Oecologia
DOI 10.1007/s00442-007-0745-8

ECOPHYSIOLOGY

# Linking water uptake with rooting patterns in grassland species

**Jesse B. Nippert · Alan K. Knapp**

Received: 20 April 2006 / Accepted: 28 March 2007


**Abstract** Water availability strongly governs grassland primary productivity, yet this resource varies dramatically in time (seasonally) and space (with soil depth and topography). It has long been assumed that co-occurring species differ in their partitioning of water use by depth, but direct evidence is lacking. We report data from two growing seasons (2004–2005) in which we measured the isotopic signature of plant xylem water from seven species (including $C_3$ forbs and shrubs and $C_4$ grasses) growing along a topographic gradient at the Konza Prairie Biological Station. Plant xylem stable oxygen isotope ratio ($\delta^{18}O$) values were compared to soil water $\delta^{18}O$ profiles, recent rainfall events, and groundwater. Species varied in both their temporal patterns of water use and their responses to seasonal droughts in both years. During wet periods, species differences in water use were minimal, with common dependency on recent rainfall events stored in the upper soil layers. However, during dry periods, most $C_3$ species used proportionally more water from deeper portions of the soil profile relative to the $C_4$ grasses. Plants in uplands used more shallow soil water compared to those in lowlands, with the greatest differences across the topographic gradient occurring during dry periods. While the documented vertical root distribution varies by species and growth form in this grassland, each of the species we measured appeared to compete for the same surface layer soil moisture when water was not limiting. Thus, our results suggest that variation in precipitation history and landscape positions are greater determinants of water-use patterns than would be expected based on absolute rooting depth.

**Keywords** $C_3$ plants · $C_4$ plants · Stable oxygen isotope ratio · Tallgrass prairie · Mixing model

Communicated by Zoe Cardon.

A. K. Knapp
Department of Biology, Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO 80523, USA

J. B. Nippert (✉)
Division of Biology, Kansas State University, Manhattan, KS 66506, USA
e-mail: gostate@ku.edu

## Introduction

Much of our present-day ecological understanding of grassland root structure and function is based on the seminal research of Prof. John Weaver and his students. From the 1920s to 1950s, Weaver mapped root distribution by depth of grassland plant assemblages from Iowa to Kansas (Weaver and Albertson 1943). Weaver determined that 65% of ''true prairie'' species (tallgrass) had rooting depths greater than 1.5 m (up to 7 m deep), and of these deep-rooted species, only 20% relied on shallower (<1.5 m) soil for water and nutrients (Weaver 1966). The ability of forb populations to persist through prolonged droughts reinforced his speculation that sub-dominant $C_3$ species must rely on deeper soil water sources than co-occurring grass species (Weaver 1966). After decades of research, Weaver concluded that the vertical layering of roots within the soil profile permitted the co-occurrence of many species in the tallgrass prairie (Weaver 1966).

Understanding landscape patterns of species occurrence requires information on functional root distribution and the responses of species or growth forms to environmental drivers that vary by soil depth, landscape position, and time (Turner and Knapp 1996). $C_4$ grasses, which are the dominant growth form in tallgrass prairie (Smith and

Knapp 2003), concentrate total root biomass in the shallow soil layers (0–10 cm), but can have roots to depths > 2 m (Albertson 1937). Shrub and forb species also concentrate roots in surface soil layers (0–30 cm) and have reduced root densities throughout the soil profile compared to grasses, but have greater root diameters and a greater proportion of roots at depth (up to 3 m) (Albertson 1937; Weaver 1954; Turner et al. 1995; Sun et al. 1997). Differences in the proportion of root biomass by depth may permit $C_3$ herbaceous and woody species to supplement total water used with deeper soil water during dry periods and use shallow soil water after rain events (Boutton et al. 1999).

Despite the recognized importance of water and its influence on patterns of aboveground net primary production (ANPP) in grasslands (Knapp and Smith 2001), ecological studies addressing belowground plant activity and functional rooting depth are rare, primarily because of the difficulty in assessing root growth, turnover and activity (Polley et al. 1992; Craine et al. 2002). An alternate approach to soil excavation and root mapping for inferring functional rooting depth and resource use has been to measure stable isotopic signatures of water. Natural fractionation alters the stable isotopic composition of water as a function of season, location, rainfall event size, and evaporative demand (see Ehleringer and Dawson 1992). These effects lead to an isotopic signature of plant xylem water that is unique to the source from which the water was acquired (White et al. 1985). This technique has several advantages over descriptive analyses of rooting depth. Previous research has shown that the occurrence of live roots at a particular depth does not equate to uptake of water by those roots, regardless of whether the water supply is deep (Thorburn and Ehleringer 1995) or shallow (Dawson and Ehleringer 1991). Similarly, comparisons of water uptake under controlled conditions (growth chamber or pot studies) often fail to incorporate competitive ecological interactions that are common in plant communities (Chapin 1991; Craine et al. 2002). The use of the stable isotope ratios of water allows for interpretation of water uptake among natural community assemblages in relation to precipitation history, providing a direct assessment of water uptake among co-existing species.

Our goal was to link belowground structure, as documented by Weaver nearly a century ago (Table 1), with function (water uptake). Specifically, we hypothesized that if species differences in water uptake exist within the tallgrass prairie, these would vary according to temporal patterns in precipitation and with patterns of soil wetting and drying. Additionally, because grass species have greater total belowground biomass in the upper soil layers compared to other grassland species (Canadell et al. 1996), we proposed that grasses would consistently rely more on soil water in the shallowest soil layers compared to $C_3$ forbs and shrubs. If $C_3$ species have greater reliance on deeper (>30 cm) soil water than the $C_4$ grasses regardless of time period, this response may reduce belowground competition, permitting $C_3$ forb and shrub species to persist over time within an ecosystem dominated by $C_4$ species. Greater reliance on deeper soil moisture by $C_3$ species would also support Weaver's observations of $C_3$ persistence through drought when $C_4$ species were previously lost from this ecosystem (Weaver 1966). Finally, we predicted that differences in water-use patterns would likely be most pronounced at upland sites, which have shallower soils and experience greater evaporational demands. Therefore, species growing in uplands were expected to be more reliant on shallow soil water compared to lowland and hillside locations with deeper soils.

## Materials and methods

Research was conducted at the Konza Prairie Biological Station (KPBS), a 3,487-ha unplowed native tallgrass prairie managed within the framework of the Long-Term Ecological Research Network. KPBS is located in the Flint Hills of eastern Kansas, USA (39°05′N, 96°35′W), a region

**Table 1** Hypothesized differences in water use from different soil depths based on Weaver's published reports of maximum rooting depth for the seven species measured in this study

| Plant type | Species | Maximum root depth (m) | Citation | Predicted water use |
|---|---|---|---|---|
| $C_4$ grass | *Andropogon gerardii* | 2.13 | Weaver and Darland (1949) | Water use will be concentrated in surface soil layers (top 30 cm) |
| | *Schizachyrium scoparium* | 1.22 | | |
| | *Sorghastrum nutans* | 1.83 | | |
| $C_3$ forb | *Lespedeza capitata* | 2.74 | Weaver (1926) | Rely on surface layer water early, and deep soil during late season |
| | *Vernonia baldwinii* | 3.35 | | |
| $C_3$ shrub | *Amorpha canescens* | 4.87 | Weaver (1919) | Consistent reliance on deep soil water (>30 cm) |
| | *Ceanothus* spp. | 4.57 | Weaver and Fitzpatrick (1934) | |

characterized by a mid-continental climate consisting of cool, dry winters and warm, wet summers. The site is divided into drainage basins (watersheds) with varying strata of Permian chert-bearing shales and limestones. The effect of long-term weathering and erosion has created a non-uniform topography, with relief of 20–50 m within watersheds, consisting of flat upland ridges, steep intermediate hillsides, and lowlands with deep soils (Oviatt 1998). Soil depth varies by location with thin, rocky upland soils characteristic of the surficial Florence limestone bedrock (<0.5 m), while lowland soils are silty-clay loams (Tully soils) and can be relatively deep (>2 m) (Schimel et al. 1991; Ransom et al. 1998).

In April 2003, two randomly located transects were established in east–west directions in two annually burned, ungrazed watersheds (watersheds 1B and 1D, respectively). These watersheds were selected because annual burning results in the greatest water limitation (Briggs and Knapp 1995), and these watersheds had been burned in late April of each year for the past 24 years. Within each watershed, two transects > 100 m long were permanently marked and spanned the topographic gradient from upland to lowland. Therefore, samples were collected monthly from June to August 2004 and 2005 from four transects containing the three characteristic topographic locations on site: upland, hillside, and lowland.

## Plant sampling

Plant species composition from each sampling location was surveyed in June 2003. The seven most abundant perennial species were chosen as the target species from the 53 total species encountered over all locations. These species include three $C_4$ grasses (*Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium*), two $C_3$ forbs (*Lespedeza capitata* and *Vernonia baldwinii*) and two small $C_3$ shrubs (*Amorpha canescens* and *Ceanothus americanus*). *L. capitata* and *A. canescens* are also both legumes. Only the grass species and *A. canescens* were present at every location, but all seven species co-occurred at over half of the sampling locations (Table 2).

For the collection of plant xylem water, approximately 20–30 g of plant tissue was collected from the crown region of each species. The crown region was non-photosynthetic and is located immediately below ground level, but above the rhizomes. Due to the destructive nature of the sampling, different individuals for each species were collected for subsequent sampling periods. Samples were cut into 1- to 3-cm lengths and placed into sealed exetainer vials (Labco, UK) and immediately stored on ice until transferred to a 1–2°C refrigerator. For each grass species, each vial was a composite sample of five to 15 co-located tillers to provide enough water for the $CO_2$ equilibration process. For the forb and shrub species, one individual provided enough water per sample vial. At each sampling period, three replicates per species were collected for each replicate location and stored in separate exetainers. These replicates constitute the sampling unit. Replicate individuals for each species were collected at least 1 m apart to capture more of the variability present per location.

Xylem water was extracted from plant samples using cryogenic vacuum distillation (Ehleringer and Osmond 1989; Webb and Longstaffe 2003). To ensure that water samples did not fractionate during the extraction process, water standards were also run through the line and processed. Variation in these standards was lower than the known precision of the instrument (~0.1‰). Additionally, samples of plant tissue were routinely weighed and oven dried to ensure the extraction time was sufficient to vaporize all xylem water within the plant stem. The stable oxygen isotope ratio ($\delta^{18}O$) of the collected xylem water was measured using direct equilibration with $CO_2$ (Epstein and Mayeda 1953). Stable isotopic analyses were performed using a Finnigan Delta-plus (Bremen, Germany)

**Table 2** Species occurrence[a] along the permanent sampling transects. Sampling locations indicate the hierarchy of topographic positions [upland (*Up*), hillside (*Hill*), and lowland (*Low*)] within transects within watersheds

| Growth form | Species | 1B | | | | | | 1D | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | | 2 | | | 1 | | | 2 | | |
| | | Up | Hill | Low | Up | Hill | Low | Up | Hill | Low | Up | Hill | Low |
| $C_3$ shrub | *A. canescens* | + | + | + | + | + | + | + | + | + | + | + | + |
| | *C. americanus* | + | – | – | + | + | – | + | + | – | + | + | – |
| $C_3$ forb | *L. capitata* | + | + | + | – | + | + | + | + | + | + | – | + |
| | *V. baldwinii* | + | + | + | – | + | + | + | + | + | + | + | + |
| $C_4$ grass | *A. gerardii* | + | + | + | + | + | + | + | + | + | + | + | + |
| | *S. scoparium* | + | + | + | + | + | + | + | + | + | + | + | + |
| | *S. nutans* | + | + | + | + | + | + | + | + | + | + | + | + |

[a] Presence (+) or absence (–) for each species for each sampling location

and a Micromass VG Optima (Manchester, UK) isotope ratio mass spectrometer (IRMS) in the Stable Isotope Laboratories at Kansas State and Colorado State Universities, respectively. Analyses were cross-calibrated between locations using a subset of identical samples and variation was minimal (0.18‰). Both instruments operate in continuous-flow with peripheral gas bench microgas injectors. Isotopic abundance is expressed in $\delta$ notation as parts per mil (‰) according to:

$$\delta^{18}\mathrm{O} = [(R_{\mathrm{sample}} / R_{\mathrm{standard}}) - 1] \times 1000 \quad (1)$$

where $R_{\mathrm{sample}}$ and $R_{\mathrm{standard}}$ are the molar abundance ratios, $^{18}\mathrm{O}$:$^{16}\mathrm{O}$ of the sample and standard (Vienna standard mean ocean water), respectively. Raw values were corrected based on calibration curves developed using internal laboratory standards. The working laboratory standard (deionized water, $\delta = -7.64$) averaged (±1 SD) –7.61(0.24), and –7.62(0.26)‰ when measured within and across runs, respectively. Arbitrarily selected blind replicates were sent to the Idaho Stable Isotope Laboratory (Moscow, Idaho), and differed by <0.15‰ compared to the values obtained in our analyses.

## Soil sampling

Soil samples were collected adjacent to plant tissues using a 5-cm-diameter sliding hammer corer (AMS Samplers, USA). One soil core was taken per location/sampling date to 35 cm or the maximum depth achievable through the extremely rocky soils. Because most of the sampling locations were inaccessible by vehicle, frequent soil coring to deep depths with a hydraulic corer was not possible, and frequent digging of large soil pits was not consistent with site management plans. Therefore, to determine the $\delta^{18}$O profile of soil water at greater depth, we used a Geoprobe hydraulic direct push corer (model 6600; Geoprobe Systems, Kan.) on the north transects of watershed 1B during the final sampling period in August 2005. This instrument extracted soil samples to 2 m deep (or bedrock). Cores were immediately separated into 5-cm increments, stored in two-layer plastic bags and placed on ice. Long-term sample storage was in a freezer at –4°C until they were analyzed. The stable isotopic signature of soil water was quantified in 5-cm increments for the first 30 cm and in 10-cm increments for the remainder of the core (Fig. 1). Groundwater $\delta^{18}$O was determined from water samples collected from five wells located on site, and adjacent to the watersheds sampled (wells maintained and sampled by Dr Gwen Macpherson, University of Kansas). While the groundwater $\delta^{18}$O incorporates old and new water inputs, annual recharge on site generally results during periods of high rainfall or when the soil is frozen. Water runoff through limestone fissures and stream contributions are largely responsible for recharge without direct infiltration through the soil profile. Samples were collected from May to September and the average value (–6.5‰) is reported as the integrated meteoric water value for comparison to soil water values at this site (Figs. 1, 2).

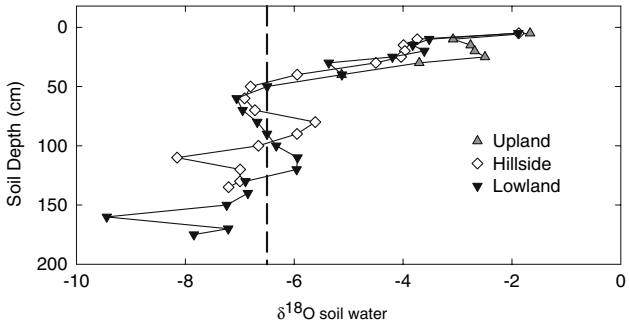


**Fig. 1** Soil water stable oxygen isotope ratio ($\delta^{18}O$) profile plots to maximum achievable depth using a hydraulic soil corer (Geoprobe Systems, Kans.). Samples were collected on the north transect of watershed 1B on 30 August 2005, concurrent to the final plant stem/soil sampling period. *Vertical dashed line* indicates the average groundwater $\delta^{18}$O and serves as the integrated meteoric water value for this site

Isotopic analyses of soil water were performed using direct equilibration techniques (Epstein and Mayeda 1953; Scrimgeour 1995). Briefly, 5 g homogenized, root-free sample was swept with a known $CO_2$/He mix and incubated at 92°F for 14 h before direct injection into the IRMS. This period of time has been shown to be adequate for complete equilibration with $CO_2$ using similar soils (Hsieh et al. 1998). Because the degree of equilibration between soil water and the headspace $CO_2$ can vary based on the gravimetric soil moisture of the original sample, our soil water signatures were corrected using regression analyses relating the isotopic signature of bulk soil at varying degrees of wetness. Textual comparison of soils from varying topographic location did not influence equilibration times, and therefore they were only adjusted for soil moisture content.

## Analyses

Statistical analyses of the data were based on a split strip-plot design, where watershed was the blocking variable and species within topographic position were replicated by transects (see Table 2). This design allowed for factor levels within a plot (e.g., all species in uplands) to overlay the levels of a separate factor level (all species in lowlands). The effects of sampling date, topographic position, species, and their interactions were used in the model to predict the isotopic signature of plant xylem water using mixed-effects models (SAS version 9.1.3; SAS Institute,

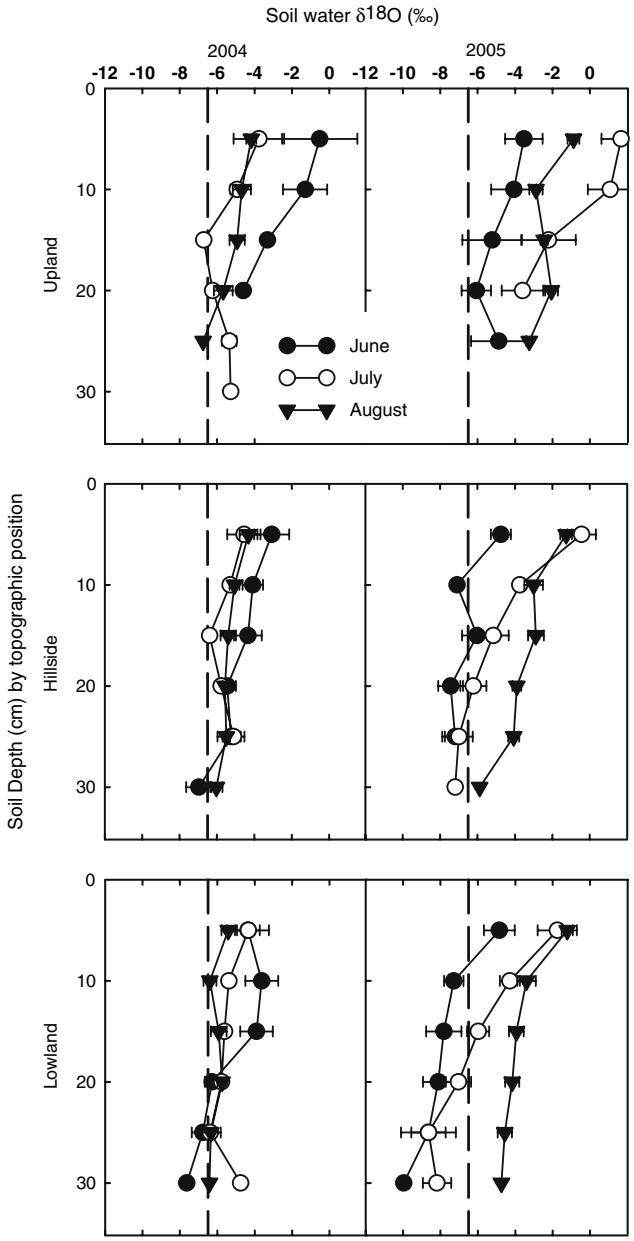


**Fig. 2** Change in the $\delta^{18}O$ values of soil water by depth for each topographic position over all sampling dates. Each point is an average value for the four transects with corresponding variance (±1 SE). *Vertical dashed lines* indicate the summer signature ($\delta^{18}O$) of groundwater collected from wells on site

Cary, N.C.). The Satterthwaite approximation was used in the estimate of denominator *df*. The random effects structure of the model included multiple separate error terms: watershed, the interaction of watershed with transect and species individually, and watershed × transect × species. Sample date was not used as a repeated measure since different individuals per species were randomly selected and sampled at successive intervals. The assumptions of homogeneity of variance and the residual normality were tested and met in all analyses.

To determine the percent contribution of soil water at depth to the signature of water within the plant's xylem, an isotopic mixing model was used (Phillips et al. 2005). For the xylem water mixture for each species × time period, we calculated all possible solutions consistent with isotopic mass balance from each soil water source (0–30 cm, in 5-cm increments) and a proxy of deep soil water. This proxy was winter precipitation (October–March: –10.3 and –10.1‰ in 2003–2004 and 2004–2005, respectively; Fig. 3). Winter precipitation infiltrates to deeper soil layers without the evapotranspirational demand common in shallow soil layers during the growing season. Because the uncertainty associated with the frequency distributions from seven contributing sources was high, we summed all feasible solutions from each model iteration for the surface soil layers (0–30 cm) using the a posteriori approach outlined by Phillips et al. (2005). Based on these new ''aggregate'' sources (0–30 cm soil and ''deep soil''), new frequency distributions were calculated in 2% increments for proportional water source use for each species × time period. The frequency distributions were normalized to make species comparisons more interpretable by multiplying each frequency by 100 and dividing by the total number of possible solutions (commonly over 45,000).

## Results

Analysis of soil water $\delta^{18}O$ values to the maximum achievable sample depth (Fig. 1), showed a progressively lighter isotopic signature of water with increasing depth. However, the majority of change in this signature occurred in the top 50 cm for all topographic positions (approximately 5‰ variation across this depth for hillside and lowland positions; Fig. 1). Of the soil cores collected to the maximum achievable depth, the profile in the top 30 cm had trends similar to samples collected over all four topographic transects at this sampling date (August 2005; Fig. 2). For shallower soil profiles sampled next to the study plants, variation in the $\delta^{18}O$ values of soil water was greater by time and by topographic position than soil depth (Fig. 2). Exceptions to this trend occurred in June 2004, and July 2005, the two driest periods sampled. Collectively, sample periods in 2005 had greater variance than 2004 due to the dry period in July, and the comparatively heavier precipitation event preceding the August sample period (rainfall $\delta^{18}O$ = –2.19‰, Fig. 3). For all topographic positions, surface soil layers had generally heavier water signatures. Signatures of soil water during the aforementioned dry periods corresponded to low gravimetric soil water content in the soil.

Statistical analyses comparing differences in plant xylem water indicated significant ($P < 0.001$) effects of

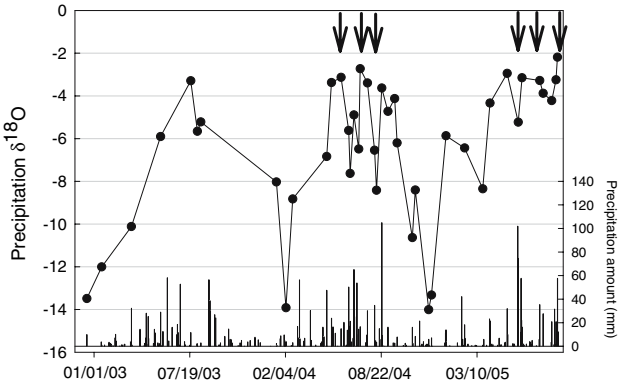


**Fig. 3** Isotopic signature of ambient precipitation events from rainfall collected on Konza Prairie Biological Station between 17 December 2002 and 25 August 2005. *Arrows* indicate sampling dates for plant stem and soil water collections. *Bar height* on the second *x*-axis corresponds to precipitation amount over time on the right *y*-axis

sampling date, topographic position and species main effects on the isotopic signature of water within the plant stems. The interactions of date × topography and date × species were also highly significant ($P < 0.001$). Henceforth, interpretations of water use and xylem water differences are expressed in terms of these two-way interactions. A significant ($P = 0.004$) three-way interaction between predictor variables was present, but did not provide any further relevance to the hypotheses tested.

The average xylem $\delta^{18}O$ values varied by nearly 5‰ over both growing seasons (Fig. 4). This variation in the xylem signature reflects the precipitation history and differences between topographic positions over time (Fig. 4). Precipitation was collected regularly on KPBS and analyzed for $\delta^{18}O$ (Fig. 3). The $\delta^{18}O$ of the rainfall exhibited clear seasonal variations that reflect the influence of temperature and storm size as ambient fractionation factors in the hydrologic cycle (Gat 1996). When analyzed by topographic position, samples collected soon after rainfall events had xylem water signatures that were more similar compared to drier periods (Fig. 4). Following several weeks with minimal rainfall, uplands had collectively heavier isotopic signatures than hillside and lowlands, respectively. Heavier signatures reflect increased evaporative enrichment of soil water resulting at upland locations from reduced plant cover and shallower soils. Plants growing in the hillside and lowland positions had similar signatures indicative of precipitation inputs and deeper water use (Fig. 4).

When patterns of xylem $\delta^{18}O$ values were analyzed by growth form, differences in the magnitude of $\delta^{18}O$ varied over time (Fig. 4). $C_4$ grass species had a collective xylem signature that was heavier than that of the $C_3$ shrubs and forbs following dry periods (June 2004, July 2005). However, following major precipitation events, xylem signatures were more similar among growth forms, suggesting greater convergence on similar water sources. The plasticity of altering water source use appears greatest during June and early July, as relative shifts in water use between growth forms were marginal from July to August of both years, despite varying precipitation patterns between 2004 and 2005 (Fig. 4).

Soil water and winter precipitation $\delta^{18}O$ values were used in an isotopic mixing model to determine water sources contributing to the $\delta^{18}O$ value of xylem water. Generally, this approach provided discrete frequency distributions for the sources of water used by species and time period (Figs. 5, 6, 7). The only exception was July 2005, when dry conditions led to more uncertainty for the $C_3$ species predictions (Figs. 6, 7). Except for the sampling performed during June 2004, water originating from the surface 30 cm was the most common source for all species (Figs. 5, 6, 7). Sampling periods following large rainfall events (4 July, 5 June, 5 August) show a high proportional dependence on surface soil water by all species (Figs. 5, 6, 7). Sampling periods conducted after several weeks of minimal rainfall show more distinct species and growth form differences in the proportional source of water used (4 June, 5 July) with $C_3$ shrubs and the $C_3$ forb *V. baldwinii* having greater proportional reliance on deep water compared to the grasses and *L. capitata*. (Figs. 6, 7). The source of water use was generally similar by species within growth forms. $C_4$ species had a greater average reliance on shallow soil water and were the least dependent on deep soil water (Fig. 5). The two $C_3$ shrubs responded similarly by sampling date, while the response of the $C_3$ forbs was more varied. *V. baldwinii* had a stronger reliance on deep soil water more similar to that of the shrubs than *L. capitata*, which responded more like the $C_4$ grasses.

## Discussion

The use of $\delta^{18}O$ values as a natural tracer to assess patterns of water use among co-occurring species is now common in ecological studies (Le Roux et al. 1995; Thorburn and Ehleringer 1995; Schulze et al. 1996). Our study used natural variation in the isotopic signature of precipitation and soil moisture to provide insight into the potential link between root function (water uptake) and previously documented patterns of root deployment in this grassland (Weaver 1966). We asked the question, do differences in root distribution among co-occurring tallgrass prairie species translate to functional differences in water use as spatial and temporal patterns in soil moisture vary?

Our results identified a range of species-dependence on soil water pools, with the most consistent differences occurring between growth forms (Fig. 4). In general, the

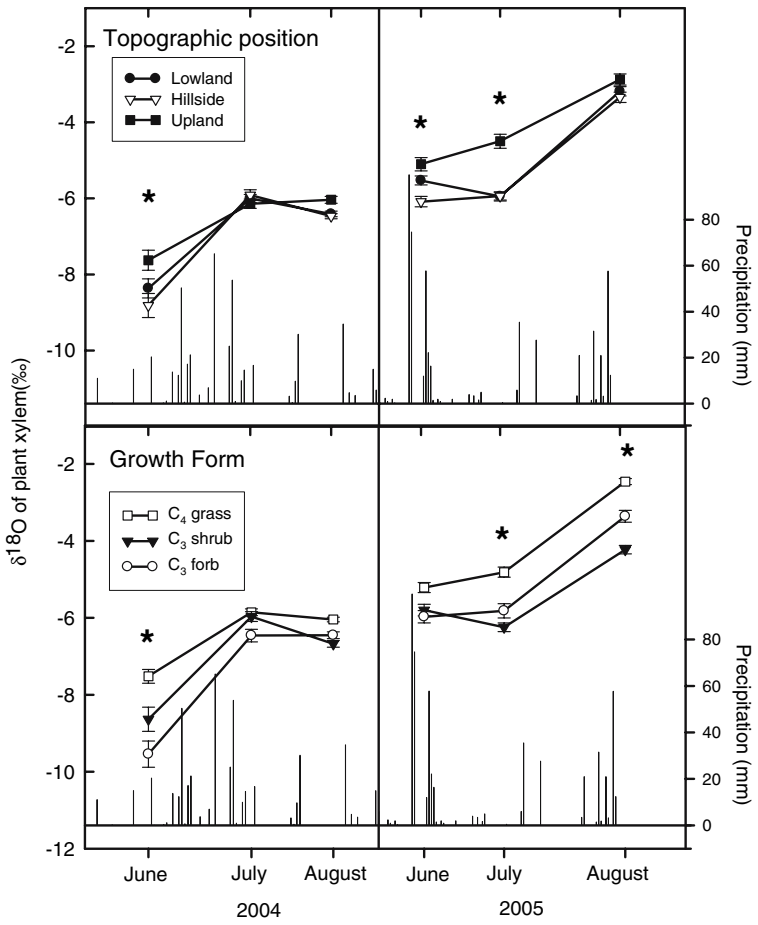


**Fig. 4** Change in the stable isotopic signature of plant xylem over time. *Top panel* reflects the average $\delta^{18}O$ value of all seven species plus error (±1 SE) by topographic position. *Bottom panel* is arranged by growth form. *Vertical bar height* is indicative of precipitation amount across day of year 2004–2005. *Asterisks* indicate a significant comparative difference using Tukey's honest significant difference test ($P < 0.05$) in the xylem $\delta^{18}O$ within a given month

$C_4$ grasses showed greater collective dependence on water in the top 30 cm of the soil profile while $C_3$ forbs and shrubs relied proportionally more on deep soil water sources (but see *L. capitata*; Fig. 7). The greatest separation by growth form occurred during dry periods (June 2004, July 2005), with overall greater reliance on soil water deeper than 30 cm as reflected by lighter stemwater $\delta^{18}O$ values in the $C_3$ shrubs and *V. baldwinii* (Figs. 4, 6, 7). Even though total water uptake and loss is likely to be low in these drought periods, this pattern suggests that when water was most limiting, the greatest degree of niche separation existed between species and growth forms.

Based on Weaver's original contention of root depth partitioning among species to minimize competition (Weaver 1966) and Walter's two-layer niche hypothesis for savannas (Walter 1971), we predicted that the $C_3$ forbs and shrubs would consistently depend on deeper water than grasses to avoid overlap for a common limiting resource. However, our results show that following major precipitation events, all species used the same surface soil water sources in similar proportions (July 2004, June, August 2005; Figs. 5, 6, 7). Distinct soil isotopic signatures existed even after large rain events and the $\delta^{18}O$ of xylem water indicates the preferential use of soil water from the 0- to 10-cm profile (compare June 2005; Figs. 2, 4). However, when water availability was reduced following prolonged dry periods (June 2004, July 2005), most $C_3$ species used proportionally more deeper soil moisture while $C_4$ grasses continued to rely predominantly on surface soil water. Inter-specific competition for water largely results from species differences in water demand and flux, root density and depth (Fitter and Hay 2002). As competition for water depletes moisture from a given portion of the soil, species must either tolerate low availability or rely upon other unexploited regions of the soil (Grime 1994). These results suggest that the potential for belowground competition for water between grassland species is highest when water is most abundant, but $C_3$ forb and shrub species are more likely to avoid direct competition with the $C_4$ species by using more water from greater depths when water becomes limiting. These patterns of reliance on soil moisture by depth and time may be key to the stable coexistence of grasses and forbs in this grassland (Weaver and Fitzpatrick 1934; Tilman 1987).

Species dependence on water use also varied by topographic position throughout the season and this pattern can

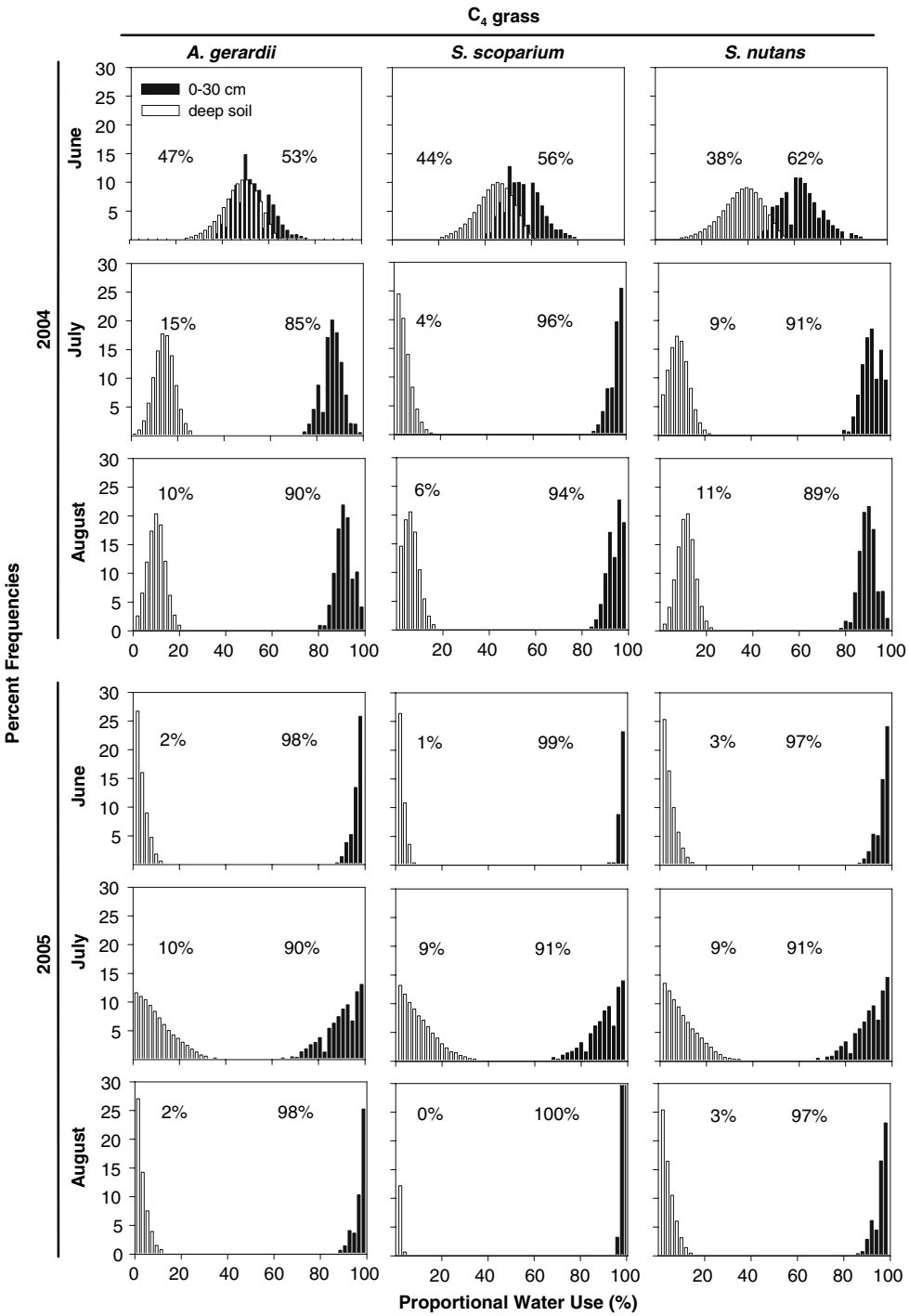


**Fig. 5** Water use proportions for $C_4$ grasses from two aggregate sources: surface soil layers (1–30 cm in 5-cm increments, *black bars*) and deep soil layers (*white bars*) inferred using winter precipitation. Distributions for aggregate sources are the sum of all solutions from the original multi-source mixing model analysis (Phillips et al. 2005). These solutions were combined a posteriori to calculate a new aggregated frequency distribution. The data are presented as percent frequency for the proportion of water used from the two combined sources for each $C_4$ species × time period. Values indicate the mean proportion of water used from each source. For species names, see Table 1

be interpreted best by considering differences in soil depth and precipitation history. Plants growing in upland positions had heavier mean plant $\delta^{18}O$ signatures than those in other positions following periodic drought, but they converged on similar water pools following rainfall (Fig. 4). Soil water $\delta^{18}O$ signatures were nearly always heavier and had lower gravimetric soil water contents in the 0- to 10-cm portion of uplands, especially during dry periods (see June 2004, July 2005). The patterns of isotopically heavier water in uplands following dry periods suggest greater dependence on evaporatively enriched surface soil. The shallow soils and increased dependence on all available water in annually burned upland prairie may contribute to the well-documented strong correlation between ANPP and precipitation amount (Knapp et al. 1993; Briggs and Knapp 1995, 2001; Nippert et al. 2006). Furthermore, species persistence and increased upland diversity may result from reduced water availability in the upper-30-cm

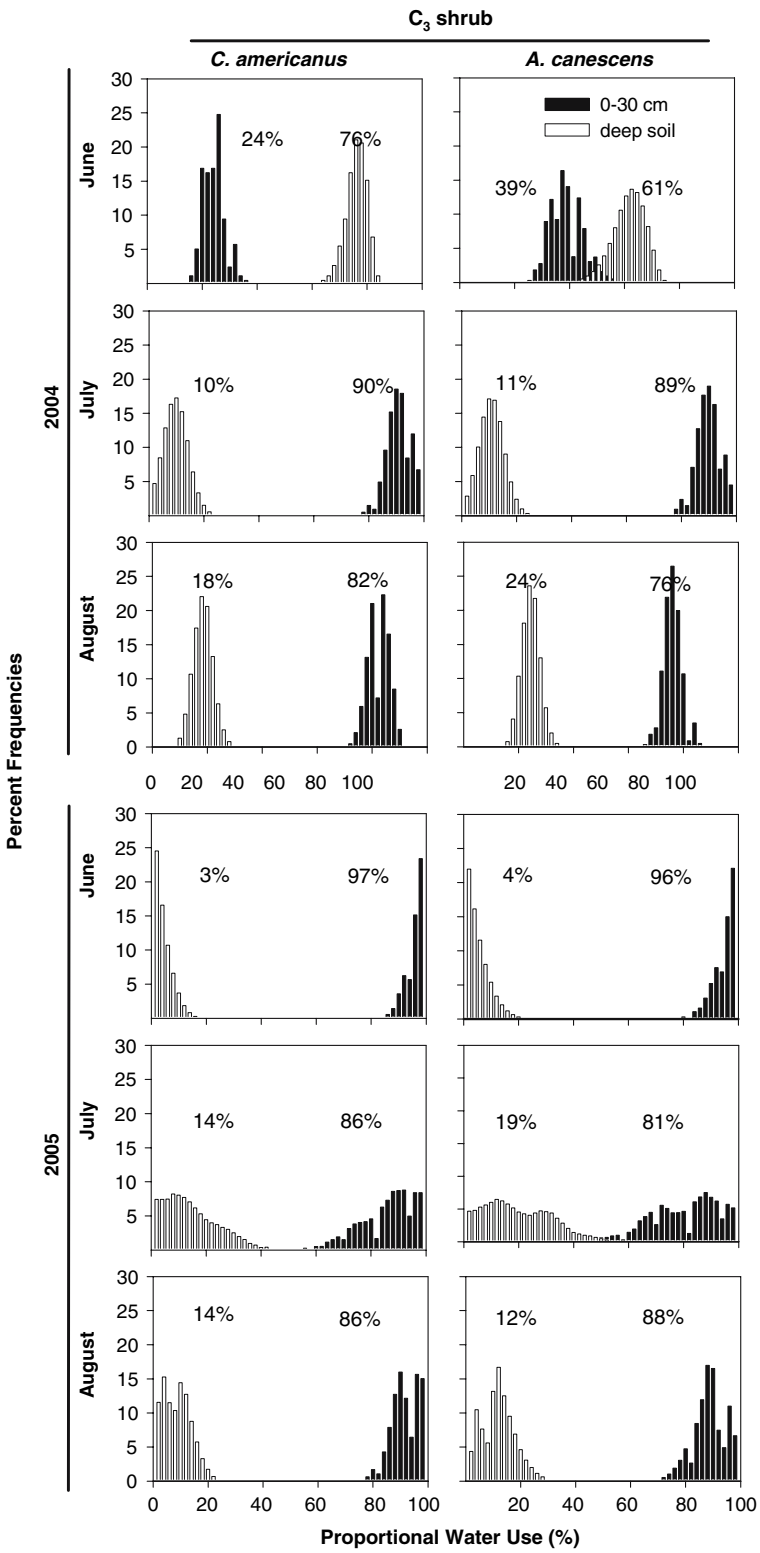


**Fig. 6** Water use proportions for $C_3$ shrubs from two aggregate sources: surface soil layers (1–30 cm in 5-cm increments, *black bars*) and deep soil layers (*white bars*) inferred using winter precipitation. Distributions for aggregate sources are the sum of all solutions from the original multi-source mixing model analysis (Phillips et al. 2005). These solutions were combined a posteriori to calculate a new aggregated frequency distribution. The data are presented as percent frequency for the proportion of water used from the two combined sources for each $C_3$ shrub × time period. Values indicate the mean proportion of water used from each source. For species names, see Table 1

soil profile having greater negative impacts on $C_4$ grasses than on some $C_3$ species (Collins 1992).

Other studies of community water use over time have reported strong temporal trends in soil water use. Indeed, our original predictions were made based on the assumption that plants would show greater dependence on surface soil water early in the growing season when rainfall events are more frequent, but we expected greater reliance on deep water as soils dried through July and August. However the unpredictability of precipitation patterns and

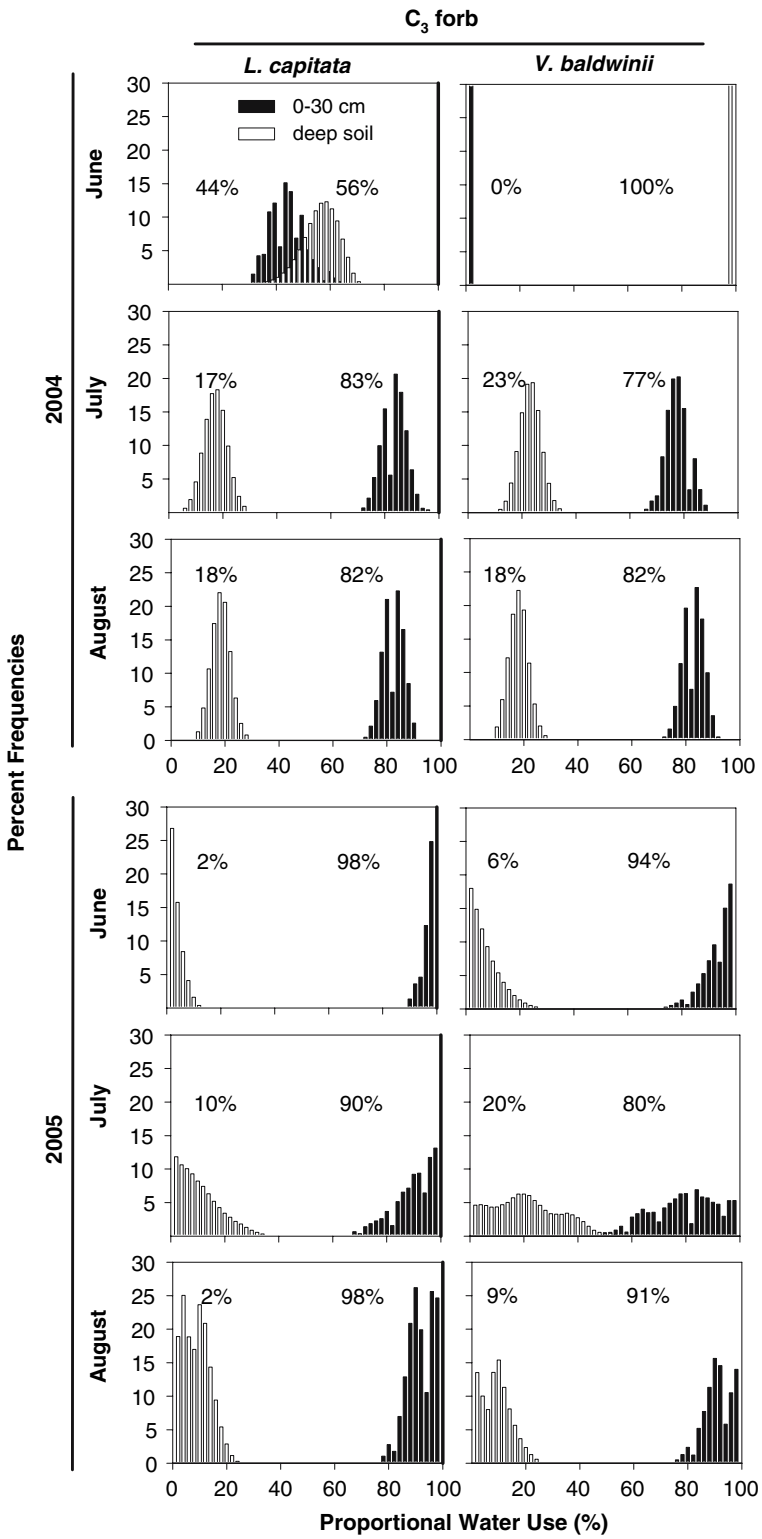


**Fig. 7** Water use proportions for $C_3$ forbs from two aggregate sources: surface soil layers (1–30 cm in 5-cm increments, *black bars*) and deep soil layers (*white bars*) inferred using winter precipitation. Distributions for aggregate sources are the sum of all solutions from the original multi-source mixing model analysis (Phillips et al. 2005). These solutions were combined a posteriori to calculate a new aggregated frequency distribution. The data are presented as percent frequency for the proportion of water used from the two combined sources for each $C_3$ forb × time period. Values indicate the mean proportion of water used from each source. For species names, see Table 1

periodic drought in this ecosystem prevented absolute ''day of year'' predictions of water use. Other studies reporting seasonal patterns in water use have been conducted in semi-arid systems (Schwinning et al. 2002) or environments with distinct wet/dry seasons (Zencich et al. 2002). Two years of water-use patterns in the tallgrass prairie show significant seasonal variation in the signature of the water used, and this variation had distinct seasonal trends with plants earlier in the season having lighter signatures, which became progressively heavier as the grow-

ing season progressed (Fig. 4). However, these differences appeared to be regulated predominantly by prevailing isotopic signatures in precipitation and the preceding drought conditions prior to rainfall. We would have expected greater temporal symmetry between years, if plant water use was influenced more by other factors (such as seasonal patterns of plant development).

In summary, our results support the conditional acceptance of Weaver's contention that differences in rooting pattern and depth can provide insight into functional differences among grassland species with respect to their reliance on varying soil water sources. For common tallgrass prairie species, the functional difference in water use among $C_4$ grasses and $C_3$ forbs and shrubs varies based on water availability in surface soils driven by recent precipitation history. As Weaver speculated, most $C_3$ species are using proportionally more water from depth than the $C_4$ grasses during drought. However, when water in the 0- to 30-cm soil depth was plentiful, each of the species we measured used proportionally similar amounts of this resource, contrary to Weaver's speculation. Therefore, the greatest potential for competition in this grassland exists when water is most available, while the greatest degree of resource partitioning occurs when water is most limiting.

**Acknowledgements** We thank John Blair, Mike Ryan, and Gene Kelly for revisions, which significantly improved this manuscript. We also acknowledge Roxane Fagan, Danielle Bilyeu, Sanjay Advani, Dan Reuss and Bob Brander for analytical assistance. Don Phillips provided advice improving our mixing-model analysis. Juan de Dios Miranda Lopez Marin, Michelle Lett, Amy Seery, Shane Neal, Aaron Heuter, Grover Reitz, and Jobie Carlisle provided outstanding field expertise. We received financial and technical support from the NSF Long-Term Ecological Research Program, the Division of Biology at Kansas State University, and the Department of Biology at Colorado State University.

## References

Albertson FW (1937) Ecology of mixed prairie in west central Kansas. Ecol Monogr 7:481–547

Boutton TW, Archer SW, Midwood AJ (1999) Stable isotopes in ecosystem science: structure, function and dynamics of a subtropical savanna. Rapid Commun Mass Spectrom 13: 1263–1277

Briggs JM, Knapp AK (1995) Interannual variability in primary productivity in tallgrass prairie: climate, soil moisture, topographic position, and fire as determinants of aboveground biomass. Am J Bot 82:1024–1030

Briggs JM, Knapp AK (2001) Determinants of $C_3$ forb growth and production in a $C_4$ dominated grassland. Plant Ecol 152:93–100

Canadell J, Jackson RB, Ehleringer JR, Mooney HA, Sala OE, Schulze E-D (1996) Maximum rooting depth of vegetation at the global scale. Oecologia 108:583–595

Chapin FS (1991) Integrated responses of plants to stress. BioScience 41:29–36

Collins SL (1992) Fire frequency and community heterogeneity in tallgrass prairie vegetation. Ecology 73:2001–2006

Craine JM, Wedin DA, Chapin FS III, Reich PB (2002) Relationship beween the structure of root systems and resource use for 11 North American grassland plants. Plant Ecol 165:85–100

Dawson TE, Ehleringer JR (1991) Streamside trees that do not use stream water: evidence from hydrogen isotope ratios. Nature 350:335–337

Ehleringer JR, Dawson TE (1992) Water uptake by plants: perspectives from stable isotope composition. Plant Cell Environ 15:1073–1082

Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) Plant physiological ecology field methods and instrumentation. Chapman & Hall, London, pp 281–300

Epstein S, Mayeda T (1953) Variation of $^{18}O$ content of waters from natural sources. Geochim Cosmochim Acta 4:213–224

Fitter AH, Hay RKM (2002) Environmental physiology of plants. Academic Press, San Diego, Calif.

Gat JR. (1996) Oxygen and hydrogen isotopes in the hydrologic cycle. Annu Rev Earth Planet Sci 24:225–262

Grime JP (1994) The role of plasticity in exploiting environmental heterogeneity. In: Caldwell MM, Pearcy RW (eds) Exploitation of environmental heterogeneity by plants. Academic Press, San Diego, Calif., pp 1–19

Hsieh JCC, Savin SM, Kelly EF, Chadwick OA (1998) Measurement of soil–water $\delta^{18}O$ by direct equilibration with $CO_2$. Geoderma 82:255–268

Knapp AK, Smith MD (2001) Variation among biomes in temporal dynamics of aboveground primary production. Science 291:481–484

Knapp AK, Fahnestock JT, Hamburg SP, Statland LB, Seastedt TR, Schimel DS (1993) Landscape patterns in soil–plant water relations and primary productivity in tallgrass prairie. Ecology 74:549–560

Le Roux X, Bariac T, Mariotti A (1995) Spatial partitioning of the soil water resource between grass and shrub components in a West African humid savanna. Oecologia 104:147–155

Nippert JB, Knapp AK, Briggs JM (2006) Intra-annual rainfall variability and grassland productivity: can the past predict the future? Plant Ecol 184:65–74

Oviatt CG (1998) Geomorphology of Konza prairie. In: Knapp AK, Briggs JM, Hartnett DC, Collins SL (eds) Grassland dynamics: long-term ecological research in tallgrass prairie. LTER, New York, pp 35–47

Phillips DL, Newsome SD, Gregg JW (2005) Combining sources in stable isotope mixing models: alternative methods. Oecologia 144:520–527

Polley HW, Johnson HB, Mayeux HS (1992) Determination of root biomasses of three species grown in a mixture using stable isotopes of carbon and nitrogen. Plant Soil 142:97–106

Ransom MD, Rice CW, Todd TC, Wehmueller WA (1998) Soils and soil biota. In: Knapp AK, Briggs JM, Hartnett DC, Collins SL (eds) Grassland dynamics: long-term ecological research in tallgrass prairie. LTER, New York, pp 48–68

Schimel DS, Kittel TGF, Knapp AK, Seastedt TR, Parton WJ, Brown VB (1991) Physiological interactions along resource gradients in tallgrass prairie. Ecology 72:672–684

Schulze E-D, Mooney HA, Sala OE, Jobbagy E, Buchmann N, Bauer G, Canadell J, Jackson RB, Loreti J, Oesterheld M, Ehleringer JR (1996) Rooting depth, water availability, and vegetation cover along an aridity gradient in Patagonia. Oecologia 108:503–511

Schwinning S, Davis K, Richardson L, Ehleringer JR (2002) Deuterium enriched irrigation indicates different forms of rain use in shrub/grasss species of the Colorado Plateau. Oecologia 130:345–355

Scrimgeour CM (1995) Measurement of plant and soil water isotope composition by direct equilibration methods. J Hydrol 172:261–274

Smith MD, Knapp AK (2003) Dominant species maintain ecosystem function with non-random species loss. Ecol Lett 6:509–517

Sun G, Coffin DP, Lauenroth WK (1997) Comparison of root distributions of species in North American grasslands using GIS. J Veg Sci 8:587–596

Thorburn PJ, Ehleringer JR (1995) Root water uptake of field-growing plants indicated by measurements of natural-abundance deuterium. Plant Soil 177:225–233

Tilman D (1987) The importance of the mechanisms of interspecific competition. Am Nat 129:769–774

Turner CL, Knapp AK (1996) Responses of a $C_4$ grass and three $C_3$ forbs to variation in nitrogen and light in tallgrass prairie. Ecology 77:1738–1749

Turner CL, Kneisler JR, Knapp AK (1995) Comparative gas exchange and nitrogen responses of the dominant $C_4$ grass *Andropogon gerardii* and five $C_3$ forbs to fire and topographic position in tallgrass prairie during a wet year. Int J Plant Sci 156:216–226

Walter H (1971) Ecology of tropical and subtropical vegetation In: Burnett JH (ed) Oliver and Boyd, Edinburgh

Weaver JE (1919) The ecological relations of roots. Carnegie Institute Washington publication no 286. Carnegie Institute, Washington, D.C.

Weaver JE (1926) Root development of field crops. McGraw-Hill, New York

Weaver JE (1954) A seventeen-year study of plant succession in prairie. Am J Bot 41:31–38

Weaver JE (1966) Prairie plants and their environment: a fifty-year study in the Midwest. University of Nebraska Press, Lincoln, Neb.

Weaver JE, Albertson FW (1943) Resurvey of grasses forbs, and the underground plant parts at the end of the great drought. Ecol Monogr 13:63–117

Weaver JE, Darland RW (1949) Quantitative study of root systems in different soil types. Science 110:164–165

Weaver JE, Fitzpatrick TJ (1934) The prairie. Ecol Monogr 4:111–295

Webb EA, Longstaffe FJ (2003) The relationship between phytolith- and plant-water $\delta^{18}O$ values in grasses. Geochim Cosmochim Acta 67:1437–1449

White JWC, Cook ER, Lawrence JR, Broecker WS (1985) The *D/H* ratios of sap in trees: implications for water sources and tree ring *D/H* ratios. Geochim Cosmochim Acta 49:237–246

Zencich SJ, Froend RH, Turner JV, Gailitis V (2002) Influence of groundwater depth on the seasonal sources of water accessed by *Banksia* tree species on a shallow, sandy coastal aquifer. Oecologia 131:8–19