Defoliation alters water uptake by deep and shallow roots of *Prosopis velutina* (Velvet Mesquite)

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Summary

1. *Prosopis velutina* Woot. (Velvet Mesquite) at a site with limited groundwater availability derived a greater percentage of water from shallow soil at the onset of the summer rainy season than did trees at a site with greater availability of groundwater. Predawn leaf water potentials (Ψ_{pd}) were not a strong indicator of shallow water use for this species with roots in multiple soil layers.

2. We experimentally defoliated *P. velutina* plants to determine if reduced-canopy photosynthesis would alter vertical patterns of root activity. After natural rain events, hydrogen isotope ratios of xylem sap indicated that defoliated *P. velutina* took up a greater percentage of its water from shallow soils than did undefoliated plants.

3. Irrigation with deuterium-labelled water further demonstrated that undefoliated plants were able to use shallow soil water. Defoliation appeared to affect the ability of trees to use deep-water sources.

4. Reduced carbon assimilation limited water uptake from deep soil layers. These data highlight that there are internal physiological controls on carbon allocation that may limit water uptake from different soil layers. During periods of high vapour pressure deficit or soil drought, when leaf gas exchange and carbon assimilation decline, this may create positive feedbacks where plants are unable to forage for deep water due to carbon limitations.

Key-words: Carbon assimilation, hydrogen isotopes, oxygen isotopes, plant water sources, sap flow

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Introduction

In arid and semiarid ecosystems, water is the limiting resource for plant growth (Noy-Meir 1979). In these habitats, water sources are often characterized by deep groundwater (recharged by non-growing-season precipitation) and pulses of shallow soil water derived from growing-season precipitation. This provides an opportunity to investigate whether plants maintain shallow and deep roots for water uptake in a manner consistent with optimal allocation theory, by using experimental manipulations of plant carbon assimilation capacity at sites with different ground-water access and similar amounts of growing-season precipitation.

Allocation can be viewed in terms of optimality theory (Bloom, Chapin & Mooney 1985; Bazzaz 1997).

Plants have a finite amount of carbon to allocate to above- and below-ground functions. Many woody plant species in arid and semiarid environments develop a dimorphic root system that is capable of accessing water stored in shallow aquifers as well as the pulses of water that appear in near-surface soil layers during rain events. Because these deep and shallow roots draw on the same internal carbon, allocation patterns below-ground of woody plants in water-limited ecosystems may reflect optimal foraging for water. If so, roots should preferentially grow into water-rich patches (Fitter 1994; Williams & Snyder 2003). Specifically, in arid and semiarid regions a stable source of groundwater should encourage long-lived perennials to produce deep roots instead of shallow lateral roots, due to the ephemeral nature of growing season precipitation (Ehleringer & Dawson 1992; Williams & Ehleringer 2000).

Many studies have surveyed the natural abundance of stable isotope ratios in xylem sap to determine the fractional use of different water sources by plants (see reviews by Ehleringer & Dawson 1992; Dawson & Ehleringer 1998). Isotopic analyses of xylem sap of

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various tree species have produced mixed results as to whether long-lived woody plants respond opportunistically to precipitation (i.e. maintain roots in multiple soils) (Smith *et al.* 1991; Mensforth *et al.* 1994; Thorburn & Walker 1994; Dawson & Pate 1996; Jolly & Walker 1996; Kolb, Hart & Amundson 1997) or exist exclusively on groundwater (Dawson & Ehleringer 1991; Busch, Ingraham & Smith 1992). There are species-specific variations within functional types, and intraspecific variation in water use across habitats and precipitation gradients that appears to be related to the relative availability of different water sources (Snyder & Williams 2000; Williams & Ehleringer 2000). Also, plants may be genetically and ontogenetically constrained in their ability to develop roots for water uptake (Donovan & Ehleringer 1994; Gedroc, Connaughay & Coleman 1996; Weltzin & McPherson 1997).

There are still gaps in our knowledge on linking stable isotope ratios of plant xylem sap with plant function. Understanding how multiple stresses affect roots and water uptake from various sources is crucial if we wish to predict plant performance under changing conditions (Eissenstat *et al.* 2000). Irrigation experiments with isotopically enriched water have been used to examine the responses of plants to additional pulses of summer rainfall and nutrients (Lin, Phillips & Ehleringer 1996; Gebauer & Ehleringer 2000; Williams & Ehleringer 2000; Schwinning *et al.* 2002), but to our knowledge no study has investigated how experimental stress imposed on the plant may affect an individual's ability to extract water from different soil layers. This type of experimental manipulation may reveal internal controls on water source use. We used defoliation treatments at sites with different groundwater availability to determine if environmental and/or physiological stress would produce changes in the water source use of a long-lived woody species.

There are many natural causes of carbon loss (e.g. drought, herbivory and fire) that stimulate plants to reallocate carbon. Plant reallocation of carbon after these events may have important feedbacks with the abiotic environment. For example, during drought, leaf gas exchange becomes limited by shedding of leaves and/or reductions in leaf conductance (Schulze 1986). If prolonged drought reduces growth belowground, which in turn limits the ability of plants to extract water, this may generate positive feedbacks between drought and plant water uptake.

Trade-offs associated with producing an extensive or deep root system or maintaining small lateral roots in the upper soil layers are not well quantified (but see Fitter 1994; Dawson & Pate 1996). However, the dynamic nature of root growth and proliferation in resource-rich zones of the soil is linked to carbon assimilation by leaves (Eissenstat & Yanai 1997). Consequently, when a plant is 'stressed' by limiting carbon assimilation capacity (e.g. through defoliation) it may face trade-offs in above-ground and below-ground

growth. Below-ground response to carbon limitation can produce a variety of plant responses such as decreasing root respiration, slowing or cessation of root growth, or reallocation of carbon between roots and shoots (Eissenstat & Yanai 1997). Plants with dimorphic root systems, therefore, may have to limit root proliferation in one soil layer at the expense of root proliferation in other soil layers when carbon uptake is limited.

Prosopis velutina (Woot.), a facultative phreatophyte native to southern Arizona, USA and Sonora, Mexico, develops deep roots and shallow lateral roots. *Prosopis* species express considerable morphological and physiological variation across different habitats (Midwood *et al.* 1993; Stromberg, Wilkins & Tress 1993; Atchley, de Soyza & Whitford 1999). The first objective of this study was to determine the variation in sources of water used by natural populations of *P. velutina* occurring at two sites where the availability of groundwater differed. We predicted that *P. velutina* at a site where groundwater is more stable would favour deep roots and use water from groundwater over shallow lateral roots that access ephemeral pulses of precipitation, in comparison to *P. velutina* at a site where groundwater levels fluctuate and are less stable. The second objective was to determine how carbon limitations affect water source use; we predicted that when tissue production and maintenance is constrained by limited carbon supply from shoots, the fractional water uptake by deep roots would be enhanced at the site with stable groundwater. In contrast, we predicted that limited carbon supply at the site with unstable groundwater would favour water uptake from shallow soil layers.

Methods

SITE DESCRIPTION

Two sites, Lewis Springs (altitude 1250 m) and Escapule Wash (1207 m), 31°33′ N, 110°07′ W and 31°36′ N, 110°09′ W, respectively, were selected within the San Pedro Riparian National Conservation Area. The Lewis Springs site is along a perennial reach of the San Pedro River. Depth to groundwater is temporally stable at this site. The Escapule Wash site is on an ephemeral tributary to the San Pedro River and is characterized by variable depth to groundwater. The sites are referred to here as the perennial and ephemeral reaches, respectively.

Mean precipitation at both sites is 330 mm per year, with 60% falling between July and September and most of the remainder falling in the winter months. At each site, *P. velutina* trees were selected on secondary alluvial terraces that no longer receive flood flow. *Prosopis velutina* was the dominant woody species on both sites, and the understorey was dominated by the perennial grass *Sporobolus wrightii* Munro ex Scribn. and the woody shrub *Acacia greggii* Gray.

Oxygen isotope ratios ($^{18}O/^{16}O$) of plants, soils and groundwater were measured during the 1997 growing season to determine sources of water used by *P. velutina* at the ephemeral and perennial reach sites (Ehleringer & Dawson 1992; Brunel, Walker & Kennett-Smith 1995). Four to six trees were selected randomly at each site and sampled repeatedly (June 7–11, July 9–11, August 8–11, September 20–27) through the growing season. Plant stems approximately 0·5 cm in diameter, including bark and phloem, were collected at midday from a sunlit branch, placed in airtight glass vials, and stored in a laboratory refrigerator for subsequent analysis of $\delta^{18}O$. Soil samples for $\delta^{18}O$ and gravimetric water content (θ_g) analyses were collected from 5, 10, 25, 50 and 100 cm depths using a soil auger. Local floodplain groundwater was collected from a well at the ephemeral reach, and regional groundwater was collected from a well at the perennial reach at each sampling period. Monthly integrated precipitation samples were collected in standard rain gauges that contained a layer of mineral oil to prevent evaporation. Water was extracted from plant stems and soils by cryogenic vacuum distillation (Ehleringer & Osmond 1989; Smith *et al.* 1991). Water from plants, soils, precipitation and groundwater samples were analysed for $\delta^{18}O$ using CO_2 equilibration (Compston & Epstein 1958) on a Finnigan Delta-S isotope ratio mass spectrometer with a precision of $\pm0.08‰$ at the University of Arizona Geosciences Stable Isotope Facility. $\delta^{18}O$ values are reported relative to V-SMOW, the international standard for oxygen.

A Scholander-type pressure chamber (PMS, Corvalis, OR, USA) was used to measure predawn leaf water potentials (Ψ_{pd}) on the same days that samples were taken for isotopic analysis. To determine the percentage of xylem water derived from shallow soil layers we used a two-ended linear mixing equation of the form:

$$\delta X_p = f(\delta X_s) + (1 - f)(\delta X_{gw}) \qquad \text{eqn 1}$$

where δX_p was the measured $\delta^{18}O$ value of the plant xylem water, and δX_s was a weighted average of the $\delta^{18}O$ values of soils sampled at 10, 25 and 50 cm depths. Soil $\delta^{18}O$ values were weighted by dividing mean θ_g at each depth by the sum of θ_g at all depths. δX_{gw} was the measured $\delta^{18}O$ value of groundwater at each site. The equation was solved for f, the fraction of plant stem water obtained from shallow soil layers, and expressed as a percentage. The occasional values obtained from the mixing model that were larger than 100% were set to 100%, and negative mixing values were set to 0%. Differences in the average percentage of stem water derived from shallow soil layers between the two sites were tested for statistical significance using *t*-tests.

Repeated-measures analysis of variance in a multivariate framework (MANOVAR; $\alpha = 0.05$) was used to

analyse the effects of site and sampling date (June, July and August, September) on Ψ_{pd} in 1997 (Von Ende 1993). Student *t*-tests ($\alpha = 0.05$) were used to compare sites within sampling dates in the presence of a significant interaction between these two factors. This same analysis was used to determine the effects of site and sampling date on the percentage of shallow soil water use.

DEFOLIATION EXPERIMENT

A defoliation experiment was conducted during the growing season of 1998 at the ephemeral and perennial reach sites. Twenty trees were selected within a large rectangular plot (approximately 120×25 m) at each site, and assigned randomly to defoliation and control treatments. Selected trees were 1–2 m tall on flat ground. The defoliation treatment consisted of hand removal of all leaves at weekly or biweekly intervals, depending on the amount of regrowth. Leaf removal began in June just after the first leaf flush, and continued through the growing season until early August. Removed leaves were collected and deposited off-site. Ten of the 20 trees were undefoliated controls.

During the onset of the rainy season, we chose three periods when defoliation was halted for between 2 and 3 weeks until there was a natural rainfall event. This allowed defoliated trees to regrow leaves prior to isotope sampling, to permit transpirational movement of xylem sap. Trees were sampled for hydrogen isotope ratios (δD) in xylem sap 4–6 days after natural rainfall events in July, August and September, to determine the fractional use of shallow soil water and groundwater. Stems, precipitation and regional groundwater were collected for isotopic analysis as above. Soils for isotopic analysis and gravimetric soil moisture were collected using a hand auger from 5, 10, 25, 50 and 100 cm depths in July; 10, 25, 50 and 75 cm in August; and 10, 20 and 30 cm depths in September. Soils were sampled from a variety of depths in an effort to minimize the number of samples collected for accurate determination of water source use. Summer precipitation rarely percolates below 50 cm in these systems (Scott *et al.* 2000), so because storm events were smaller, samples were taken from fewer depths in September. Hydrogen isotope ratios rather than $\delta^{18}O$ were measured in this experiment, to be consistent with the deuterium-labelling experiment (described below).

We irrigated with isotopically enriched water to trace that pulse over a longer period, to control for potential differences in precipitation quantity and isotopic ratios between sites, and to enable comparisons between non-irrigated and irrigated trees. Irrigation water was applied during the third week of August; five trees at the ephemeral reach and four trees at the perennial reach in each treatment group (defoliated and undefoliated) were irrigated with 305 l each of deuterium-labelled irrigation water ($\delta D = 66‰$, $\delta^{18}O = -9.0‰$), while the reminder of the trees were left as

non-irrigated controls. Water was applied evenly in 5 m diameter circles around target plants. The irrigation was equivalent to a 14 mm rainfall event. Trees and soils were sampled just prior to irrigation, and 2, 4 and 6 days afterwards. Soils were sampled near irrigated plants from 10, 20, 25, 50 and 75 cm depths just prior to irrigation and 2 days after irrigation, and from 10 and 20 cm depths 4 days after irrigation. Sap flow through stems was measured continuously with heat balance collars on irrigated plants (Dynamax Inc., Houston, TX, USA), and predawn leaf water potential was assessed as above on all plants.

Plant, soil and groundwater samples in 1998 were analysed for hydrogen isotope ratios using a dual-inlet isotope ratio mass spectrometer with a precision of ±0·9‰ (Delta-S, Finnigan, Bremen, Germany). A chromium reduction furnace attached online to the mass spectrometer was used to convert liquid water to hydrogen gas (HD-Device, Finnigan-MAT, Bremen, Germany). δD values are reported relative to V-SMOW, the international standard for hydrogen. A subset of the 1998 samples was analysed for $\delta^{18}O$ (see above).

The percentage of plant water derived from shallow soil layers was determined with the two-compartment linear mixing equation (equation 1), however, δD rather than $\delta^{18}O$ values were used. Soils were sampled at various depths in 1998, but for consistency in the mixing models only soils sampled from the 10, 25 and 50 cm depths were used in the weighted soil average. In September, due to sampling limitations, soils sampled from 30 cm depth were substituted for 25 cm. Trees at the ephemeral reach appeared to have access to regional groundwater during 1998, as evidenced by the δD of tree xylem sap during dry periods, therefore regional groundwater was considered the main groundwater source at both sites. Sap flow per unit sapwood area, measured 2 days following irrigation in August, was partitioned between deep and shallow sources using results of the mixing model. Sap flow rates were summed over the entire day and multiplied by the proportion of water derived from shallow and deep soil to obtain the absolute volume of water extracted from these soil layers.

Two-way ANOVA was used to assess the effects of site and defoliation at each sampling date in 1998 on the percentage of shallow soil water use calculated with the two-compartment mixing model. If there was a significant interaction between site and defoliation, contrasts ($\alpha = 0·05$) were used to determine if a treatment (defoliation or control) had a similar effect on the percentage of shallow soil water used by trees across sites.

Repeated-measures analysis of variance in a multivariate framework (MANOVAR) was used to determine the effects of defoliation and irrigation on δD of P. velutina xylem sap at each site during the irrigation experiment. ANOVA was used to determine the effect of defoliation and irrigation within a site on initial Ψ_{pd} and 2 days after irrigation (post-Ψ_{pd}). The difference

between initial and post-Ψ_{pd} was used as the response variable, to avoid violating the assumptions of independence. Differences between sites in Ψ_{pd} before irrigation were assessed using ANOVA with site and defoliation as factors.

Data were transformed as necessary to meet the assumptions of MANOVAR and ANOVA based on inspection of residual plots. Non-transformed values are presented in the figures and text.

Results

1997 WATER SOURCE STUDY

The $\delta^{18}O$ value of regional groundwater at the perennial reach was −9·7 ± 0·01‰ throughout the 1997 growing season (Fig. 1). The $\delta^{18}O$ value of groundwater at the ephemeral reach site was also stable throughout much of the growing season ($\delta^{18}O = -8·4 ± 0·1‰$), but in September shifted to −7·8‰ due to recharge of the shallow aquifer by evaporatively enriched streamflow generated by monsoon precipitation. Predawn water potentials (Ψ_{pd}) of P. velutina at the ephemeral reach site were more negative than at the perennial
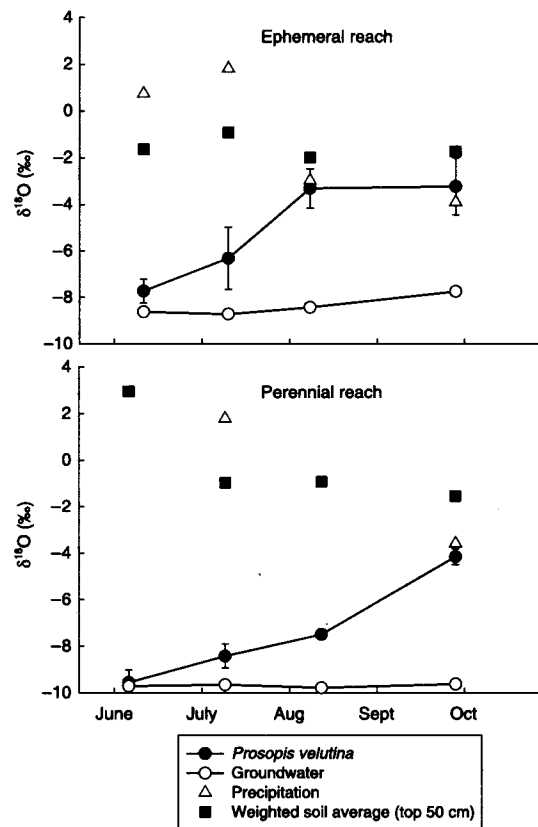

Fig. 1. Mean $\delta^{18}O$ (±1 SE) values of Prosopis velutina sampled in 1997 on secondary terraces along a perennial reach and ephemeral tributary of the San Pedro River, Arizona. $\delta^{18}O$ values of floodplain groundwater, soil (average of top 50 cm values weighted by gravimetric soil moisture) and rainfall (when available) sampled throughout the growing season are shown.
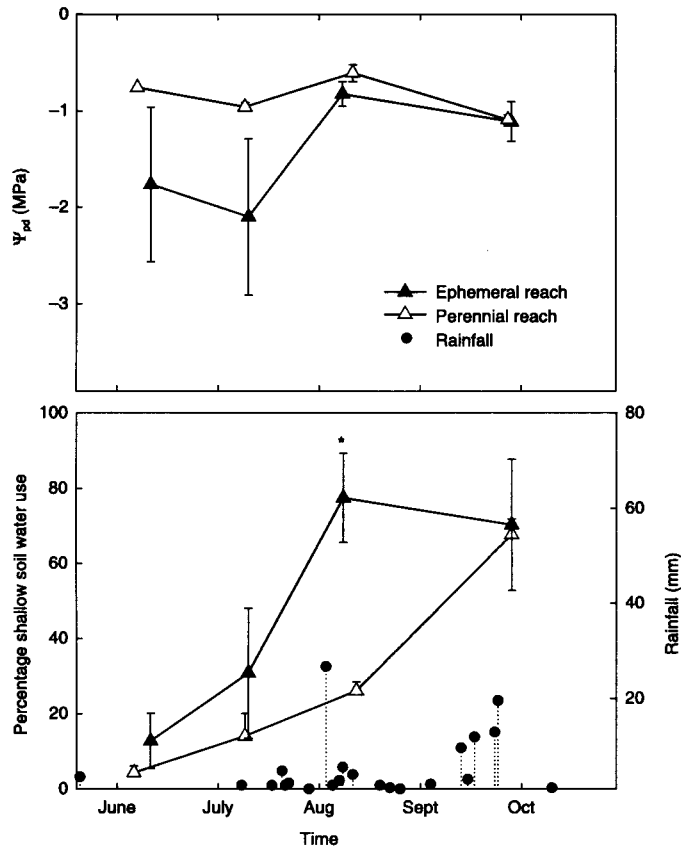
Fig. 2. Mean (±1 SE) Ψ_{pd} and percentage of shallow soil water use by *Prosopis velutina* throughout the 1997 growing season. Percentage shallow soil water use was calculated using a two-compartment linear mixing model; asterisks indicate differences between sites using t-tests. Rainfall amounts (mm) and timing are shown in the bottom panel (●).

reach site (MANOVAR, $P = 0.07$), indicating that access to groundwater was more limited at the ephemeral than at the perennial reach site. The difference in Ψ_{pd} was reduced after the onset of summer rains (Fig. 2).

There was a significant interaction between site and sampling date on the percentage of shallow soil water used (MANOVAR, $P = 0.007$). Therefore differences between sites within a sampling date were assessed using t-tests. The percentage of xylem water derived from shallow soil layers did not differ between trees at the two sites in June, July and September ($P > 0.19$). Site differences were present in August following the period of greatest water stress at the ephemeral reach site ($P = 0.0005$). During the dry period between June and July, $\delta^{18}O$ values of *P. velutina* xylem sap resembled groundwater (Fig. 1), and results of the mixing model indicate that less than 30% of the water used by trees was derived from shallow soil layers (Fig. 2). The $\delta^{18}O$ values of tree xylem sap increased at both sites in August, shifting toward that of summer rainfall (Fig. 1). Trees along the ephemeral reach had a greater shift in $\delta^{18}O$, suggesting that they obtained a greater percentage of their water from shallow soil than those at the perennial reach. By September, more than 60% of tree water was derived from shallow soil at both sites.

DEFOLIATION EXPERIMENT

Uptake of natural rainfall

Total rainfall for the 7 days preceding sampling in July, August and September 1998 was 31, 91 and 12 mm; and 28, 63 and 14 mm, at the perennial and ephemeral reaches, respectively. Gravimetric soil water contents at all depths were similar at the two sites in July, but soil at the perennial reach was wetter than at the ephemeral reach later in the season (Fig. 3). A large rain event in August and the small rain event in September affected soil moisture less at the ephemeral reach. Soils at the ephemeral reach are coarser textured than those at the perennial reach, which may explain this difference. Data from previous years indicate that gravimetric soil moisture content is generally less at this site, and during dry periods may decline to 2% (Snyder & Williams 2000). The difference in soil moisture at 10 cm relative to that at 25 cm is approximately the same magnitude at both sites during all sampling periods, and δD values at 10 cm are similar to those of rainfall. It is reasonable to assume that rainfall increased soil water availability and created another potential source of plant water. Due to unusual weather patterns which resulted in relatively cold rainfall, the δD of rainfall in July and September resembled groundwater and was characteristic of winter rainfall. However, due to residual water stored in the upper 50 cm of soil, δD of shallow soil was typically evaporatively enriched and isotopically distinct from deeper soil layers.

δD values of stems on defoliated trees at both sites following natural rainfall events were similar to those of shallow soil water. δD values of undefoliated trees resembled groundwater δD in July and September, but shifted towards shallow soil water in August (Fig. 4). The percentage of water derived from shallow soil layers was altered by defoliation. In general, defoliated trees used a greater percentage of shallow soil water than undefoliated trees (Fig. 5). However, the percentage of shallow water used by trees varied by site in July and August, producing a significant interaction (ANOVA, $P < 0.05$). The effect of defoliation on trees at the perennial reach site became more pronounced as the growing season progressed. Initially in July, defoliated trees at the perennial reach used a smaller percentage of shallow soil water than defoliated trees at the ephemeral reach (contrasts, $P < 0.0004$). By August, after continued defoliation treatment defoliated trees at both sites used a similar percentage of shallow soil water (contrasts, $P > 0.11$). By September there was only an effect of defoliation, with defoliated trees using a greater percentage of shallow soil water than undefoliated controls at both sites (ANOVA, $P < 0.0001$). Undefoliated trees at both sites used similar percentages of shallow soil water after rain events in July and August (contrasts, $P > 0.12$) and in September (ANOVA, $P < 0.0001$).
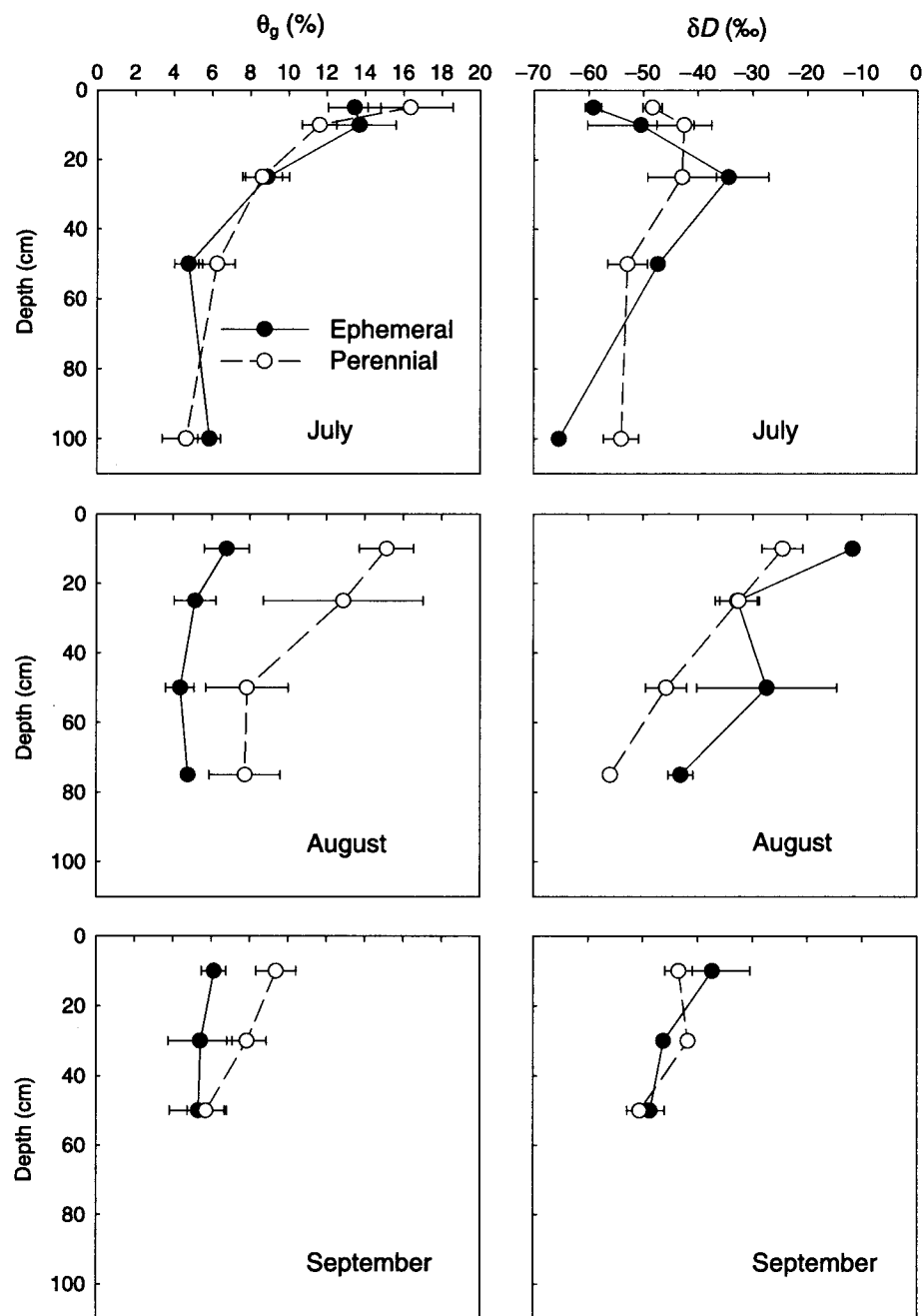
Fig. 3. Mean gravimetric soil moisture contents (±1 SE) and mean δD values (±1 SE) of soils after natural rainfall events.

Uptake of irrigation water

Irrigation wetted the upper 10 cm of soil at the perennial reach and penetrated to 20 cm at the ephemeral reach (Fig. 6). A 12‰ increase in the soil δD value was observed in the 10 cm soil layer at both sites. We analysed data from the two sites separately because of a three-way statistical interaction between defoliation, site and time ($P = 0.047$). Plots of $\delta^{18}O$–δD (Clark & Fritz 1998) were used to evaluate whether changes in δD resulted from evaporative enrichment in stems, rather than from uptake of the label (Lin, Phillips & Ehleringer 1996). There was no evidence of evaporative enrichment (data not shown), therefore changes in δD values are indicators of water source use.

At the ephemeral reach, undefoliated trees clearly responded to irrigation water, with increased δD values 2 and 4 days after irrigation compared with non-irrigated controls (Fig. 7). However, defoliated trees did not respond specifically to the irrigation water, because δD of non-irrigated trees indicated a similar reliance on shallow soil water to that of irrigated trees. Defoliated trees had consistently more positive δD values throughout the 6-day period, as did defoliated trees that did not receive irrigation. This was reflected in a significant main effect of leaf treatment, with defoliated trees having more positive δD values throughout the irrigation experiment, indicating that these trees derived a greater percentage of water from shallow soil ($P = 0.002$). There was a main effect of irrigation on
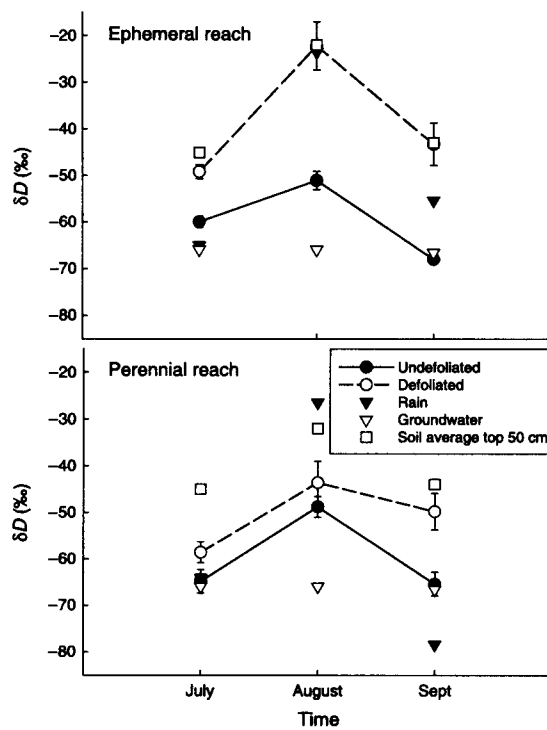
Fig. 4. Mean δD (± 1 SE) values of *Prosopis velutina* xylem sap of undefoliated trees and defoliated trees sampled after natural rain events in 1998. δD values of groundwater, precipitation and the upper 50 cm of soil weighted by gravimetric soil moisture are also shown.

the δD of *P. velutina* at the ephemeral reach ($P = 0.02$); irrigated trees obtained a greater percentage of their water from shallow soil layers compared to non-irrigated plants, but this pattern was driven mainly by the strong response to irrigation of undefoliated trees.

Uptake of the labelled irrigation water at the perennial reach was masked by the large natural rainfall event (91 mm) that preceded the watering treatment.
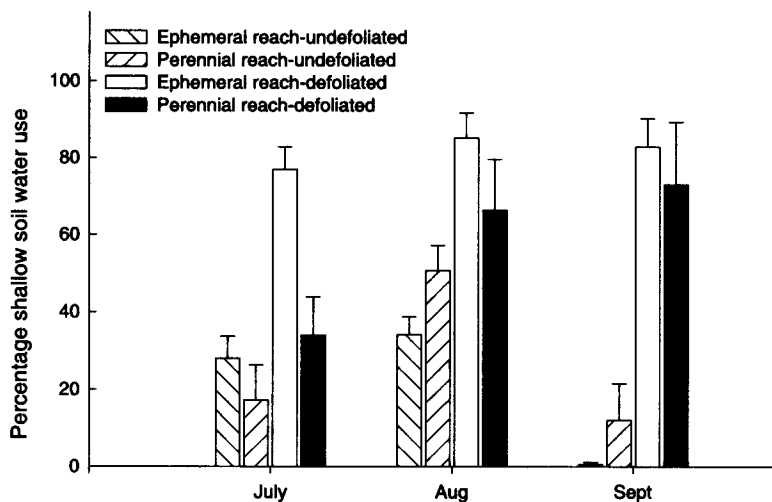

Fig. 5. Percentage shallow soil water use from the upper 50 cm of soil calculated with a two-compartment linear mixing model for defoliated and undefoliated *Prosopis velutina* trees in 1998.

δD values for all trees at this site increased from day 0 to day 2. However, defoliated trees maintained more positive δD values than undefoliated trees as the experiment progressed (until day 6; $P < 0.02$), suggesting that defoliated trees had diminished use of deeper water or used shallow water more slowly.

Defoliated trees at the ephemeral reach had the lowest sap flow rates of all trees at both sites (Fig. 8). Defoliation at the perennial reach reduced sap flow rates, but not as dramatically as at the ephemeral reach. To compare the response of irrigated trees at the different sites, the percentage of water derived from shallow soil water was calculated with the mixing model 2 days after irrigation. Defoliated and undefoliated trees that received irrigation derived similar percentages of their water from shallow soil layers 2 days after irrigation ($P > 0.44$; Fig. 8, values in parentheses). Defoliation did reduce the absolute amount of water derived from deep and shallow water sources (Fig. 8).

Ψ_{pd} of trees just prior to irrigation was affected only by site, not by defoliation treatment, and was more negative at the ephemeral reach (-1.0 ± 0.05 MPa) than at the perennial reach (-0.8 ± 0.04 MPa) ($P < 0.003$). Ψ_{pd} exhibited a slight response to irrigation at the ephemeral reach; Ψ_{pd} of irrigated trees 2 days after irrigation was less negative than that of non-irrigated trees ($P < 0.09$, data not shown). There was no effect of defoliation, watering, or their interaction on Ψ_{pd} at the perennial reach.

Discussion

We hypothesized that vertical patterns of resource availability and short-term carbon dynamics account for the substantial interpopulation variation in water sources used by perennial woody plants in arid and semiarid environments. We predicted that *P. velutina* would: (1) favour activity of deep roots at a site where groundwater was more available as a source for transpiration; and (2) enhance water uptake by deep roots when the amount of labile carbon was limited by defoliation.

Vertical patterns of water uptake at the two sites used in this study varied with the availability of groundwater during the 1997 growing season (Fig. 2). These data are consistent with previous data for this species, and for *Populus fremontii* sampled along the San Pedro but at locations within the primary flood-plains that had shallower depths to groundwater (Snyder & Williams 2000). Natural patterns of water use illuminated site-specific differences and provided evidence that differences in plant water use might be expected at these two sites. However, there were no significant site differences for the control trees after natural rainfall in 1998 (Fig. 4). Different trees were sampled in 1998, so it is not clear if interannual variability or variations in individual tree locations are responsible.
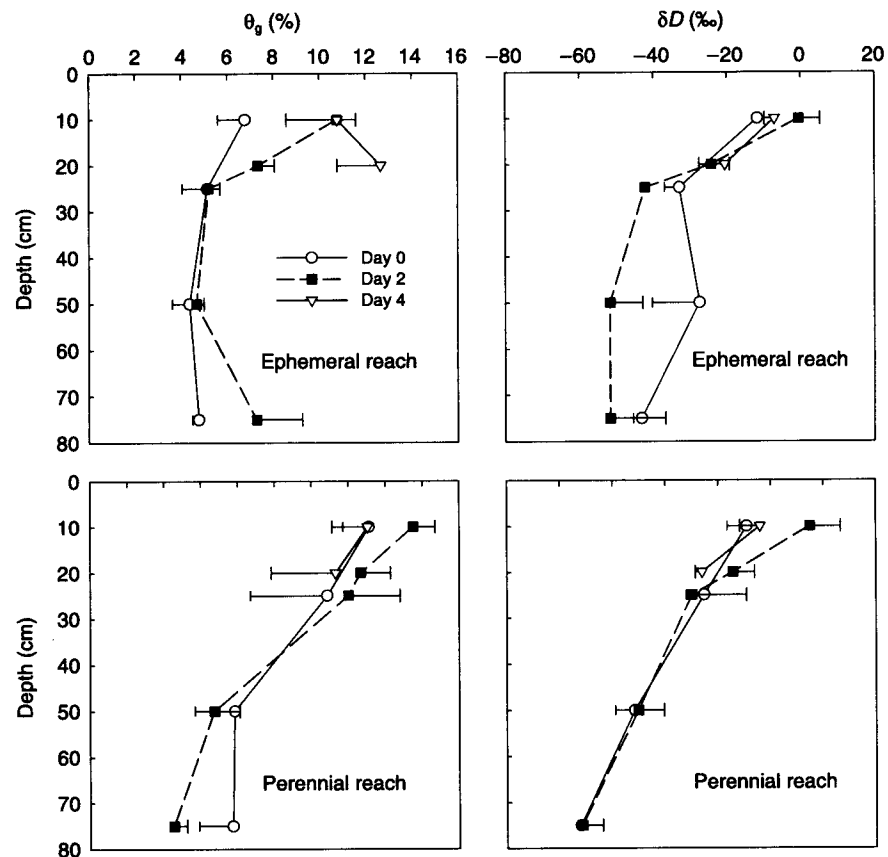
Fig. 6. Mean gravimetric soil moisture contents (±1 SE) and mean δD values (±1 SE) of soils sampled at 10, 20, 25, 50 and 75 cm depths. Soils were sampled just prior to irrigation (day 0) and 2 and 4 days after irrigation with deuterium-labelled water under watered trees.
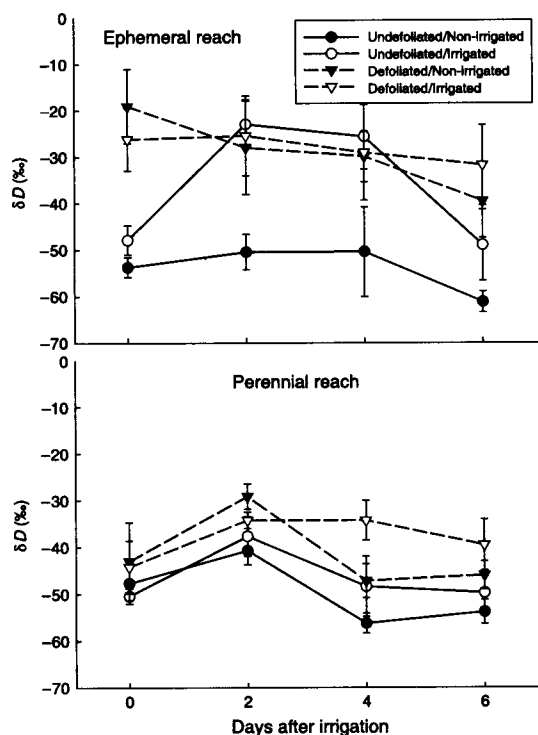
Fig. 7. Mean δD (±1 SE) values of *Prosopis velutina* xylem sap sampled just prior to irrigation (day 0) and 2, 4 and 6 days after irrigation with deuterium-labelled water.

Our results were surprising in terms of the second prediction. Defoliation promoted use of shallow soil water, even at a site where groundwater was stable (Fig. 4). Both defoliated and undefoliated *P. velutina* used water from shallow soil (Fig. 7). Defoliation reduced deep groundwater use by *P. velutina*. Defoliated *P. velutina* consistently took up fractionally more of its water from shallow soil layers than did undefoliated plants at both sites, suggesting that short-term carbon pools in plants can rapidly alter water source use. Defoliation appeared to have an increasing impact on water source use of trees throughout the season at the perennial reach, in contrast to the fast response of trees at the ephemeral reach. This is consistent with our field observations of leaf regrowth. Trees at the ephemeral reach had less regrowth and required less frequent defoliation. At the perennial reach, trees required more frequent defoliation initially, but after repeated defoliation leaf regrowth slowed. This may be because trees with less access to groundwater had smaller carbon reserves and/or decreased carbon assimilation, and therefore the effects of defoliation were more immediate.

There are several potential explanations for the observed effects of defoliation on water source use. It is possible that the water potential gradient from soil to leaf in undefoliated plants may have been insufficient
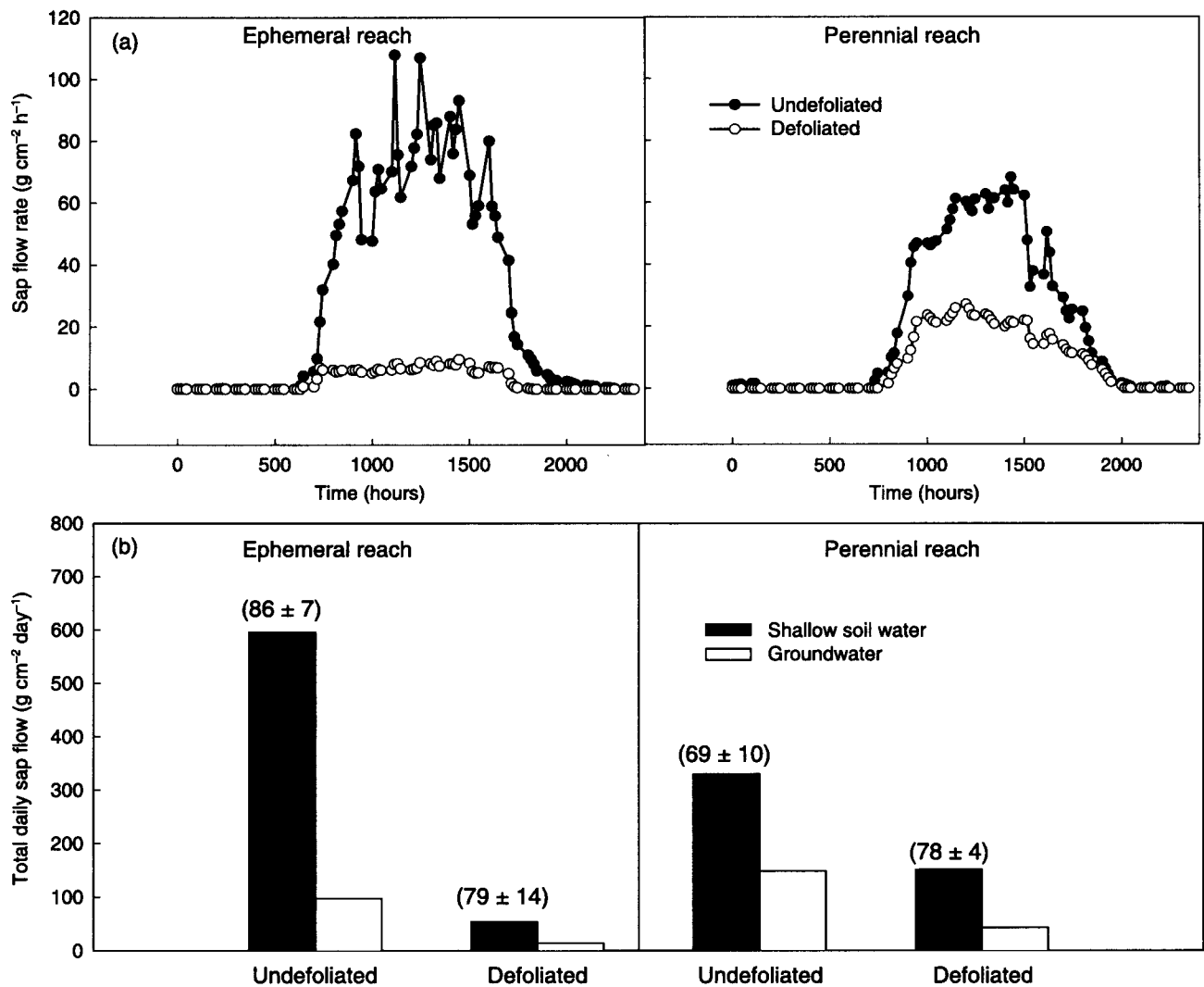
Fig. 8. (a) Daily course of sap flow rate per unit sapwood area of *Prosopis velutina* measured along the ephemeral reach 2 days after irrigation (date of year (DOY) = 233) and along the perennial reach 1 day after irrigation (DOY = 231) in 1998. (b) Amount of total daily sap flow derived from groundwater and soil water in August 1998 for two populations of *P. velutina* after irrigation with deuterium-labelled water. Data are expressed as transpiration flux (g H_2O per unit xylem area per unit time) at the scale of single stems, rather than for whole canopies or per unit ground area. Values were derived by summing sap flow rates over an entire day and multiplying by the percentage of water used from deep and shallow layers derived from isotopic data and the two-compartment linear mixing model. Values in parentheses are mean percentage shallow soil water use (±1 SE) calculated with the two-compartment linear mixing model for irrigated trees. There was no significant effect of site or defoliation or their interaction on percentage shallow soil water used by trees (ANOVA, $P > 0.44$).

to pull water from deep groundwater. Groundwater depth was around 10 m, which requires a minimum gradient of 0·1 MPa to overcome gravitational force (Nobel 1991). However, midday water potentials measured on basal shoots in defoliated and undefoliated trees were more than sufficient to establish the necessary Ψ gradient to overcome this gravitational force. Therefore it is unlikely that this alone accounted for any shift in water source use between defoliated and undefoliated plants.

Alternatively, the effect of reduced photosynthetic assimilation caused by defoliation may have been manifested first in deep roots. In this study, it may be that fractional water use from deep soil layers declined because the proliferation or maintenance of deep roots near groundwater may have been preferentially reduced in comparison to shallow lateral roots. *Prosopis*

velutina exists in a variety of habitats, including more xeric uplands where deep groundwater is not available, therefore this species may be adapted to retaining shallow roots during periods of water shortage. We can only speculate about why shallow roots might be retained preferentially; optimality theory predicts greater root longevity in resource-poor environments (Eissenstat & Yanai 1997; Espeleta & Donovan 2002). How this relates to roots of a single plant remains to be explored. There may be similarities with roots in resource-poor layers having slower turnover than roots in resource-rich layers, and this may be a function of carbon costs. Different carbon costs (Poorter & Villar 1997) may be associated with different root types. More costly root tissue necessitates maintaining roots for a longer period to maximize the ratio of costs and benefits (Hunt, Zakir & Nobel 1987). In a separate

study from the same region, the construction costs of shallow lateral roots for three obligate and two facultative phreatophytes were measured (Williams & Snyder 2003). Because shallow water use was greater in facultative phreatophytes, it was predicted that construction costs of shallow lateral roots would be smaller than those of obligate phreatophytes. This is analogous to the ephemeral rain roots produced by cacti that allow these species to exploit ephemeral pulses of shallow soil moisture (Nobel, Alm & Cavalier 1992). Contrary to this prediction, shallow root costs of facultative phreatophytes, including *P. velutina*, were greater than those of obligate phreatophytes. The costs and benefits of dimorphic root systems, especially in N_2-fixing species such as *Prosopis*, need to be investigated further.

An alternative explanation is that all new root growth may have been inhibited by defoliation (Eissenstat & Duncan 1992), and the reduction in deep root growth was sufficient to disconnect these plants from groundwater, while shallow roots remained in contact with soil water. Research on rooting patterns has found that this species, while capable of growing roots to great depths (Phillips 1963), generally is rooted just into the capillary zone above the water table (Stromberg 1993). Therefore reduced root growth in a potentially limited capillary fringe could disconnect these trees from groundwater. In addition, deep roots are more susceptible to cavitation at less negative water potentials than shallow roots (Jackson, Sperry & Dawson 2000). Consequently, disconnection from the capillary fringe may result in cavitation of tap roots, thereby reducing their ability to conduct water.

Finally, defoliation may have reduced plant demand for water but increased the demand for nutrients to continually rebuild leaves. Therefore plants may have maintained activity of surface roots not for water uptake, but for uptake of other resources concentrated in shallow soil layers.

Ψ_{pd} indicated site differences, but did not appear to be a good indicator of shallow soil water use because plants used shallow soil water without changes in Ψ_{pd}. This result could be a function of *P. velutina* having roots that span multiple soil layers and having at least limited access to groundwater. Split-pot experiments with walnut saplings found only 20% of the soil volume needed to be well-watered for plant water potentials to recover to levels similar to that of wet soil (Améglio *et al.* 1999). Although the specific root fraction is likely to vary by species and with absolute soil moisture, it appears that for *P. velutina* maintenance of roots in multiple soil layers may buffer changes in Ψ_{pd}.

Results from the irrigation experiment indicated that all plants used similar percentages, but different volumes of shallow soil water (Fig. 8). However, results after natural rainfall events were quite different, suggesting that undefoliated plants did not use shallow soil water. Our transpiration data indicate that this may be explained by the rate of soil water extraction

and sampling date. Undefoliated plants had greater transpiration rates and used the irrigation water more rapidly, returning to groundwater use by day 4 or 6 at the perennial and ephemeral site, respectively (Fig. 7). This site difference is probably due to the coarser textured soil at the ephemeral reach that allowed for deeper percolation of irrigation water. As we sampled 4–6 days after natural rainfall events, it may be that we missed the uptake of the smaller rainfall events in July and September by sampling after the shallow soil water had already been extracted. In August we sampled 4 days after a rain event, but the rain event was much larger and non-defoliated plants exhibited greater use of shallow soil water. These data indicate the importance of sampling date for isotopic studies, and also highlight that the isotopic composition of xylem water is highly variable over short time scales.

When plants are water-limited, either by atmospheric drought (high vapour pressure deficits) or soil drought, gas exchange is limited by declining stomatal conductance (Schulze 1986). If plants become carbon-limited, certain water sources may be accessed preferentially instead of foraging across all soil layers. This could have a positive feedback on plant water stress, if plants reduce root activity in layers where soil moisture is relatively stable, as well having effects on ecosystem hydrological functioning. Defoliation reduced sap flow in *P. velutina*, but a greater fraction of water was derived from shallow soil water compared with undefoliated trees. These results suggest that the function of deep roots was influenced more than shallow roots when photosynthate pools were diminished by defoliation. Manipulation of carbon uptake capacity at the canopy level by defoliation produced significant changes in the sources of water used by *P. velutina*, indicating that shifts in sources of water can be expressed rapidly in response to carbon limitation. These data suggest that changes in isotopic ratios of plant xylem sap need to be interpreted not only in terms of changes in the abiotic environment, but also in terms of internal physiological controls that may limit water uptake from different soil layers.

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