Research

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Soil water partitioning contributes to species coexistence in tallgrass prairie

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The majority of tallgrass prairie root biomass is located in the upper soil layers (0–25 cm), but species differences exist in reliance on soil water at varying depths. These differences have led to the hypothesis that resource partitioning belowground facilitates species co-existence in this mesic grassland. To determine if plant water relations can be linked to soil water partitioning as a potential mechanism allowing C_3 species to persist among the more dominant C_4 grasses, we measured differences in the source of water-use using the isotopic signature of xylem water, volumetric soil water content at 4 depths, and leaf water potentials. Data were collected for seven species representing C_4 grasses, C_3 forbs and C_3 shrubs over three growing seasons at the Konza Prairie (Kansas, USA) to encompass a range of natural climatic conditions. C_4 grasses relied on shallow soil water (5 cm) across the growing season and had midday leaf water potentials that were highly correlated with shallow soil water regardless of soil water availability at other portions of the soil profile (20, 40 and 90 cm). In contrast, C_3 species only used shallow soil water when plentiful at this depth; these species increased their dependence on soil water from greater depths as the upper soil layers dried. Structural equation models describing plant water relations were very similar for the three C_4 species, whereas a unique set of models and drivers were identified for each of the C_3 species. These results support soil water partitioning as a mechanism for species coexistence, as C_4 species in this grassland have relatively consistent dependence on water in shallow soil layers, whereas C_3 species show niche differentiation in water use strategies to avoid competition with C_4 grasses for water in shallow soil layers when this resource is limiting and leaf water stress is high.

Perennial grasslands have been referred to as inverted forests, because a defining characteristic of these ecosystems is the high proportion of biomass and annual productivity that occurs belowground (Kucera 1991). Within the tallgrass prairie, 2/3 of total plant biomass is belowground, yet species differences in belowground biomass and the consequences of differences in biomass distribution by depth are poorly documented (Rice et al. 1998). In general, as much as 80% of total belowground biomass is located in the surface 25 cm, with up to 44% of that total at 0–10 cm (Kucera and Dahlman 1968, Sims and Singh 1978, Rice et al. 1998). This surface concentration of roots suggests that plant species of the tallgrass prairie, especially the dominant C_4 grasses, rely heavily on shallow portions of the soil profile for water and nutrients (Sun et al. 1997, Knapp et al. 2002).

Total root biomass varies seasonally as root growth, length, and turnover respond phenologically to the plant carbon balance and changing soil water and precipitation patterns (Hays and Seastedt 1987, Rice et al. 1998, Schenk and Jackson 2002). When water is not limiting, root occurrence and root function may be a direct product of increased competition among co-existing species for available soil water and increased nutrient availability following rainfall (Weaver 1966). Species responses to changes in water availability are generally linked to varying capacities for water uptake and tolerance of water stress (Chaves et al. 2002). Drought may initiate differential species responses in root depth distribution via increased root branching, maximum depth obtained, and root turnover (Weaver 1966, Hays and Seastedt 1987, Turner et al. 1995, Sun et al. 1997). Additionally, drought may induce seasonal and multi-year effects on root growth dynamics (Kucera et al. 1967, Rice et al. 1998, Ogle and Reynolds 2004). For these reasons, assessments of root structure and function must account for the temporal and spatial dynamics in soil water availability.

Driven by water potential gradients from the surrounding soils, plants primarily use the water source most easily accessible (Thorburn and Ehleringer 1995), but grasses may focus water uptake in surface soils due to their fibrous roots and a greater ability to respond to pulses in water availability (Bunce et al. 1977, Caldwell and Richards 1986, D'Antonio and Mahall 1991). Competition with grasses for water in the surface soil layers may constrain C_3 forbs and shrubs from utilizing the same proportion of this source of water. Thus, competition may force the subdominant C_3 species to use deeper soil water when demand for surface soil water is high (Silverton 2004). Inter-specific variation in water use resulting from competition has been posited as a mechanism of species coexistence in tallgrass prairie for many years (Weaver 1966), though empirical verification is lacking. The ability of co-existing species to acquire water from sources different than their competitors may aid in explaining patterns of community structure, spatial distribution at varying landscape positions, or productivity–diversity relationships (Walter 1971, Knapp 1993, Turner et al. 1995).

Previously, we have shown that the precipitation history and the topographic position on the landscape are better predictors of species water-use patterns, than structural characteristics in rooting pattern (Nippert and Knapp 2007). Using the stable isotopic signature of water in the plant, soil, and recent precipitation, we reported common water-use for all species from shallow soil layers following rainfall, but greater exploitation of water in deeper soil layers for C_3 species during drought (Nippert and Knapp 2007). Because partitioning of water based solely on differences in rooting depth does not explain patterns of water uptake for these species, here we explore the mechanisms contributing to soil water partitioning and the potential implications for community structure and species coexistence in this grassland. We tested the following hypotheses: (1) species and growth form differences in the source of water used will vary in response to changes in soil water availability at different depths, (2) leaf water potential will vary by species and growth form in response to water availability at varying depths, and (3) the patterns of response among variables in multivariate models when developed by species will show growth-form specific responses.

Material and methods

Research was conducted on the Konza Prairie Biological Station (KPBS), a mesic grassland located in northeastern Kansas, USA (39°05′N, 96°35′W). This region experiences high climatic variability in both temperature and precipitation within and between years (Borchert 1950, Hayden 1998). KPBS receives 835 mm average annual precipitation, 75% of which falls during the growing season (May-September). The average annual air temperature is 13°C with mean January and July ranging between -9 to 3°C and 20 to 33°C, respectively. Aboveground productivity on KPBS is dominated by a relatively few perennial C_4 species, but a diverse community of C_3 species (>300 species) co-occur within this grassland (Freeman and Hulbert 1985, Towne 2002).

Two permanent east-west transects of 100 m were established in April, 2003 within two ungrazed watersheds. These watersheds are burned each year in April but rapidly re-establish complete foliage canopy cover by late May. Each transect spanned a topographic gradient from uplands to lowlands (ca 50 m) common within KPBS. Within each transect, one permanent location was established for three distinct topographic positions: upland, hillside, and lowland (Ransom et al. 1998). At each position, two 30 cm TDR (time-domain reflectometry) probes were inserted horizontally into an intact soil profile at 5, 20, 40 and 90 cm in the hillside ($n=32$) and lowlands ($n=32$) and at 5 and 20 cm in the uplands ($n=16$). The extreme rockiness of upland soil prevented the placement of TDR probes at comparable depths to hillsides and lowlands. Volumetric soil water content (θ_v,%) was recorded at mid-morning from all probes on a weekly basis during the summer sampling seasons of 2003–2005 (late May to late August).

Plant sampling

The distribution of species along the sampled transects has been previously described (Nippert and Knapp 2007). The seven most abundant perennial species over all topographic positions by transects included three C_4 grasses (*Andropogon gerardii*, *Sorghastrum nutans* and *Schizachyrium scoparium*), a leguminous and non-leguminous C_3 forb (*Lespedeza capitata* and *Vernonia baldwinii*, respectively), a leguminous C_3 sub-shrub (*Amorpha canescens*) and a C_3 shrub (*Ceanothus americanus*).

To link species water uptake to the source of water used, plant xylem tissue was harvested monthly for stable isotopic analysis from June to August of 2004 and 2005 from all transects/topographical positions. Tissue samples were collected from the non-photosynthetic crown region, which lies immediately belowground, but above the rhizomes if present. Three replicates per species were collected for each sampling location and date. These replicates were collected from spatially non-adjacent regions (>3 m apart) within each topographic position in order to capture more of the inherent site variability. At each sampling date, different individuals per position were collected, cut into 1–3 cm lengths, sealed inside Exetainer vials and placed on ice until the samples were returned to the laboratory and stored at 1°C. Plant water extraction was performed using cryogenic vacuum distillation (Ehleringer and Osmond 1989, Webb and Longstaffe 2003), and the stable isotopic analysis for $\delta^{18}O$ was performed using direct equilibration with headspace CO_2 (Epstein and Mayeda 1953) analyzed on a gas bench in continuous flow with a stable isotope ratio mass spectrometer (IRMS). Further analytical details of the technique, as well as within and across-run sample variation have been previously reported (Nippert and Knapp 2007).

Leaf water potentials were measured at all locations over the growing season for all three years. Both predawn (Ψ_p) and midday (Ψ_m) water potentials were measured to estimate soil water potential in the zones of total root distribution and active uptake, respectively. At each location, 5 to 10 replicates leaves from separate individuals per species were measured with a Scholander-type pressure chamber.

Statistical analysis of the data for water potentials and volumetric soil water were performed using mixed-effects model in Proc Mixed (SAS 9.1.3). Watershed was a blocking variable and species within topographic position were replicated by transects (two transects per watershed). This analysis allowed for all species at one topographic location to be compared to all species at a separate location. Fixed-effects included date, topographic position, species, and their interactions. The random effects included the error associated with watershed, watershed by transect, watershed by species within a given topographic position, and the 3-way interaction of watershed × transect × species within topographic position. In this analysis, sampling date was not used as a repeated measure since different individuals were randomly selected and sampled at successive dates. The analysis of volumetric soil water was similar to the water potential analysis, except soil depth was nested within topographic position rather than species, and sampling date was used as a repeated measure and not as a predictor variable because the TDR probes record soil water at the same location over time.

Structural equation modeling

Structural equation modeling (SEM), as an alternative to traditional multivariate statistical methodology, uses a hypothesis-testing framework to examine relationships among interacting variables based on prior information about the system (Byrne 1998, Shipley 2000). Once the predicted relationships amongst the suite of variables are specified, a series of structured equations describing the system are tested simultaneously to assess the model as an appropriate hypothesis that is consistent with the data (Grace and Bollen 2005).

The initial hypotheses used in our SEM development included a comprehensive suite of causal relationships anticipated among multiple parameters (Mitchell 2001, Stoner and Joern 2004). The parameters identified within each model included volumetric soil water at four depths (θ_v at 5, 20, 40, 90 cm), leaf water potentials (Ψ_p and Ψ_m), $\delta^{18}O$ of xylem water, and a 'seasonality' variable. Time of year (season) was treated as a simple categorical variable representing the progressive sampling order across the summer for the three years of the study (1 = June, 2 = July, 3 = August). This variable was included because this analysis was conducted on annually-burned watersheds and the regrowth of a new aboveground canopy each spring results in predictable phenological and leaf area development patterns, independent of abiotic drivers. We focused our analyses on the predictive relationships for xylem $\delta^{18}O$ (proxy for the source of water uptake) and Ψ_m (leaf water stress). We predicted that the xylem $\delta^{18}O$ would be related to changes in θ_v at all depths, Ψ_p and Ψ_m, and season. Similarly, we predicted that Ψ_m would be related to changes in θ_v at the soil depths 5, 20 and 40 cm, and would vary by season. Covariance matrices between parameters were developed using maximum-likelihood estimation in LISREL 8.72 (Jöreskog and Sörbom, SSI Scientific Software Inc. 2005).

Identification of the best-fit model was based on several criteria describing the fit of the model to the data, model parsimony, and constrained by the aforementioned relationships predicting $\delta^{18}O$ and Ψ_m (Table 1). The sample size of each model was species-specific, from the uneven distributions of species occurrence by location. Species estimates from each location × time used in the model reflect an average of multiple measurements ($n = 3$ ($\delta^{18}O$), 7–10 ($\Psi_{p,m}$), and 2 (θ_v per depth). Because sample size varied by species, we included goodness of fit statistics which reflect the stability of our data based on these sample sizes (Table 1).

Results

ANOVA analysis

Water availability

Volumetric soil water (θ_v) varied significantly among topographic positions ($p = 0.0025$) and soil depth (0.0186). Differences between watersheds, and all interactions were not significant ($p > 0.05$). For the 5 and 20 cm depths, upland soils dried more rapidly, had

Table 1. Best-fit model statistics for all species. Low χ^2 and $p > 0.05$ suggest no evidence to reject the model based on a lack of fit with the data. Values of root mean square error of approximation (RMSEA) and standardized root mean square residual (RMR) <0.05 indicates a good fit between model and data. To determine the effects of a small sample size on model development, comparative fit index (CFI) was calculated. CFI accounts for sample size and values near 1.0 indicate a good fit. Once a candidate set of acceptable models that fit the data were characterized for each species, AICc was used to select the most parsimonious model (Burnham and Anderson 2002). Models presented here had the lowest AICc value of the candidate set per species.

	Species	N	X^2	P	df	RMSEA	RMR	CFI	AIC_c
	A. canescens	45	6.29	0.710	9	0.000	0.046	1.00	101.66
C_3	*C. americanus*	32	1.26	0.532	2	0.000	0.029	1.00	86.11
	L. capitata	33	3.56	0.893	8	0.000	0.046	1.00	93.51
	V. baldwinii	45	4.22	0.897	9	0.000	0.025	1.00	104.48
	A. gerardii	47	6.95	0.643	9	0.000	0.056	0.98	121.46
C_4	*S. nutans*	46	2.83	0.900	7	0.000	0.029	1.00	104.23
	S. scoparium	70	2.06	0.724	4	0.000	0.020	1.00	91.29

more variable θ_v, and the absolute maximum θ_v by depth was lower than in the other topographic positions (Fig. 1). When analyzed by depth for all positions, variability in θ_v over time decreased with increasing depth. In general, θ_v responded quickly to soil drying and wetting at 5 cm depths for all locations, while θ_v varied minimally at the 90 cm depth for both the hillside and lowland positions. However, after multiple weeks without rain (see 7/08/05), θ_v at 90 cm decreased with only minor recovery following rainfall compared to more shallow soil depths (Fig. 1).

Leaf water stress

Both predawn and midday leaf water potential (Ψ_p and Ψ_m) varied significantly by sampling date, species, topographic position, and date × species ($p < 0.0001$). The interaction of date × topographic position was significant for Ψ_p and Ψ_m ($p < 0.0001$ and $p = 0.0073$, respectively). The three-way interaction of date, species and topographic position was also significant for Ψ_p ($p < 0.0001$). Although statistically significant, trends among species and photosynthetic types were similar for both Ψ_p and Ψ_m measurements

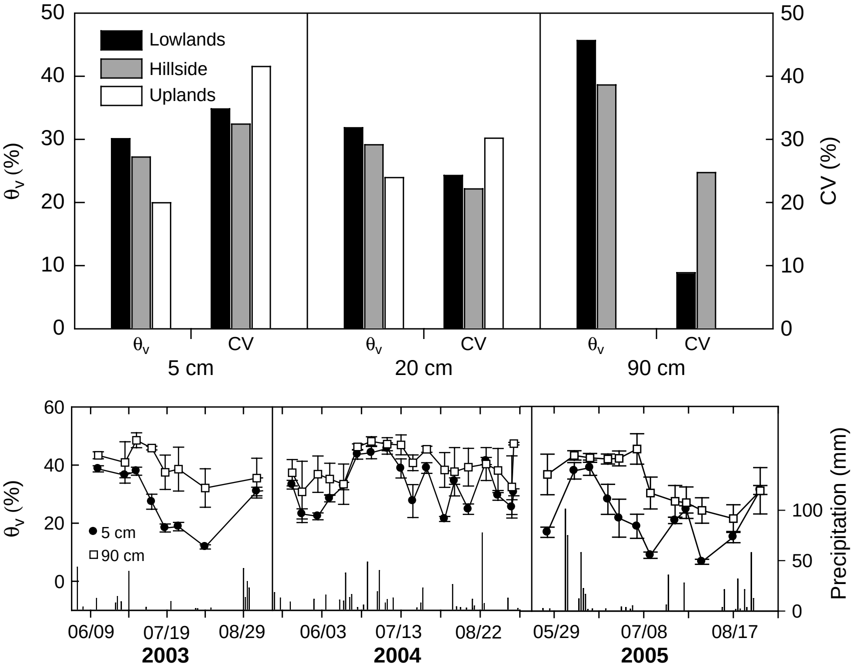

Fig. 1. Changes in volumetric soil water (θ_v) by depth and topographic position for the summers of 2003–2005. In the top panel, the left group of bars for each soil depth is the average θ_v by location (left y-axis), and the right group of bars is the corresponding coefficient of variation (CV) (right y-axis). θ_v was not measured at 90 cm in upland locations (Methods). The bottom panel shows the dynamics of soil water at two depths (5 cm, 90 cm) averaged across all four lowland positions measured (±1 SE) from late May to late August from 2003–2005. Vertical bars in the bottom panel (right y-axis) depict the ambient precipitation record over the same time period.

(Fig. 2). As expected, trends in water potential for all species followed the precipitation history with decreased values (greater leaf water stress) during periodic drought and consistently higher values (reduced leaf water stress) following rainfall. Ψ_p values were lowest for all species during the late summer of 2003, following 50 days with only 1 rainfall event over 10 mm. In general C_4 species had lower Ψ_m following dry periods (late 2003, mid 2005), but the general magnitude and pattern were similar among growth forms. Rainfall occurred consistently in 2004, and water potentials varied little over time for any of the species measured (Fig. 2). Ψ_p and Ψ_m were generally synchronous, but Ψ_m responded more rapidly to dry periods with proportionally greater change than Ψ_p (Fig. 2).

Source of water used

Xylem $\delta^{18}O$ varied significantly among species during the summers of 2004–2005 (Fig. 3). In general, heavier

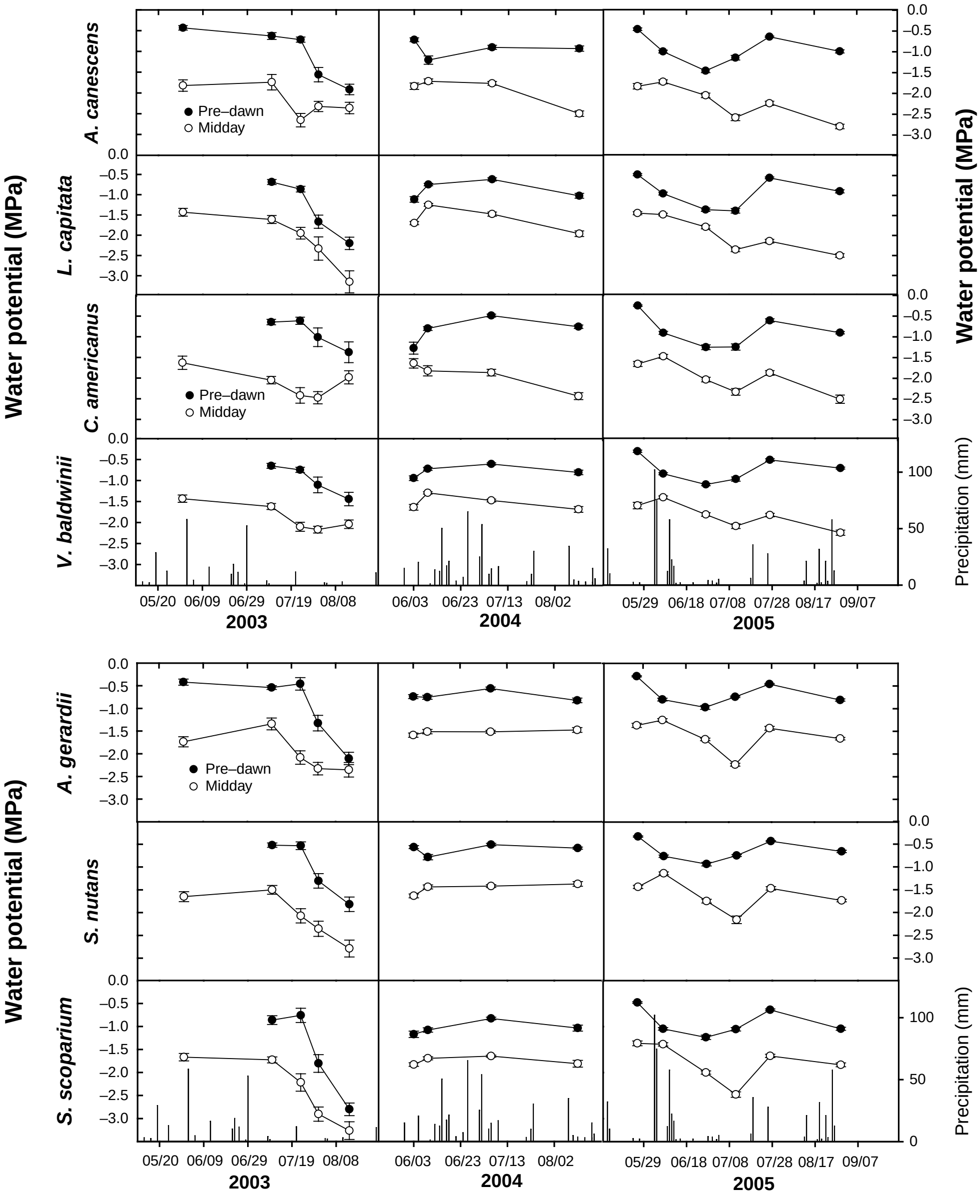

Fig. 2. Dynamics of mean leaf water potential (Ψ_p and $\Psi_m \pm 1SE$) for C_4 grass and C_3 forb and shrub species for the 2003–2005 growing seasons on KPBS. Species grouped in the top panel are C_3, and the bottom panel contains the C_4 species. The lower right y-axis of the C_3 and C_4 species groupings depicts the ambient precipitation record over the same time period.

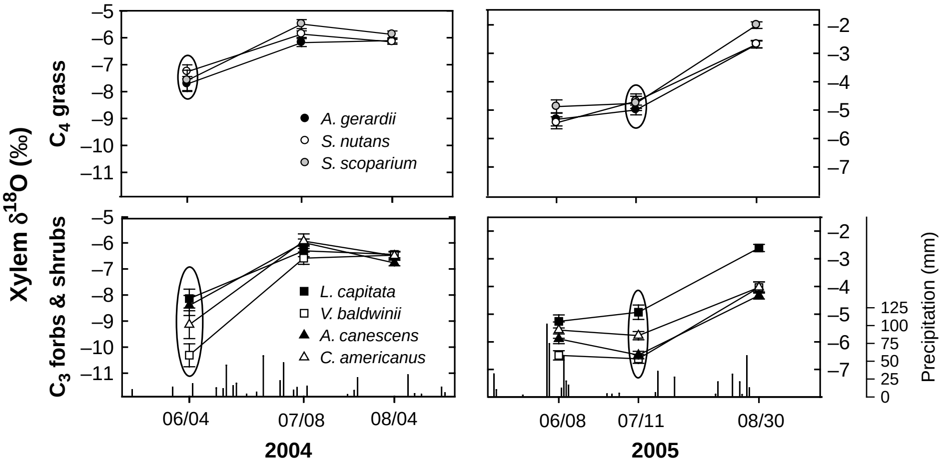

Fig. 3. Average species differences (±1 SE) in the $\delta^{18}O$ of xylem water during the summers of 2004 and 2005. Species are grouped in rows based on photosynthetic pathway (C_4, C_3). C_3 species are additionally classified by growth form (■ = forbs, ▲ = shrubs). Values in 6/04 and 07/11 are circled to highlight similarity of values among C_4 species and divergence among C_3 following several weeks of drought. Note the shift in the y-axis range between years. The lower offset right y-axis depicts the ambient precipitation record over the same time period.

isotopic signatures indicate water-use from surface soil layers subject to evaporative enrichment, while lighter signatures suggest water-use from deeper portions of the soil profile (Dawson 1993, Dawson et al. 1998). A trend towards heavier isotopic water signatures from early summer 2004 to late summer 2005 was noted across this sampling period. The three C_4 grasses responded similarly for all sampling periods whereas the C_3 species were more variable in trends and magnitude of $\delta^{18}O$, even when comparisons were restricted to similar growth forms (forb/shrub). The greatest species differences in $\delta^{18}O$ among C_3 plants were noted following several weeks of low rainfall and dry soils (June, 2004; July, 2005, Fig. 3). In general, the xylem $\delta^{18}O$ of C_3 species was several ‰ lower than C_4 species, indicating greater use of water from depth.

SEM analysis

The response variables Ψ_p, Ψ_m, $\delta^{18}O$ and θ_v, each had significantly different responses to topographic location when analyzed via standard ANOVA, thus we were unable to develop a candidate set of SEMs to describe the topographic response when all species were combined ($p < 0.05$). This result is unsurprising given the wide range of species studied. Thus, our SEM analyses focused on relationships at the species level. Path models with all constructs for the best-fit model (Fig. 4) and the covariance associated with each multivariate hypothesis (Table 2) are shown for each species.

C_3 species

For *A. canescens*, *L. capitata*, and *V. baldwinii*, θ_v at 5 cm and Ψ_p had significant positive direct effects on $\delta^{18}O$. The most likely interpretation for these relationships suggests greater uptake of surface soil water (isotopically-heavier) when soil water content is higher and predawn water stress is lowest, and the use of deeper, isotopically-lighter soils when surface water availability declines, and predawn water stress is higher. Ψ_m had a significant direct effect on $\delta^{18}O$ for most C_3 species, but the effect was negative. Therefore, increased midday water stress (noted by decreases in Ψ_m) corresponds with greater water uptake from shallow soil layers (heavier $\delta^{18}O$ water signature). Indirect effects on $\delta^{18}O$ were present for each of these C_3 species, but they varied by predictor variable. θ_v at 20, 40, or 90 cm did not have a significant direct effect on $\delta^{18}O$ for any of the C_3 species measured. The shrub *C. americanus* had distinctly different patterns compared to the other C_3 species. For example, predictions of $\delta^{18}O$ by θ_v and Ψ_p were negative for *C. americanus*. This species was also the only C_3 species to show a significant effect of season on $\delta^{18}O$.

For the C_3 species, Ψ_m had fewer significant predictors and a lower amount of explained variance compared to predictions of $\delta^{18}O$ (Table 2). We expected water content throughout the soil profile to be a greater predictor of Ψ_m for C_3 species, and not just surface water. *L. capitata* and *C. americanus* were the only species in which θ_v (5 and 20 cm) had a significant effect on Ψ_m. In general, the strongest predictor of Ψ_m for C_3 species was season. This variable was included to

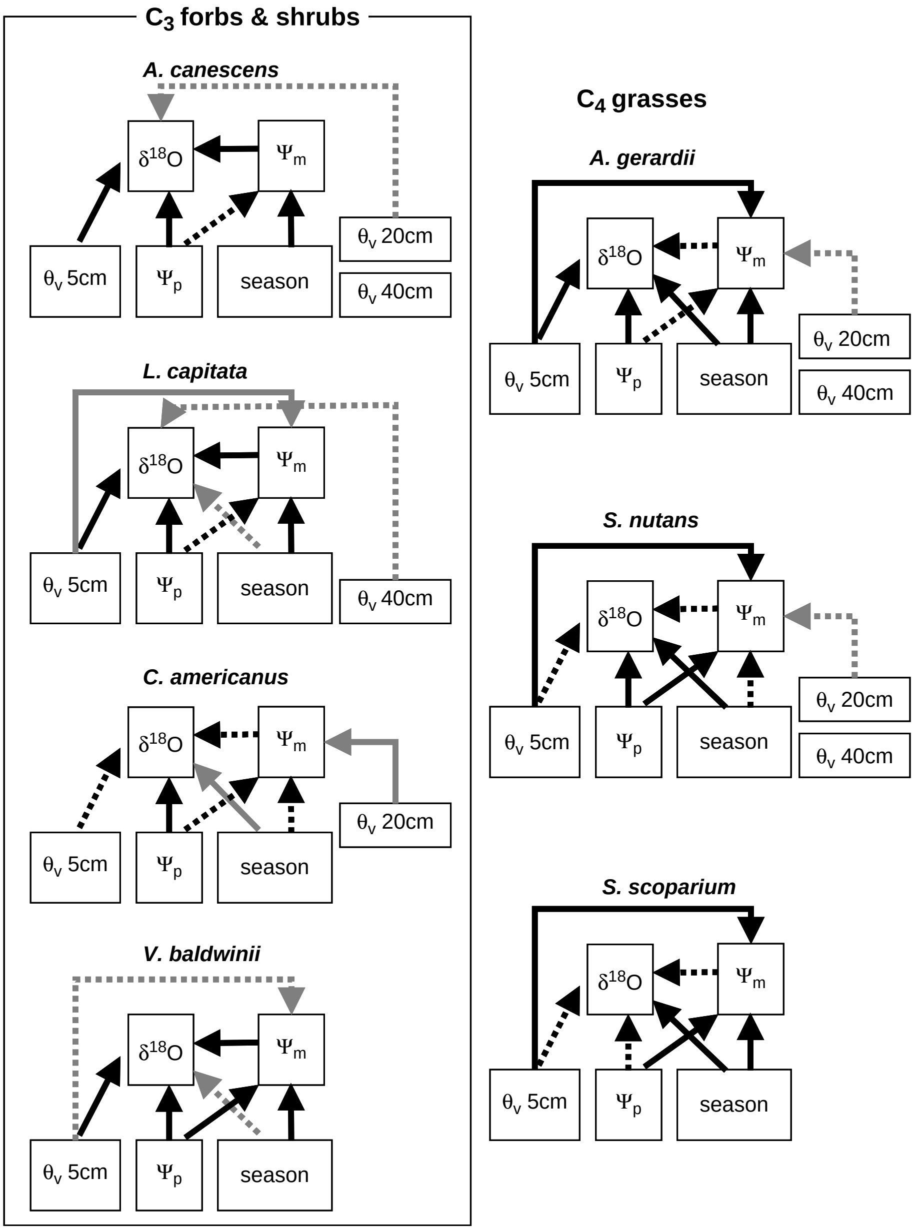

Fig. 4. Path models for all species depicting the relationships between water availability by depth, plant water stress, water-use, and season. Boxes depict variables comprising the overall SEM (Table 2). 'θ_v 5, 20 and 40 cm' reflect volumetric soil water by depth, '$\delta^{18}O$' is the isotopic signature of water used by the plant, 'season' is a categorical variable reflecting phenological development, and 'Ψ_p and Ψ_m' are predawn and midday water potentials, respectively. Solid arrows indicate a significant direct relationship ($p < 0.05$), while dashed arrows indicate the direct relationship between variables was insignificant ($p > 0.05$), yet a required construct for the overall best model fit (Table 1). Grey arrows indicate that this model construct varied among other species of the same photosynthetic pathway (C_3 forbs and shrubs vs C_4 grasses).

reflect phenological species differences occurring independent of average seasonal climatic patterns (e.g. wet spring, dry summer). This effect was negative, suggesting Ψ_m declines as the summer progressed, potentially from greater whole-plant transpiration.

C_4 species

In contrast to the C_3 species, the models developed for the C_4 grasses had very similar combinations of parameters and directional effects for the response variables we tested (Table 2). θ_v at any depth had little

Table 2. Unstandardized coefficients (covariance) associated with the prediction of each multivariate hypothesis for each species. C_3 species are presented in the top table, while C_4 species are in the bottom table. Values are direct and (indirect) effects of the suite of predictors on the dependent variable. Significant relationships ($p < 0.05$) are in bold. The complete model is the aggregation of each structural equation (each of the predictors of $\delta^{18}O$ and Ψ_m, respectively) and the associated error variance for a given species. θ_v at 90 cm depth did not have additional explanatory power for any species in the SEM analysis.

Response variable		Predictor variables						Error	R^2
	C_3 species	5 cm	Ψp	season	Ψm	20 cm	40 cm		
$\delta^{18}O$	*A. canescens*	**0.076** (0.01)	**2.05** (0.08)	**(0.44)**	**−1.08**	0.016 (0.014)	0.012	1.11	0.74
	L. capitata	**0.09 (−0.06)**	**2.63** (−0.08)	0.34 (0.57)	**−1.60**	(−0.06)	−0.075	3.38	0.51
	C. americanus	−1.70	**−0.70** (0.01)	**0.25** (0.01)	−2.07	(−0.04)		6.19	0.31
	V. baldwinii	**0.092 (−0.02)**	**1.69** (0.22)	0.40 **(0.55)**	**−2.08**			2.07	0.55
Ψ_m	*A. canescens*		−0.077	**−0.41**				0.20	0.35
	L. capitata	**0.025**	0.049	−0.36				0.21	0.54
	C. americanus		−0.004	−0.003		**.019**		0.08	0.30
	V. baldwinii	0.010	**−0.110**	**−0.27**				0.086	0.44
	C_4 species	5 cm	Ψp	season	Ψm	20 cm	40 cm	Error	R^2
$\delta^{18}O$	*A. gerardii*	**0.056** (−0.01)	**2.38** (−0.06)	**1.12** (0.08)	−0.50			2.35	0.49
	S. nutans	0.042 (−0.01)	**2.46** (−0.75)	**0.63**	−0.84			2.03	0.44
	S. scoparium	0.014 (0.01)	1.03 (0.34)	**1.05** (−0.11)	0.67			3.45	0.27
Ψ_m	*A. gerardii*	**0.022** (0.001)	0.13	**−0.17**		0.001	−0.006	0.16	0.42
	S. nutans	**0.19** (−0.01)	**0.89**	0.004		−0.026	0.0088	0.20	0.58
	S. scoparium	**0.016**	**0.50**	**−0.17**				0.16	0.57

effect on $\delta^{18}O$ for any of the C_4 species (except see θ_v at 5 cm for *A. gerardii*). Ψ_p and season were better predictors of $\delta^{18}O$ for each of the C_4 species. Similar to the relationship previously noted for C_3 species, when Ψ_p values are higher changes in xylem $\delta^{18}O$ values indicate water-use from isotopically-heavier soils. The positive relationship between xylem $\delta^{18}O$ and season was stronger for all three C_4 species than reported for the C_3 species and may reflect a tighter correspondence between plant water uptake and the signature of rainfall occurring across this study. Unlike C_3 species, Ψ_m was not a significant predictor of C_4 $\delta^{18}O$.

Almost half of the variance associated with predictions of Ψ_m for C_4 species was explained by θ_v at 5 cm, Ψ_p, and season (Table 2). As θ_v at 5 cm fluctuated over this study, Ψ_m mirrored these changes in surface soil water content. This effect was common for all C_4 species, but the magnitude of the effect was greatest for *S. nutans*. Seasonal effects on Ψ_m were negative for *A. gerardii* and *S. scoparium*, but no seasonal change in Ψ_m occurred for *S. nutans*. For models describing *A. gerardii* and *S. nutans*, θ_v at 20 and 40 cm was an important model construct for predictions of Ψ_m. However, the best-fit model for *S. scoparium* did not include this variable, suggesting reduced effects of deeper soil water on *S. scoparium* compared to *A. gerardii* and *S. nutans*. The relationship between 20 and 40 cm θ_v and Ψ_m for *A. gerardii* and *S. nutans* (and not *S. scoparium*) was the only difference in model structure for these three species, despite each model being developed individually. Further, few indirect effects were noted for any of the variables for these C_4 species (Table 2).

Discussion

Species and growth form differences in patterns of water use may further our understanding of patterns of community structure, diversity, and productivity within temperate grasslands (Fowler 1986, Weltzin and McPherson 1997, Köchy and Wilson 2000, Tsialtas et al. 2001, Fay et al. 2002). In many terrestrial ecosystems, belowground plant traits such as root distribution, water-use by depth, and plant water stress exhibit similar patterns between species, photosynthetic types, or landscape position because these traits are all strongly correlated and driven by water availability (Dawson 1993, Dawson et al. 1998, Jackson et al. 2000, Meinzer et al. 2001). However, species may also exhibit multiple strategies, purportedly to reduce competition and facilitate resource acquisition (Schenk 2006). We linked leaf water stress and the source of water uptake to seasonal and spatial changes in soil water content in order to better understand how competition for water may influence resource partitioning between species in the tallgrass prairie.

Forb productivity is not strongly correlated with precipitation amount within or between years, which distinguishes this growth form from the grasses in the tallgrass prairie (Briggs and Knapp 2001, Nippert et al. 2006). In fact, the strongest recorded predictor of forb abundance is an inverse relationship with grass abundance, suggesting the forb response is modulated by grass productivity (Briggs and Knapp 2001). While microclimate changes in temperature and light during years of high and low grass productivity would influence the C_3 forb community, dependence on water for nutrient uptake and CO_2 assimilation would be predicted to be commensurate or even greater than for C_4 grasses (Black 1973, Pearcy and Ehleringer 1984). The lack of a correlation between rainfall amount and productivity for forbs and shrubs suggests that demand rarely exceeds supply or that these species have proportionally greater access to water sources that vary less than the surface soil layers than C_4 species. Greater dependence on deep water by forbs than grasses has been posited for tallgrass prairie communities repeatedly over the past 70 years (Albertson 1937, Weaver and Albertson 1943, Sims and Singh 1978, Knapp 1985, Abrams et al. 1986, Kucera 1991, Knapp et al. 1998, Fay et al. 2002, Nippert et al. 2006) and this difference in spatial root partitioning may be a response to avoid competition with the more competitive C_4 grasses.

Our results indicate that differences in leaf water potential by species and growth form can be linked primarily to dynamic changes in soil water content and the temporal development of the plant community (Fig. 4). We hypothesized that midday leaf water potential (Ψ_m) would vary by species based on water availability at depth. Accordingly, the best predictor of Ψ_m for C_4 species was 5 cm θ_v, (Table 2, Fig. 4), suggesting that as surface soil water increases, midday plant water stress decreases and vice versa. This relationship between surface soil water and leaf water stress for C_4 species was predictable, as these species are reported to have nearly half of their root biomass in the surface 10 cm (Rice et al. 1998). However, Ψ_m was not significantly related to changes in surface soil water for most C_3 species, where the best predictor of Ψ_m was the class variable 'season' (Table 2, Fig. 4). Like C_4 grasses, these C_3 species also have the majority of root biomass in upper soil layers but the relationship between water stress and water availability was less clearly defined. The lack of a relationship between 5 cm θ_v and Ψ_m for most C_3 species suggests a shifting reliance away from surface soil layers to deeper sources of soil water when water availability declines, without corresponding changes in Ψ_p or Ψ_m (Smith et al. 1991). Only the shallow-rooted *L. capitata* showed a significant influence of changes in

5 cm θ_v on Ψ_m. Previous studies measuring gas exchange responses to seasonal declines in water availability showed a greater response for C_4 than C_3 species in this grassland (McAllister et al. 1998), and our Ψ_m measurements corroborate this differential response among growth forms.

We predicted these grassland species would shift the source of water used based on changes in water availability by depth. For the C_3 species, 5 cm θ_v, Ψ_p, and Ψ_m all had significant effects on water source (using $\delta^{18}O$ as a proxy). These predictors explained over half of the variation in the water source for most C_3 species, with *A. canescens* having a $R^2 = 0.74$. This positive relationship suggests that as the water content at 5 cm increases (θ_v), the isotopic signature of water ($\delta^{18}O$) in the plant stem increased, indicative of increased reliance on isotopically heavier water (Brunel et al. 1991, Midwood et al. 1998). When 5 cm θ_v declines, the stable isotopic signature of water in C_3 plant xylem becomes lighter, indicative of increased reliance on water from deeper portions in the soil profile. Unlike the C_3 species, changes in water content in surface soil layers do not have corresponding changes in the source of water-used for C_4 species (except see weak relationship for *A. gerardii*). This non-response reinforces previous assertions that C_4 grasses relied more on water in surface soil layers, irrespective of supply than the co-occurring C_3 species (Nippert and Knapp 2007). The primary drivers of xylem $\delta^{18}O$ for the C_4 grass species were season and Ψ_p. The effect of season was especially large on *A. gerardii* and *S. scoparium* with heavier xylem $\delta^{18}O$ signature of 1‰ per month as the growing season advanced (Table 2). Similar to C_3 species, Ψ_p was positively correlated with xylem $\delta^{18}O$ in C_4 species, suggesting the wettest portion of the plant's rooting zone is in the surface soil layers when Ψ_p is higher, and in deeper soil layers when Ψ_p is lower.

Distinct growth form trends are present when the patterns and responses of water relations variables are compared by species within the entire SEM (Fig. 4). Each of the models for the C_4 grasses we measured had similar responses and relationships among variables. The best-fit models for *A. gerardii* and *S. nutans* were identical, and *S. scoparium* only differed by the lack of a θ_v relationship with Ψ_p at 20 and 40 cm (Table 2, Fig. 4). The relationships among model parameters were also similar in response (positive/negative) and magnitude among these grasses (Table 2). These models show a common response among C_4 grasses, with dependence on surface soil water despite changes in leaf water stress or soil water availability. Models describing water relations for the C_3 species had similar relationships among model parameters (positive or negative correlations), but the model structure varied by species (Table 2, Fig. 4). This response was not growth-form specific, as comparisons of legumes, forbs, or shrubs all responded uniquely. The response of *L. capitata* was more similar to the C_4 grasses than the other C_3 species in terms of absolute values of Ψ_m and $\delta^{18}O$ and the amount of variance explained in each structured equation. *L. capitata* has been shown to have a more shallow (60 cm) maximum rooting depth compared to 15 other common forbs in this system (Zajicek et al. 1986), and the response may be related to a reduced capacity of this species to utilize deeper sources of soil water as the surface dries. The shrub *C. americanus* also had responses and best-fit models divergent from the other C_3 species for nearly every response variable. Tallgrass prairie shrubs have previously been shown to exhibit variable responses to water availability both within and across growth form (McCarron and Knapp 2001). *C. americanus* has coarse woody roots in the surface soil and may be an inferior competitor for surface soil water, using proportionally more water from depth (Nippert and Knapp 2007).

Previous results from North American tallgrass prairies have shown co-limitation among multiple resources including nitrogen, water, and light (Knapp et al. 1998). Because this study focused on responses in annually-burned prairie only, light was likely not a significant limitation (Knapp and Seastedt 1986), while the effects of high light and temperature further exacerbate water limitation (Chaves et al. 2002). However, ambient seasonal N limitations have been shown to be strong determinants of ecosystem processes such as plant productivity and decomposition (Seastedt et al. 1991, Turner and Knapp 1996, Blair 1997, Nippert et al. 2007). N fertilization studies in tallgrass prairie have shown immediate and substantial changes in community structure with decreases in species richness following fertilization (Collins et al. 1998, Pennings et al. 2005, Suding et al. 2005). Because N uptake is inherently related to water availability in terms of location in the soil profile and timing of seasonal availability (Seastedt and Hayes 1988, Seastedt et al. 1991), our results that show seasonal responses of niche differentiation and resource partitioning as drivers of species coexistence maybe just as relevant for the co-varying role of N limitation on community structure in this grassland.

While niche differentiation as a mechanism to reduce competition for limiting resources and contribute to patterns of coexistence in community structure has been long supposed in this grassland ecosystem (Weaver 1966), empirical verification has been lacking. Our results link theory with experimental data, showing soil water partitioning among species of C_3 forbs/shrubs and C_4 grasses in response to changes in water content at depth, leaf water stress, and the source of water uptake. Despite similar rooting depths among species in this grassland (Albertson 1937, Weaver 1954, Turner et al. 1995), the dominant C_4 grasses we measured show reliance on water in surface soil layers, while C_3 forbs and shrubs exhibited greater plasticity by shifting the source of water-used in response to leaf water stress and water availability. Based on models linking water relations variables among species and growth forms, we were able to infer a common strategy among C_4 grasses to compete for water in surface soil layers even during periodic drought, while C_3 forbs and shrubs show distinct species strategies to avoid competition with grasses for water in surface soil layers, when this resource is limiting.

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