker PB, Nye PH. 2000. *Solute movement in the rhizosphere*. New York: Oxford University Press.
- Turner BL, Driessen JP, Haygarth PM, Mckelvie ID. 2003. Potential contribution of lysed bacterial cells to phosphorus solubilisation in two rewetted Australian pasture soils. *Soil Biology and Biochemistry* 35, 187-189.
- Vitousek P. 1982. Nutrient cycling and nutrient use efficiency. *American Naturalist* 119, 553–572.
- Wedin D, Tilman D. 1993. Competition among grasses along a nitrogen gradient: initial conditions and mechanisms of competition. *Ecological Monographs* 63, 199–229.
- White PJ, George TS, Dupuy LX, Karley AJ, Valentine TA, Wiesel L, Wishart J. 2013. Root traits for infertile soils. *Frontiers in plant science* 4. doi: 10.3389/fpls.2013.00193

- Wilkinson S, Bacon MAZ, Davies WJ. 2007. Nitrate signalling to stomata and growing leaves: interactions with soil drying, ABA, and xylem sap pH in maize. *Journal of Experimental Botany* 58, 1705–1716.
- Wilkinson S, Corlett JE, Oger L, Davies WJ. 1998. Effects of xylem pH on transpiration from wild-type and flacca tomato leaves: a vital role for abscisic acid in preventing excessive water loss even from well-watered plants. *Plant Physiology* 117, 703–709.
- Wilkinson S, Davies WJ. 1997. Xylem sap pH increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. *Plant Physiology* 113, 559–573.
- Wilkinson S. 2004. Water use efficiency and chemical signalling. In: Bacon MA, editor. *Water use efficiency in plant biology*. Oxford, UK: Blackwell Publishing Ltd; p. 75-112.
- Witkowski ETF, Mitchell DT. 1987. Variations in soil phosphorus in the fynbos biome. *South African Journal of Ecology* 75, 1159–1171.
- Woodward J. 1699. Some thoughts and experiments concerning vegetation. *Philosophical Transactions of Royal Society of London* 21, 193–227.
- Yates MJ, Verboom GA, Rebelo AG, Cramer MD. 2010. Ecophysiological significance of leaf size variation in Proteaceae from the Cape Floristic Region. *Functional Ecology* 24, 485-492.