

# Hydraulic redistribution by *Protea* ‘Sylvia’ (Proteaceae) facilitates soil water replenishment and water acquisition by an understorey grass and shrub

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**Abstract.** Proteaceae of the Cape Floristic Region, South Africa, transpire throughout the summer drought, implying access to deep water. Hydraulic redistribution by *Protea* ‘Sylvia’ [*P. susannae* E. Phillips × *P. exima* (Salisb. Ex Knight) Fourc.; Proteaceae] was investigated in overnight pot and field experiments, where it was hypothesised that (1) Proteaceae replenish water in upper soil layers, (2) hydraulic redistribution facilitates nutrient uptake and (3) shallow-rooted understorey plants ‘parasitise’ water from proteas. Potted Sylvias redistributed ~17% of the tritiated water supplied, equating to  $34 \pm 1.2$  mL plant<sup>-1</sup>. Shallow-rooted *Cynodon dactylon* (L.) Pers. (Poaceae), plants growing in the same pots as Sylvia contained amounts of labelled water similar to those found in Sylvia, indicating water parasitism. In the field, Sylvia plants growing in aeolian sands took up the deuterated water applied at 1.2 m depth as indicated by increased  $\delta^2\text{H}$  of plant xylem water from  $-38 \pm 0.8$  to  $334 \pm 157\text{‰}$ . This deuterated water was then redistributed to the upper soil layer (0.2 and 0.4 m), as indicated by increased  $\delta^2\text{H}$  of soil water from  $-24.5 \pm 0.7$  to  $-8.0 \pm 3.0\text{‰}$  and soil moisture from 0.48 to 0.89%. Lithium, as a K-analogue, was taken up equally by plants watered with deep water and those not watered, probably since both had access to naturally-occurring deep water. Water in stems of the shallow-rooted understorey shrub, *Lysiera gnaphalodes* (L.) L. (Asteraceae) had similar  $\delta^2\text{H}$  values to stems of Sylvia ( $P=0.939$ ), again indicating water parasitism was tightly coupled to the protea. We conclude that hydraulic redistribution by Proteaceae plays an important role in soil water replenishment, water supply to shallow-rooted plants, and, thus, ecosystem structure and function during the summer drought of the Cape Floristic Region.

**Additional keywords:** *Cynodon dactylon*, deuterium, *Lysiera gnaphalodes*, Proteaceae, stable isotopes, summer drought, tritium.

## Introduction

Transpiration is driven by a gradient in water potential from soil to atmosphere ( $\Delta\psi$ ) and is controlled by vascular hydraulic architecture and leaf conductance. At night, or whenever evaporative demand or stomatal conductance is minimal, roots growing in dry soil become an alternative sink for available water (Burgess *et al.* 1998). This passive process is called hydraulic redistribution and has been documented for more than 50 taxa including trees, shrubs and grasses, particularly in summer-drought Mediterranean areas, but also in deserts, tropical forests (Jackson *et al.* 2000) and crop plants (Sekiya and Yano 2004). Hydraulic redistribution even occurs via senesced grass roots that have intact xylem conduits (Leffler *et al.* 2005), further supporting the idea that hydraulic redistribution is passive and only requires a gradient in  $\psi$  to drive the process, although aquaporins have been implicated in facilitating the process (e.g. McElrone *et al.* 2007). Although hydraulic redistribution commonly occurs from lower to upper soil layers, it has also been observed to occur downwards and laterally (Burgess *et al.* 1998).

Increased water content in upper soil layers is thought to facilitate nutrient uptake by lateral roots (Richards and Caldwell 1987; Caldwell and Richards 1989; Caldwell *et al.* 1998; Burgess *et al.* 2001; Ryel *et al.* 2003; Ryel 2004) and microbial degradation of organic matter. Hydraulic redistribution is, thus, likely to be of particular importance in environments that are water-limited or experience dry summer months (e.g. the Great Basin of the western United States; Leffler *et al.* 2004) and mediterranean-type ecosystems such as the Australian kwongan (Dawson and Pate 1996; Burgess *et al.* 2000), the Californian chaparral (e.g. Querejeta *et al.* 2007) and the Cape Floristic Region (CFR) of South Africa. However, the role of hydraulic redistribution on the ecosystem level is poorly understood, and not all hypotheses for the role of hydraulic redistribution in ecosystem functioning have been supported. For instance, it was recently found that hydraulic redistribution of water did not increase nitrate uptake (Snyder *et al.* 2008).

The climate of the CFR is typically described as Mediterranean and delimited by rainfall of more than 300 mm. Winters are wet but summers are hot and dry; although there is

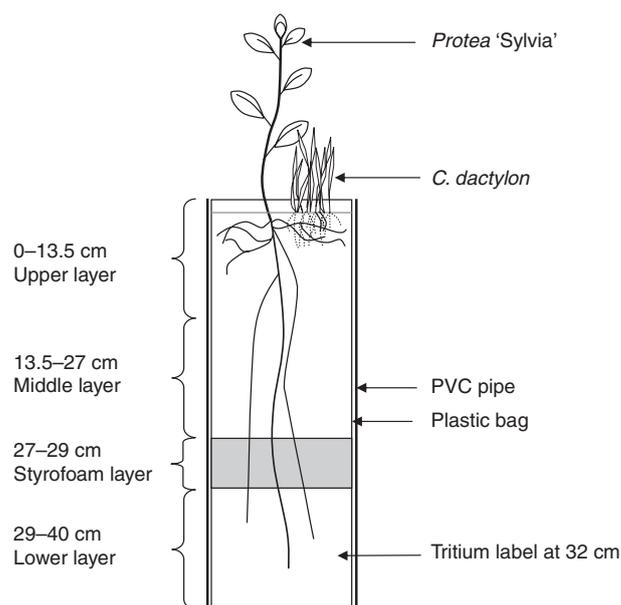
an increase in summer precipitation towards the south-east of the region (Proches *et al.* 2005). Soils are ancient, highly weathered, leached and generally acidic (pH 3–5; Witkowski and Mitchell 1987) with small amounts of inorganic nutrients, especially N ( $2 \mu\text{g NO}_3^- \text{g}^{-1}$  soil and  $13\text{--}27 \mu\text{g NH}_4^+ \text{g}^{-1}$  soil; Hawkins *et al.* 2005) and P (P-Bray II  $0.8\text{--}8.0 \mu\text{g P g}^{-1}$  soil; Witkowski and Mitchell 1987). Proteaceae are often the largest shrubs in this vegetation (usually 2–5 m but up to 10 m, Rebelo 2001). Roots of CFR Proteaceae are dimorphic with deep ‘sinker’ roots and shallow lateral roots (Mortimer *et al.* 2003) similar to Australian Proteaceae (Pate *et al.* 1995). Hydraulic conductivities and vessel lengths in sinker roots (2–4 m deep) of Australian Proteaceae were 2- and 5-fold greater, respectively, than in lateral roots (Pate *et al.* 1995) implying that sinker roots play the major role in water acquisition. Rooting depths for shrub-forms of SA Proteaceae are known to reach 3.6 m (Higgins *et al.* 1987), and have been shown to continue to transpire throughout the summer drought in the west of the CFR, while other shallower-rooted plants cease to transpire over this period (van der Heyden and Lewis 1988). This implies that the deeper rooted Proteaceae have access to deep water reserves which sustain photosynthetic and transpiration capacity through the summer. Not only do Proteaceae continue to transpire throughout summer, but they also grow predominantly during the hot, dry summer months (Rebelo 2001).

Since proteas have a dimorphic rooting system, we hypothesised that these plants are able to hydraulically redistribute water from deeper moist soil layers to upper drier soil layers where nutrients occur. Redistributed water may aid in improving the water and nutrient status of the proteas, but it could also positively influence surrounding plants (Dawson 1993) and, thus, may be an important component not only in soil water replenishment in summer but also in maintaining diversity in these systems. We hypothesised that (1) *Protea* ‘Sylvia’ plants would redistribute water from sinker roots to lateral roots near the soil surface in both a pot and field study; (2) this redistributed water would be available to both proteas and understorey plants, and (3) redistributed water would contribute to plant uptake of limiting nutrients, as traced by the K-analogue, Li.

## Materials and methods

### Pot culture

In the pot experiment, hydraulic redistribution from sinker roots to lateral roots and soil in upper soil layers was determined in *Protea* ‘Sylvia’ plants [*Protea susannae* E. Phillips  $\times$  *Protea exima* (Salisb. ex Knight) Fourc.; Proteaceae  $n = 10$ ] as well as to roots and shoots of a shallow-rooted (2–6 cm) understorey grass, *Cyanodon dactylon* (L.) Pers. of the Poaceae ( $n = 10$ ) using  $^3\text{H}_2\text{O}$  as a tracer (Fig. 1). Plants were grown in a glasshouse from August to December (spring to summer) where the day/night temperature varied between  $28^\circ\text{C}/15^\circ\text{C}$ . *Protea* ‘Sylvia’ (hereafter called Sylvia) cuttings were transplanted into 1-L pots with a mixture of 2 peat:2 styrofoam:1 sand (1-mm grain size), grown for 1 year, and subsequently transplanted into 400-mm long, clear plastic bags perforated at the bottom



**Fig. 1.** Schematic of pot culture comprising a PVC pipe and removable plastic bag containing growth medium and a capillary barrier of styrofoam between lower and middle/upper soil layers supporting *Protea* ‘Sylvia’ and *Cyanodon dactylon*. Tritiated water was injected 6 cm above the bag base (32 cm depth) into lower soil layers as indicated. *Protea* and grass roots are depicted as solid and dotted lines, respectively.

with several 2-mm holes. The bags filled supporting PVC pipes (40 cm long, 15 cm wide) and contained 930 g of sand (1 : 1 ratio of 1-mm and 0.25-mm grain) per bag. Sand was divided into upper, middle and lower sand layers with 310 g of sand each. A 20-mm layer of styrofoam beads was placed between the lower and middle/upper layers of sand and acted as a capillary barrier to prevent water movement from the lower to the middle and upper sand layers (Fig. 1). Control pots contained sand and capillary barrier only. The plastic bags were the same diameter as the PVC pipes and allowed the columns of sand and plants to be lifted out of the pipes for measurement of soil water content and subsequently re-inserted with the column intact. Plants were watered every fourth day with 500 mL of low-phosphate, 1/4-strength Hoaglands nutrient solution (pH 6.5) containing the following nutrient concentrations ( $\mu\text{M}$ ):  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  (200),  $\text{KH}_2\text{PO}_4$  (3.8),  $\text{CaSO}_4$  (200),  $\text{K}_2\text{SO}_4$  (100),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (54),  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$  (0.24),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (0.1),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.02),  $\text{H}_3\text{BO}_3$  (2.4),  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (0.03) and Fe-EDTA (10). Seeds of *C. dactylon* were sown into pots 30 days after transplanting proteas. Seventy days after transplanting, protea roots had reached a depth of 20–30 cm below the soil surface close to the bottom of the bag and were ready for use in the tracer experiment.

### Water supply in pots

In the pot experiment, the water content of the substrate in the top of bags was kept drier than that at the bottom. Drier top layers were intended to mimic natural conditions in a soil

column where sinker roots would grow towards lower, wetter soil layers. A time domain reflectometry (TDR) probe (Hydrosense, 120-mm probe, Campbell Scientific, Townsville, Qld, Australia) was used to measure volumetric soil water content ( $\theta_v$ ) of the sand. The TDR measurements were calibrated using gravimetric determinations of soil water content. Soil water potentials ( $\psi$ ) were measured from soil water retention curves of the sand. Once the plant and substrate column had been lifted clear of the supporting PVC pipe, the TDR probe was inserted horizontally through the plastic bags at 6, 14 and 29 cm from the base of the bag to determine percentage volumetric water content in the lower, middle and upper substrate layers, respectively. Plants were maintained at an average of 25% volumetric water content or  $-5$  kPa. Before supplying tritiated water, lower and upper soil layers were allowed to dry to 12 and 7% volumetric water content, respectively, over 7 days. Soils were dried so that sufficient tritiated water could be supplied to plants.

### $^3\text{H}_2\text{O}$ supply in pots

Since hydraulic redistribution is most prominent in the absence of transpirational demand, plants were supplied with labelled water overnight from dusk (1900 hours) and harvested 12 h later, just after dawn (0700 hours). Planted and unplanted pots were supplied with 180 mL  $25 \mu\text{Ci } ^3\text{H-H}_2\text{O}$  ( $^3\text{H-H}_2\text{O}$ , AEC-Amersham, Buckinghamshire, UK) via a syringe inserted through the lower part of the plastic bag, 6 cm above the bag base (Fig. 1). The plastic bags were then secured around the stem of each plant to reduce  $^3\text{H}_2\text{O}$  loss through evaporation.

### $^3\text{H}_2\text{O}$ detection in potted plants and soil

Protea roots and soil surrounding protea roots from the pot experiment were harvested from the lower, middle and upper areas of each bag, or from the upper soil layer in the case of grass roots ( $n=4$ ), after laying pots horizontal, withdrawing bags and slitting the bag around the soil column within each pot. The first fully expanded protea leaves and the grass leaves were harvested, weighed, frozen in liquid nitrogen and stored at  $-20^\circ\text{C}$  until extraction. Plant samples were homogenised in liquid nitrogen, and  $\sim 0.01$  g of each homogenised plant sample was mixed with 3 mL scintillation liquid (Readygel, Beckman Coulter Inc., Fullerton, CA, USA), to which 0.6 mL water was added to form a gel. Soil samples (2 g) were extracted into 20 mL 1 M KCl by shaking at 180 rpm for 1 h, centrifuging at 10 000g for 10 min and filtering through Whatman No. 1 filter paper (Whatman, Kent, UK). A 0.1-mL subsample of the extracted water was withdrawn and mixed with 1 mL scintillation fluid. Both plant and soil samples were counted on a liquid scintillation counter (Tri-carb 2100TR, Packard, Fullerton, CA, USA) for 60 min. The fraction ( $F$ ) of tritium label (in kBq) redistributed to upper soil layers was calculated as:

$$F = \frac{\text{kBq}_{\text{Upper+Mid soil}}}{\text{kBq}_{\text{Label}}} \quad (1)$$

Soil dry mass in upper, middle and lower soil layers was then subtracted from the respective soil wet masses to obtain the

amount of water in each 310-g soil layer, and the volume of water redistributed ( $HR$ ) from lower to upper and middle soil layers was simply calculated as:

$$HR(\text{mL}) = F(\text{mL}_{\text{Upper+Mid soil}}) \quad (2)$$

### Autoradiography of roots

After supplying labelled water overnight in the pot experiment, a single sinker root and the attached lateral roots were harvested from *Sylvia* and autoradiographed on an X-ray film (Hyperfilm  $\beta$ -max, Amersham). The film was exposed for 45 h at  $-80^\circ\text{C}$  between two intensifying screens (Hyperscreen; GE Healthcare, Piscataway, NY, USA) and then developed for 1 min using Kodak (Perkin Elmer, Inc., Waltham, MA, USA) reagents.

### Field experiment location and set-up

The field site (Arnelia Farms, Hopefield, on the west coast of the CFR,  $33^\circ 2' 49''\text{S}$ ,  $18^\circ 20' 30''\text{E}$ ) has an annual rainfall of  $399 \pm 38$  mm ( $n=9$ ) where a total of 62 mm fell between December 2007 and March 2008 when the field experiment was conducted. No rain fell during March when the labelled water experiment was conducted. The site was selected as the deep aeolian sands facilitated insertion of access tubes supplying water at 1.2-m depth, intended to simulate water supply from a watertable. Tap roots of *Sylvia* were observed to exceed 2 m depth from exploratory trenches while the rooting depth of feeder roots, determined from 0.5-m trenches adjacent to sixteen plants similar to those used in the experiment, were between 0.2 and 0.4 m. Insertion tubes were placed in two rows next to farmed 5-year-old *Sylvia* plants ( $n=8$ ). Plants within rows were spaced 1 m apart and between rows 1.5 m apart. Water was supplied at depth for an 8-week period over summer (December–March) to encourage root growth near the tubes. Following this, deuterated water was supplied to plants in one row but not the other. A shallow-rooted (0.10–0.15 m deep) shrub growing under the deep-rooted proteas, *Leysera gnaphalodes* (L.) L. (Asteraceae;  $n=5$ ), was used to determine whether small shrubs may benefit from redistributed water, i.e. water parasitism.

### Soil moisture profiles

Two soil moisture profiles per treatment were established in the field in order to confirm that a water potential gradient between surface soil with lateral feeder roots (0.2 to 0.4 m) and deeper soils with tap roots (0 to  $>1$  m) of *Sylvia* existed. Field soil moisture was measured as percent volumetric water content ( $\theta_v$ ) using a frequency domain reflectometry (FDR) soil water profile sensor (Diviner 2000, Sentek Sensor Technologies, Stepney, SA, Australia). The Diviner sensor measured soil moisture every 0.1 m for 1 m. Access tubes for the Diviner sensor (5 cm  $\emptyset$ ) were installed by excavating a 9-cm  $\emptyset$  hole in sand and inserting the pipe with slurry of native sand and water. Soil moisture profiles equilibrated 2 weeks after inserting access tubes and the experiment was performed 2 weeks after that. The results from Diviner 2000 were confirmed with gravimetric measurements of soil water in the upper 0.2–0.4 m layer, which were similar to those given

by the diviner probe. Therefore, the default calibration equation of the Sentek software was considered adequate for all measurements using the Diviner probe.

#### Water supply in the field

Water was supplied to 16 adult field-grown *Sylvia* plants for 8 weeks at a rate of 90 L week<sup>-1</sup>. Half of this water was supplied via PVC access tubes sunk to 1.2 m (25 cm  $\emptyset$ ) with sealed bottoms and gauze covered, 4-mm holes drilled into the distal 20 cm) and half from surface drippers next to each plant. Water was supplied at depth to encourage root growth near the access tubes, which simulated a deep water supply as well as a means to supply the deuterated water (see 'Deuterium labelling in the field'). After 8 weeks, water was supplied for 2 weeks via access tubes only. Access tubes for watering were installed by excavating and backfilling a 90-mm  $\emptyset$  hole in sand. After 10 weeks, the water supply was stopped and the soil dried for 1 week before supplying the labelled water. Drying was necessary in order to reduce dilution of the label. After drying the soil, half of these plants ( $n=8$ ) were fed with the labelled water at 1.2-m depth, while plants in the adjacent row received no water, i.e. were out of the labelling zone. Watering at depth (90 L week<sup>-1</sup>) was resumed for 3 weeks subsequent to the labelling experiment as part of the experiment using Li as a K-analogue (see below).

#### Deuterium labelling in the field

Deuterated water was supplied at 1.2 m depth at dusk to eight protea plants, while no water was supplied to eight other protea plants in an adjacent row. Each watered plant received 12.5 mL 99 atom% D<sub>2</sub>O (Sigma-Aldrich, St Louis, MO, USA) in 25 L tap water giving a calculated  $\delta^2\text{H}$  of 3173‰ and an actual  $\delta^2\text{H}$  of  $2575 \pm 1\text{‰}$  (relative to V-SMOW).

#### $\delta^2\text{H}$ determination in field plants and soil

Field soil samples around plants watered with the deuterated water and from soil equidistant between the watered and unwatered plants were taken from the 0.2 to 0.4 m soil layer both before watering and 12 h later at dawn, and were subsequently frozen. Soil samples taken between rows had no lateral roots and functioned as a control for water redistribution by capillary rise. Likewise, fully suberised stem samples were taken from the same plants both before and after supplying the label water. Stems were debarked, sealed with Parafilm to eliminate evaporation, placed in sealed vials and kept frozen at 4°C until water extraction and analysis. Water was extracted from soils and stems by cryogenic distillation (West *et al.* 2006). Extracted water was reduced to H<sub>2</sub> gas using the closed tube zinc reduction method (Coleman *et al.* 1982). Isotope ratios were measured with a Delta XP mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) at the University of Cape Town and were expressed as  $\delta^2\text{H}$  (in ‰) relative to the standard V-SMOW, following the equation:

$$\delta^2\text{H} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (3)$$

where  $R$  is the ratio of  $^2\text{H}/^1\text{H}$ . Analytical precision on internal standards was 1.4‰. The fraction ( $f$ ) of label ( $\delta_{\text{label}}$ )

contributing to the  $\delta^2\text{H}$  signal in the plant stems after labelling ( $\delta_{\text{after}}$ ) relative to the  $\delta^2\text{H}$  before labelling ( $\delta_{\text{before}}$ ) was calculated using a two-component mixing model:

$$\text{Fraction}(f) = \frac{(\delta_{\text{after}} - \delta_{\text{before}})}{(\delta_{\text{label}} - \delta_{\text{before}})} \quad (4)$$

The fractional increase in soil water ( $f\text{SW}$ ) after labelling/watering was calculated as:

$$\text{Fractional increase in soil water } (f\text{SW}) = \frac{(\text{SW}_{\text{after}} - \text{SW}_{\text{before}})}{\text{SW}_{\text{after}}} \quad (5)$$

where SW was calculated as from soil dry bulk density and soil moisture content (MC) as:

$$\text{Soil water } (\text{SW g cm}^{-3}) = \frac{\text{Soil bulk density } (\text{g cm}^{-3})}{1 - \text{MC}} \quad (6)$$

#### LiCl supply and detection in field-grown plants

Lithium (1 g LiCl mixed in 10 mL sand per plant) was inserted into small holes in the sand at the level of feeder roots (~0.4 m depth) in the soil surrounding both watered and unwatered protea plants. Leaf samples for Li determination were taken before LiCl supply, 12 h after LiCl supply and then 1 and 3 weeks later ( $n=8$ ). Lithium in pulverised leaf samples was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian Vista MPX, Melbourne, Vic., Australia).

#### Statistical analyses

Treatments were compared using Newman Keuls multiple range tests after one-way ANOVAs, and Student  $t$ -tests (Statistica 8, Statsoft Inc., Tulsa, OK, USA). Standard errors for the linear mixing model were calculated according to Phillips and Gregg (2001).

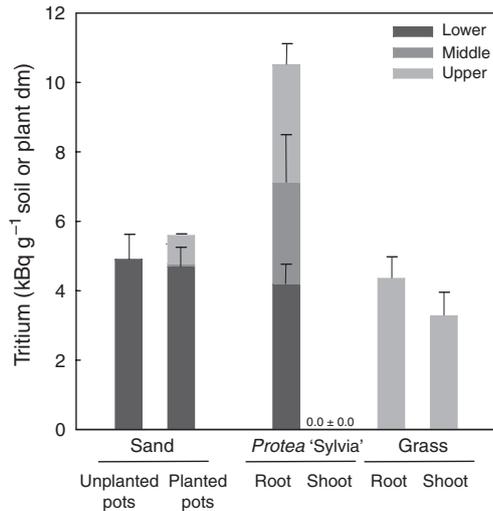
## Results

### Hydraulic redistribution in pot culture

Roots of *Sylvia* in the upper, middle and lower soil layers were labelled to a similar extent (3–4 kBq g<sup>-1</sup> dry mass, Fig. 2) as would be expected of roots acting as conduits. That roots acted as conduits for the tritiated water was clearly evident from the even distribution of  $^3\text{H}$  between sinker and lateral roots on the autoradiogram (Fig. 3a). However, one lateral root situated midway between the lower and upper layers (arrow on root-autoradiogram overlay, Fig. 3b) was not labelled, suggesting little labelling of root laterals in the middle soil layer. The presence of tritium label in the upper soil layer clearly indicated that hydraulic redistribution had occurred (Fig. 2). However, middle soil layers remained poorly labelled (Fig. 2), indicating that most of the tritiated water had been redistributed from lower to upper soil layers with little leakage of water from roots to middle layers, supporting what was observed on the autoradiogram (Fig. 3). Based on the  $^3\text{H}$  in the upper and middle soil layers, *Sylvia* lifted 17% of the supplied labelled water from lower to upper and middle soil layers

( $274 \pm 7$  kBq), but mostly to the upper layers (Fig. 2), equating to  $34 \pm 1.2$  mL plant<sup>-1</sup> or pot<sup>-1</sup>. There was no redistribution of labelled water from the lower soil layers in unplanted pots, indicating that <sup>3</sup>H<sub>2</sub>O redistribution was exclusively through roots and not by capillary rise in the soil (Fig. 2).

Soil volumetric water contents determined in the week prior (dehydration phase) and for 1 day after supply of the tritiated water (rehydration phase) also indicated that hydraulic



**Fig. 2.** Radioactivity in sand, *Protea* 'Sylvia' roots, and roots and shoots of the grass *C. dactylon* after supplying tritium label overnight to lower sand layers. Values are means ( $n = 10$ )  $\pm$  standard errors.



**Fig. 3.** (a) Autoradiogram, and (b) autoradiogram with root overlay of a sinker and lateral roots of a *Protea* 'Sylvia' plant that had been supplied with 180 mL tritium label overnight. Note that radioactivity is present in sinker roots and upper lateral roots but not in a side root occurring near the middle of the pot (arrow).

redistribution had occurred (Fig. 4). An increase in volumetric water content of lower soil layers after tritium labelling was less in the planted pots than in unplanted pots after labelling, implying a net movement of water from lower to upper soil layers in planted, but not in unplanted pots (Fig. 4). This pot experiment was repeated with similar results (data not shown).

#### Water parasitism in pot culture

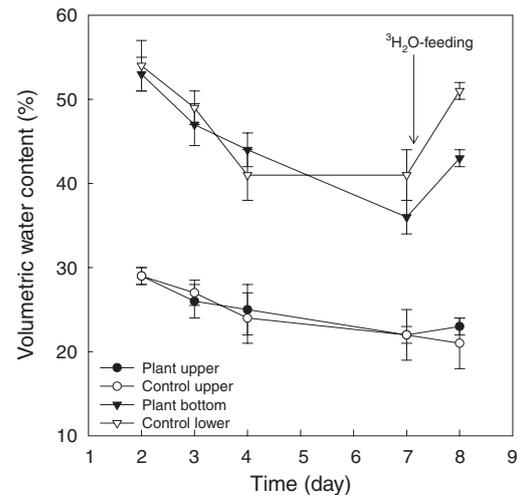
Not only were *Sylvia* roots labelled, but label was also present in both the shoots ( $3.3 \pm 0.7$  kBq g<sup>-1</sup>) and roots ( $4.3 \pm 0.7$  kBq g<sup>-1</sup>) of the understorey grass, *C. dactylon* (Fig. 2). The grass roots were shallow ( $4.5 \pm 2.3$  cm long) and confined to the upper layers of the sand and did not penetrate the styrofoam layer in the pots. Since the labelled water was supplied below this layer, the grass roots could only have accessed <sup>3</sup>H<sub>2</sub>O hydraulically lifted by the *Sylvia* roots. Thus, labelling of *C. dactylon* indicated water parasitism by this grass. Labelling in the grass was similar to that in the *Sylvia*, and was considerably greater than that in the surface soil.

#### Field soil characterisation and capillary rise

Soils at Hopefield were fine- to coarse-grained aeolian sand, ranging in grain size between 30 and 700  $\mu$ m with an average of  $300 \mu$ m  $\pm$  45 ( $n = 30$ ). The sand comprised  $55.2\% \pm 1.1$  Si sand and  $44.8\% \pm 1.1$  shell fragments ( $n = 4$ ). There was no clay present and the percentage organic matter, measured as carbon, was low ( $1.57 \pm 0.6\%$ ). Models of capillary rise in a sand similar to that in our experiment (Lu and Likos 2004), estimated a capillary rise of 0.25 m over 1 day, which would exclude labelled water rising above 0.95 m, i.e. well below the soil sampling zone.

#### Hydraulic redistribution in field plants

Stem water extracted from *Sylvia* plants that had access to the deuterium label at 1.2 m depth was highly enriched with <sup>2</sup>H, but



**Fig. 4.** Soil volumetric water contents in upper and lower sand layers in unplanted pots or pots planted with *Protea* 'Sylvia' from 7 days before and 1 day after supplying a tritium label. Values are means  $\pm$  s.e. ( $n = 10$ ).

Sylvia plants with no roots in the labelling zone had  $\delta^2\text{H}$  values similar to stem samples collected before labelling (Table 1). According to the linear mixing model,  $14.4 \pm 0.03\%$  of the  $\delta^2\text{H}$  found in the plant stems could be attributed to the supplied labelled water. Roots were not sampled.

Hydraulic redistribution was clearly indicated by gravimetric determinations of upper soil (0.2–0.4 m depth) moisture where soil moisture had increased by  $52 \pm 6\%$  the morning after supplying 25 L of deuterated water compared with soil before supplying labelled water or soil that had not been supplied with labelled water (Table 1,  $P=0.009$ ). Confirming this, the  $\delta^2\text{H}$  in the upper soil layer surrounding Sylvia plants that had received labelled water at depth was higher ( $P<0.0001$ ) than that of soil before watering with labelled water and also higher than that of surface soil equidistant from the label source, but out of the rooting zone of Sylvia (Table 1). There was no difference between the  $\delta^2\text{H}$  of the latter soil and soil sampled before labelling (Table 1;  $P=0.546$ ,  $t$ -test), confirming that capillary rise could not have accounted for the change in soil moisture in the upper soil layer.

Soil moisture profiles (0–1 m) taken before labelling supported the isotope data (Fig. 5). These profiles showed that soil moisture content was highest at 1 m depth and decreased towards the surface, but increased again between 0.4 and 0.2 m below the surface. Since lateral roots were also found at 0.2 to 0.4 m depth: this pattern suggests hydraulic redistribution was occurring over the weeks before the labelling experiment. During this period, no precipitation occurred and surface irrigation had been discontinued.

#### Water parasitism in field-grown plants

The Asteraceous shrub (*L. gnaphalodes*) growing under Sylvia that had been supplied with labelled water at 1.2 m depth were highly enriched with  $^2\text{H}$  compared with those sampled before watering with label (Table 1). Since *L. gnaphalodes*

roots were shallow ( $12.3 \pm 4.5$  cm), as directly verified by excavation, these shrubs could only have accessed the labelled water from hydraulically-lifted water, demonstrating that the shallow shrub could take up or ‘parasitise’ water lifted by Sylvia. As in the pot experiment in which the protea and grass were equally labelled, the  $\delta^2\text{H}$  of *L. gnaphalodes* was similar to that of the ‘lifting’ protea plant, both before and after labelling (paired  $t$ -tests,  $P=0.528$  and  $0.939$ , respectively, Table 1). Close correlation ( $r^2=0.98$ ) between  $\delta^2\text{H}$  of the *L. gnaphalodes* and the protea after labelling supported the notion that the spatial coupling between water uptake by the parasitising plant and water release by the protea roots was very close.

#### Lithium uptake

The 25 L of water supplied at depth to Sylvia plants made no apparent contribution to Li uptake via hydraulic redistribution. Roots of plants either supplied or not supplied with deuterated water took up the Li as shown by increasing leaf [Li] over the 3 week sampling period post-labelling/watering. There was, however, no difference in leaf [Li] between watered ( $4.66 \pm 2.00 \mu\text{mol Li g}^{-1}$  leaf dry mass) and unwatered plants ( $7.46 \pm 3.26 \mu\text{mol Li g}^{-1}$  leaf dry mass,  $P>0.05$ ) after 3 weeks. Leaves sampled from plants in both treatments before labelling had a [Li] of  $0.08 \pm 0.006 \mu\text{mol Li g}^{-1}$  leaf dry mass. This indicated that both treatments had sufficient soil water supply to acquire the Li spread around the lateral root zone. There was no precipitation or irrigation during the 3 week sampling period.

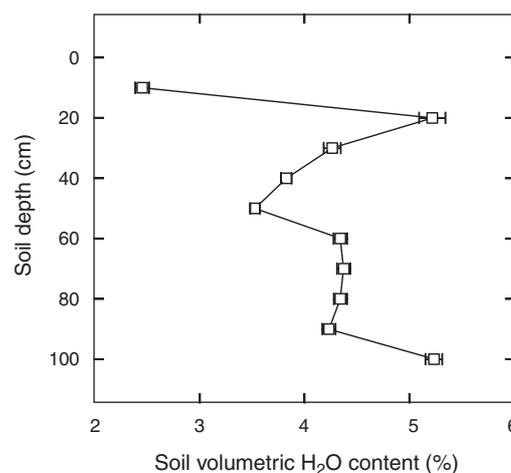
#### Discussion

Proteas are known to continue photosynthetic activity throughout the summer drought (van der Heyden and Lewis 1988). This is likely because deep-rooted Proteaceae have a strongly dimorphic root system with deep sinker roots that have relatively higher hydraulic conductivities and longer vessel lengths than lateral roots (Australian Proteaceae, Pate *et al.* 1995). These root

**Table 1.**  $\delta^2\text{H}$  of *Protea* ‘Sylvia’, *Leysera gnaphalodes* and soil (20–40 cm depth) as well as gravimetric soil water content the morning after supplying 25 L deuterated water at 1.2 m depth to *Protea* ‘Sylvia’

Values are means  $\pm$  s.e. ( $n=8$  for protea,  $n=5$  for *L. gnaphalodes*,  $n=8$  for soil). Different letters indicate difference at the  $P<0.05$  level after one-way ANOVA and Newman–Keuls multiple range test (protea, soils) or  $t$ -tests (*L. gnaphalodes*)

	$\delta^2\text{H}(\text{‰})$	Gravimetric soil H <sub>2</sub> O content (%)
D <sub>2</sub> O labelling solution	$2575 \pm 1$	–
Sylvia before labelling	$-38.0 \pm 0.8^a$	–
Sylvia after labelling, no roots in labelling zone	$-38.0 \pm 0.3^a$	–
Sylvia after labelling, roots in labelling zone	$338 \pm 157^b$	–
<i>L. gnaphalodes</i> before labelling	$-37 \pm 1^a$	–
<i>L. gnaphalodes</i> after labelling	$361 \pm 276^b$	–
Soil before labelling	$-25.4 \pm 1.6^a$	$0.50 \pm 0.06^a$
Soil after labelling, out of Sylvia root zone	$-24.5 \pm 0.7^a$	$0.48 \pm 0.05^a$
Soil after labelling, in Sylvia root zone	$-8.0 \pm 3.0^b$	$0.89 \pm 0.12^b$



**Fig. 5.** Soil moisture profiles adjacent to *Protea* ‘Sylvia’ plants grown on aeolian sand at Arnelia Farms, Hopefield, South Africa, before supply of deuterium label. Values are means  $\pm$  s.e. ( $n=4$ ).

characteristics enable proteas access to water throughout the year. From the work presented here and that by Pate *et al.* (1995), it is evident that these plants access water deep in the soil profile. In addition, we show that proteas accessing deep water redistributed this water to upper soil layers. *Protea* 'Sylvia' redistributed water from deep tap roots to surface lateral roots in both a pot and field study and this redistribution both replenished the soil water in upper soil layers and was available to understorey plants. This redistribution was measurable overnight ( $\sim 34 \text{ mL plant}^{-1}$  in the pot experiment and a 52% increase in soil water in the upper soil of the field experiment). By redistributing water during the night, proteas created a water pool in upper soil layers in addition to that present in lower soil layers. Two distributed water pools may ensure water supply during peak transpiration and so prevent cavitation (Caldwell *et al.* 1998), and water stress (Horton and Hart 1998) as well as potentially aiding in nutrient uptake (Ryel 2004). Although the amounts of labelled water redistributed by the field plants in our study were small, these amounts were redistributed over a short time period (12 h) and were sufficient to demonstrate hydraulic redistribution. This is the first report of hydraulic redistribution and water parasitism in the CFR. Hydraulic redistribution by large shrubs such as proteas has important implications for hydrology in the CFR, which has relatively few species with tree habits. It is likely that many Proteaceae shrubs have a dimorphic rooting system similar to that found in *Sylvia* (e.g. Higgins *et al.* 1987) and since Proteaceae are a common and characteristic feature of the fynbos, it is likely that these medium to large shrubs are essential for replenishment of surface soil water during the mediterranean summer drought.

The similarity in  $\delta^2\text{H}$  values of xylem water between *Sylvia* and *L. gnapholodes*, both before and after supplying labelled water, indicates the tight coupling between the plant responsible for hydraulic redistribution and the water 'parasite'. Before supply of labelled water, *Sylvia* and *L. gnapholodes* had indistinguishable  $\delta^2\text{H}$  values, which differed from those of the upper soil. Following labelling, the  $\delta^2\text{H}$  of individual *L. gnapholodes* plants again tracked that of the *Sylvia* plant under which it was growing, rather than that of the bulk upper soil. This suggests that *L. gnapholodes* was preferentially taking up redistributed water from directly adjacent to the protea roots, rather than being more generally rooted in the upper soil layer. The smaller isotopic enrichment of the upper soil water was very likely due to a dilution effect, where only a fraction of the soil sampled was in the sphere of influence of leakage from *Sylvia* roots. An alternative explanation for the similarity in  $\delta^2\text{H}$  between *Sylvia* and *L. gnapholodes* is that *L. gnapholodes* was able to directly access the labelled water via deep roots. However, this possibility was excluded by excavating the plants to examine for roots extending below the upper surface soil. Furthermore, a similar result was also observed in the pot experiment, where the tritium label in *C. dactylon* was more similar to that seen in *Sylvia* than of that in the upper soil. Again, we verified that *C. dactylon* roots were not able to directly access the labelled water. This close coupling between 'lifter' and parasite' suggests the potential for tight ecological links between Proteaceae and shallow rooted understorey species in the fynbos.

Our data highlighted that different species and tissues may become enriched with isotopes to varying extents. In the pot experiment, for example, grass shoots absorbed labelled water although the *Sylvia* shoots did not. A possible explanation for this is that *Sylvia* shoots grow in 'flushes' during summer with little leaf expansion (and therefore minimal water incorporation into tissues) between flushes when leaves are suberised. At the time of tritium labelling, the *Sylvia* flushes were already fully expanded and would have only required water for tissue maintenance, but not cell expansion. Conversely, the grass shoots were actively growing and evidently incorporating the labelled water into leaf tissue. Night-time transpiration (e.g. Snyder *et al.* 2008) could also account for the  $^3\text{H}_2\text{O}$  incorporation in the grass. Vasculature between grasses and proteas/shrubs are also likely to differ and may influence root-shoot water transport.

Redistribution of soil water into drier surface soils by plants has potential implications for nutrient acquisition by plants. Water redistributed to lateral roots in topsoils, where most nutrient concentrations are relatively high compared with subsoils (Marschner 1995), would likely aid nutrient transport into the rhizosphere by either diffusion or mass flow, both of which require soil moisture (Barber 1995). In this field study however, deep water supply for 3 weeks subsequent to the labelling experiment (and resultant hydraulic redistribution as shown by the overnight experiment) did not affect the amount of Li (used as a K-analogue) taken up by *Sylvia*. This might be taken to suggest that hydraulic redistribution did not make a measurable contribution to ion uptake. However, it is more likely that plants in both of our treatments made use of hydraulically-lifted water, besides the deuterated water supplied once-off overnight, so that the 25 L of deuterated water added did not make a significant difference to the diffusion or mass flow of Li in soil solution or to that taken up by the plants.

Hydraulic redistribution also has potentially profound consequences for plant community dynamics. Hydraulic redistribution does not always facilitate water use by shallow rooted plants, as some plants redistribute water downwards after rains (e.g. Burgess *et al.* 1998) resulting in the removal of water resources away from shallower rooted understorey plants. However, the present study demonstrated water parasitism by a grass (pot experiment) and a shrub (field experiment), supporting previous evidence for water parasitism in a mediterranean ecosystem (Peñuelas and Filella 2003). Shallow rooted plants are likely to be dependent on occasional rainfall events during summer or dew as well as on water redistributed by large shrubs. Competitive interactions between overstorey Proteaceae and understorey plants occur in the fynbos, where the presence of grass and Proteaceae reciprocally reduce growth of the other (Vlok and Yeaton 2000). Although competitive interactions determining recruitment of overstorey and understorey plants, particularly after fire, are complex, species-specific (Bond *et al.* 1992; Vlok and Yeaton 2000; Heelemann *et al.* 2008) and dependent on soil (Richards *et al.* 1997) and environmental factors (Thuiller *et al.* 2004), assemblages of proteas with  $\text{C}_3$  and  $\text{C}_4$  grasses and other understorey plants commonly occur in fynbos. Whatever the competitive interactions between protea and grass understorey during recruitment, once

established, the persistence of grass and small shrubs such as the *L. gnaphalodes* under the canopies of Proteaceae shrubs in fynbos is likely facilitated by water lifted by proteas during summer drought. Threats to Proteaceae, such as alien plant invasion and agriculture, thus, may have severe effects on water availability to plants in the CFR.

## Conclusion

Hydraulic redistribution by proteas plays an important role in soil water replenishment, water supply to shallow-rooted plants, and, thus, is likely to influence ecosystem functioning and plant community dynamics during the summer drought in the CFR. Hydraulic redistribution by proteas also has consequences for commercial protea growers. After plant establishment, irrigation scheduled to re-charge deep soil water would encourage deep-rooted growth, enabling access to moist soil or the watertable, making these plants less dependent on irrigation events during periods of summer drought. Drip irrigation that wets only surface soils could increase the dependence of the plants on surface irrigation making them susceptible to short periods of drought and more dependent upon sustained irrigation. Further work is required to elucidate potential nutritional benefits of hydraulic redistribution.

## Acknowledgements

The authors thank Molteno Brothers Pty Ltd, Elgin Glen, South Africa for providing *Sylvia* cuttings, as well as the National Research Foundation of South Africa and the South African Protea Producers and Exporters Association for financial support during this project. The authors also thank Carin Basson for technical assistance.

## References

- Barber SA (1995) 'Soil nutrient bioavailability, a mechanistic approach.' 2nd edn. (John Wiley & Sons: New York)
- Bond WJ, Cowling RM, Richards MB (1992) Competition and coexistence. In 'Fynbos nutrients, fire and diversity'. (Ed. RM Cowling) pp. 206–225. (Oxford University Press: Oxford)
- Burgess SSO, Adams MA, Turner NC, Ong CK (1998) The redistribution of soil water by tree root systems. *Oecologia* **115**, 306–311. doi: 10.1007/s004420050521
- Burgess SSO, Pate JS, Adams MA, Dawson TE (2000) Seasonal water acquisition and redistribution in the Australian woody phreatophyte, *Banksia prionotes*. *Annals of Botany* **85**, 215–224. doi: 10.1006/anbo.1999.1019
- Burgess SSO, Adams MA, Turner NC, White DA, Ong CK (2001) Tree roots: conduits for deep recharge of soil water. *Oecologia* **126**, 158–165. doi: 10.1007/s004420000501
- Caldwell MM, Richards JH (1989) Hydraulic lift: water efflux from upper roots improves effectiveness of water uptake by deep roots. *Oecologia* **79**, 1–5. doi: 10.1007/BF00378231
- Caldwell MM, Dawson TE, Richards JH (1998) Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia* **113**, 151–161. doi: 10.1007/s004420050363
- 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. doi: 10.1021/ac00243a035
- 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, Pate JS (1996) Seasonal water uptake and movement in root systems of Australian phreatophytic plants of dimorphic root morphology: a stable isotope investigation. *Oecologia* **107**, 13–20. doi: 10.1007/BF00582230
- Hawkins H-J, Wolf G, Stock WD (2005) Cluster roots of *Leucadendron lauroleum* (Proteaceae) and *Lupinus albus* (Fabaceae) take up glycine intact: an adaptive strategy to low mineral N in soils? *Annals of Botany* **96**, 1275–1282. doi: 10.1093/aob/mci279
- Heelemann S, Proches S, Rebelo AG, van Wilgen B, Poremski S, Cowling RM (2008) Fire season effects on the recruitment of non-sprouting serotinous Proteaceae in the eastern (bimodal rainfall) fynbos biome, South Africa. *Austral Ecology* **33**, 119–127. doi: 10.1111/j.1442-9993.2007.01797.x
- van der Heyden F, Lewis OAM (1988) Seasonal variation in the photosynthetic capacity with respect to plant water status of five species of the mediterranean climate region of South Africa. *South African Journal of Botany* **55**, 509–515.
- Higgins KB, Lamb AJ, Van Wilgen BW (1987) Root systems of selected plant species in mesic mountain fynbos in the Jonkershoek Valley, south-western Cape Province, South Africa. *South African Journal of Botany* **53**, 249–258.
- Horton JL, Hart SC (1998) Hydraulic lift: a potentially important ecosystem process. *Trends in Ecology & Evolution* **13**, 232–235. doi: 10.1016/S0169-5347(98)01328-7
- 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. doi: 10.1016/S1360-1385(00)01766-0
- Leffler AJ, Ivans CY, Ryel RJ, Caldwell MM (2004) Gas exchange and growth responses of the desert shrub *Artemisia tridentata* and *Chrysothamnus nauseosus* to shallow- vs. deep-soil water in a glasshouse experiment. *Environmental and Experimental Botany* **51**, 9–19. doi: 10.1016/S0098-8472(03)00041-8
- Leffler AJ, Peek MS, Ryel RJ, Ivans CY, Caldwell MM (2005) Hydraulic redistribution through the root systems of senesced plants. *Ecology* **86**, 633–642. doi: 10.1890/04-0854
- Lu N, Likos WJ (2004) Rate of capillary rise in soil. *Journal of Geotechnical and Geoenvironmental Engineering* **130**, 646–650. doi: 10.1061/(ASCE)1090-0241(2004)130:6(646)
- Marschner H (1995) 'Mineral nutrition of higher plants.' (Academic Press: London)
- McElrone AJ, Bichler J, Pockman WT, Addington RN, Linder CR, Jackson RB (2007) Aquaporin-mediated changes in hydraulic conductivity of deep tree roots accessed via caves. *Plant, Cell & Environment* **30**, 1411–1421. doi: 10.1111/j.1365-3040.2007.01714.x
- Mortimer P, Swart JC, Valentine AG, Jacobs G, Cramer MD (2003) Does irrigation influence the growth, yield and water use efficiency of the *Sylvia* hybrid 'Sylvia' (*Protea susannae* × *Protea eximia*)? *South African Journal of Botany* **69**, 135–143.
- Pate JS, Jeschke WD, Aylward MJ (1995) Hydraulic architecture and xylem structure of the dimorphic root systems of south-West Australian species of Proteaceae. *Journal of Experimental Botany* **46**, 907–915. doi: 10.1093/jxb/46.8.907
- Peñuelas J, Filella I (2003) Deuterium labelling of roots provide evidence of deep water access and hydraulic lift by *Pinus nigra* in a Mediterranean forest of NE Spain. *Environmental and Experimental Botany* **49**, 201–208. doi: 10.1016/S0098-8472(02)00070-9
- Proches S, Cowling RM, Du Preez DR (2005) Patterns of geophyte diversity and storage organ size in the winter-rainfall region of southern Africa. *Diversity & Distributions* **11**, 101–109. doi: 10.1111/j.1366-9516.2005.00132.x
- Querejeta JI, Egerton-Warburton LM, Allen MF (2007) Hydraulic lift may buffer rhizosphere hyphae against the negative effects of severe soil drying in a California oak savanna. *Soil Biology & Biochemistry* **39**, 409–417. doi: 10.1016/j.soilbio.2006.08.008

- Rebello AG (2001) 'Proteas. A field guide to the Proteas of Southern Africa.' (Fernwood Press: Cape Town, South Africa)
- Richards JH, Caldwell MM (1987) Hydraulic lift: substantial nocturnal water transport between soil layers by *Artemisia tridentata* roots. *Oecologia* **73**, 486–489. doi: 10.1007/BF00379405
- Richards MB, Cowling RM, Stock WD (1997) Soil factors and competition as determinants of the distribution of six fynbos Proteaceae species. *Oikos* **79**, 394–406. doi: 10.2307/3546024
- Ryel RJ (2004) Hydraulic redistribution. *Progress in Botany* **65**, 413–435.
- Ryel RJ, Caldwell MM, Leffler AJ, Yoder CK (2003) Rapid soil moisture recharge to depth by roots in a stand of *Artemisia tridentata*. *Ecology* **84**, 757–764. doi: 10.1890/0012-9658(2003)084[0757:RSMRTD]2.0.CO;2
- 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 Research* **86**, 167–173. doi: 10.1016/j.fcr.2003.08.007
- Snyder KA, James JJ, Richards JH, Donovan LA (2008) Does hydraulic lift or nighttime transpiration facilitate nitrogen acquisition? *Plant and Soil* **306**, 159–166. doi: 10.1007/s11104-008-9567-7
- Thuiller W, Lavorel S, Midgley G, Lavergne S, Rebello T (2004) Relating plant traits and species distributions along bioclimatic gradients for 88 *Leucadendron* taxa. *Ecology* **85**, 1688–1699. doi: 10.1890/03-0148
- Vlok JHJ, Yeaton RI (2000) Competitive interactions between overstorey proteas and sprouting understorey species in South African mountain fynbos. *Diversity & Distributions* **6**, 273–281. doi: 10.1046/j.1472-4642.2000.00090.x
- 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. doi: 10.1002/rcm.2456
- Witkowski ETF, Mitchell DT (1987) Variations in soil phosphorus in the fynbos biome, South Africa. *Journal of Ecology* **75**, 1159–1171.

Manuscript received 26 February 2009, accepted 16 May 2009