

Patterns and ecological consequences of water uptake, redistribution, and loss in tallgrass prairie

by

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B.S., The College of New Jersey, 2010

M.S., Saint Joseph's University, 2012

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## Abstract

Water availability is a key driver of many plant and ecosystem processes in tallgrass prairies, yet we have a limited understanding of how grassland plants utilize water through space and time. Considering that tallgrass prairies experience tremendous heterogeneity in soil resources, identifying spatiotemporal variation in plant ecohydrology is critical for understanding current drivers of plant responses to water and for predicting ecosystem responses to future changes in climate. Here, I investigated the patterns, drivers, and ecological consequences of plant water use (e.g., water uptake, water redistribution, and water loss) in a native tallgrass prairie located in northeastern Kansas, USA. Using a combination of leaf gas exchange, sap flow, and isotopic techniques, I addressed four main questions: 1) How does fire and grazing by bison impact use of water from different sources and niche overlap for common grasses, forbs, and shrubs? 2) Does hydraulic lift occur in grazed and ungrazed tallgrass prairie, and does this impact facilitation for water within grassland communities? 3) What are the patterns and drivers of nocturnal transpiration in common grassland species? 4) How does diel stem sap flow and canopy transpiration vary among common grassland species?

I found that bison grazing increased the depth of water uptake by *Andropogon gerardii* and *Rhus glabra*, reducing niche overlap with co-occurring species. Conversely, grazing did not affect hydraulic lift, which was generally uncommon and likely limited by nocturnal transpiration. Further, leaf gas exchange measurements indicated that nocturnal transpiration occurred commonly in tallgrass prairie plants and was greatest among grasses and early in the growing season. Nocturnal transpiration was not driven by vapor pressure deficit or soil moisture, as commonly observed in other systems, but was regulated by nocturnal stomatal conductance in most species. Finally, I found that daytime sap flow rates were variable among

species and functional types, with larger flux rates among woody species. Nocturnal sap flow rates were more consistent across species, which caused nighttime sap flow and transpiration to account for a larger proportion of daily flux rates in grasses than in forbs or shrubs. These results show that water uptake, water redistribution, and water loss are all influenced by different biotic and abiotic drivers and have varying ecological impacts across a heterogeneous landscape. Additionally, extensive differences in water flux exist among co-occurring species and plant functional groups, which likely reflect varying strategies to tolerate water limitation. These results suggest that shifts in the abundance of these species with future climate changes, or with ecosystem state changes, will likely impact ecosystem-level water balance.

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## **Dedication**

I dedicate this dissertation to my parents, Diane and Jimmy O’Keefe, for their love and support through this crazy endeavor.

# **Chapter 1 - Introduction**

## **Overview**

Although considered relatively mesic ecosystems, tallgrass prairies are often water-limited for much of the year. The tallgrass prairies of North America, for example, receive the majority of annual precipitation primarily early (May-June) and late (September) in the growing season, and are typically dry during July and August. When growing season precipitation does occur, rain events are intermittent and fall in large, infrequent events, resulting in long dry periods during which shallow soil water rapidly declines (Abrams and Knapp 1986; Williams and Rice 2007; Zeglin et al. 2013). Soil water recharge occurs during the non-growing season, when water is able to infiltrate to deep soil layers (> 2 m deep) (Ransom 1998; Nippert and Knapp 2007a). Deep water is typically available during the entire year, which helps develop a moisture gradient throughout the soil profile (e.g., dry shallow soil – wet deep soil). Further complicating this system, soil water content is patchy across the landscape and is modulated by topography (Nippert et al. 2011) and disturbance regimes such as fire (Hulbert 1969; McMurphy and Anderson 1965) and grazing (Archer and Detling 1986; Harrison et al. 2010; Greenwood and McKenzie 2001; Hamza and Anderson 2005).

Numerous aspects of tallgrass prairie structure and function are sensitive to variation in soil water content. Considering that water limitation is most prevalent during the middle of the growing season, when the majority of vegetative growth occurs (Nippert et al. 2011), precipitation and soil water are positively correlated with aboveground net primary productivity (Briggs and Knapp 1995; Knapp and Smith 2001). Soil water availability can also impact plant community composition and structure, having variable effects on species diversity (Knapp et al. 2002; Jones et al. 2016) and intraspecific variation in genetic diversity (Avolio et al. 2013).

Others have shown that soil water variability shifts microbial communities (Zeglin et al. 2013) and alters many ecosystem processes such as soil CO<sub>2</sub> flux (Knapp et al. 2002; Harper et al. 2005), decomposition rates (Schuster 2016), and nitrogen cycling (Groffman et al. 1993). At larger scales, precipitation drives earth-atmosphere exchange of landscape carbon and water fluxes (Petrie et al. 2012; Logan and Brunsell 2015).

Despite the recognized importance of water in tallgrass prairies we still have a limited understanding of the mechanistic processes that underpin community and ecosystem responses to water availability. While leaf physiological responses to water have been well studied in grasslands, the vast majority of research examining mechanisms of plant water use does not consider how these processes vary across the landscape or at different temporal scales. For example, previous studies have shown that leaf gas exchange and water potential are sensitive to changes in soil water, and this varies both inter-specifically (Knapp 1984; Nippert et al. 2007, 2009) and intra-specifically (Avoilio et al. 2013; Hartman et al. 2011; O’Keefe et al. 2013). Different species and plant functional types also exhibit varying water uptake strategies (Nippert et al. 2007a,b), drought tolerance traits (Tucker et al. 2011), and hydraulic strategies (Ocheltree et al. 2013) to persist through periods of water limitation. However, plant water-use is typically only studied in one or a few locations, or in common garden experiments, which does not reveal potential variation in these responses across a heterogeneous landscape (Knapp et al. 1993). These studies also utilize instantaneous measurements across a growing season, which do not fully describe the potential dynamic variation in these responses through time. Finally, most research on tallgrass prairie ecohydrology has only investigated water relations in dominant grasses; comparisons among various growth forms and subdominant species are rarely considered. Thus, previous investigations of grassland ecohydrology have not captured the full

extent of potential patterns across a heterogeneous landscape or through time. Considering that tallgrass prairies experience tremendous spatiotemporal variability in soil moisture and other resources, identifying spatial and temporal variation in plant ecohydrology will be critical for predicting ecosystem responses to future changes in climate.

Many sources of spatiotemporal variation in plant ecohydrology exist in tallgrass prairies. First, water use may vary across the landscape as a function of topography, fire frequency, or large herbivore grazing. Upland soils are shallower, rockier, and drier than lowland soils (Jantz et al. 1975; Oviatt 1998), which could induce variation in plant water-use across topographic gradients (Nippert et al. 2007a,b; Nippert et al. 2011). Numerous factors associated with fire and grazing, including changes in root growth (Johnson and Matchett 2001; Klumpp et al. 2009; Nippert et al. 2012) and soil moisture (Archer and Detling 1986; Harrison et al. 2010; McMurphy and Anderson 1965), could also alter water-use dynamics in grasslands. Assessing how plant water-use varies across the landscape in response to topography or common grassland management practices could provide mechanistic insight into how various ecological processes such as niche partitioning, interspecific competition, and community composition vary across space, and could also improve predictions of how these processes may change in the future.

Second, patterns of water use may vary within individual plants as water redistribution across roots (i.e. hydraulic lift). Hydraulic lift, the passive movement of water across soil water potential gradients through plant roots (Breazeale 1934; Richards and Caldwell 1987; Caldwell et al. 1998), has been observed in a wide range of arid and semi-arid ecosystems (reviewed in Neumann and Cardon 2012; Prieto et al. 2012; Sardans and Penuelas 2014). Although not previously observed in grasslands, hydraulic lift may play an important role in tallgrass prairies because most tallgrass prairies plants have deep roots (Weaver 1919) that extend between areas

of dry, shallow soil and deep, wet soil (Abrams and Knapp 1986; Williams and Rice 2007; Zeglin et al. 2013). Understanding if, when, and where water redistribution occurs in tallgrass prairies is important because this process could represent an unidentified drought avoidance strategy, with potential consequences for community interactions such as facilitation (Caldwell 1990; Dawson 1993; Moreira et al. 2003; Warren et al. 2007) and competition (Ludwig et al. 2003, 2004) among neighboring plants.

Third, water use may vary through time, at daily, intra-annual, and inter-annual time scales. Spot measurements of leaf gas exchange across a growing season and continuous eddy covariance measurements across the landscape show that water fluxes do indeed vary across time in tallgrass prairies (Nippert et al. 2007; Logan and Brunsell 2015). However, instantaneous leaf level measurements do not capture high-resolution temporal flux dynamics, while eddy covariance measurements integrate water fluxes over large spatial scales and cannot be used to identify species-specific drivers of these fluxes. Thus, we lack an understanding of continuous water use in individual species. Additionally, we have no knowledge of how diel water fluxes vary throughout a single day. While many studies have shown that substantial rates of nocturnal transpiration occur in other ecosystems (Caird et al. 2007; Dawson et al. 2007), whether nocturnal water loss occurs in tallgrass prairie is unknown. Evaluating diel patterns of water loss will therefore improve estimates of tallgrass prairie water budgets and also provide insight into whether this phenomenon exists as an important ecohydrological strategy in diverse grassland communities.

## **Objectives and Hypotheses**

The primary goal of this dissertation was to better understand the patterns, drivers, and ecological consequences of grassland water fluxes at fine spatial and temporal scales, focusing

on water uptake (Chapter 2), water redistribution (Chapter 3), and water loss (Chapters 4-5). Using a combination of leaf gas exchange, sap flow, and isotopic techniques, I addressed the following questions: 1) How does fire and grazing by bison impact water source use and niche overlap for common grassland grasses, forbs, and shrubs? 2) Does hydraulic lift occur in grazed and ungrazed tallgrass prairie, and does this impact facilitation for water within grassland communities? 3) What are the patterns and drivers of nocturnal transpiration in common grassland species? 4) What are the patterns and drivers of stem sap flow and canopy transpiration in common grassland species?

## **Chapter 2: Water Uptake**

Fire and grazing are important disturbances in grasslands, yet we know little about how they impact plant physiological processes, such as plant water uptake. To address this knowledge gap, I evaluated whether fire and grazing alter water source use in common grassland species, if changes in source water alter niche overlap among species, and how these patterns vary across topographic gradients and through time. Using stable isotope techniques I tested the following hypotheses:

- 1) Because grazing can reduce shallow grass root biomass, the presence of bison will lower the depth of water utilized by a dominant grass (*Andropogon gerardii*).
- 2) The presence of bison will also lower the depth of water utilized by forb and woody species because grazing reduces soil moisture and because these species can shift dependence on water source according to availability.
- 3) Forbs and woody species will use deeper water in recently burned areas because fire can also reduce surface soil moisture.

- 4) Shifts in water uptake associated with varying combinations of grazing and fire treatments will alter niche overlap among species.
- 5) Responses to low soil moisture associated with fire and grazing would be exacerbated in xeric uplands and later in the growing season, when soil water is more limiting.

### **Chapter 3: Water Redistribution**

Hydraulic lift is a key ecohydrological process in many water-limited ecosystems, though the occurrence and relevance of this phenomenon in grasslands is unknown. However, hydraulic lift may be an important drought avoidance strategy in tallgrass prairies because these ecosystems are often limited by water and because many prairie plants have deep roots that extend between wet and dry soil layers. Here, I assessed whether hydraulic lift occurs in a tallgrass prairie, if bison grazing or nocturnal transpiration limits hydraulic lift, and if water redistribution results in facilitation for neighboring grasses. I tested four hypotheses:

- 1) Common grass, forb, and woody species will all exhibit hydraulic lift because they are all deep-rooted ( $\geq 2$  m deep).
- 2) Because grazing reduces shallow root biomass, the presence of bison will reduce water efflux from shallow roots and ultimately limit hydraulic lift.
- 3) Reductions in hydraulic lift will also be associated with higher rates of nocturnal transpiration.
- 4) If a dominant grass, *A. gerardii*, does not exhibit hydraulic lift, it will instead utilize water lifted to shallow soil by neighboring shrubs or forbs.

## **Chapters 4 & 5: Water Loss**

Tallgrass prairies are diverse ecosystems that are currently undergoing extensive landscape changes. However, predicting corresponding changes in ecosystem fluxes is a major challenge because we do not know how transpiration dynamics vary at high-resolution temporal scales or among co-existing growth forms. In Chapter 4 I measured diel patterns of leaf gas exchange to assess whether nocturnal transpiration occurs among coexisting tallgrass prairie species, how patterns of nocturnal transpiration change over time, and whether nocturnal transpiration is regulated by plant stomata. Specifically, I tested the following hypotheses:

- 1) Nocturnal transpiration will occur among coexisting plant functional types including grasses, forbs, and shrubs.
- 2) Daytime and nocturnal transpiration will vary intra-annually and will be greatest early in the growing season, during periods with the highest soil water availability.
- 3) Nocturnal water loss will be actively regulated by nocturnal stomatal conductance.

In Chapter 5 I use external heat balance sap flow sensors and a Bayesian modeling technique to investigate continuous patterns of diel whole-plant water use in a grassland community. I tested two hypotheses:

- 1) Diurnal and nocturnal fluxes will vary among common grasses, forbs, and woody species, with the highest rates observed among woody species.
- 2) Flux rates will vary across a growing season and will be greatest during periods of high soil moisture.



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## **Chapter 2 - Grazing by bison is a stronger driver of plant ecohydrology than fire history**

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### **Abstract**

Fire and grazing are important disturbances in grasslands, yet we know little about how they impact a variety of plant physiological processes such as plant ecohydrology. Here, we assessed the impact of fire history and grazing by *Bison bison* on the source of water uptake and niche overlap in common grassland species at the Konza Prairie Biological Station, a temperate mesic grassland located in northeastern Kansas, USA. We used the stable isotopic signature of soil and xylem water to evaluate water uptake in *Andropogon gerardii*, *Vernonia baldwinii*, *Amorpha canescens*, and *Rhus glabra* within varying grazing (grazed, ungrazed), fire (0,1,2 or 3 years since last burn), topography (upland, lowland), and month (July, August) contrasts over three years (2013-2015). The presence of grazers, not fire history, altered water uptake patterns in these common grassland species. Particularly, grazing increased the proportion of shallow water utilized by *A. gerardii* and *R. glabra*, reducing niche overlap with other co-occurring species. However, these responses varied intra-annually and were often modulated by topography. These results suggest that grazing can alter aspects of grassland ecohydrology at small scales, which may extend to impact community and ecosystem processes at larger spatial scales.

## Introduction

Fire and grazing are important drivers of ecosystem structure and function in grasslands and savannas worldwide (Bond and Keeley 2005; Knapp et al. 1999). In mesic grasslands, grazing and fire can have strong but contrasting effects on vegetation dynamics such as primary productivity, plant diversity, and vegetation heterogeneity. Depending on the evolutionary history of grazing (Cingolani et al. 2005; Milchunas et al. 1988), large herbivores can increase species diversity and structural heterogeneity (Belsky 1992; Collins et al. 1998; Eby et al. 2014; Hartnett et al. 1996; Knapp et al. 1999) because they preferentially graze on dominant C<sub>4</sub> grasses over subdominant C<sub>3</sub> grasses, forb, and shrubs (Fahnestock and Knapp 1993; Veen et al. 2008). Conversely, fire reduces diversity and increases productivity by creating high light, low nitrogen environments that favor the growth of dominant C<sub>4</sub> grasses (Briggs and Knapp 1995; Collins 1992; Collins and Calabrese 2012; Collins and Smith 2006; Seastedt et al. 1991; Veen et al. 2008). However when fire and grazing occur together, these drivers interact to create highly productive, diverse, and heterogeneous ecosystems (Anderson et al. 2006; Archibald et al. 2005; Collins et al. 1998; Collins and Calabrese 2012; Collins and Smith 2006; Hartnett et al. 1996; Noy-Meir 1995; Veen et al. 2008; Vinton et al. 1993). Consequently, these disturbances have been extensively studied and are often used as management tools to maintain grassland biodiversity (Fuhlendorf and Engle 2004; Hamilton 2007) and ecosystem structure (Briggs et al. 2002; Briggs et al. 2005; Uys et al. 2004).

Although vegetation responses to fire and grazing have been well studied at the community and ecosystem levels, comparatively little is known about the physiological mechanisms that underpin these patterns. Most research detailing physiological responses to fire and grazing have been limited to studies of leaf-level gas exchange and water potential. These

studies have shown that grazing generally increases photosynthetic rates following defoliation, allowing for rapid regrowth (Detling et al. 1979; Harrison et al. 2010; Painter and Detling 1981; Peng et al. 2007; Wallace 1990). Defoliation also reduces leaf area and thus, the amount of water transpired per ground area, which can increase soil moisture and leaf water potential of both the grazed plants (Archer and Detling 1986; Harrison et al. 2010; Svejcar and Christiansen 1987) and neighboring ungrazed plants (Fahnestock and Knapp 1993,1994). Conversely, fire increases photosynthesis soon after burning but reduces leaf water potential later in the growing season due to enhanced transpiration during hot, dry periods (Knapp 1985), although responses are often species specific and can vary across ecosystems (Hodgkinson 1992; Knapp 1985; Potts et al. 2012; Turner et al. 1995).

While many studies show sensitive leaf-level responses to grassland disturbances, it is unclear how fire and grazing affect other aspects of plant functioning such as whole-plant ecohydrology. Grassland ecohydrology is complex, even without considering the effects of fire and grazing, because ecohydrological processes are often influenced by environmental heterogeneity. For example, shallow and deeply rooted grassland species commonly exhibit vertical niche partitioning of water from contrasting soil depths (Ward et al. 2013; Weaver 1968; Weaver and Albertson 1943). However, the extent of niche partitioning can vary through space and time, as  $C_4$  grasses typically have a fixed reliance on water from the shallowest soil layers, while  $C_3$  forbs and shrubs exhibit more opportunistic plasticity to utilize water from multiple soil layers depending upon availability (Asbjornsen et al. 2008; Kulmatiski and Beard 2013; Nippert and Knapp 2007a,b; Priyadarshini et al. 2015). The observation that plant water uptake shifts in response to changing resources suggests that water use is likely sensitive to environmental changes associated with grassland disturbances as well.

Multiple factors associated with fire and grazing, including changes in root growth and soil moisture, could alter water use dynamics in grasslands. Grazing often reduces shallow root biomass as plants allocate carbon aboveground for regrowth (Johnson and Matchett 2001; Klumpp et al. 2009; Nippert et al. 2012), which may limit shallow water uptake in grasses. Grazing can also increase soil moisture by reducing transpiration (Archer and Detling 1986; Harrison et al. 2010; Svejcar and Christiansen 1987) or reduce soil moisture by compacting soil and limiting infiltration (Greenwood and McKenzie 2001; Hamza and Anderson 2005; Naeth et al. 1991). Similarly, fire can reduce soil moisture by removing detrital layers and exposing soil to higher temperatures and evaporation rates (Hulbert 1969; McMurphy and Anderson 1965). Drier soil resulting from either disturbance may shift plant dependence on deeper water, as has been observed in certain grassland species during seasonal drought (Asbjornsen et al. 2008; Nippert and Knapp 2007a,b). Water uptake should therefore be responsive to fire and grazing, which could have compounding impacts on other ecological processes such as niche partitioning, interspecific competition, and community composition. Thus, assessing how water uptake varies across a range of disturbance regimes will improve our understanding of how ecological interactions respond to common grassland management practices.

Here, we evaluated the role of common grassland disturbances on vertical water partitioning within a tallgrass prairie community. Specifically, we asked: 1) Does grazing by *Bison bison* (bison) alter the depth of water uptake in common grass, forb, and woody species? Grazing can reduce shallow grass root biomass (Johnson and Matchett 2001; Nippert et al. 2012), so we predicted that the presence of bison would lower the depth of water utilized by a dominant grass (*Andropogon gerardii* Vitman, big bluestem; Figure 2.1). Because grazing can also reduce soil moisture (Archer and Detling 1986; Greenwood and McKenzie 2001; Hulbert

1969), and because forbs and shrubs can shift dependence on water source according to availability (Asbjornsen et al. 2008; Nippert and Knapp 2007a,b), we predicted that bison would also lower the depth of water utilized by forb and woody species (Figure 2.1). 2) Does time since last prescribed fire modify the depth of water uptake in these species? Fire can also reduce soil moisture, so we predicted that forbs and woody species would use deeper water in recently burned areas compared to areas that were burned less recently (Figure 2.1). 3) Do shifts in water uptake alter the degree of niche overlap among species? We predicted that shifts in water associated with varying combinations of grazing and fire treatments would alter niche overlap among species (Figure 2.1). 4) Are these responses modulated by topography or by time of year? Finally, we predicted that responses to low soil moisture associated with fire and grazing would be exacerbated in xeric uplands and later in the growing season, when soil water is more limiting.

## **Methods**

### **Site Description**

Research was conducted at the Konza Prairie Biological Station (KPBS), a 3,487-ha native tallgrass prairie supported by the NSF Long Term Ecological Research Network. KPBS is located within the Flint Hills of northeastern Kansas, USA (39.1°N, 96.9°W), where long-term weathering has created a topographically complex landscape consisting of flat upland ridges, deep-soiled lowlands, and steep slopes that span a relief of 40-70 m within watershed basins (Oviatt 1998). Layers of limestone and Permian shale alternate across the terrain (Jantz et al. 1975) and soil depth varies by topography, with shallow (<0.5 m), rocky soil in the uplands and deep (>2.0 m) silty-clay loams in the lowlands. Lowland soils are generally wetter than uplands

and deep lowlands soils have water available throughout the growing season (Figure A.1). KPBS is divided into watersheds that receive varying combinations of fire frequency (burned every 1, 2, 4, or 20 years) and grazing (grazed by cattle, bison, or ungrazed) treatments. The landscape is dominated by a few perennial C<sub>4</sub> grass species and also contains numerous sub-dominant C<sub>3</sub> grass, forb, and woody species (Smith and Knapp 2003).

The Flint Hills region experiences a mid-continental climate, with cool, dry winters and warm, wet summers. Mean annual precipitation for the region (1982-2014) is 829 mm, of which 73% occurs during the growing season (April – September). Precipitation was 783 mm in 2013 (77% of which occurred during the growing season), 706 mm in 2014 (68% of which occurred during the growing season), and 1002 mm in 2015 (75% of which occurred during the growing season). The average coldest month of the year (1982-2014) is January, with mean minimum and maximum air temperatures of -7.14°C and 4.87 °C, respectively. The average warmest month (1982-2014) is July, with mean minimum and maximum air temperatures of 19.78 °C and 32.69 °C, respectively. Mean minimum air temperatures during July 2013, 2014, and 2015 were 18.46°C, 17.06°C, and 20.67°C respectively. Maximum air temperatures during July 2013, 2014, and 2015 were 30.99°C, 31.7°C, and 32.02°C, respectively.

## **Sampling Description**

We sampled four species representative of different plant functional types, including one C<sub>4</sub> grass (*A. gerardii*), one C<sub>3</sub> forb (*Vernonia baldwinii* Torr., Baldwin's ironweed), one leguminous C<sub>3</sub> sub-shrub (*Amorpha canescens* Pursh., leadplant), and one C<sub>3</sub> shrub (*Rhus glabra* L., smooth sumac). These species are deep-rooted (Weaver 1968; Weaver and Albertson 1943) and are common across KPBS. Additionally, *A. gerardii* is the only of these species that is

grazed by bison, as bison prefer the highly nutritious, palatable new growth of C<sub>4</sub> grasses (Coppedge and Shaw 1998; Fuhlendorf and Engle 2001; Plumb and Dodd 1993;). Although white-tailed deer (*Odocoileus virginianus*) are also present at the site, they also prefer to browse on herbaceous growth rather than woody species (van der Hoek et al. 2002). Sampling occurred in two grazed (N4B and N4D) and two ungrazed (4B and 4F) watersheds at KPBS. All watersheds were burned once every four years, but not in the same calendar year (Table 2.1). Samples were collected from three spatially separate 50 x 50 m permanent plots (>100 meters apart) in both topographic positions within each watershed, and one sample per species was collected from a random location within each plot, resulting in n=3 for each species. *R. glabra* was not present in N4D lowlands. Due to the destructive nature of collecting plant tissue for isotopic analyses, separate but similarly sized individuals were randomly sampled within each sampling location during each collection period.

Samples were collected two times per growing season, during early July (mid growing season) and during late August (late growing season) for three consecutive years (2013-2015). During each sampling period, all samples were collected when no precipitation had occurred for at least five days to prevent contamination of soil water with the isotopic signature of rainwater.

### **Isotope Samples**

To assess the source of water uptake in each species, approximately 20-30 g of non-photosynthetic crown tissue was collected from each species. Plant tissue was collected from one stem per species at each sampling location excluding *A. gerardii*, which required 5-10 tillers to produce enough water for analyses. Samples were immediately stored in exetainer vials (Labco, UK) on ice until transferred to a 1-2 °C refrigerator. One 25 cm deep soil core per replicate



sampling location was also collected with a hand corer. Soil cores were split into 5 cm sections (0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, and 20-25 cm depths), placed in double-layered plastic bags, and stored on ice until transferred to a -5 °C freezer. The frozen soil was later transferred to exetainer vials for water extraction. All isotope samples were collected on two consecutive days within each sampling period due to time constraints, on climatically similar days during which no precipitation occurred. Water was extracted from plant and soil tissue using the cryogenic vacuum distillation method (Ehleringer and Osmond 1989; Nippert and Knapp 2007a). The stable hydrogen ( $\delta\text{D}$ ) and oxygen ( $\delta^{18}\text{O}$ ) ratios of water samples were then analyzed using a Picarro WS-CRDS isotopic water analyzer and possible interference or contamination were checked using ChemCorrect software. The  $\delta\text{D}$  and  $\delta^{18}\text{O}$  ratios are reported as deviations from an international standard in parts per thousand (‰) using  $\delta$ -notation:

$$\delta = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \right] \quad (1)$$

where R is the absolute ratio of the rare and common isotope for the sample and standard.

### **Leaf Water Potential**

Midday leaf water potential ( $\Psi_{\text{md}}$ ) was measured on one individual per species in every sampling location and during every sampling period. Measurements were made on different days with similar weather conditions to those when isotope samples were collected. The youngest, fully developed leaf was collected from similarly sized individuals, stored in a darkened, humidified plastic bag, and allowed to equilibrate for an hour. Leaf water potential was then measured with a Scholander pressure chamber (PMS Instrument Company, Albany, OR).

## Soil Moisture

The relative water content (RWC) of the top 10 cm soil was measured with a Hydra Probe II Soil Sensor (Stevens Water Monitoring Systems, Portland, OR, USA) in units of water fraction by volume (wfv). Five subsample measurements were made randomly within each sampling location during days on which isotope samples were collected. These subsamples were then averaged to calculate a mean shallow soil moisture value for each sampling location.

## Statistics

All analyses were conducted using the statistical program R V3.1.0 (R Core Team 2012). We used Information Theoretic (IT) model averaging (Burnham and Anderson 2004) to assess the influence of grazing, time since last fire, month, and topographic position on xylem  $\delta^{18}\text{O}$ , midday leaf water potential, and soil RWC. For all response variables, model averaging was performed for each species individually. First, we created a linear mixed effects global model including all factors using the *lmer* function of the *lme4* package V1.1-7 (Bates *et al.* 2014). In each global model, grazing treatment, time since fire, month, and topography were fixed effects, and sampling year and sampling location within watershed were random effects. After the global model was defined we standardized the input variables with the *standardize* function in the *arm* package (Gelman et al. 2009). We then created a full submodel set with the *dredge* function in the *MuMIn* package (Barton 2009) and obtained the top model set using 4AICc as a cutoff with the *get.models* function in the *MuMIn* package, according to Grueber et al. (2011). The top model set was then averaged using the zero method with the *model.avg* function in the *MuMIn* package. Relative importance was determined for each parameter included in the final average model. Relative importance is a unitless metric that sums Akaike

weights for all top models in which the parameter appears and ranges from 0 (indicating that the parameter has no explanatory weight) to 1 (indicating that the parameter is included in all top models) (Burnham and Anderson 2004).

Stable Isotope Analysis in R (SIAR), a Bayesian mixing model, was used to estimate the proportion of surface and deep water sources in plant tissue (Parnell 2010). We ran two sets of analyses; the first set ran individual models for each grazing x month x topography combination and the second set ran individual models for each fire x month x topography combination. Each analysis ran for 500,000 iterations, of which the first 50,000 iterations were discarded. Surface water sources were obtained from the 5-10 cm deep section of the soil cores collected at each location and the corresponding sample for each watershed x topography x month contrast was used for each analysis (Table A.1). A deep soil core (1-2 m deep) was collected from five random lowland locations across KPBS with a 540MT Geoprobe Systems hydraulic-push corer (Salina, KS, USA) and was used as the deep source in all analyses.

To evaluate whether shifts in source water alter niche overlap with other species, we calculated the proportional similarity index (PS) (Colwell and Futuyma 1971) of source water between all possible species pairs using the following equation:

$$PS = 1 - 0.05 \sum_{i=1}^n |p_{1i} - p_{2i}| \quad (2)$$

where  $p$  is the proportional contribution of source  $i$  for species 1 and 2, and  $n=2$  sources (surface and deep). Proportional similarity values range from 0 (no niche overlap) to 1 (complete niche overlap).

## Results

### Isotope Samples

Variability in xylem water  $\delta^{18}\text{O}$  was best described by the individual effects of grazing and month for *A. gerardii*, *V. baldwinii*, and *R. glabra* (importance  $\geq 0.98$  for both predictors in each species; Figure 2.2; Tables A.2,3,5). Xylem  $\delta^{18}\text{O}$  was lower (more depleted) in ungrazed areas compared to grazed areas for *A. gerardii*, *V. baldwinii*, and *R. glabra* (Table A.6). Xylem  $\delta^{18}\text{O}$  was also lower in July for *A. gerardii* and *V. baldwinii*, and lower in August for *R. glabra* (Table A.6). Conversely, *A. canescens*  $\delta^{18}\text{O}$  was lower in ungrazed areas, but only in August (grazing x month importance = 1.0; Figure 2.2; Tables A.4,6). Topography and fire were generally less important in describing xylem water  $\delta^{18}\text{O}$  for all species (Figure 2.2; Tables A.2-5). However, xylem  $\delta^{18}\text{O}$  was lower in lowland locations for all species except *R. glabra* (Table A.6), and lower during years 1 and 3 post-fire (Table A.7).

We estimated the proportion of surface and deep water used by each species within each month, grazing, and topography contrast and found that the effects of month, grazing treatment, and topography on source water were variable across species. *A. gerardii* generally used more surface water (5-10 cm deep) than the other species and used more surface water in August (63-90%) than in July (53-72%) (Figure 2.3). Grazing generally increased the proportion of surface water used by *A. gerardii*, although this shift primarily occurred in lowland locations. *A. gerardii* used 12% more shallow water in grazed areas compared to ungrazed areas during July (Figure 2.3c-d) and 16% more in grazed areas during August (Figure 2.3g-h). No shift in water source occurred in upland locations during July, and *A. gerardii* used 11% less shallow water in upland grazed areas than in upland ungrazed areas during August.

Although the effects of grazing and month on *V. baldwinii* and *A. canescens*  $\delta^{18}\text{O}$  had high importance values (Figure 2.2), the modeled estimates of surface and deep water sources used by these species were generally consistent across treatments (Table 2.2). These species used  $\geq 90\%$  deep water across all treatment contrasts, and any detectable variation typically occurred in the shape of the posterior density distributions (Figure A.2-3), rather than in mean source values. *R. glabra* also primarily used deep water, although the proportion of surface and deep water occurring in *R. glabra* xylem water varied by months and grazing treatments (Figure 2.4). *R. glabra* used proportionally more surface water in July (4-19%) than in August (2-4%). Like *A. gerardii*, *R. glabra* also used more surface water in grazed watersheds, both in upland (8% increase) and lowland locations (9% increase) during July.

We also estimated the proportion of shallow and deep water used by each species within each month, topography, and fire contrast, and found that source water varied little by time since previous fire. *V. baldwinii*, *A. canescens*, and *R. glabra* used proportionally more shallow water during 1 and 3 years since the previous fire, but these trends were variable across months and topographic positions (Table A.8).

Finally, we used the modeled estimates of source water contribution to evaluate niche overlap between each species pair (Table 2.3). *A. gerardii* had lower niche overlap with all other species in lowland grazed areas (0.279-0.323 in July and 0.210-0.303 in August) compared to lowland ungrazed areas (0.402-0.462 in July and 0.370-0.423 in August). *R. glabra* also had lower niche overlap with other species in lowland grazed areas (0.270-0.854) than in ungrazed areas (0.402-0.954), but only in July. *V. baldwinii* and *A. canescens* consistently had high niche overlap with each other (0.950-0.999).

## Leaf Water Potential

Midday leaf water potential was best explained by different variables for each species (Tables A.9-12). Generally, leaf water potential values were higher in July than in August, particularly for *A. gerardii* (importance = 1.0; Table A.9; Figure 2.5a) and *A. canescens* (importance = 1.0; Table A.11; Figure 2.5c). Leaf water potential values were also greater in ungrazed areas compared to grazed areas during July (Figure 2.7a,b,d) for *V. baldwinii* (importance = 1.0; Table A.10) and *R. glabra* (importance = 1.0; Table A.12). Finally, *A. gerardii* had lower leaf water potential values in upland ( $-1.738 \text{ MPa} \pm 0.085 \text{ SE}$ ) than in lowland ( $1.442 \text{ MPa} \pm 0.065 \text{ SE}$ ) areas, as well as in watersheds that were burned the previous year ( $-2.123 \text{ MPa} \pm 0.177 \text{ SE}$ ) than in years 0, 2, or 3 post-fire ( $-1.476 \text{ MPa} \pm 0.088 \text{ SE}$ ,  $-1.358 \text{ MPa} \pm 0.508 \text{ SE}$ , and  $-1.746 \text{ MPa} \pm 0.091 \text{ SE}$ , respectively).

## Soil Moisture

Variability in soil moisture was best described by month x topography, grazing x topography, and month x fire interactions in the average model for soil RWC (importance = 1.0 for each interaction; Table A.13). Soil RWC was lower in upland locations than lowland locations, and ungrazed upland locations had lower soil RWC than grazed upland locations during July (Figure 2.6). Finally, soil RWC was lower during years 1 and 3 since the previous burn, but only during August (Table A.14).

## Discussion

We used the stable isotopic signatures of soil and xylem water to assess the impacts of bison grazing and fire history on water uptake and hydrological niche overlap in common

grassland species of North America. Our results are consistent with previous observations of source water partitioning in grasslands, where dominant C<sub>4</sub> grasses generally rely on water from shallow depths while some C<sub>3</sub> forbs and shrubs exhibit plasticity in water use depending on availability. However, we also found that grazing, not fire, impacted water uptake in certain species (*A. gerardii* and *R. glabra*), and that changes in the depth of water uptake were associated with shifts in niche overlap among co-occurring species. Grazing responses also varied intra-annually and were altered by topography. Our results suggest that grazing can impact grassland ecohydrology at small scales, although responses are species-specific and are modulated by other spatial and temporal factors.

We expected that grazing-induced reductions in shallow root biomass (Kitchen et al. 2009; Nippert et al. 2012) and soil moisture (Greenwood and McKenzie 2001; Hamza and Anderson 2005; Naeth et al. 1991) would cause grasses to rely on deeper water in grazed areas than in ungrazed areas, increasing niche overlap with co-occurring species (Figure 2.1). Contrary to our hypothesis, we found that grazing actually increased the proportion of shallow water used by both *A. gerardii* and *R. glabra*, and this resulted in reduced niche overlap (Figures 2.3,4; Table 2.3). Soil moisture availability did not likely drive this trend, since soil RWC did not differ between grazing treatments (Figure 2.6). It is also unlikely that changes in shallow root biomass altered depth of water uptake because our data show increased reliance on water from shallow depths. These results suggest that *A. gerardii* can effectively use shallow water in grazed locations, despite reduction in root biomass, and is likely able to maintain constant water use due to physiological drought tolerance (Knapp 1984; Nippert et al. 2009; Tucker et al. 2011).

Rather than the amount of soil moisture or root biomass, we suggest that the shift in proportional water uptake is related to varying plant communities in grazed and ungrazed

watersheds. Bison preferentially graze on C<sub>4</sub> grasses such as *A. gerardii*, which reduces dominant grass cover and increases the cover of forb and woody species. For example, the presence of bison at the Konza Prairie have been shown to reduce the percent cover of *A. gerardii* (Hartnett et al. 1996; Vinton et al. 1993) and increase the percent cover of *V. baldwinii* (Vinton et al. 1993), *A. canescens* (Hartnett et al. 1996), and common shrubs and trees (Briggs et al. 2002). As a result, locations that are grazed also have greater plant diversity, richness, and evenness (Hartnett et al. 1996). Previous work has shown that local diversity can impact resource acquisition and niche space within communities (Grossiord et al. 2014; Kunert et al. 2012; Verheyen et al. 2008). Niche partitioning of resources results from either: 1) interspecific differences in traits that allow plants to inherently exploit different resources, or 2) interspecific interactions that cause plastic shifts in resource use as species avoid competition (Valverde-Barrantes et al. 2013; von Felton et al. 2009). When plasticity arises from interspecific interactions, niche breadth and overlap are often reduced in an effort to reduce competition (Silvertown et al. 1999). In this case, depth of water uptake may shift as plants alter niche space to avoid competition in diverse communities (Hoekstra et al. 2014; Grossiord et al. 2014; Meißner et al. 2012).

Our results suggest that the high plant diversity resulting from the presence of grazers (Belsky 1992; Collins et al. 1998; Eby et al. 2014; Hartnett et al. 1996; Knapp et al. 1999) may create more numerous and smaller hydrological niches in this system. Reduced niche space per species may influence *A. gerardii* towards increased reliance on surface water to avoid niche overlap with species that use water at deeper depths (Figure 2.3; Table 2.3), particularly because this grass species can tolerate drier soils than the other abundant species at this site (Knapp 1984; Nippert et al. 2009; Tucker et al. 2011). Previously, Hoekstra et al. (2014) found that grasses



shift reliance to shallow water when grown in mixtures with deep-rooted species. We also found that *R. glabra* used proportionally less deep water in grazed areas, which reduced niche overlap with other deep-rooted species, while *V. baldwinii* and *A. canescens* continued to rely primarily on deep water (Figure 2.4; Tables 2.2-3). These results indicate that plasticity and tolerance of niche overlap are species-specific. Shifts in niche overlap due to grazing also suggest that competition for water may be lower in high diversity, grazed locations, although further investigation comparing community diversity with competition for water is required.

Grazers also impacted leaf-level physiology, as midday leaf water potential was lower in grazed areas than in ungrazed areas for *A. gerardii*, *V. baldwinii*, and *R. glabra* during July (Figure 2.5). This result may have occurred despite the higher soil RWC measured in grazed watersheds during July (Figure 2.6) because grazed areas are often characterized by more bare ground than ungrazed areas. Depending on the amount of solar radiation, the albedo of soil and plant cover, and the roughness of the terrain, bare ground can increase sensible heat and may consequently increase leaf temperatures and reduce leaf water potential (Nippert et al. 2013). This result is contrary to the commonly observed increases in leaf water potential with grazing (Harrison et al. 2010; Svejcar and Christiansen 1987), and may explain why others have also reported conflicting results (Archer and Detling 1986). Thus, grazing can impact additional aspects of plant ecohydrology independent of changes in soil moisture or source water use.

Although grazing can alter depth of water uptake and niche overlap in some species, fire history was generally not an important predictor of source water, particularly for *A. gerardii* and *R. glabra* (Figure 2.2; Tables A.2-5). The SIAR mixing models showed that surface water contributed proportionally more to *V. baldwinii*, *A. canescens*, and *R. glabra* xylem water in years 1 and 3 since last burn (Table A.8). However, “time since burn” had low importance values

for all species except *A. canescens*, suggesting that, when the variability associated with “year” was accounted for as a random effect, this trend was simply an artifact of the calendar year in which the “time since burn” treatment occurred (i.e., years 1 and 3 since last burn both occurred in 2014, while years 0 and 2 occurred in both 2013 and 2015; Figure 2.2; Table 2.1; Tables A.2-5). If plant diversity of the local community is indeed the primary driver of differences in water uptake between grazed and ungrazed locations, we would not expect fire history to impact source water because all watersheds studied here are burned every four years and therefore have similar plant diversity within grazing treatments despite being burned in different calendar years.

Finally, we investigated seasonal patterns and topographic differences in water uptake. Seasonal differences occurred in *R. glabra*, as source water varied among grazing treatments only in July (Figure 2.4). As is typical in other studies of C<sub>3</sub> woody species, (Asbjornsen et al. 2008; Kulmatiski and Beard 2013; Nippert and Knapp 2007a,b; Priyadarshini et al. 2015), *R. glabra* shifted reliance to deep water during drier periods, regardless of grazing treatment (Figures 2.4,6). Thus, soil water availability is still an important mediator of vertical niche partitioning for water, even when differences in partitioning are associated with varying plant communities. We also observed topographic differences in water uptake, particularly in *A. gerardii*. Whereas lowland *A. gerardii* consistently increased its reliance on surface water in grazed areas, upland *A. gerardii* did not use different water sources among grazing treatments during July and used proportionally less shallow water in grazed areas during August (Figure 2.3).

To conclude, the results from this study provide novel insights into the role of disturbance-driven heterogeneity in water uptake patterns and niche overlap in a diverse grassland community. Grazers modified functional niches in this tallgrass prairie plant

community by altering the depth of water uptake and degree of niche overlap. These results highlight that *A. gerardii*, a dominant C<sub>4</sub> grass in the region, is more plastic in its water use than previously described, and it suggests that niche partitioning may be very sensitive to changes in plant diversity within this heterogeneous ecosystem. These responses to grazing varied within growing seasons and are dependent on topography, suggesting that spatiotemporal variation in resource heterogeneity is an important driver of plant functioning in mesic grasslands. These results clearly show that grassland disturbance can have strong impacts on plant functioning at small scales, with important consequences for local ecohydrology.

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**Table 2.1 Fire histories for the grazed and ungrazed watersheds burned in different calendar years. Numbers indicate years since last burn within each sampling year. 0 = burned that year, 1 = 1 year since previous burn, 2 = 2 years since previous burn, and 3 = 3 years since previous burn.**

	2013	2014	2015
<b>Ungrazed</b>			
4B	0	1	2
4F	2	3	0
<b>Grazed</b>			
N4B	2	3	0
N4D	0	1	2

**Table 2.2 SIAR estimates of the proportion of shallow and deep water used by *V. baldwinii* and *A. canescens* within each month, topography, and grazing contrast. Shown are mean estimates for shallow and deep sources, with the 95% credible interval in parentheses.**

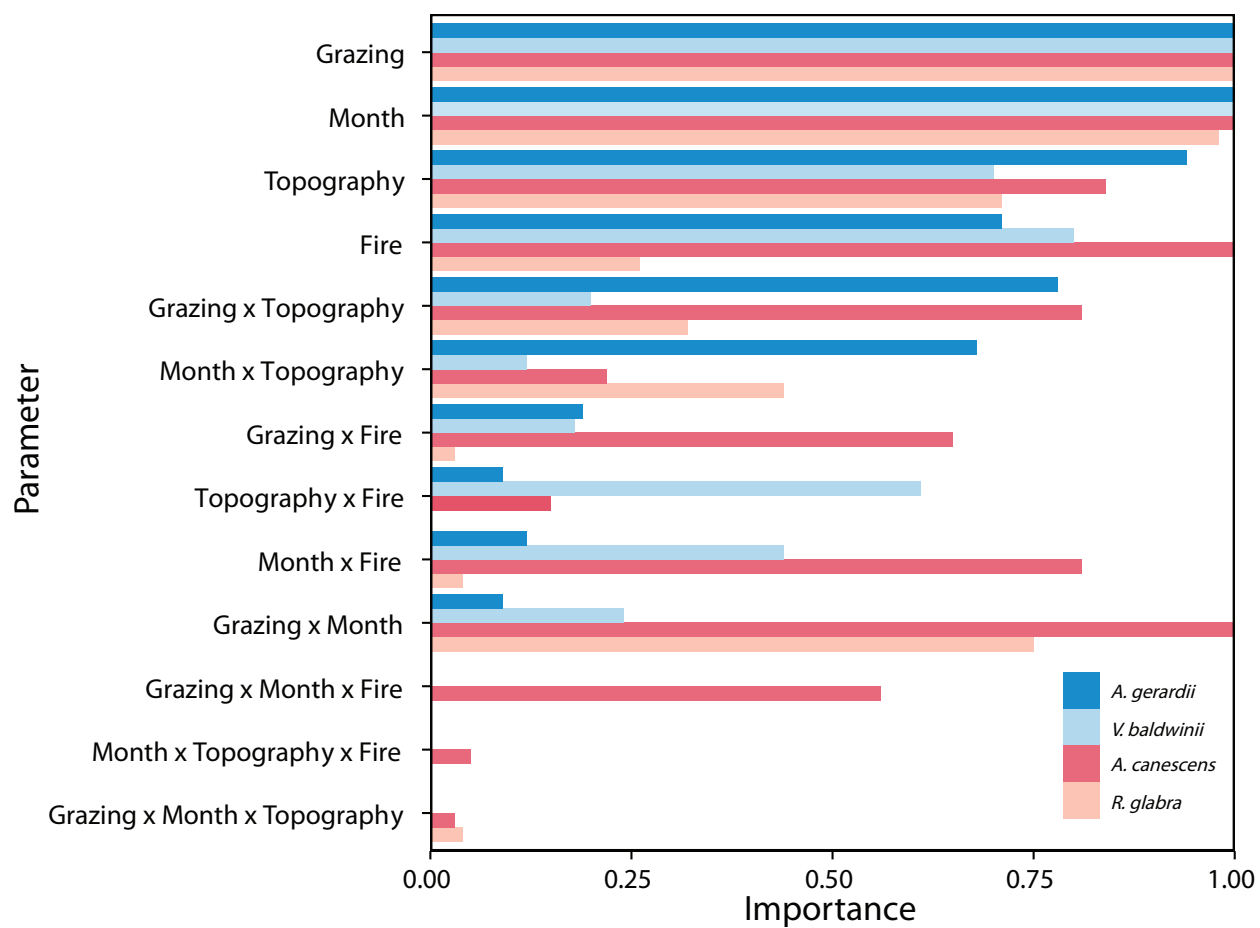
		July		August	
		Surface	Deep	Surface	Deep
<b><i>V. baldwinii</i></b>					
Grazed	Upland	0.038 (0.000, 0.116)	0.962 (0.884, 1.006)	0.045 (0.000, 0.134)	0.955 (0.866, 1.006)
	Lowland	0.045 (0.000, 0.136)	0.955 (0.864, 1.007)	0.043 (0.000, 0.125)	0.957 (0.875, 1.006)
Ungrazed	Upland	0.047 (0.000, 0.140)	0.953 (0.860, 1.007)	0.050 (0.000, 0.149)	0.950 (0.851, 1.007)
	Lowland	0.048 (0.000, 0.143)	0.952 (0.857, 1.007)	0.043 (0.000, 0.127)	0.957 (0.873, 1.006)
<b><i>A. canescens</i></b>					
Grazed	Upland	0.050 (0.000, 0.150)	0.950 (0.850, 1.007)	0.038 (0.000, 0.110)	0.962 (0.890, 1.005)
	Lowland	0.044 (0.000, 0.134)	0.956 (0.866, 1.006)	0.093 (0.000, 0.249)	0.907 (0.751, 1.011)
Ungrazed	Upland	0.050 (0.000, 0.147)	0.950 (0.853, 1.007)	0.042 (0.000, 0.120)	0.958 (0.880, 1.006)
	Lowland	0.059 (0.000, 0.183)	0.940 (0.817, 1.009)	0.053 (0.000, 0.163)	0.947 (0.837, 1.008)

**Table 2.3 Proportional similarity of source water use between different species pairs. Shown are comparisons between combinations of all *A. gerardii* (AG), *V. baldwinii* (VB), *A. canescens* (AC), and *R. glabra* (RG) pairs.**

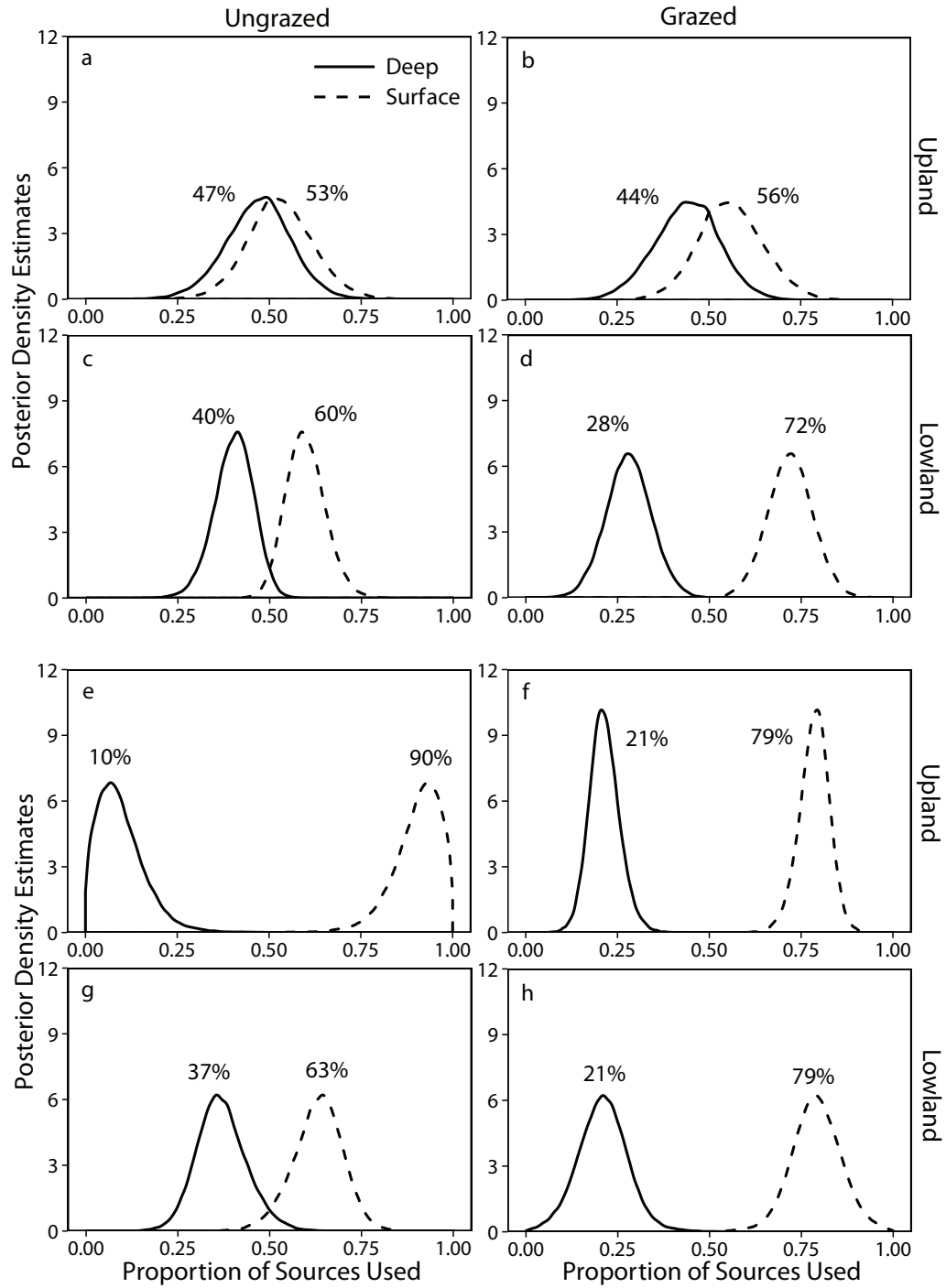
Comparison	July				August			
	Ungrazed		Grazed		Ungrazed		Grazed	
	Upland	Lowland	Upland	Lowland	Upland	Lowland	Upland	Lowland
AG – VB	0.520	0.462	0.491	0.323	0.138	0.423	0.252	0.303
AG – RG	0.470	0.402	0.441	0.279	0.096	0.370	0.214	0.210
AG – AC	0.517	0.450	0.479	0.324	0.146	0.413	0.259	0.253
VB – AC	0.997	0.989	0.998	0.999	0.992	0.990	0.993	0.95
VB – RG	0.989	0.954	0.918	0.854	0.981	0.980	0.981	0.996
AC – RG	0.986	0.966	0.930	0.853	0.989	0.970	0.988	0.946

	Grazed	Ungrazed
Burned Recently	<b>Prediction: low niche overlap</b> <ul style="list-style-type: none"> <li>• Low soil moisture = forbs &amp; woody use deep water</li> <li>• Reduced shallow grass root biomass = grasses use intermediate water</li> </ul>	<b>Prediction: low niche overlap</b> <ul style="list-style-type: none"> <li>• Low soil moisture = forbs &amp; woody use deep water</li> <li>• High shallow grass root biomass = grasses use shallow water</li> </ul>
Not Burned	<b>Prediction: niche overlap</b> <ul style="list-style-type: none"> <li>• Intermediate soil moisture = forbs &amp; woody use intermediate water</li> <li>• Reduced shallow grass root biomass = grasses use intermediate water</li> </ul>	<b>Prediction: niche overlap</b> <ul style="list-style-type: none"> <li>• High soil moisture = forbs &amp; woody use shallow water</li> <li>• High shallow grass root biomass = grasses use shallow water</li> </ul>

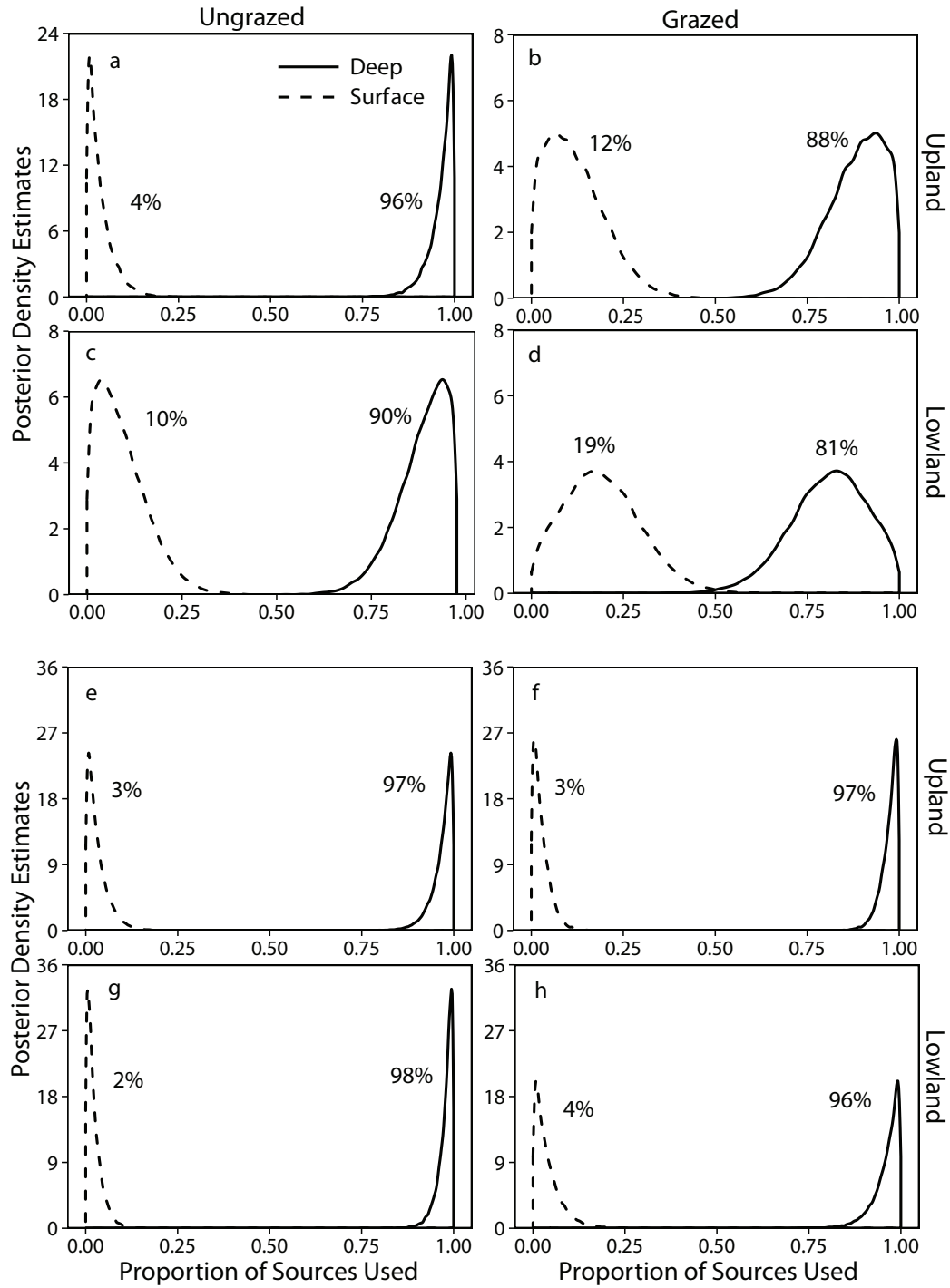
**Figure 2.1 Hypothesized changes in source water use and niche partitioning for grass, forb, and woody species with changes in grazing and fire.**



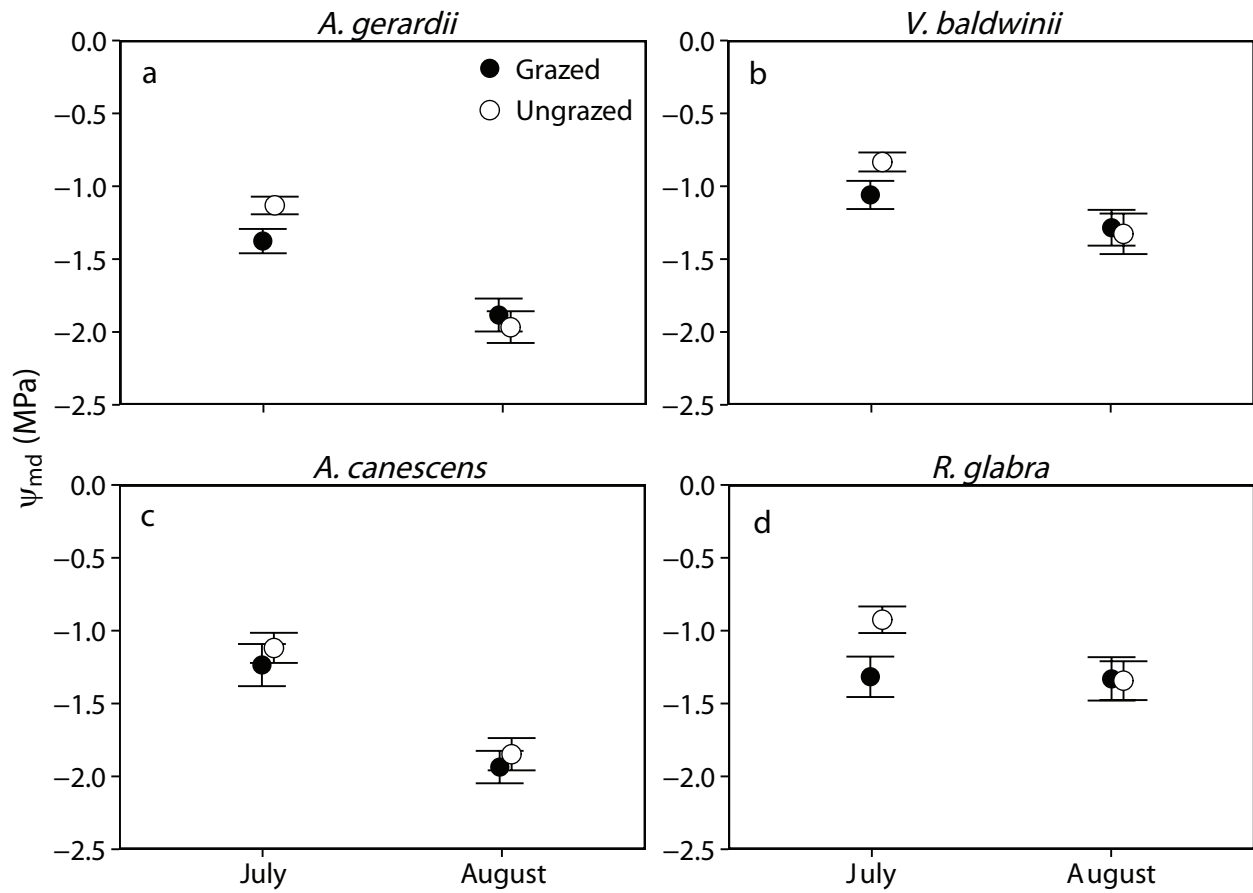
**Figure 2.2 Relative importance of all parameters included in the average  $\delta^{18}\text{O}$  models for all species.**



**Figure 2.3** Posterior density estimates for the proportion of surface and deep water sources used by *A. gerardii* in ungrazed (a,c,e,g) and grazed (b,d,f,h) watersheds, upland (a,b,e,f) and lowland (c,d,g,h) topographic positions, and during July (a-d) and August (e-h).

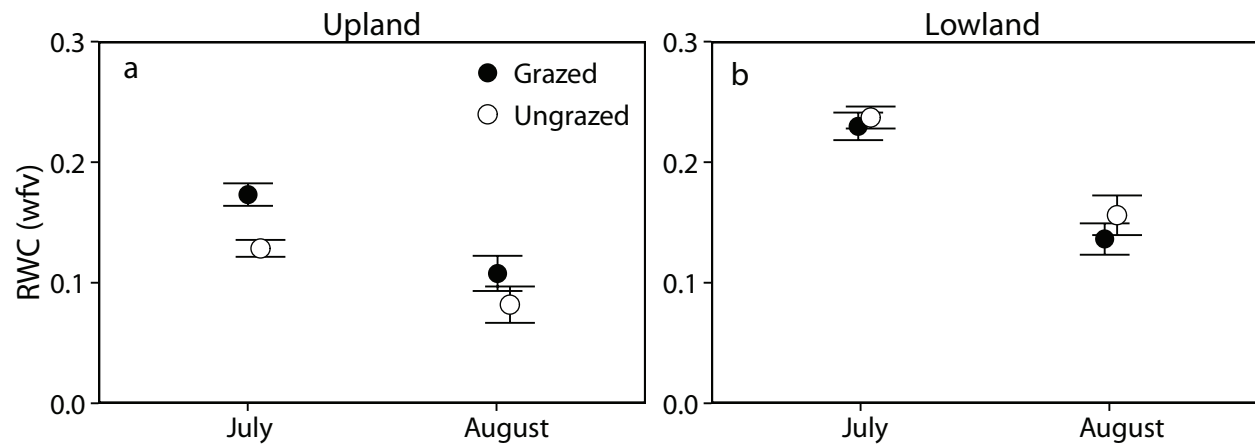


**Figure 2.4** Posterior density estimates for the proportion of surface and deep water sources used by *R. glabra* in ungrazed (a,c,e,g) and grazed (b,d,f,h) watersheds, upland (a,b,e,f) and lowland (c,d,g,h) topographic positions, and during July (a-d) and August (e-h).



**Figure 2.5** Midday leaf water potential ( $\Psi_{md}$ ) measured on *A. gerardii* (a), *V. baldwinii* (b), *A. canescens* (c), and *R. glabra* (d) in ungrazed and grazed locations. Shown are mean  $\pm$  1SE.





**Figure 2.6 Soil relative water content (RWC), measured in units of water fraction by volume (wfv) within the top 10 cm of the soil in all grazing treatments, months, and topographic locations. Shown are mean  $\pm$  1SE.**

## **Chapter 3 - An assessment of diurnal water uptake in a mesic prairie: evidence for hydraulic lift?**

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### **Abstract**

Hydraulic lift, the passive movement of water through plant roots from wet to dry soil, is an important ecohydrological process in a wide range of water-limited ecosystems. This phenomenon may also alter plant functioning, growth, and survival in mesic grasslands, where soil moisture is spatially and temporally variable. Here, we monitored diurnal changes in the isotopic signature of soil and plant xylem water to assess (1) whether hydraulic lift occurs in woody and herbaceous tallgrass prairie species (*Rhus glabra*, *Amorpha canescens*, *Vernonia baldwinii*, and *Andropogon gerardii*), (2) if nocturnal transpiration or grazing by large ungulates limits hydraulic lift, and (3) if a dominant grass, *A. gerardii*, utilizes water lifted by other tallgrass prairie species. Broadly, hydraulic lift does not appear to be widespread or common in this system, but isolated instances suggest that this process does occur within tallgrass prairie. Grazing did not impact patterns of hydraulic lift, nor did neighboring grasses utilize water lifted by target plants. We suggest that the topographic complexity of this tallgrass prairie and the high rates of nocturnal transpiration observed in this study likely limit the frequency and occurrence of hydraulic lift. These results suggest that hydraulic lift is not likely an important ecohydrological process in tallgrass prairie and that hydraulic lift is a patchy process, particularly in heterogeneous landscapes.

## Introduction

Tallgrass prairies are dynamic ecosystems that experience tremendous spatial variability in water availability through time (Knapp et al. 1993; Nippert et al. 2011). Precipitation occurs intermittently in these systems, resulting in long dry periods during which plants must either tolerate water stress (Tucker et al. 2011) or access deep water sources (Nippert and Knapp 2007a,b; Asbjornsen et al. 2008) to persist through drought. Another strategy that may allow deeply rooted prairie plants to survive drought is hydraulic lift, the passive movement of water across soil water potential gradients through plant roots (Breazeale 1934; Richards and Caldwell 1987; Caldwell et al. 1998). Hydraulic lift typically occurs at night when plant stomata close and the water potential gradient between shallow and deep soil is more pronounced than the gradient between deep soil and plant leaves. This gradient redistributes deep water to shallow soil, reducing water limitation in the rhizosphere for both the plant lifting the water and for neighboring plants that grow within the zone of lifted water (Dawson 1993; Moreira et al. 2003).

Hydraulic lift is most commonly observed in arid and semi-arid ecosystems (reviewed in Neumann and Cardon 2012; Prieto et al. 2012a; Sardans and Penuelas 2014). Few examples of hydraulic lift exist in grasslands or herbaceous plants and these instances have only been observed in semi-arid regions (Espeleta et al. 2004; McCulley et al. 2004), deserts (Schulze et al. 1998; Yoder and Nowak 1999), and greenhouses (Armas et al. 2012). However, hydraulic lift may play an important role in tallgrass prairies because the long dry periods that occur during the growing season cause significant drying within the shallow soil layers (0-30 cm; Abrams and Knapp 1986; Williams and Rice 2007; Zeglin et al. 2013), and should therefore produce the soil water potential gradient necessary to drive hydraulic lift. Most tallgrass prairies plants are also deep-rooted (typically  $\geq 2\text{m}$  deep; Weaver 1919) and utilize at least some deep soil water during

the growing season (up to 47% deep water in grasses, 56% deep water in forbs, and 76% deep water in shrubs; Nippert and Knapp 2007a). Thus, these species should have the capability to utilize deep water via hydraulic lift, if environmental conditions are favorable.

However, certain characteristics of the prairie may also limit water redistribution. In grasslands that are grazed, large ungulates such as *Bison bison* (American bison) reduce shallow root biomass (Johnson and Matchett 2001; Klumpp et al. 2009; Nippert et al. 2012), which may reduce water efflux from the shallow roots of grazed grasses. Grazers also reduce leaf area, and thus, transpiration, which can increase soil moisture (Archer and Detling 1986; Svejcar and Christiansen 1987; Harrison et al. 2010) and eliminate the driving gradient necessary for hydraulic lift for both grazed and nearby ungrazed plants. Additionally, the substantial rates of nocturnal transpiration that occur in grassland plants (O’Keefe and Nippert, unpublished data) may create a competing sink for water movement and reduce the magnitude of hydraulic lift (Donovan et al. 2001, 2003; Kavanagh et al. 2007; Scholz et al. 2008; Howard et al. 2009). Considering the heterogeneous nature of the tallgrass prairie, as well as the dependence of hydraulic lift on certain biotic and abiotic features, it seems likely that hydraulic lift may be spatially and temporally variable within a tallgrass prairie. However, it is unknown if hydraulic lift occurs in tallgrass prairie ecosystems or how this process may change along a grassland landscape.

Here, we assessed whether hydraulic lift occurs in a native tallgrass prairie located in northeastern Kansas, USA. Previous studies have identified hydraulic lift with methods that are typically time consuming, destructive, or require expensive equipment (e.g., time-domain reflectometry probes, soil psychrometers, sap flow sensors, enriched stable isotope tracers, or radioactive dye; reviewed in Prieto et al. 2012a). In this study, however, we used the natural

variation in the isotopic signature of soil water to identify hydraulic lift, which is a relatively cheap and non-destructive approach. The stable isotopic signature of soil water varies predictably by depth due to evaporation-driven fractionation, with enriched water found near the soil surface and depleted water found in deep soil (Ehleringer and Dawson 1992). If hydraulic lift occurs, shallow soil water should develop a depleted  $\delta^{18}\text{O}$  signature overnight as deep water is redistributed (Figure 1). Furthermore, this shift in  $\delta^{18}\text{O}$  should be reflected in plant stem water because tallgrass prairie plants typically utilize some water overnight (O’Keefe and Nippert, unpublished data) and because the isotopic signature of water within the stem reflects the water source used (White et al. 1985).

By analyzing diurnal changes in the isotopic signature of plant and soil water, we addressed the following questions: (1) Does hydraulic lift occur in common woody, forb, and grass species in a tallgrass prairie? We hypothesized that each species will exhibit hydraulic lift because they are all deep-rooted ( $\geq 2$  m deep) and have the capability to conduct deep soil water (Nippert and Knapp 2007a). (2) Is hydraulic lift limited by bison grazing or nocturnal transpiration? Bison can reduce shallow root biomass (Johnson and Matchett 2001; Klumpp et al. 2009; Nippert et al. 2012) and increase soil moisture (Archer and Detling 1986; Svejcar and Christiansen 1987; Harrison et al. 2010), which may limit water efflux in shallow soil or reduce the soil water potential driving gradient required for hydraulic lift. Thus, we hypothesized that the presence of bison will limit hydraulic lift. We also hypothesized that reductions in hydraulic lift will be associated with higher rates of nocturnal transpiration, as nocturnal transpiration can create a competing water sink for hydraulic lift (Donovan et al. 2001, 2003; Kavanagh et al. 2007; Scholz et al. 2008; Howard et al. 2009). (3) If a dominant grass, *Andropogon gerardii* Vitman, does not exhibit hydraulic lift, does it benefit by using water lifted by neighboring deep-

rooted forbs and shrubs? Although *A. gerardii* is deep-rooted (maximum rooting depth = 1.8 m; Weaver 1919), this grass also has a greater reliance on water from shallow soil layers using a fibrous root system (Nippert and Knapp 2007a,b). Previous studies have also shown that grasses with fibrous shallow roots can utilize water lifted by nearby plants (Dawson 1993; Moreira et al. 2003). Thus, if *A. gerardii* does not exhibit hydraulic lift, we hypothesized that it will instead utilize water lifted to shallow soil by neighboring shrubs or forbs.

## **Methods**

### **Site Description**

Field work was conducted at the Konza Prairie Biological Station (KPBS), a Long-Term Ecological Research (LTER) site located in the Flint Hills region of northeastern Kansas, USA (39.1°N, 96.9°W). KPBS is a 3487 ha tallgrass prairie that is dominated by a few perennial C<sub>4</sub> grass species along with numerous sub-dominant C<sub>3</sub> grass, forb, and woody species (Smith and Knapp 2003). The site is divided into watershed-level plots that are burned at different frequencies (every 1, 2, 4 or 20 years) and are either grazed or ungrazed by cattle and the ungulate *Bison bison*. KPBS is also topographically heterogeneous, with layers of Permian shale and limestone alternating across a non-uniform terrain of flat upland ridges, steep slopes, and lowland valleys (Jantz et al. 1975; Oviatt 1998). Soil depth varies by topographic position; upland ridges are characterized by shallow, rocky soils (< 0.5 m) derived from the chert-bearing Florence limestone, while lowlands are characterized by deep (> 2 m) silty-clay loams (Tully soil series) derived from alluvial-colluvial deposits (Oviatt 1998; Ransom 1998). Clay content in lowland soils ranges from 35-40% at the surface and 45-60% in the lower subsoil (Ransom 1998). Although lowland soils are generally deep, many areas are shallower and contain

numerous rock fragments (up to 15% rock fragment by soil volume; Ransom 1998). Lowland soils are also moderately well-drained, and deep soil water is recharged during the non-growing season when precipitation events infiltrate to a greater depth in the soil and move laterally between layers of shale and limestone (Ransom 1998; Nippert and Knapp 2007a). Due to these topographic differences, the deep lowland soils are typically more mesic and have higher primary productivity than shallow upland soils (Knapp et al. 1993; Nippert et al. 2011).

The Flint Hills region of Kansas experiences a mid-continental climate, characterized by warm, dry summers and cool, wet winters. Long-term mean annual precipitation at KPBS is 829 mm (1982-2014), with 73% occurring during the growing season (April – September). Precipitation was 783 mm in 2013 and 706 mm in 2014 (77% and 68% of which occurred during each respective growing season; Figure B1). July is the warmest average month of the year (1982-2014), with mean maximum and minimum air temperatures of 32.69 °C and 19.78 °C, respectively. January is the coldest average month (1982-2014), with mean maximum and minimum air temperatures of 4.87 °C and -7.14°C. Maximum air temperatures during July 2013 and 2014 were 30.99°C and 31.7°C, respectively. Minimum air temperatures during July 2013 and 2014 were 18.46°C and 17.06°C, respectively.

## **Sampling Description**

We sampled from two watersheds that are burned in 4-year intervals, as these locations are most representative of historic burn frequencies for tallgrass prairie in northeastern Kansas (Frost 1998), and they also contain more forb and woody plant diversity than annually or biannually burned plots (Collins and Calabrese 2012; Koerner and Collins 2014). To assess the impact of large herbivore grazing on hydraulic lift, we sampled in one watershed grazed by bison

and one ungrazed watershed. While watershed treatments were not replicated due to their large size and low availability at KPBS, we sampled from three spatially separated lowland sites (>100 m apart) within each watershed to achieve spatial independence among samples, as is common in large experimental landscapes.

We sampled four different species that have widespread distribution and abundance at KPBS: one clonal C<sub>3</sub> shrub (*Rhus glabra* L., smooth sumac), one leguminous C<sub>3</sub> sub-shrub (*Amorpha canescens* Pursh., leadplant), one C<sub>3</sub> forb (*Vernonia baldwinii* Torr., Baldwin's ironweed), and one C<sub>4</sub> grass (*A. gerardii*, big bluestem). These species have the potential to exhibit hydraulic lift because they are all deeply rooted, having maximum rooting depths of 2.3 m (*R. glabra*), 2.3 m (*A. canescens*), 3.3 m (*V. baldwinii*), and 1.8 m (*A. gerardii*) (Weaver 1919). Additionally, *R. glabra*, *A. canescens*, and *V. baldwinii* utilize deep water as a primary water source in this system (Nippert and Knapp 2007a,b; O'Keefe and Nippert, unpublished data). Although grazing typically increases the abundance of forbs and shrubs relative to ungrazed sites, all four species were present at each study site included here.

We conducted four sampling campaigns throughout 2013 (DOY 187 and 241) and 2014 (DOY 188 and 233). In each year we sampled once in early July (mid growing season) and once in late August (late growing season). These sampling times were chosen because the majority of growing season precipitation occurs earlier in the year (April-June) at this site and thus, strong water potential gradients do not typically exist throughout the soil until later in the growing season (Abrams and Knapp 1986; Williams and Rice 2007; Zeglin et al. 2013). Additionally, each sampling campaign occurred at least 5-7 days after a rainfall event to promote the development of a soil water potential gradient across different depths (Figure B1).



During each campaign we collected plant samples for isotopic analysis at 18:00 h (“night”) and again at 05:00 h the following morning (“morning”). We sampled individuals that were located at least 1 m apart from other deep-rooted plants to reduce the possibility of detecting hydraulic lift from neighbors. Due to the destructive nature of isotope sampling (see below), we could not sample the same individual more than once and thus had to sample similarly sized individuals for each time point. One individual per species was sampled at each site within a watershed for all time points (n=3 plants per species in each watershed at each sampling time point; total n=24 per time point). To assess whether *A. gerardii* utilizes lifted water by shrubs or forbs, we sampled one “isolated” *A. gerardii* and one “neighboring” *A. gerardii* adjacent to each “target plant” (*R. glabra*, *V. baldwinii*, and *A. canescens*) at each sampling site during each time point (n=3 isolated/adjacent *A. gerardii* plants in each watershed and time point; total n=24). Isolated *A. gerardii* functioned as a control and were located at least 1 m from other deep-rooted forbs or shrubs, and the neighboring *A. gerardii* were located within a 0.25 m radius from the main stem of the target plant.

## **Water Isotope Samples**

Plant tissue and soil cores were collected for isotope analyses during each night and morning sampling time points. For plant isotope samples, we collected 20-30 g of non-photosynthetic stem or crown tissue, which is located immediately below ground level but above any rhizomes or root tissue. We collected one stem for all species except *A. gerardii*, which required 5-10 tillers to provide enough water for the extraction and analysis processes. Plant tissues were immediately sealed in exetainer vials (Labco, Ltd., UK) and stored on ice until transferred to a 1-2°C refrigerator. We collected soil samples adjacent to the stems sampled for

plant tissue using a 25 cm hand corer. The core was split up into 5 cm deep segments (0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, and 20-25 cm depths), stored in double-layered plastic bags, and placed on ice until transferred to a -5°C freezer. The soil was then later removed from the freezer and immediately placed in exetainer vials for water extraction. Deep soil cores (1-2 m deep) were also collected for use as a “deep” endpoint in an isotopic mixing model (see *Statistics*). Cores were collected at five random lowland locations using a 540MT Geoprobe Systems hydraulic-push corer (Salina, KS, USA). The soil from the very bottom of these cores was collected and saved in exetainer vials for later water extraction and analysis.

We extracted xylem and soil water using the cryogenic vacuum distillation method (Ehleringer and Osmond 1989; Nippert and Knapp 2007a) and analyzed the stable hydrogen ( $\delta D$ ) and oxygen ( $\delta^{18}O$ ) signatures of these water samples using a Picarro WS-CRDS isotopic water analyzer.

### **Leaf Water Potential Measurements**

To assess whether hydraulic lift is associated with differences in leaf pressure potential, we measured predawn ( $\Psi_{pd}$ ) and midday ( $\Psi_{md}$ ) leaf water potential on the same plants collected for isotope analyses.  $\Psi_{md}$  was measured on each night individual at 12:00 h the day of night measurements.  $\Psi_{pd}$  was measured on each morning individual immediately before each plant was harvested for morning isotope samples. For all measurements, the youngest, most fully developed leaf was cut and placed in a dark, humidified plastic bag for approximately one hour. After the equilibration period, leaf water potential was measured using a Scholander pressure chamber (PMS Instrument Company, Albany, OR). We made  $\Psi_{pd}$  and  $\Psi_{md}$  measurements on all

four study species at each sampling site (n=3 plants) as well as *A. gerardii* adjacent to *R. glabra*, *V. baldwinii*, and *A. canescens* (n=3 plants).

### **Nighttime Transpiration Measurements**

To assess whether nocturnal water loss is associated with a potential absence of hydraulic lift, as has been observed in other species (Howard et al. 2009), we measured nocturnal transpiration using an Li-6400xt open gas exchange system (Li-Cor, Inc., Lincoln, NE). Measurements were made during 2014, between 22:00 – 01:00 h the night before isotope samples were collected. The youngest, fully developed leaf of each plant was allowed to stabilize in the Li-6400xt cuvette for 2-3 minutes and then measurements were logged every 10 seconds for 3 minutes. The last minute of data was averaged as a representative measurement for each plant. Measurements were made on all four study species at each sampling site (n=3 plants), as well as one *A. gerardii* adjacent to each target plant (n=3 plants).

### **Soil Water Content Measurements**

In addition to stable isotope measurements, we measured soil water content adjacent to each night and morning plant to evaluate whether hydraulic lift could be detected using soil moisture measurements alone. Soil water content was measured using two methods. First, we measured the relative water content (RWC) in the top 10 cm soil within a 10 cm radius from the main stem of each plant at the time it was sampled for isotope analyses. RWC was measured in situ with a Hydra Probe II Soil Sensor (Stevens Water Monitoring Systems, Portland, OR, USA) as a proportion in units of water fraction by volume (wfv). Second, we measured the gravimetric soil moisture content (GWC,  $\theta_g$ ) of each soil core depth using subsamples of the soil collected

for isotopic analysis. The wet weight of the soil was determined with a microbalance ( $\pm 0.1$  mg; Ohaus Pioneer, Ohaus Corporation, Parsippany, NJ, USA). The soil was dried at 60°C for 48 hours, reweighed to measure the soil dry weight, and gravimetric water content was calculated following Black (1965).

## **Statistics**

The Bayesian isotopic mixing model Stable Isotope Analysis in R (SIAR) was used to quantify the proportion of deep and shallow water sources to the isotopic signature of plant xylem water (Parnell 2010). We used separate SIAR analyses for each sampling campaign (July 2013, August 2013, July 2014, and August 2014) and ran each model for 500,000 iterations, of which we discarded the first 50,000 iterations. We used shallow (0-5 cm deep) and deep soil water (1-2 m deep) as the two source endpoints in all analyses. For the shallow sources, we averaged the isotopic signatures of 0-5 cm soil collected at night for each year x month combination and used that average value ( $n=24$ ) for the corresponding SIAR model analysis. For the deep soil water source, we used the mean isotopic signature of soil water extracted from the bottom of 1-2 m deep soil cores ( $n=5$ ) for all SIAR analyses. Each analysis produced a posterior distribution predicting the proportion of shallow and deep source water contribution for each species at every sampling location and time point. We then used the mean value of each distribution to calculate the difference in deep water used by each species between night and morning time points for every grazing treatment x month x year combination. See Parnell (2010) for a full description of the model.

We compared leaf water potential data among treatment combinations using a linear mixed-effects model in a completely randomized design (CRD) with year (2013 and 2014),

month (July and August), grazing treatment (grazed and ungrazed), and species (*R. glabra*, *A. canescens*, *V. baldwinii*, and isolated *A. gerardii*) as fixed effects. Sampling site within watershed was included as a random effect to account for variability associated with grouped data (Pinheiro and Bates 2000). Nighttime transpiration data were analyzed using a linear mixed-effects model in a CRD with month, grazing treatment, and species as fixed effects and site within watershed as a random effect. We also conducted separate analyses comparing leaf water potential and nocturnal transpiration among differently positioned *A. gerardii* using year, month, grazing treatment, and *A. gerardii* location (isolated *A. gerardii*, *A. gerardii* near *R. glabra*, *A. gerardii* near *A. canescens*, and *A. gerardii* near *V. baldwinii*) as fixed effects and site within watershed as a random effect.

Soil water isotope data were analyzed using a linear mixed-effects model in a CRD with year, month, grazing treatment, sampling time (night, morning), species, and soil depth as fixed effects and site within watershed as a random effect. Soil RWC data were analyzed using a linear mixed-effects model in a CRD with year, month, grazing treatment, sampling time (night, morning), and species as fixed effects and site within watershed as a random effect. Finally, soil GWC data were analyzed using a linear mixed-effects model in a CRD with month, grazing treatment, sampling time, and soil depth as fixed effects and site within watershed as a random effect. Homogeneity of variances was assessed by examining residuals versus fitted plots, all data were checked for normality by examining normal qq-plots (Faraway 2005), and post-hoc multiple comparisons were calculated using Tukey's Honest Significant Difference test (Tukey 1949). All analyses were conducted using the '*lmer*' function of the '*lme4*' package V1.1-7 (Bates *et al.* 2014) in the statistical program R V3.1.0 (R Core Team 2012).

## Results

### Soil Moisture Data

We found a significant year x month x grazing x species interaction ( $p=0.02$ ) among RWC values, and significant month x grazing ( $p=0.03$ ) and species x grazing ( $p=0.01$ ) interactions among GWC values (Table B1). RWC was greater in July than August and lower in ungrazed locations than in grazed locations, while GWC was greater in July and greater in ungrazed locations (Tables B2-3). We did not detect evidence of hydraulic lift with either method, as there were no significant differences between night and morning sampling times ( $p>0.05$ ; Table B1).

### Soil Isotope Data

We found a significant year x month x grazing x sampling time x species interaction ( $p=0.01$ ) for soil core  $\delta^{18}\text{O}$  signatures, indicating considerable variation in the isotopic signatures of soil cores (Table B4; Figures 3.2-3). Due to the complexity of interpreting a significant five-way interaction, we chose to report significant lower-order interactions as well, which include significant year x month x depth ( $p<0.01$ ), year x grazing x species ( $p=0.03$ ), grazing x depth ( $p<0.01$ ), grazing x sampling time ( $p=0.04$ ), and month x grazing ( $p<0.001$ ) interactions. Of the soil cores that exhibited shifts to deeper water signatures, only six instances were statistically different (Figure 3.2e,h,l,n; Figure 3.3h,l). However, non-significant shifts to deeper  $\delta^{18}\text{O}$  signatures were also observed in additional soil cores representative of all species, in all grazing treatments, months, and years (Figures 3.2-3). Positive overnight shifts in the isotopic signature of soil water were generally not observed.

## Relative Deep Soil Water Use

SIAR analyses showed that the proportion of deep water in plant xylem tissue varied between night and morning samples, and that these changes were variable across species and sampling periods (Figure 3.4). Most species typically used less deep water during the morning time point compared to night, suggesting that hydraulic lift did not occur (indicated by negative values in Figure 3.4). Several exceptions were observed when species used deeper water during morning than night (indicated by positive values in Figure 3.4). These instances included grazed *V. baldwinii* and ungrazed *A. canescens* collected during July 2013, as well as ungrazed *R. glabra* collected during all time periods except 2013. We found no evidence of hydraulic lift or use of water lifted by target plants in neighboring *A. gerardii* (Table B5).

## Nighttime Transpiration Data

Nocturnal transpiration ( $E_{\text{night}}$ ) was observed in all species and locations, in both July and August 2014 (Table 3.1). Mean  $E_{\text{night}}$  rates ranged from 0.21 mmol H<sub>2</sub>O m<sup>2</sup>s<sup>-1</sup> to 0.84 mmol H<sub>2</sub>O m<sup>2</sup>s<sup>-1</sup> (Table 3.1, Table B6). However, there were no significant differences in  $E_{\text{night}}$  among months, grazing treatments, or species ( $p>0.05$ ; Table B7). There were also no differences in  $E_{\text{night}}$  between isolated *A. gerardii* and *A. gerardii* near target plants ( $p>0.05$ ; Table B7).

## Leaf Water Potential Measurements

Predawn leaf water potential ( $\Psi_{\text{pd}}$ ) showed a significant year x month x species x grazing interaction ( $p=0.02$ ; Table A8).  $\Psi_{\text{pd}}$  was lower in August than in July and lower in 2014 than 2013 (Table B9).  $\Psi_{\text{pd}}$  was also lower in grazed areas than in ungrazed areas in August 2013 and

July 2014, for all species with the exception of *A. gerardii* (Table B9). Similarly, we found a significant year x month x species interaction ( $p < 0.01$ ; Table B8) for midday leaf water potential ( $\Psi_{md}$ ).  $\Psi_{md}$  was lower in August than in July and also lower in 2014 than 2013 (Table B10).  $\Psi_{md}$  was similar among species, with the exception of the low values observed for *A. gerardii* and *A. canescens* in August 2013 (Table B10). There was no significant effect of grazing treatment on  $\Psi_{md}$  ( $p > 0.05$ ; Table B8).

We found no differences in  $\Psi_{pd}$  or  $\Psi_{md}$  between isolated *A. gerardii* and *A. gerardii* near target plants ( $p > 0.05$ ; Table B8). There were significant year ( $p < 0.01$ ) and month ( $p < 0.01$ ) effects when comparing  $\Psi_{pd}$  among *A. gerardii* samples (Table B8), as  $\Psi_{pd}$  was lower in 2014 than in 2013 and lower in August than in July (Table B11). Similarly, there were significant year ( $p < 0.01$ ) and month x grazing ( $p = 0.02$ ) effects observed for  $\Psi_{md}$  compared across *A. gerardii* samples (Table B8).  $\Psi_{md}$  was lower in 2014 than 2013 and lower in August than in July, although this was more prevalent in ungrazed areas than in grazed areas (Table B12).

## Discussion

Hydraulic lift is an important ecohydrological process in water-limited ecosystems, though the occurrence and relevance of this phenomenon in mesic grasslands is unknown. Here, we tested the hypotheses that (1) hydraulic lift does occur in deep-rooted grass, forb, and woody species in a tallgrass prairie, (2) any reductions in hydraulic lift will be associated with bison grazing or nocturnal transpiration, and (3) a dominant grass, *A. gerardii*, will utilize water lifted by neighboring forbs and shrubs if it does not lift water itself. We observed overnight shifts in the isotopic signature of soil water adjacent to deep-rooted plants, suggesting that our method was sufficient to detect hydraulic lift in a native tallgrass prairie. However despite our



predictions, we found that hydraulic lift was uncommon, did not differ between grazing treatments, and did not facilitate water use by neighboring grasses. We did measure substantial rates of nocturnal transpiration among all species, suggesting that nocturnal water loss may limit hydraulic lift in this system. Overall, our work demonstrates that hydraulic lift can occur, but is generally not widespread or common in tallgrass prairie.

### **(1) Does hydraulic lift occur in common woody, forb, and grass species in a tallgrass prairie?**

Using the stable isotopic signature of soil and plant water, we tested the hypothesis that all four deep-rooted species utilize hydraulic lift in a tallgrass prairie. We found evidence of short-term shifts in the stable isotopic signature of soil and plant water, indicating that hydraulic lift does occur in this system. These changes occurred rapidly between dawn and dusk and could not be explained by other slower processes such as capillary action. However, instances of hydraulic lift were rare and inconsistent across treatment combinations. For example, hydraulic lift occurred in ungrazed *R. glabra* throughout all sampling months with the exception of July 2013, whereas July 2013 was the only month that hydraulic lift occurred in grazed *V. baldwinii* (Figure 3.4). Additionally, diurnal shifts in  $\delta^{18}\text{O}$  did not always occur simultaneously in both soil and plant tissue. Of the plants that experienced overnight  $\delta^{18}\text{O}$  shifts, only two instances showed corresponding shifts in soil  $\delta^{18}\text{O}$  (Figures 3.2-4), and there were several instances of overnight shifts in soil  $\delta^{18}\text{O}$  without associated shifts in plant  $\delta^{18}\text{O}$  (Figures 3.2-4). Consistent with these results, hydraulic lift did not correspond with shifts in leaf water potential, as has been observed in previous studies (Kurz-Besson et al. 2006; Cardon et al. 2013).

The inconsistent isotopic shifts we observed between plant and soil water may have occurred for several reasons. In cases when  $\delta^{18}\text{O}$  shifts were observed only in plant tissue, the deeply rooted plants may have lifted water to a soil depth below that of the 25 cm soil cores. A similar phenomenon has been previously reported by Bleby et al. (2010), illustrating that water can be redistributed to intermediate root depths by deep lateral roots. Alternatively, the shift in xylem  $\delta^{18}\text{O}$  could represent a shift in the relative contribution of different soil layers to direct water uptake associated with nocturnal transpiration, rather than hydraulic lift. In the instances when  $\delta^{18}\text{O}$  shifts were observed only in soil water, plants may have lifted water to shallow soil, where the soil water potential driving gradient was greatest, but concomitantly used water from greater depths, where water content was higher. This mechanism would not be surprising considering that forbs and shrubs typically utilize water from soil zones with the highest availability (Nippert and Knapp 2007a,b; Asbjornsen et al. 2008; Kulmatiski and Beard 2013; Priyadarshini et al. 2015).

The rarity of hydraulic lift observed here is interesting, considering that this system is characterized by deep lowland soils (Knapp et al. 1993), plants with deep-rooted dimorphic roots (Weaver 1919; Weaver and Albertson 1943), and seasonal drought that reduces shallow soil moisture (Nippert et al. 2011). The fact that hydraulic lift generally does not occur under conditions that should promote this process raises several questions. Specifically, why does hydraulic lift not occur when conditions are favorable? What other environmental or biotic factors limit hydraulic lift? Does spatial and temporal heterogeneity within an ecosystem impact the occurrence of hydraulic lift? We propose several alternative explanations for the lack of hydraulic lift observed here. First, the heterogeneous structure of the tallgrass prairie may prevent hydraulic lift from occurring uniformly across the landscape. Although lowland areas are

generally characterized by deep silt-clay loams, patches of shallower soil and rocks are common throughout these locations (Ransom et al. 1998). Thus, it is possible that hydraulic lift only occurs when soil conditions are appropriate (e.g., the soil is sufficiently deep and free of rocks). If this is true, our results suggest that hydraulic lift is a patchy process and that topographic features should be considered when observing and modeling hydraulic lift in heterogeneous environments. Second, hydraulic lift may not have been detected in *R. glabra* if the individuals we monitored transferred lifted water through its clonal network before being released into shallow soil, as has been observed by Ye et al. (2016). Finally, the low replication of this study ( $n=3$ ), as well as the limited spatial and temporal sampling design, may have limited our ability to detect hydraulic lift, particularly if soil water potential gradients were not strong. Had we sampled with greater intensity, or directly measured soil water potential gradients, we may have detected stronger evidence for this variable process.

## **(2) Is hydraulic lift limited by grazing or nocturnal transpiration?**

Considering that a variety of factors can influence the pattern and magnitude of hydraulic lift (Caldwell et al. 1998; Burgess et al. 2000; Egerton-Warburton et al. 2007; Scholz et al. 2008; Nadezhidina et al. 2009; Bleby et al. 2010; Prieto et al. 2012b; Wang et al. 2011; Neumann et al. 2012; Priyadarshini et al. 2015), we hypothesized that grazing and nocturnal transpiration would limit hydraulic lift in this system. Grazing by bison can have significant impacts on plant diversity, vegetation structure, soil physical properties, and nutrient cycling (Knapp et al. 1999). Grazing has also been shown to reduce shallow root biomass, as carbon allocation is shifted aboveground during regrowth following defoliation (Johnson and Matchett 2001; Klumpp et al. 2009; Nippert et al. 2012). Reduced shallow root biomass due to grazing may therefore reduce

hydraulic lift because fewer shallow roots should reduce root-soil contact and limit water efflux from roots. Grazing also increases shallow soil moisture due to reduced transpirational water loss (Archer and Detling 1986; Svejcar and Christiansen 1987; Harrison et al. 2010), which could reduce the soil water potential gradient necessary to drive hydraulic lift. However, we did not find that grazing impacts hydraulic lift in this system, as the few instances of hydraulic lift occurred in both grazed and ungrazed locations. Despite this weak relationship, we should still consider land management practices such as grazing in future hydraulic lift studies, particularly in ecosystems where hydraulic lift may be more prevalent.

Previous studies have shown that incomplete stomatal closure and transpiration at night reduces hydraulic lift by creating a competing sink for water movement through plant canopies (Donovan et al. 2001, 2003; Kavanagh et al. 2007; Scholz et al. 2008; Howard et al. 2009). Reductions in hydraulic lift by nocturnal transpiration can be substantial, in some cases limiting the amount of water released by roots into soil by 73% (Howard et al. 2009). We found few instances of hydraulic lift but observed consistent rates of nocturnal transpiration in all species (Table 3.1). These results suggest that nocturnal transpiration likely limits hydraulic lift to some extent, although both processes can occur depending on how the environment drives competing sinks between the plant and soil (Scholz et al. 2002; Donovan et al. 2003; Howard et al. 2009). Nocturnal transpiration may also explain why high predawn leaf water potential values were not always observed when hydraulic lift occurred, since nocturnal transpiration can prevent nocturnal equilibrium between soil and xylem water potential (Donovan et al. 2001, 2003).

**(3) If a dominant grass, *A. gerardii*, does not exhibit hydraulic lift, does it benefit by using water lifted by neighboring deep-rooted forbs and shrubs?**

Finally, we tested the hypothesis that, if *A. gerardii* does not exhibit hydraulic lift, it will utilize water by neighboring forbs and shrubs. Our results show that *A. gerardii* did not exhibit hydraulic lift, nor did it utilize water lifted by neighboring target plants. The lack of hydraulic lift utilized by *A. gerardii* is somewhat surprising considering that this species is deep-rooted and utilizes a small proportion of deep water (Weaver 1919; Nippert and Knapp 2007), and because hydraulic lift has been observed in some grasses (Schulze et al. 1998; Yoder and Nowak 1999; Espeleta et al. 2004; Leffler et al. 2005; Armas et al. 2012). The lack of facilitation observed in cases where hydraulic lift occurred is also surprising considering that facilitation of hydraulically lifted water by neighboring grasses or understory shrubs is so often reported in the literature (Caldwell 1990; Dawson 1993; Moreira et al. 2003; Warren et al. 2007; Hawkins et al. 2009).

Facilitation by hydraulic lift may not occur in this system for several reasons. First, competition for lifted water occurred between the lifting plants and neighboring grasses, outweighing any facilitative impact of hydraulic lift. A similar phenomenon has been observed in semi-arid African savannas, where *Acacia* trees utilized a substantial amount of lifted water in shallow soil layers, reducing soil moisture as well as the biomass of neighboring grasses (Ludwig et al. 2003, 2004). However, *A. gerardii* has high rates of diurnal transpiration (Table 3.1; O’Keefe and Nippert, unpublished data) and has a fibrous root system (Weaver 1954) that only utilizes shallow soil water (Nippert and Knapp 2007a,b; Asbjornsen et al. 2008), suggesting that this grass should be an effective competitor for lifted water in shallow soil layers. Another explanation could be that hydraulic lift moved deep soil water to intermediate depths below which *A. gerardii* can access, as previously discussed.

## **Conclusion**

In summary, hydraulic lift occurred infrequently in a native tallgrass prairie, making it unlikely that this process is an important component of local ecohydrology under current climate conditions. However, climate models predict that precipitation patterns will become increasingly variable in the North American Great Plains over the next century which, when coupled with elevated air temperatures, will result in drier conditions throughout the region (Christensen et al. 2007). Simulations show that future climate conditions will decrease soil moisture and carbon assimilation in grasslands, with concomitant increases in growing season carbon and water flux variability (Petrie et al. 2012). Additional models have shown that hydraulic lift can maintain shallow soil moisture and transpiration during drought, which could maintain these canopy-scale water and carbon fluxes (Domec et al. 2010). Thus, hydraulic lift may buffer the negative consequences of future climate change under conditions drier than those experienced during this study.

In the few instances where we did observe hydraulic lift we found no evidence that this process is influenced by grazing, nor does it result in facilitation for neighboring grasses. The rarity of hydraulic lift found here is somewhat surprising, considering the ecology and climate of North American tallgrass prairies. However, identifying the conditions when hydraulic lift is infrequent in water-limited ecosystems provides insight as important as identifying the mechanisms and drivers of this phenomenon when it occurs. In this grassland, high rates of nocturnal transpiration, topographic complexity, and patchy resource availability likely reduce the occurrence of hydraulic lift. Ultimately, our study provides further evidence that hydraulic lift is an extremely patchy process regulated by many factors, and that multiple physiological and

environmental factors should be considered in studies of hydraulic lift in heterogeneous landscapes.

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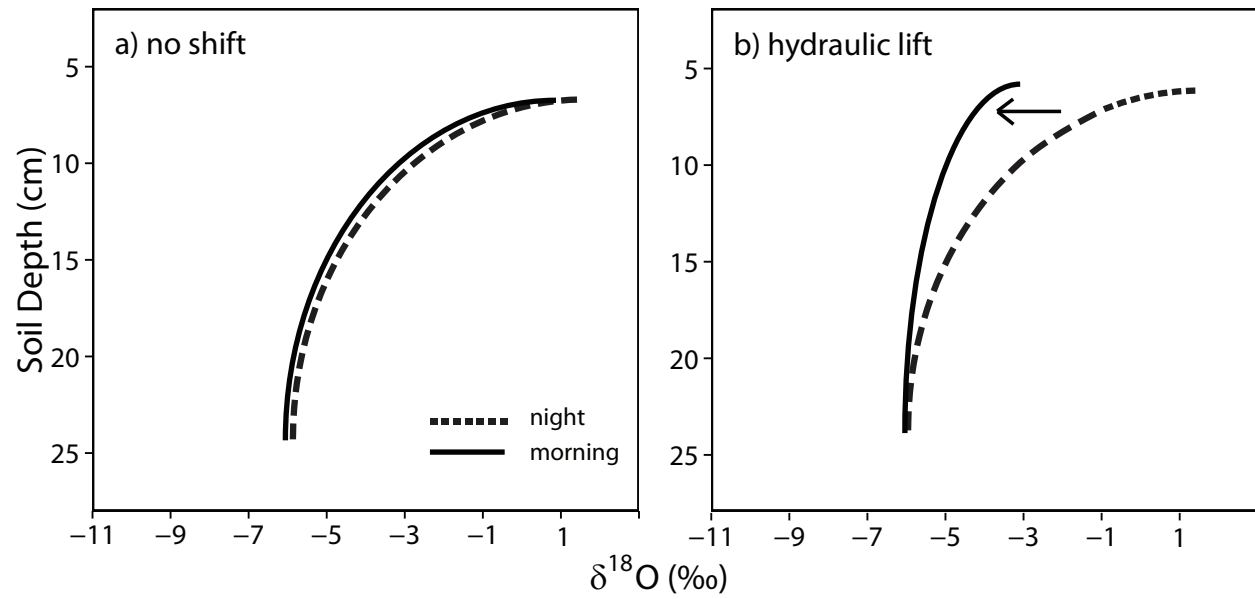
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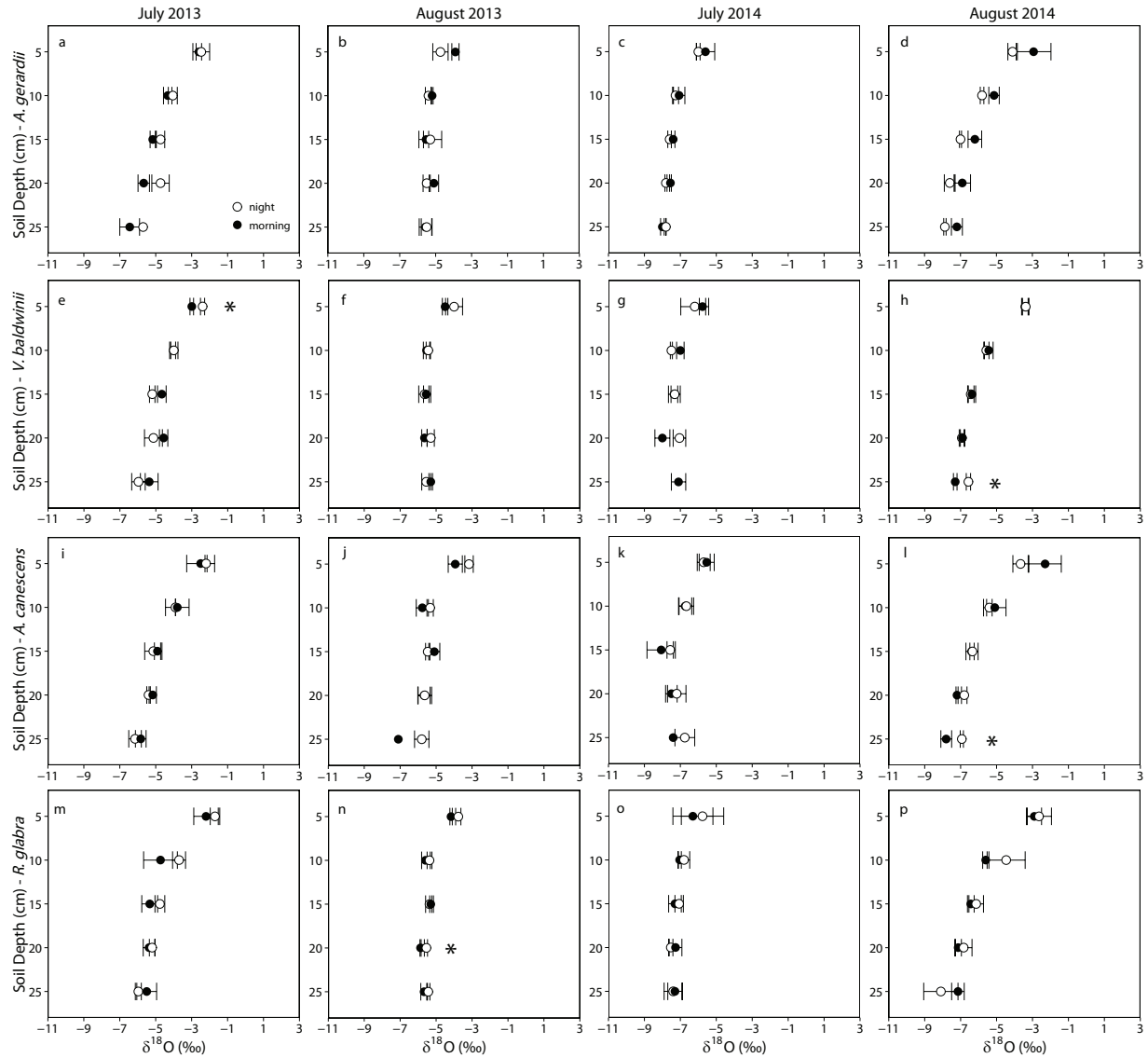
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**Table 3.1 Nocturnal transpiration ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ) during July and August 2014. Measurements were made on *A. gerardii*, *V. baldwinii*, *A. canescens*, and *R. glabra* in ungrazed and grazed locations. Shown are mean  $\pm$  1 SEM.**

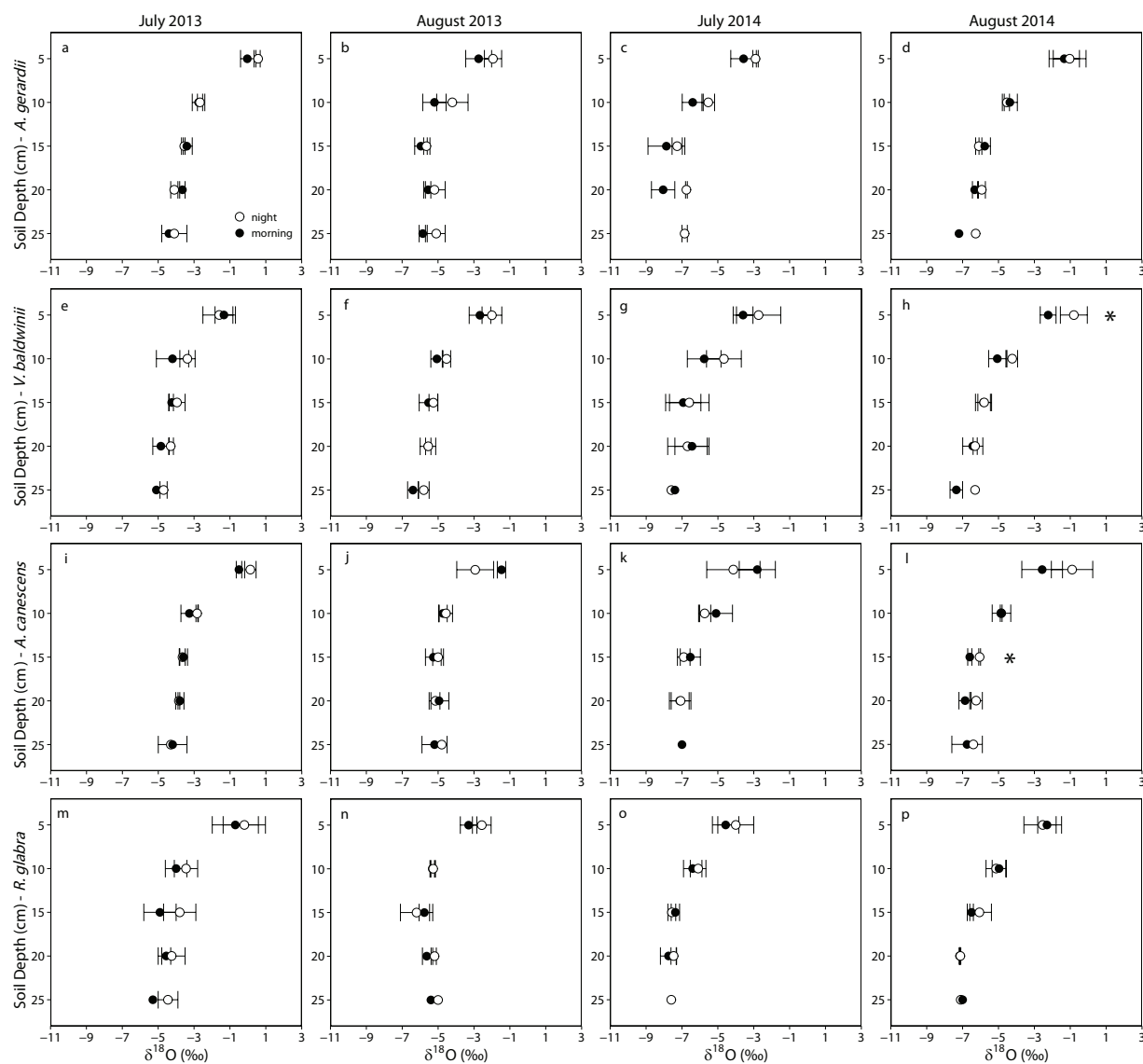
	July 2014		August 2014	
	Ungrazed	Grazed	Ungrazed	Grazed
<i>A. gerardii</i>	$0.5803 \pm 0.2181$	$0.6282 \pm 0.2520$	$0.5658 \pm 0.3256$	$0.4495 \pm 0.0869$
<i>V. baldwinii</i>	$0.3806 \pm 0.1104$	$0.5577 \pm 0.1840$	$0.4798 \pm 0.0579$	$0.4683 \pm 0.0984$
<i>A. canescens</i>	$0.4352 \pm 0.1938$	$0.4837 \pm 0.0506$	$0.3574 \pm 0.0644$	$0.4797 \pm 0.1251$
<i>R. glabra</i>	$0.2562 \pm 0.0476$	$0.3141 \pm 0.01272$	$0.2113 \pm 0.0543$	$0.3310 \pm 0.0999$



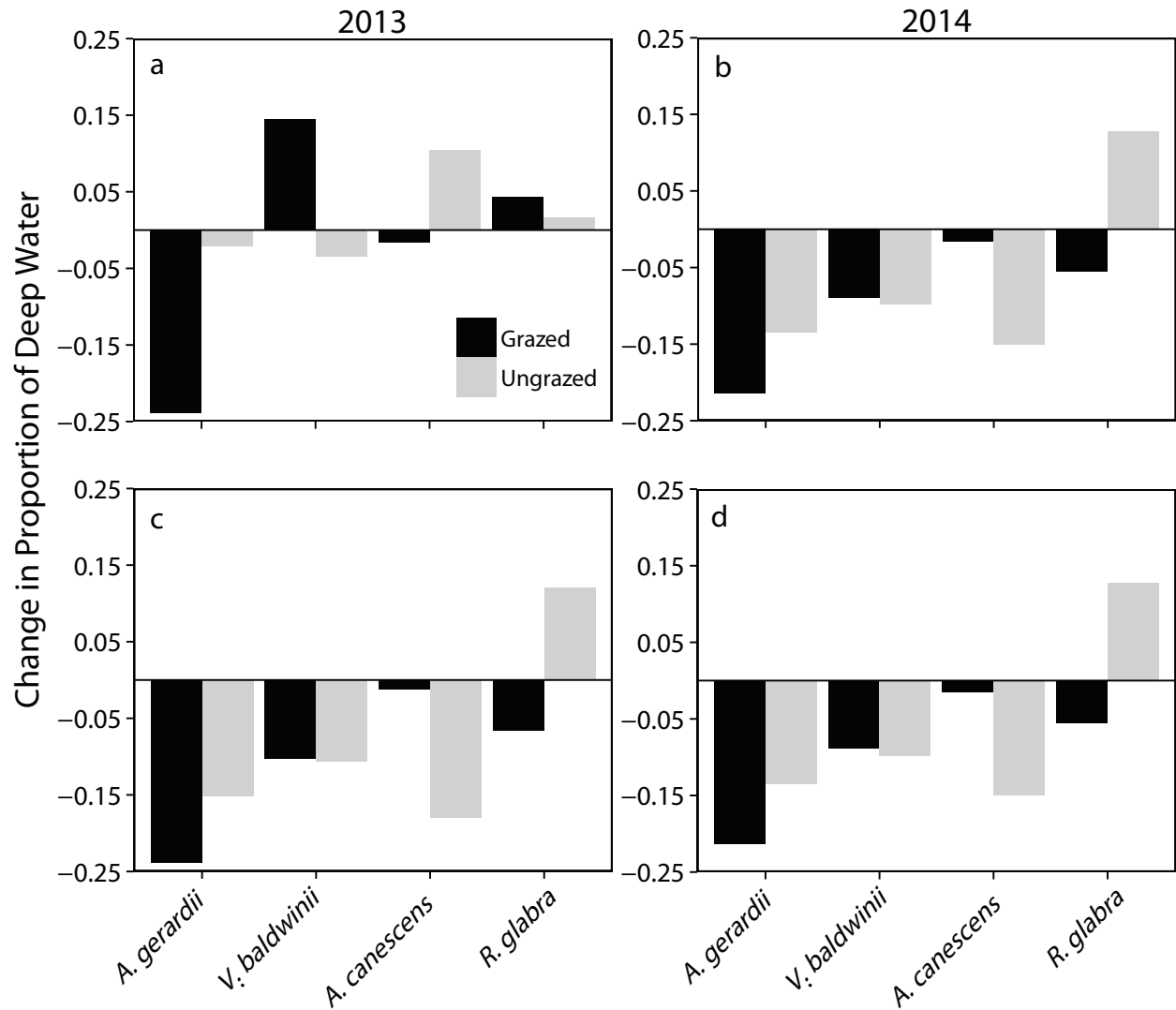
**Figure 3.1 Predicted diurnal changes in soil water  $\delta^{18}\text{O}$  (‰) within soil cores adjacent to plants. Shown are predictions for no diurnal change (a), and a negative shift indicating hydraulic lift (b).**



**Figure 3.2** Soil water  $\delta^{18}\text{O}$  (‰) measured at various depths within soil cores adjacent to each plant in ungrazed locations. Shown are soil cores collected at night and morning time points adjacent to *A. gerardii* (a-d), *V. baldwinii* (e-h), *canescens* (i-l), and *R. glabra* (m-p) during July 2013 (a, e, i, m), August 2013 (b, f, j, n), July 2014 (c, g, k, o), and August 2014 (d, h, l, p). Shown are mean  $\pm 1$  SEM.



**Figure 3.3** Soil water  $\delta^{18}\text{O}$  (‰) measured at various depths within soil cores adjacent to each plant in grazed locations. Shown are soil cores collected at night and morning time points adjacent to *A. gerardii* (a-d), *V. baldwinii* (e-h), *canescens* (i-l), and *R. glabra* (m-p) during July 2013 (a, e, i, m), August 2013 (b, f, j, n), July 2014 (c, g, k, o), and August 2014 (d, h, l, p). Shown are mean  $\pm 1$  SEM.



**Figure 3.4** Change in the proportion of deep water in plant xylem from night to morning time points, measured during July (a, b) and August (c, d) in 2013 and 2014. Positive values indicate an increase in deep water contribution to xylem water from night to morning, while negative values indicate a decrease in deep water contribution to xylem water from night to morning. Samples were collected for *A. gerardii*, *V. baldwinii*, *A. canescens*, and *R. glabra* in ungrazed and grazed locations. Values were calculated from the posterior distributions of the SIAR analyses.



## **Chapter 4 - Nocturnal water loss accounts for a substantial portion of plant water-use in a diverse grassland community**

### **Abstract**

Nocturnal transpiration can have significant consequences for plant functioning and earth-atmosphere water fluxes, yet little is known about how this process varies within diverse communities, particularly in grassland ecosystems. We measured leaf-level nighttime transpiration and daytime photosynthetic rates on eight grass, forb, and woody species in a North American tallgrass prairie. Measurements were made periodically across a two growing seasons (May-August 2014-2015) on three C<sub>4</sub> grasses (*Andropogon gerardii*, *Sorghastrum nutans*, and *Panicum virgatum*), two C<sub>3</sub> forbs (*Vernonia baldwinii* and *Solidago canadensis*), one C<sub>3</sub> subshrub (*Amorpha canescens*) and two C<sub>3</sub> shrubs (*Cornus drummondii* and *Rhus glabra*). We observed the highest rates of nocturnal transpiration in grasses and *C. drummondii*. All species showed a strong relationship between nocturnal transpiration and nocturnal stomatal conductance, suggesting that nocturnal water loss is regulated by plant stomata in this system. Additionally, nocturnal transpiration was equivalent to a large proportion of daytime transpiration rates, reaching over 35% in the C<sub>4</sub> grasses. Finally, both daytime and nighttime gas exchange rates were highly variable among species, within growing seasons, and across years. Our results suggest that patterns of nocturnal transpiration are variable within a community, are dynamic through time, and can be a considerable portion of a plant water budget. The regulation of nocturnal water loss by stomatal conductance also suggests that nocturnal transpiration may have functional significance as a competitive strategy in this ecosystem. Further characterization of this process will improve ecohydrological estimates in model forecasts of earth-atmosphere fluxes in grassland ecosystems.

## Introduction

Nocturnal transpiration has historically been ignored due to the conventional assumption that plants close their stomata at night to prevent water loss when carbon gain is absent. However, research over the past two decades has shown that nocturnal transpiration occurs often and can contribute significantly to total plant water use (Dawson et al. 2007). In fact, nighttime water flux typically ranges between 5-15% of daytime transpiration rates (Caird et al. 2007), and has even been reported to be up to 69% of total transpiration in certain species (Forster 2014). Substantial nighttime transpiration has been observed in a wide range of plant taxa and ecosystem types (Caird et al. 2007), including temperate forests (Barbour et al. 2005; Daley and Phillips 2006), tropical montane cloud forests (Alvarado-Barrientos et al. 2015), deserts (Snyder et al. 2003; Ogle et al. 2012), tropical savannas (Domec et al. 2006), and tropical rainforests (Wallace and McJannet 2010). Considering the widespread occurrence of this phenomenon, nocturnal transpiration likely contributes greatly to global-scale evapotranspiration (Zeppel et al. 2014; Resco de Dios et al. 2015).

Despite the importance of this phenomenon, the ecological advantage of nocturnal transpiration is still highly debated. Incomplete stomatal closure and transpiration at night can represent a significant cost to plant functioning and ecosystem water balance. Nocturnal water loss without concomitant carbon gain decreases water-use efficiency (Christman et al. 2008; Coupel-Ledru et al. 2016), prevents nocturnal equilibrium between soil and xylem water potential (Donovan et al. 2001, Bucci et al. 2004; Kavanagh et al. 2007), and reduces leaf water potential, water storage recharge in plant tissues, and daytime transpiration rates (Dawson et al. 2007). Nighttime plant water use also has the potential to deplete water in the rhizosphere and

accelerate the rate at which plants become drought stressed, particularly under dry conditions. These observations have led to the suggestion that nocturnal transpiration has no adaptive value and is simply the result of “leaky” stomata (Barbour et al. 2005).

Yet others argue that nocturnal transpiration is not as costly to plant function as initially proposed (Caird et al. 2007; Cirelli et al. 2015). High rates of nocturnal water flux through a plant may repair partial xylem cavitation (Snyder et al. 2003), prevent excess turgor in leaf tissue experiencing high osmotic potentials (Donovan et al. 2001), or promote bulk flow of water and nutrients through the soil (Scholz et al. 2007). Nocturnal transpiration can also stimulate early morning photosynthesis (Daley and Phillips 2006; Dawson et al. 2007), increase carbohydrate export (Marks and Lechowicz 2007), and increase oxygen concentrations in sapwood (Daley and Phillips 2006). Enhancing water, nutrient, or carbon relations can improve plant growth and survival, providing these species with a competitive advantage over species that do not transpire at night.

Elucidating whether nocturnal water loss has functional significance in ecological communities will first require a detailed understanding of how nocturnal transpiration varies among co-existing species. Although nocturnal transpiration is a widely reported phenomenon, most studies report rates of nocturnal water flux in either one or a few co-occurring species. Further, nocturnal transpiration has been primarily documented in dominant tree species of forest ecosystems (Daley and Phillips 2006; Dawson et al. 2007; Kavanagh et al. 2007; Buckley et al. 2011; Barbeta et al. 2012; Zeppel et al. 2010) or, to a lesser extent, crops (Caird et al. 2007; Fuentes et al. 2013; Rogiers and Clarke 2013) and herbaceous plants such as *Helianthus* or *Arabidopsis* grown in greenhouses (Howard and Donovan 2006; Christman et al. 2008; Escalona et al. 2013; Auchincloss et al. 2014; Neumann et al. 2014). Considerably less is known about

interspecific variation in nocturnal transpiration within the same ecosystem, particularly among different plant functional types (e.g., herbaceous versus woody species) (Ogle et al. 2012; Resco de Dios et al. 2015; Snyder et al. 2003). Here, we address this knowledge gap by observing community-level variation in nocturnal transpiration in a tallgrass prairie.

The tallgrass prairie of North America is an ideal ecosystem in which to test questions regarding the functional significance of nocturnal transpiration within plant communities. Although nocturnal transpiration has not yet been observed in grasslands, water loss occurring at night may be an important ecohydrological process here because tallgrass prairies experience tremendous temporal variability in soil moisture at small spatial scales (Knapp et al. 1993; Nippert et al. 2011). As a result, prairie grasses, forbs, and shrubs have developed an array of water-use strategies to coexist within variable and often limiting conditions. Previous research in this ecosystem has revealed considerable interspecific variation in traits such as drought tolerance (Tucker et al. 2011; Ocheltree et al. 2016) and source water uptake (Asbjornsen et al. 2008; Nippert and Knapp 2007a,b; O’Keefe and Nippert 2016; Ratajczak et al. 2011). Furthermore, interspecific variation in these traits can shift niche overlap and potentially alter competitive interactions within communities (O’Keefe and Nippert 2016). Considering the diversity of water-use traits observed in this system, as well as the role these traits play in determining interspecific interactions and species coexistence, nocturnal transpiration may be an important ecohydrological strategy here as well. However, evaluating the functional significance of nocturnal water loss in tallgrass prairie first requires a comprehensive assessment of whether nocturnal transpiration occurs in this system, if it varies among coexisting species, and how patterns of nocturnal transpiration change over time.

In the present study, we evaluated patterns of nocturnal transpiration in commonly occurring species in a tallgrass prairie. Using leaf-level measurements of diel gas exchange, we addressed four primary questions: (1) Does nocturnal transpiration occur in a tallgrass prairie? (2) How does nocturnal transpiration vary within a diverse community of grasses, forbs, and shrubs? (3) How do patterns of diel gas exchange vary intra- and inter-annually? (4) Is nocturnal transpiration regulated by nocturnal stomatal conductance or is it passively driven by nocturnal vapor pressure deficit? We hypothesized that (1) nocturnal transpiration will occur among coexisting plant functional types including grasses, forbs, and shrubs, (2) daytime and nocturnal transpiration will vary intra-annually and will be greatest early in the growing season, during periods with the highest soil water availability, and (3) nocturnal water loss will be actively regulated by nocturnal stomatal conductance.

## **Methods**

### **Study Location**

This research was conducted in 2014 and 2015 at the Konza Prairie Biological Station (KPBS), a Long Term Ecological Research (LTER) site located in the Flint Hills region of northeastern Kansas, USA (39.1°N, 96.9°W). KPBS is a 3,487-ha native tallgrass prairie that is divided into experimental watersheds, each of which receive varying combinations of grazing (grazed by *Bison bison*, cattle, or ungrazed) and prescribed fire (burned every 1, 2, 4, or 20 years) treatments. Long-term weathering has created a topographically heterogeneous landscape consisting of shallow, rocky uplands, steep slopes, and deep, loess soiled lowlands. KPBS is dominated by a few perennial C<sub>4</sub> grass species along with numerous sub-dominant C<sub>3</sub> grass, forb, and woody species (Smith and Knapp 2003).

The Flint Hills region of Kansas experiences a mid-continental climate, characterized by cool, wet winters and warm, dry summers. Long-term mean annual precipitation at KPBS is 829 mm (1982-2014), with 79% occurring during the growing season (April – September). Precipitation was 706 mm in 2014 and 1002 mm in 2015 (68% and 75% of which occurred during each respective growing season). The warmest average month of the year is July (1982-2014), with mean maximum and minimum air temperatures of 32.69 °C and 19.78 °C, respectively. The coldest average month is January (1982-2014), with mean maximum and minimum air temperatures of 4.87 °C and -7.14°C. During July 2014, mean maximum and minimum air temperatures were 31.7°C and 17.06°C, respectively. During July 2015, mean maximum and minimum air temperatures were 32.02°C and 20.67°C.

## **Experimental Design**

The current study was conducted in lowland prairie of an ungrazed watershed that is burned every four years (last burned in 2013). A four-year fire interval is similar to the historic frequency of fire for the region (Frost 1998) and results in a landscape characterized by greater forb and shrub diversity than annually burned prairie (Collins and Calabrese 2012; Koerner and Collins 2014). We sampled commonly occurring species at KPBS including three dominant C<sub>4</sub> grasses (*Andropogon gerardii* Vitman, big bluestem; *Panicum virgatum* L., switchgrass; *Sorghastrum nutans* (L.) Nash., indiangrass), two C<sub>3</sub> forbs (*Solidago canadensis* L., Canada goldenrod; *Vernonia baldwinii* Torr., Baldwin's ironweed), one leguminous C<sub>3</sub> sub-shrub (*Amorpha canescens* Pursh., leadplant), and two C<sub>3</sub> shrubs (*Rhus glabra* L., smooth sumac; *Cornus drummondii* C.A. Mey., rough-leaf dogwood). All measurements were made from May through September of each year during the study.

## **Environmental Conditions**

A micrometeorological station located at a lowland position within the same experimental watershed (< 50 m from all study plants) was used to measure air temperature, relative humidity, and soil moisture. Vapor pressure deficit was calculated from air temperature measurements made with a 100 K thermistor (Betatherm, Hampton, VA, USA) and relative humidity measurements made using a HM1500A sensor (Humierl, Hampton, VA, USA). Soil moisture was measured at 10 cm and 30 cm depths using Hydraprobe II sensors (Stevens Water Monitoring Systems, Portland, OR). Data were recorded as 30 minute averages using a CR10X datalogger (Campbell Scientific Inc. Logan, UT) throughout each growing season of the study.

## **Leaf physiology measurements**

Leaf physiology measurements (midday gas exchange, nocturnal gas exchange, midday water potential, and predawn water potential) were made six times per growing season, approximately once every 2-4 weeks. For each sampling time point, all measurements were made on the youngest, fully developed leaf from 3-5 randomly selected individuals per species. Leaf gas exchange was measured with an Li-6400XT gas analyzer (Li-Cor, Inc., Lincoln, NE, USA). Midday measurements were made on clear days between 1000-1400 hours and included maximum CO<sub>2</sub> assimilation at ambient C<sub>a</sub> (A<sub>max</sub>), daytime stomatal conductance of water vapor (g<sub>sday</sub>), and daytime transpiration rate (E<sub>day</sub>). Daytime cuvette conditions were set to [CO<sub>2</sub>] = 400 µmol CO<sub>2</sub> mol<sup>-1</sup>, relative humidity = 40-60%, and photosynthetically active radiation = 1500 µmol m<sup>-2</sup>s<sup>-1</sup> photon flux density.

Nocturnal measurements were made on the same day as daytime gas exchange measurements, approximately one hour following sunset, and typically lasted 2-3 hours. At night, cuvette conditions were also set to  $[\text{CO}_2] = 400 \mu\text{mol CO}_2 \text{ mol}^{-1}$  and relative humidity = 40-60%, but the light source was turned off. Nocturnal measurements were made on the same leaves used for daytime gas exchanges measurements and included nocturnal stomatal conductance ( $g_{\text{snight}}$ ), nocturnal transpiration rate ( $E_{\text{night}}$ ), and vapor pressure deficit at the leaf surface. In the few instances where leaves were damaged between daytime and nocturnal measurements, a morphologically and developmentally similar leaf on the same plant was chosen for the nocturnal measurement. For all gas exchange measurements, each leaf was allowed to stabilize within the cuvette for 2-5 minutes and then a single measurement was recorded. Gas exchange calculations were adjusted for leaf area within the cuvette during data processing, if necessary.

Predawn ( $\Psi_{\text{pd}}$ ) and midday ( $\Psi_{\text{md}}$ ) leaf water potentials were measured on adjacent and climatically similar days to those during which gas exchange measurements were performed. Leaves for predawn measurements were collected approximately one hour prior to sunrise and leaves for midday measurements were collected at 1200 hours the same day that predawn measurements were made. Each leaf was cut with a razor blade, sealed in dark, humidified plastic bag, and allowed to equilibrate for one hour prior to measurement with a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA).

## **Statistics**

All analyses were performed with the statistical program R V3.1.0 (R Core Team 2012). For all physiological response variables, differences among treatment contrasts were evaluated



using a three-way linear fixed-effects model in a completely randomized design with species, within-season sample period, and year as main effects. The percent nocturnal transpiration of daytime transpiration rate ( $\%E_{\text{night}}$ ) was calculated for day-night measurement pairs on each leaf and was also compared among treatment contrasts using a three-way linear fixed effects model. Post-hoc multiple comparisons were calculated using Tukey's Honest Significant Difference test (Tukey 1949). To assess whether nocturnal transpiration can be predicted from nocturnal stomatal conductance or nocturnal VPD, we compared the relationship between nocturnal transpiration and nocturnal VPD measured at both the leaf level (Leaf VPD) and at the atmospheric level (Bulk VPD) using linear and nonlinear regression analyses. Leaf VPD was recorded by the Li-6400XT during nocturnal gas exchange measurements and a single bulk VPD measurement was recorded at daily minimum (03:00 h) conditions for each measurement night by the nearby micrometeorological station. Separate regression analyses were conducted for all species, averaged over all sample periods and years. Homogeneity of variances was assessed by examining residuals versus fitted plots and all data were checked for normality by examining normal qq-plots (Faraway 2005).

## **Results**

### **Environmental Conditions**

Soil moisture measured at 10 cm and 30 cm depth declined over each growing season (Figure 4.1a-b) with highest values at DOY 161 in 2014 and DOY 156 in 2015. Lowest soil moisture values occurred at DOY 242 in 2014 and DOY 249 in 2015. Soil moisture was generally higher in 2015 than in 2014, which was associated with greater annual precipitation and larger precipitation events in 2015 compared to 2014 (Figure 4.1c-d). Vapor pressure deficit

was variable over each growing season but was generally higher in 2014 than 2015 (Figure 4.1e-f). Maximum VPD values measured during the day (15:00 h) occurred on DOY 236 in 2014 and DOY 249 in 2015, while minimum VPD values measured at night (03:00) occurred on DOY 219 in 2014 and frequently reached 0.0 kPa throughout 2015.

### Nocturnal Gas Exchange

Measurable rates of nocturnal conductance and transpiration occurred in all species, for all measurement periods and years (Figure 4.2). Nocturnal gas exchange varied significantly among species and within-growing season dates (significant species x date interactions for  $G_{\text{snight}}$  and  $E_{\text{night}}$ ; Table C.1), as well as among years and dates (significant year x date interactions for  $G_{\text{snight}}$  and  $E_{\text{night}}$ ; Table C.1). In 2014, nocturnal stomatal conductance was high on the first two and last sampling days, but declined between DOY 196-229 (Figure 4.2a). However, in 2015 nocturnal conductance was high on the first sampling day (DOY 152) and on DOY 211, and was lower between these dates as well as at the end of the growing season (Figure 4.2b). Grasses and *C. drummondii* had the highest nocturnal conductance values during these days of high overall nighttime conductance, while the remaining shrubs (*A. canescens* and *R. glabra*) had consistently low rates of nocturnal conductance (Figure 4.2a-b).

Nocturnal transpiration showed similar intra-annual trends over both growing seasons (Figure 4.2c-d). During 2014, grasses and *C. drummondii* had the highest rates of nocturnal transpiration early in the season (DOY 172 and 184), but rates declined and were comparable with other species during the remainder of the growing season. Throughout most of 2015, grasses had the highest rates of nocturnal transpiration, with *A. gerardii* having the overall

highest values on DOY 211 (Figure 4.2d). Shrubs and forbs had the lowest and intermediate nocturnal transpiration rates throughout 2015, respectively (Figure 4.2d).

The percent nocturnal transpiration of daytime transpiration rates also varied among species, years, and within-growing season dates (Table C.1). Generally, nocturnal transpiration represented a higher proportion of daytime transpiration rates early and late in the growing season, for both years (Figure 4.2e-f). During these periods, grasses had the highest proportion, with *A. gerardii* having nocturnal transpiration rates reach almost 40% of daytime rates during DOY 152 in 2015 (Figure 4.2f). Forbs also had nocturnal transpiration rates that comprised a high percent of daytime rates during DOY 212 and 229 in 2014.

Variation in nocturnal transpiration was best explained by nocturnal stomatal conductance for all species (Figure 4.3-5). Nocturnal transpiration increased nonlinearly with increasing stomatal conductance for all species, although the rate of this increase and the strength of this relationship varied among species. Conversely, nocturnal transpiration showed a very weak relationship with vapor pressure deficit at both the atmosphere and the leaf scale.

## **Daytime Gas Exchange**

Daytime gas exchange rates varied significantly among species, years, and within-growing season measurement dates (Table C.2). All gas exchange rates steadily declined throughout the 2014 growing season but increased mid-season before declining at the end of the growing season in 2015 (Figure 4.6). Grasses typically had the highest photosynthetic rates compared to forbs or shrubs, with *P. virgatum* having overall highest rates in 2014 and *S. nutans* having the highest rates in 2015 (Figure 4.6a-b). Forbs had the lowest photosynthetic rates, with *S. canescens* having the absolute lowest rates in 2014 and *V. baldwinii* having lowest rates in

2015. The woody species, *A. canescens*, *R. glabra*, and *C. drummondii*, had fairly consistent rates of photosynthesis over time, with little decline in 2014 compared to other species, little increase in 2015 compared to other species, and little difference between years.

Daytime stomatal conductance to water vapor and daytime transpiration showed similar intra- and inter-annual trends, but variation among species differed compared to photosynthesis. Grasses generally had the overall lowest stomatal conductance in both 2014 and 2015, while *S. canescens* and *R. glabra* had the highest stomatal conductance during both years (Figure 4.6c-d). Grasses and shrubs generally had consistent stomatal conductance across both years, while the forbs showed the largest decline in 2014 and increase in 2015. Daytime transpiration was less static over time than stomatal conductance (Figure 4.6e-f). *S. canadensis* had the highest transpiration rates early in the 2014 growing season and the lowest rates by mid-season, but consistently had the highest transpiration rates throughout 2015. *R. glabra* had the highest transpiration rates by mid-season in 2014. Transpiration rates were generally similar among all other species over both growing seasons.

## **Leaf Water Potential**

Leaf water potential varied significantly among species, years, and within-growing season measurement dates (Table C.3). Both predawn and midday water potential were greater in 2015 than in 2014 (Figure 4.7). Predawn leaf water potential declined during the middle of the 2014 growing season for all species, but remained consistently high throughout 2015 (Figure 4.7a-b). All species had similar predawn water potential values during both years except *R. glabra*, which consistently had lower predawn water potential values than all other species on each sampling date in 2015. Midday leaf water potential declined over both growing seasons, but

to a greater extent in 2014 than in 2015. In 2014, *R. glabra* and *S. canescens* had the highest midday water potential values earlier in the growing season, while *A. canescens* had the lowest values later in the growing season. In 2015, *A. canescens* and *R. glabra* had the lowest midday water potential values throughout most of the growing season.

## Discussion

Although nocturnal transpiration has been observed in a variety of ecosystems, whether this phenomenon occurs in grasslands or how it may vary within a diverse grassland community has not been previously studied. To address this knowledge gap we compared patterns of diel gas exchange among eight common grass, forb, and shrub species in a tallgrass prairie over two years. We present four main conclusions: 1) substantial rates of nocturnal transpiration occur in all species measured here, with the highest rates observed among grasses and the clonal shrub *C. drummondii*; 2) nocturnal transpiration is equivalent to a large proportion of daytime transpiration rates, reaching over 35% in some instances; 3) diel leaf physiology is highly variable among species, within growing seasons, and across years, and 4) nocturnal transpiration is driven by changes in stomatal conductance, not VPD. Combined, these results suggest that nocturnal transpiration is actively regulated by grassland plants and may impact plant physiology, community interactions, and landscape-scale water fluxes.

We measured substantial rates of nocturnal water loss in all grasses, forbs, and shrubs. These rates ranged from  $0.02 - 1.44 \text{ mmol m}^{-2}\text{s}^{-2}$  and were equivalent to 0.5-35.5% of daytime transpiration rates, suggesting that nocturnal loss is variable within a community and can represent a significant portion of the water lost from this ecosystem. The dominant  $C_4$  grasses in this system (*A. gerardii*, *P. virgatum*, and *S. nutans*) and the clonal shrub *C. drummondii* had the

highest rates of nocturnal transpiration throughout both years, while the remaining forbs and woody plants generally exhibited lower rates of nocturnal conductance and water loss (Figure 4.2). Additionally, interspecific differences in nocturnal conductance and transpiration were greater during periods of high soil moisture availability, which may represent species-specific responses to changes in the drivers of nocturnal transpiration, as has been observed in other studies (Cirelli et al. 2015; Daley and Phillips 2006; Ogle et al. 2012).

Nocturnal transpiration as a proportion of daytime transpiration also varied greatly among species, with maximum values ranging from up to 35.5 % in grasses, 15.5% in forbs, and 23.6% in shrubs. The significant portion of water lost at night in grasses likely occurs because these C<sub>4</sub> species maintain lower stomatal aperture during the day (Figure 4.6c-d) yet retain higher stomatal conductance at night compared to C<sub>3</sub> species (Figure 4.2a-b). Interestingly, this observation is contrary to previous evidence showing that plants with high daytime conductance also have high nocturnal conductance (Barbour et al. 2005; Synder et al. 2003). However, this discrepancy may simply result from an underrepresentation of C<sub>4</sub> grasses in the nocturnal transpiration literature (reviewed in Caird et al. 2007). Considering the overwhelming dominance of C<sub>4</sub> grasses in mesic grasslands (Epstein et al. 1998), nocturnal transpiration likely accounts for a significant proportion of the total water budget in this ecosystem.

Interspecific differences in nocturnal transpiration suggest that there may be a functional significance associated with water loss at night in mesic grasslands. One explanation may be that nocturnal transpiration promotes rapid water loss as a competitive strategy within a community. Under wet conditions, high rates of nocturnal transpiration may quickly diminish water in the rhizosphere. If the plant transpiring at night can persist through periods of low water availability, they may have a competitive advantage over neighbors that are less resistant to drought. We

found that the drought-tolerant C<sub>4</sub> grasses in this ecosystem, which grow quickly when resources are available and can tolerate periods of resource limitation (Nippert et al. 2007a; Tucker et al. 2011), had the highest rates of nocturnal transpiration. Thus, these species may take advantage of wet periods and continuously lose water to reduce the total amount of water available for coexisting species that may have greater susceptibility to drought stress. In this scenario, the loss of a community-level resource would have more significant negative consequences on species sensitive to periods of low-water availability. Another functional role of nocturnal transpiration may be to increase nutrient availability. Previous studies have shown that the bulk flow of soil water associated with nocturnal water fluxes can increase nutrient availability for the transpiring plants, particularly when soil moisture availability is high or when nutrient availability is low (Scholz et al. 2007; Kupper et al. 2012; Resco de Dios et al. 2013; Rohula et al. 2014). Considering that the tallgrass prairies of North America are fairly nutrient-limited (Fay et al. 2015; Klodd et al. 2016), and that nocturnal transpiration rates were highest under wetter conditions, nocturnal water fluxes may indeed facilitate nutrient transfer and uptake in this system. Combined, these hypotheses warrant further mechanistic evaluations of nocturnal transpiration in tallgrass prairies.

Nocturnal and diurnal leaf physiology were both highly variable within growing seasons and across years. During the day, grasses had the highest photosynthetic rates and lower rates of daytime stomatal conductance and transpiration, which is typical of plants that utilize the C<sub>4</sub> photosynthetic pathway (Kalapos et al. 1996; Knapp et al. 1993; Nippert et al. 2007; Turner et al. 1995). Daytime gas exchange rates and leaf water potential values decreased throughout the 2014 growing season for all species, which was associated with overall drier conditions including a steady decline in soil moisture (Figure 4.1a), few precipitation events throughout the

middle of the growing season (Figure 4.1c), and increasing VPD over time (Figure 4.1e). Conversely, daytime gas exchange rates increased throughout 2015, which is not surprising considering that soil moisture was generally greater throughout the growing season (Figure 4.1b), there were more frequent precipitation events throughout the entire season (Figure 4.1d), and VPD was lower than in 2014 (Figure 4.1f).

Nocturnal conductance and transpiration were also variable through time, with somewhat similar responses to environmental conditions. Previous research has shown that nocturnal transpiration rates are positively associated with environmental conditions such as high nocturnal VPD and high soil moisture (Alvarado-Barrientos et al. 2015; Benyon 1999; Dawson et al. 2007; Fisher et al. 2007; Howard and Donovan 2006). Conversely, we observed lower rates of nocturnal water loss when VPD was high (e.g., DOY 212 in 2014) and higher rates of nocturnal transpiration when VPD was low (e.g., DOY 152 in 2015), which indicates that atmospheric evaporative demand is not necessarily a driver of nocturnal transpiration in this system. We also found a weak relationship between nocturnal transpiration and VPD measured at both the leaf scale and the landscape scale for all species (Figure 4.3-5), further demonstrating that VPD is not a singular driver of nocturnal transpiration in grassland plants. Rather, nocturnal transpiration was strongly related to nocturnal stomatal conductance, suggesting that these plants may actively regulate water loss across a range of potential VPD. This result gives further support to the idea that nocturnal transpiration may have adaptive significance in tallgrass prairie.

Additionally, nocturnal transpiration appears to be modulated by precipitation and soil moisture availability. Nocturnal conductance and transpiration were typically greater early in the growing season when soil moisture was greater (Figure 4.1a, 4.3a-d) and also following precipitation events (e.g., DOY 211-212 in 2015; Figure 4.1d, 4.3d). Given that grasses exhibit



continuous use of surface soil water over time (Nippert and Knapp 2007a,b; O’Keefe and Nippert 2016), this pattern may explain why grasses generally had the highest rates of nocturnal water loss, especially during periods of high soil moisture. Forbs and shrubs, which exhibit plasticity in water source use depending on availability (Nippert and Knapp 2007a,b), have constant access to soil water and should therefore be less responsive to pulses in shallow soil moisture over time. Overall, these results indicate that both daytime and nighttime gas exchange rates are sensitive to changes in soil moisture, although responses vary among species.

The high rates of nocturnal water loss observed here likely have significant implications for this mesic grassland. Rapid reductions in shallow soil moisture associated with nocturnal transpiration have the potential to reduce leaf gas exchange and growth rates of species that are sensitive to changes in water availability (Knapp 1984, 1985; Nippert et al. 2009), which can result in lower rates of evapotranspiration and increased carbon losses at the landscape scale (Niu et al. 2008; Petrie and Brunsell 2011; Petrie et al. 2012; Zhang et al. 2011). A drier rhizosphere can also cause certain species to shift reliance to deeper soil water (Nippert and Knapp 2007a,b), which can alter niche overlap within a community (O’Keefe and Nippert 2016) or even deplete deep water stores under long periods of drought (Logan and Brunsell 2015). Finally, nocturnal transpiration may also impact hydraulic lift, the passive movement of water through plant roots from areas of deep, moist soil to shallow, drier soil (Richards and Caldwell 1987). Hydraulic lift does occur in this grassland (O’Keefe and Nippert, unpublished data), and nocturnal transpiration may reduce the magnitude of redistributed water by creating a competing sink for water movement (Donovan et al. 2003; Howard et al. 2009).

Overall, this study provides a first assessment of nocturnal transpiration in a tallgrass prairie. We show that nocturnal transpiration occurs in herbaceous and woody functional groups

and, importantly, that nocturnal water loss can represent a large fraction of total plant water use across a range of species. These results suggest that nocturnal transpiration may have significant impacts on plant physiology, community interactions, and landscape-scale water fluxes. Future measurements of tallgrass prairie evapotranspiration should therefore include nocturnal water loss to improve estimates of ecosystem water balance. Finally, these results also highlight the importance of studying community level variation in nocturnal transpiration. While most nocturnal transpiration research does not consider multiple functional groups, we demonstrate that nocturnal water loss has a greater proportional impact on C<sub>4</sub> grass water use than any other functional group. This results suggests that nocturnal transpiration may have unique functional significance as a competitive strategy in this ecosystem, and also that nocturnal water loss may contribute more greatly to ecosystem water balance in grasslands that are dominated by these species.

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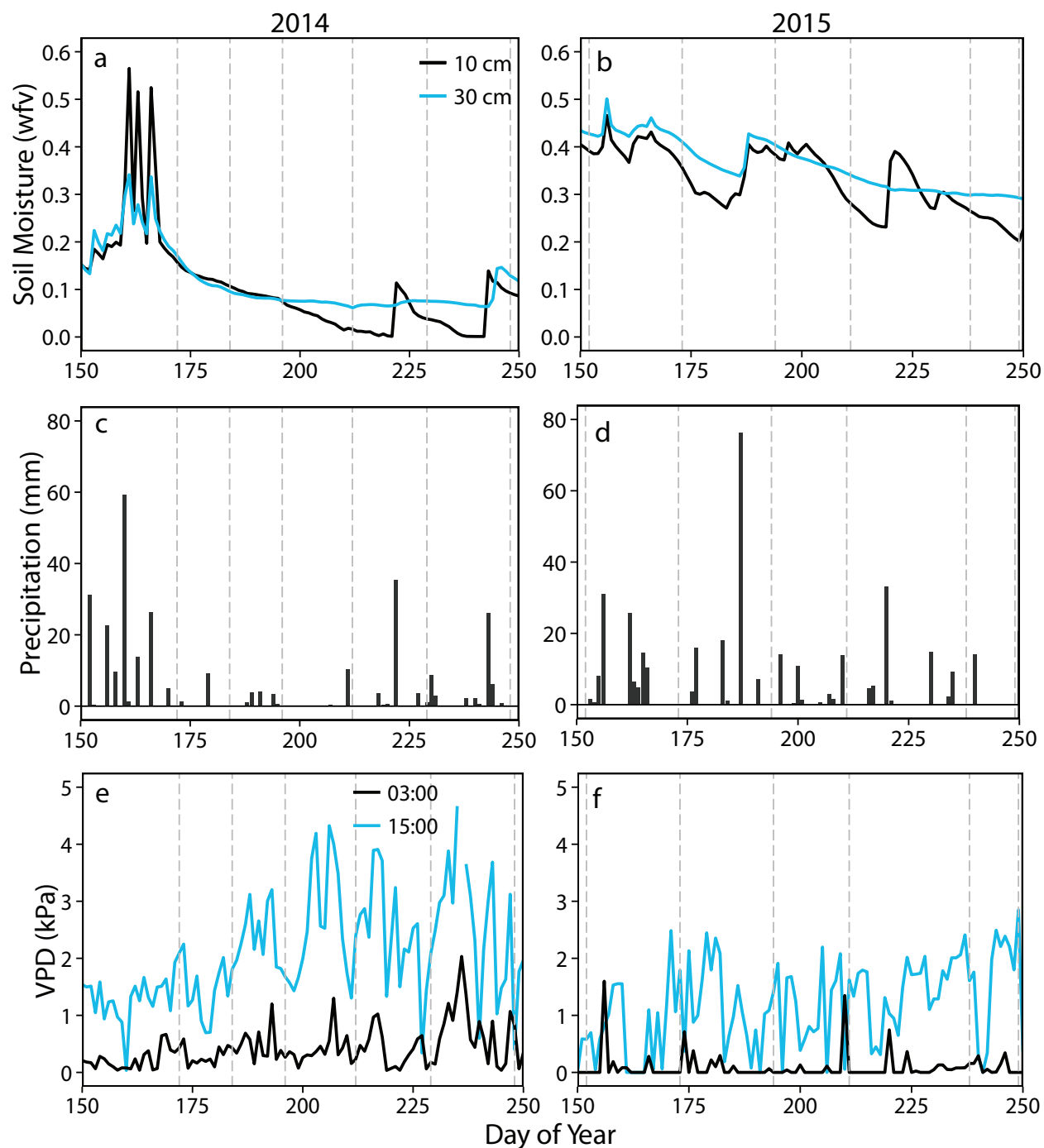
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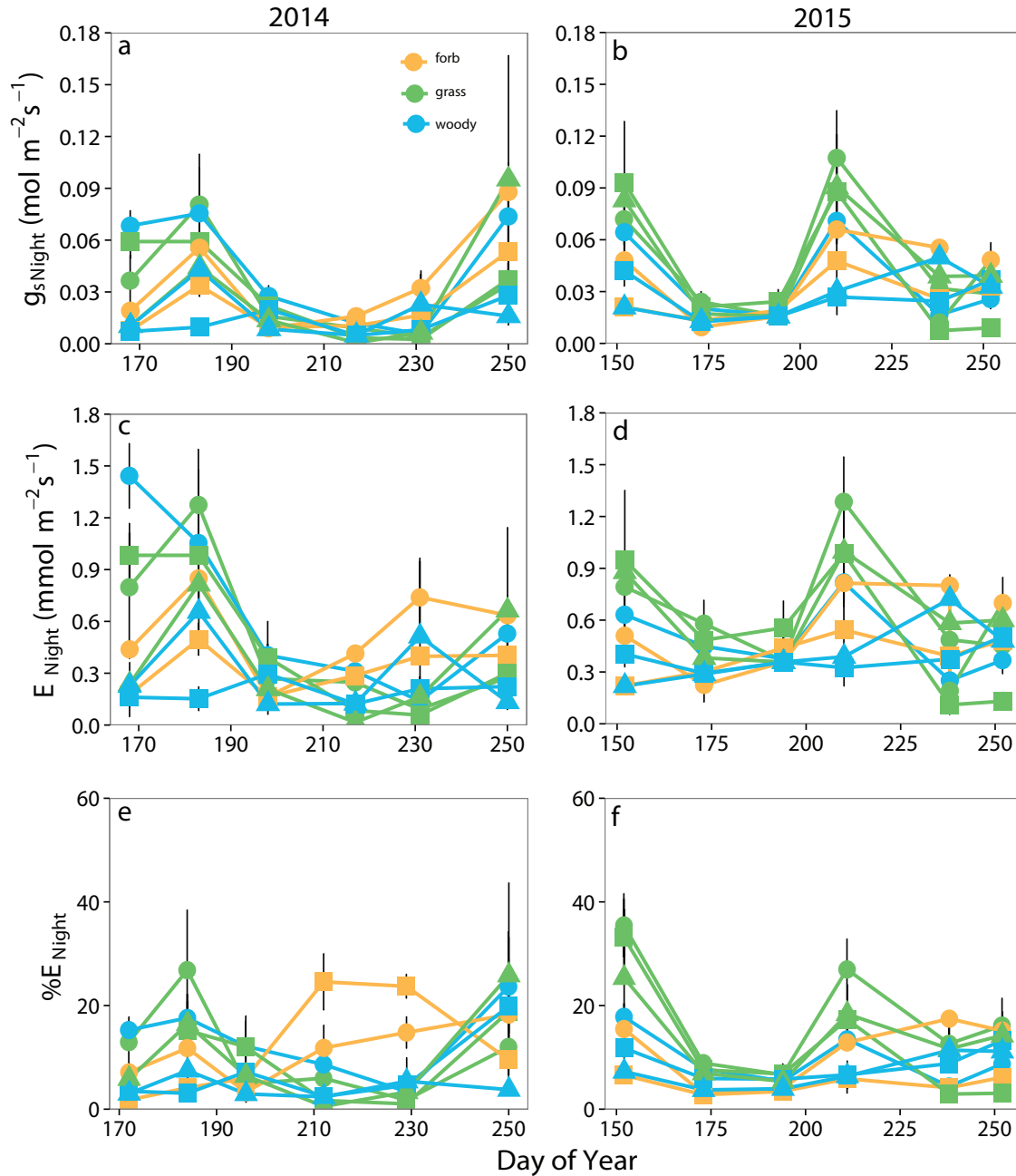
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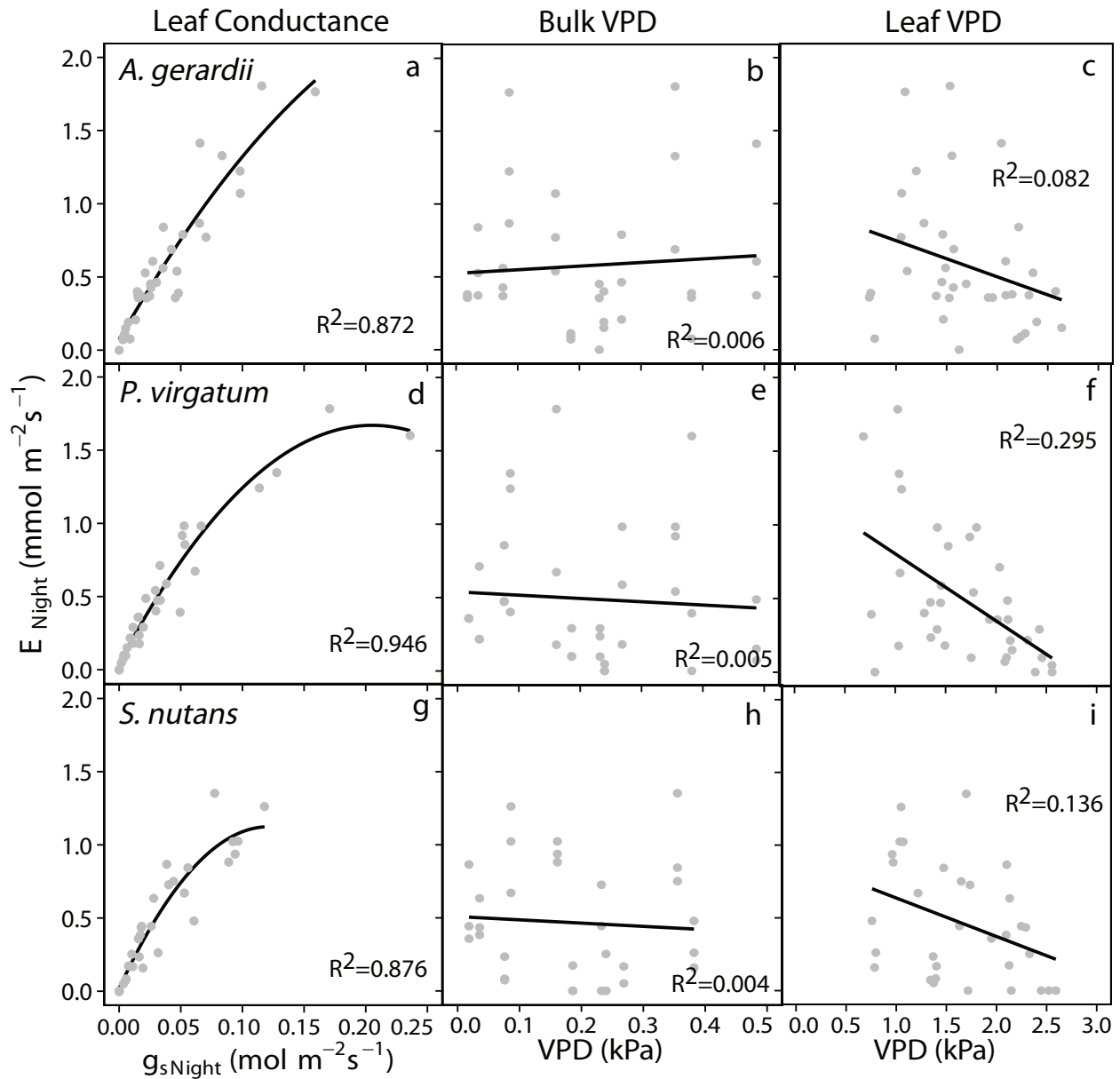
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**Figure 4.1** Environmental data measured between day of year 150-250 in 2014 and 2015. Shown are daily averages of soil moisture at 10 cm and 30 cm measured in units of water fraction by volume, wfv (a-b), cumulative daily precipitation (c-d), and vapor pressure deficit (VPD) reported at average daily maximum (15:00 h) and minimum (03:00 h) conditions (e-f). Dashed vertical lines indicate sampling days on which gas exchange measurements occurred during each growing season.

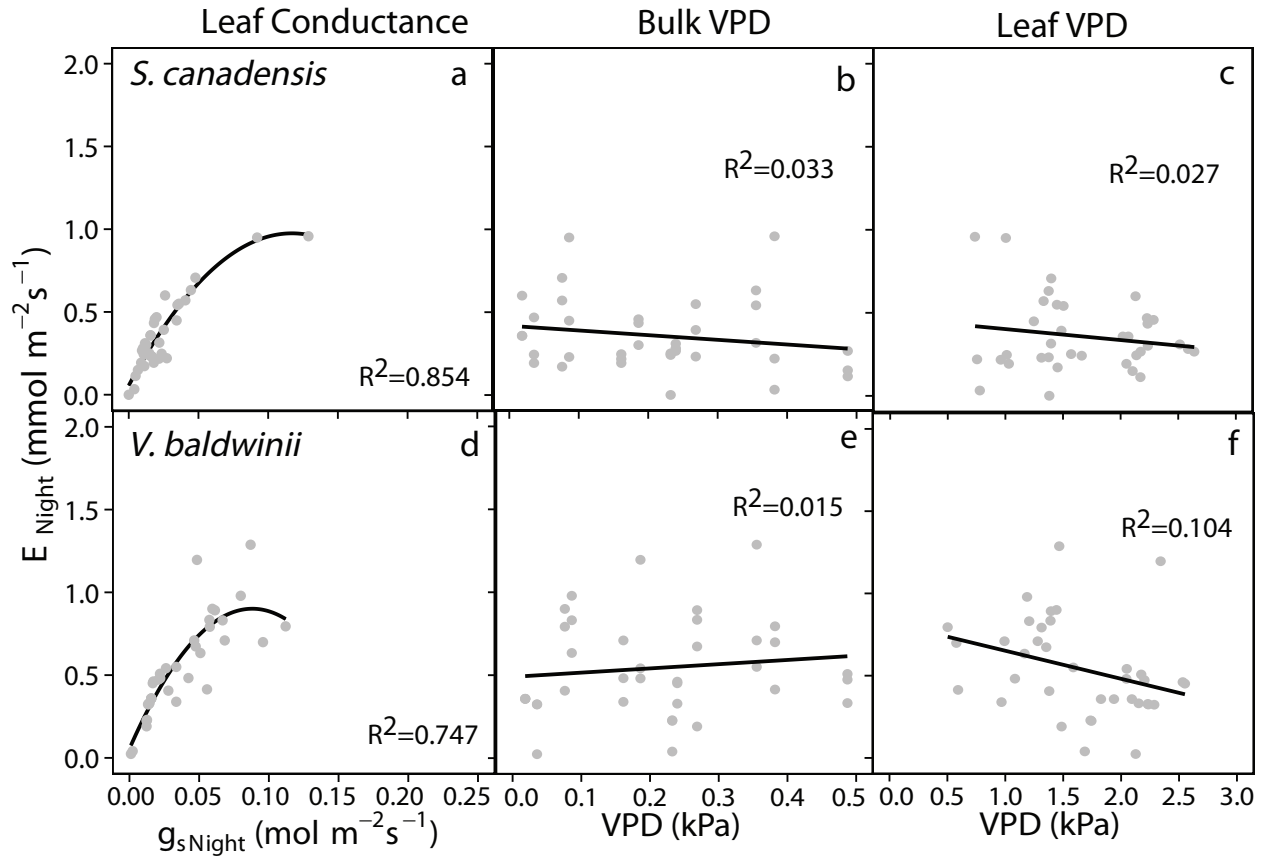


**Figure 4.2** Nighttime gas exchange traits measured from day of year 150-250 in 2014 and 2015. Shown are the mean + 1 SEM nocturnal stomatal conductance of water vapor,  $g_{snight}$  (a-b), nocturnal transpiration rate,  $E_{night}$  (c-d), and the percent nighttime of daytime transpiration rates, %  $E_{night}$  (e-f) measured for each species. Plant functional groups are distinguished by color (forbs = orange, grasses = green, and woody species = blue), and individual species are indicated by marker shape. Forbs include *S. canadensis* (square) and *V. baldwinii* (circle), grasses include *A. gerardii* (circle), *P. virgatum* (triangle), and *S. nutans* (square), and woody plants include *A. canescens* (square), *R. glabra* (triangle), and *C. drummondii* (circle).

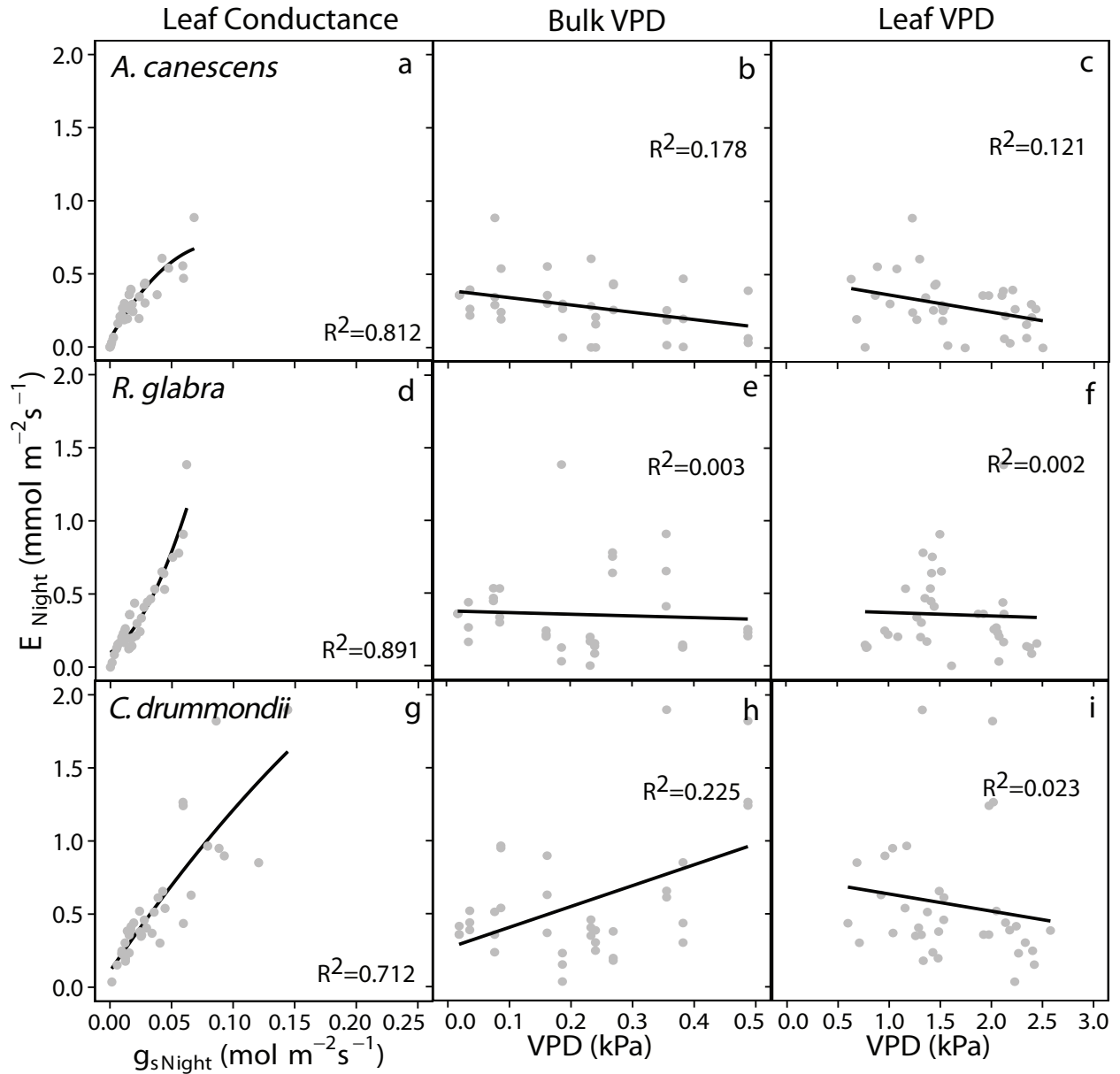


**Figure 4.3 Nonlinear regression relationships between nocturnal transpiration and nocturnal stomatal conductance (a,d,g), linear relationships between nocturnal transpiration and bulk VPD (b,e,h), and linear relationships between nocturnal transpiration and leaf VPD (c,f,i) for grasses. Shown are responses for *A. gerardii* (a-c), *P. virgatum* (d-f), and *S. nutans* (g-i).**

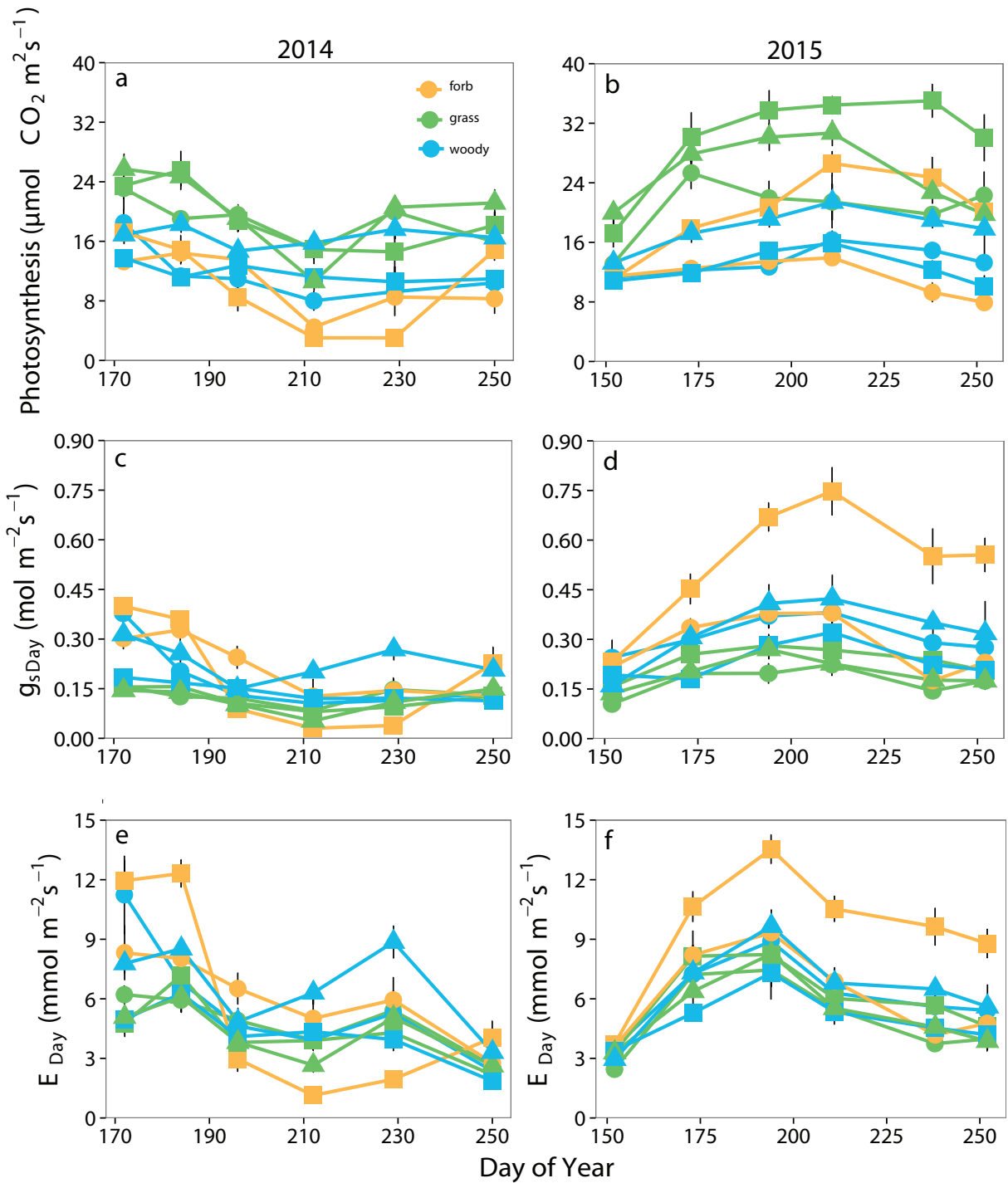




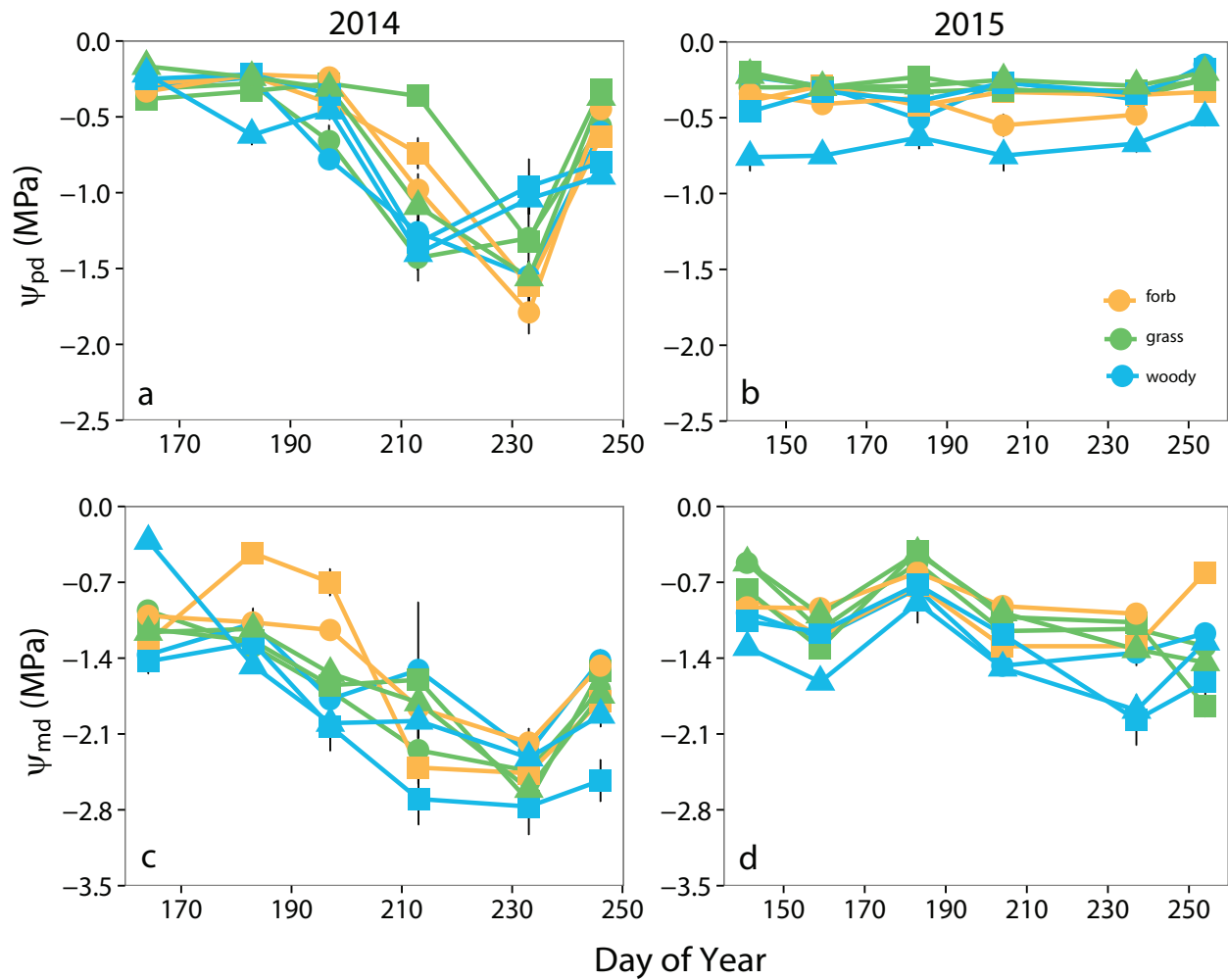
**Figure 4.4 Nonlinear regression relationships between nocturnal transpiration and nocturnal stomatal conductance (a,d), linear relationships between nocturnal transpiration and bulk VPD (b,e), and linear relationships between nocturnal transpiration and leaf VPD (c,f) for forbs. Shown are responses for *S. canadensis* (a-c) and *V. baldwinii* (d-f).**



**Figure 4.5 Nonlinear regression relationships between nocturnal transpiration and nocturnal stomatal conductance (a,d,g), linear relationships between nocturnal transpiration and bulk VPD (b,e,h), and linear relationships between nocturnal transpiration and leaf VPD (c,f,i) for woody species. Shown are responses for *A. canescens* (a-c), *R. glabra* (d-f), and *C. drummondii* (g-i).**



**Figure 4.6** Daytime gas exchange traits measured from day of year 150-250 in 2014 and 2015. Shown are the mean + 1 SEM steady-state photosynthesis (a-b), daytime stomatal conductance of water vapor,  $g_{sDay}$  (c-d), and daytime transpiration rate,  $E_{Day}$  (e-f) measured for each species. See Figure 2 for description of plant functional group and species indicators.



**Figure 4.7** Leaf water potential traits measured from day of year 150-250 in 2014 and 2015. Shown are the mean + 1 SEM predawn leaf water potential,  $\Psi_{pd}$  (a-b) and midday leaf water potential,  $\Psi_{md}$  (c-d) measured for each species. See Figure 2 for description of plant functional group and species indicators.

# **Chapter 5 - Stem sap flow in herbaceous and woody grassland plants: implications for tallgrass prairie ecohydrology**

## **Abstract**

Grasslands contribute significantly to global biogeochemical and hydrological cycling, yet we have little information about how water fluxes vary among grassland species at fine spatiotemporal scales. Using external heat balance sap flow sensors and a hierarchical Bayesian state-space modeling approach, we investigated stem sap flow and canopy transpiration in small-stemmed herbaceous and woody plants in tallgrass prairie community. We addressed the following questions: (1) How do diurnal and nocturnal fluxes differ among common grasses, forbs, and woody species in a tallgrass prairie? (2) How do flux rates vary across a growing season? (3) How do environmental variables drive patterns of nocturnal water flux? Diurnal sap flux and canopy transpiration varied among species and across the growing season. Woody species typically had the greatest rates of diurnal sap flux and canopy transpiration, and these rates were greatest early in the growing season, during periods of high soil water availability and low VPD. Nocturnal sap flux and canopy transpiration were generally consistent among species and over time, with somewhat higher rates observed among grasses. Finally, nocturnal water flux accounted for a greater proportion of daily water use in grasses than in forbs or woody species, and these rates were driven primarily by VPD. Overall, we suggest that interspecific differences in water flux may reflect species-specific strategies to tolerate water limitation and that shifts in the abundance of these species with future climate changes may impact ecosystem-level water balance.

## Introduction

Grasslands constitute a major portion of global vegetation, accounting for approximately 52.5 million km<sup>2</sup> or 40% of the terrestrial biome (White et al. 2000). Due to their widespread cover, grasslands exert tremendous influence over local and global carbon and water cycling. For instance, grasslands account for an estimated 19-24% of global evapotranspiration (Schlesinger and Jasechko 2014), 30-35% of terrestrial net primary productivity (Field et al. 1998), and 30% of stored soil carbon (Jackson et al. 1997; Lal 2004). Considering their contribution to ecosystem function, as well as their inherent sensitivity to climate perturbations (Knapp et al. 2002; Brunsell et al. 2010), grassland responses to a changing climate will likely have significant consequences for future global biogeochemical and hydrological cycles.

Despite the widespread cover of grasslands, current estimates and future predictions of grassland water fluxes are limited by our incomplete understanding of ecohydrological processes at fine spatiotemporal scales. Growing evidence suggests that local ecohydrological processes in grasslands are spatially and temporally variable, which produce large uncertainties when modeling fluxes at greater scales (Asbjornsen et al. 2011). Spatial variation in grassland ecohydrology can exist as changes in plant growth or functional traits across topographic gradients (Nippert et al. 2011; Tucker et al. 2011), variation in water uptake in response to management regimes (O’Keefe and Nippert 2016), or even as changes in hydraulic traits within individual plants (Ocheltree et al. 2013a,b). Temporal variation exists in the form of seasonal plasticity in plant water uptake (Nippert and Knapp 2007a,b), seasonal variation in leaf gas exchange and water potential (Knapp 1984, 1985; Nippert et al. 2009), or diel fluctuations in leaf transpiration (O’Keefe and Nippert, *unpublished data*). Understanding ecosystem and global water fluxes will therefore require high spatial and temporal resolution information about the

driving mechanisms and patterns of water fluxes for individual species within grassland communities.

Grassland water fluxes are typically studied at the landscape or leaf scale, each of which have inherent tradeoffs regarding spatial and temporal resolution. For instance, eddy covariance measurements provide information about carbon, water, and energy exchanges across large spatial scales. While these high frequency data are useful in describing temporal flux dynamics across the landscape, eddy flux measurements provide an integrative picture of earth-atmosphere exchanges and cannot be used to understand the mechanistic processes driving these fluxes in individual species. Additionally, nocturnal evapotranspiration measurements made with eddy covariance techniques are generally unreliable due to low turbulence that occurs at night (Goulden et al. 1996; Baldocchi 2003), which limits their utility in describing diel patterns of water loss. Conversely, leaf-level gas exchange measurements can be made quickly and can provide information about the instantaneous physiology of multiple species across a heterogeneous landscape. While leaf-level measurements can offer inference about species-level variation in carbon and water fluxes, they only provide an instantaneous snapshot of temporally dynamic processes. Thus, we have no information about how a wide range of grassland species use water at the whole-plant scale, how these patterns vary among species or within functional groups, or how plant water use responds to changes in climate.

Sap flow sensors offer a unique approach to quantify whole-plant water flux among coexisting species at fine temporal resolutions. Sap flow technology was first developed in the 1930's by Huber and colleagues (Huber 1932; Huber and Schmidt 1936) and has since become a standard approach utilized in many studies of plant-water relations (reviewed in Cermak et al. 2004). Although many sap flow methods now exist, most studies currently use thermodynamic

methods to measure the rate and volume of water ascent through plants. These techniques typically insert heater and thermocouple probes into sapwood (typically > 10 cm stem diameter), and are therefore unsuitable for use on smaller stemmed shrubs or herbaceous plants (Skelton et al. 2013). Thus, sap flow sensors have been utilized almost exclusively on trees. The external heat balance method was developed by Clearwater et al. (2009) to measure low sap flow rates on small stems, but these techniques have been primarily applied to crops such as maize (Gavloski et al. 1992), soybean, cotton (Cohen et al. 1990, 1993; Cohen & Li 1996), and wheat (Langensiepen et al. 2014). With the exception of several studies conducted during the mid-1990's (Senock and Ham 1995; Owensby et al. 1997), no work has investigated sap flux in small-stemmed, herbaceous plants *in situ*.

We present here an assessment of whole-plant water use in herbaceous and woody species common throughout the tallgrass prairies of North America. We used external heat balance sap flow microsensors to measure sap flow rates in small-stemmed plants (< 7 mm diameter) in a tallgrass prairie and then combined these data with a hierarchical Bayesian state-space model to calculate species-specific canopy transpiration. Using these data we addressed the following questions: (1) How do diurnal and nocturnal fluxes differ among common grasses, forbs, and woody species in a tallgrass prairie? (2) How do flux rates vary across a growing season? (3) How do environmental variables (e.g., vapor pressure deficit, soil moisture, and air temperature) relate to patterns of nocturnal flux rates?



## Methods

### Site Description

Research was conducted during May – September 2014 at the Konza Prairie Biological Station (KPBS), a 3,487-ha area of native tallgrass prairie located in the Flint Hills region of northeastern Kansas, USA (39.1°N, 96.9°W). The KPBS is a Long-Term Ecological Research (LTER) site that is divided into experimental watersheds, each of which receive varying combinations of prescribed fire (burned every 1, 2, 4, or 20 years) and grazing (grazed by *Bison bison*, cattle, or ungrazed) treatments. While these treatments alter vegetation diversity and productivity (Collins et al. 1998; Eby et al. 2014; Hartnett et al. 1996; Knapp et al. 1999; Briggs and Knapp 1995; Collins and Calabrese 2012; Veen et al. 2008) the landscape is generally dominated by a few perennial C<sub>4</sub> grass species along with numerous sub-dominant C<sub>3</sub> grass, forb, and woody species (Smith and Knapp 2003). Long-term weathering at the site has created a topographically heterogeneous landscape, including shallow, rocky uplands, steep slopes, and deep, loess soiled lowlands.

The Flint Hills region of Kansas is characterized by a mid-continental climate with cool, wet winters and warm, dry summers. Long-term mean annual precipitation (1982-2014) at KPBS is 829 mm, of which 79% occurs during the growing season (April – September). Precipitation was 706 mm in 2014 and 1002 mm in 2015, with 68% and 75% occurring during each respective growing season. The warmest average month of the year (1982-2014) is July, with mean maximum and minimum air temperatures of 32.69 °C and 19.78 °C. The coldest average month (1982-2014) is January, with mean maximum and minimum air temperatures of 4.87 °C and -7.14°C. Mean maximum and minimum air temperatures were 31.7°C and 17.06°C during July

2014. Mean maximum and minimum air temperatures were 32.02°C and 20.67°C during July 2015.

This study was conducted in lowland prairie of a watershed that is burned every four years and was last burned in 2013. This location was chosen because a four-year fire interval is similar to the historic frequency of fire for the region (Frost 1998) and because watersheds burned with this frequency have greater forb and shrub diversity than watersheds that are burned annually. We sampled commonly occurring herbaceous and woody species including three dominant C<sub>4</sub> grasses (*Andropogon gerardii*, big bluestem; *Panicum virgatum*, switchgrass; *Sorghastrum nutans*, Indiangrass), two C<sub>3</sub> forbs (*Solidago canadensis*, Canada goldenrod; *Vernonia baldwinii*, Baldwin's ironweed), one leguminous C<sub>3</sub> sub-shrub (*Amorpha canescens*, leadplant), and two C<sub>3</sub> shrubs (*Rhus glabra*, smooth sumac; *Cornus drummondii*, rough-leaf dogwood).

### **Sap flow measurements**

Stem sap flow was measured using the external heat balance method with commercially available sap flow sensors (SGA3 and SGA5 Dynagage Microsensors; Dynamax Inc., Houston, TX USA) connected to a Flow32 sap flow system (Dynamax Inc., Houston, TX, USA). Briefly, a thermocouple sensor was attached to the circumference of a plant stem, heat was applied to the plant stem, and the flow of sap through the stem was estimated by measuring voltage outputs from the flux of heat through the heated stem section (Sakuratani 1981; Smith and Allen 1996). Sap flow was calculated as:

$$Q = Q_r + Q_v + Q_f + S \quad (1)$$

where  $Q$  is the total power,  $Q_r$  is the radial heat flux,  $Q_v$  is the axial heat flux,  $Q_f$  is the residual heat flux, and  $S$  is the rate of change in stem heat storage (see Senock and Ham 1995 for detailed calculations of each heat flux component).

Sensors were installed on plants that fit within the stem diameter range of each sensor size (3-5 mm for SGA3 and 5-7 mm for SGA5). Prior to installation, dirt and debris were cleaned from stems and a conducting gel was applied to the stem surface to facilitate heat transfer between the stem and the sensor (Dynamax 2009). Each sensor was sealed with putty to prevent moisture damage to the sensor and insulated with multiple layers of foam and bubble foil to reduce the impact of radiation on sensor measurements.

Eight sap flow sensors were installed during the growing season (DOY 140-254). In order to alleviate potential stress associated with using sensors on small stems for prolonged periods of time, the sensors were rotated among all species throughout the growing season. The eight sensors were deployed on two individuals of four study species for a period of ten days. Following the ten-day sampling period, all sensors were switched to a different set of four species. The species chosen for each ten-day period were randomly selected, but all species were sampled for approximately an equal amount of time across the growing season.

Measurements were made every 60 seconds, averaged, and logged every 30 minutes with a CR1000x data logger and an AM 16/32 multiplexer (Campbell Scientific, Logan, Utah). The data logger and sensors were powered with a 100-W solar panel and sensor heat inputs were controlled with an AVR voltage regulator (v 3.1, Dynamax Inc., Houston, TX, USA) linked to the data logger.

## Environmental variables

The microclimate of the study site was evaluated by measuring air temperature (100 K thermistor; Betatherm, Hampton, VA, USA), soil moisture at 10 cm (Hydraprobe II; Stevens Water Monitoring Systems, Portland, OR, USA), relative humidity (HM1500; Humierl, Hampton, VA, USA), and wind speed (3-cup anemometer; Gill Instruments, Lymington, UK). A radiation shield (41003; RM Young, Traverse City, MI, USA) was used to protect the relative humidity sensor, and air temperature and relative humidity measurements were used to calculate vapor pressure deficit at the site. All measurements were logged every 10 s with a CR10X data logger (Campbell Scientific, Logan, UT, USA). Missing data due to inclement weather or equipment failure were gap-filled using a moving diurnal mean (Falge et al. 2001).

## Model description

The hierarchical Bayesian State-Space Canopy Conductance (StaCC) model was used to gap-fill missing sap flow data and to estimate leaf transpiration, canopy conductance, and canopy transpiration for each species (Bell et al. 2015). The StaCC model combines a sap flux data model with a canopy conductance model, while applying prior knowledge of the model parameters and accounting for random error associated with individual sap flow sensors. The data model utilizes sap flow measurements made by a set of sensors to calculate average species sap flux across the time series. Sap flux of probe  $i$  at time  $t$  was modeled as:

$$J_{it} \sim N(J_t Z(d_i) a_i, S) \quad (2)$$

where  $J_t$  is the average sap flux at time  $t$ ,  $Z(d_i)$  is a sapwood depth model,  $a_i$  is the random effect associated with probe  $i$  and  $S$  is the Gaussian observation variance. Because sap flux was measured on herbaceous and small-stemmed woody plants ( $< 7$  mm diameter), we did not utilize

the sapwood depth submodel. Similarly, because these small-stemmed plants do not likely store an appreciable amount of stem water (Nobel and Jordan 1983), we also did not utilize the additional capacitance submodel described in Bell et al. (2015).

The canopy conductance process model was used to estimate canopy conductance ( $G_t$ ), transpiration per  $m^2$  leaf area ( $E_L$ ), and transpiration per  $m^2$  ground area ( $E_t$ ). First, steady-state conductance at time  $t$  ( $G_{s,t}$ ,  $mmol\ m^{-2}\ s^{-1}$ ) was modeled as a function of vapor pressure deficit ( $D_t$ ), photosynthetically active radiation ( $Q_t$ ), and volumetric soil moisture ( $M_t$ ) according to Jarvis (1976):

$$G_{s,t} = f(D_t)g(Q_t)h(M_t) \quad (3)$$

Within this model, the effect of  $D_t$  on  $G_{s,t}$  was calculated as:

$$f(D_t) = G_{ref} - \lambda \ln(D_t) \quad (4)$$

where  $G_{ref}$  is the reference conductance ( $G_{s,t}$  when  $D_t = 1$  kPa) and  $\lambda$  is the stomatal sensitivity to  $D_t$  (Oren et al. 1999). Second, the effect of  $Q_t$  on  $G_{s,t}$  was calculated as:

$$g(Q_t) = 1 - \alpha_1 \exp(Q_t / \alpha_2) \quad (5)$$

where  $\alpha_1$  is nocturnal stomatal conductance and  $\alpha_2$  is the sensitivity to  $Q_t$ . Third, the effect of  $M_t$  on  $G_{s,t}$  was modeled as:

$$h(M_t) = \exp(-0.5(M_t - \alpha_3)^2 / \alpha_4^2) \text{ if } M_t \leq \alpha_3 \text{ or} \quad (6)$$

$$h(M_t) = 1 \text{ if } M_t > \alpha_3$$

where  $\alpha_3$  is the threshold below which  $M_t$  reduces  $G_{s,t}$  and  $\alpha_4$  describes the sensitivity of the decline in  $G_{s,t}$  with declines in  $M_t$  below that threshold. Next,  $G_{s,t}$  was used to calculate actual canopy conductance ( $G_t$ ), assuming that  $G_t$  is dependent on previous conductance and a time interval,  $d_t$ : (Rayment et al. 2000; Ward et al. 2008):

$$G_t = G_{t-dt} + (G_{st} - G_{t-dt})V_t \quad (7)$$

where  $d_t = 30$  min and  $V_t = 1 - \exp(-d_t/\tau)$ . The  $V_t$  term accounts for stomatal lags and  $\tau = 10$  min, following Bell et al. (2015). Canopy conductance was then used to calculate  $E_L$  ( $\text{kg m}^{-2}\text{s}^{-1}$ ) as:

$$E_{L,t} = G_t q_t \quad (8)$$

where  $q_t$  is a composite variable that incorporates air temperature and vapor pressure deficit, as well as several constant terms (Bell et al. 2015). Finally,  $E_L$  was scaled to  $E_t$  with the following equation:

$$E_t = E_L A_L (A_s R_s)^{-1} \quad (9)$$

where  $A_L$  is the leaf area index,  $A_s$  is the sapwood area index, and  $R_s$  is a scaling constant. Leaf area index data for grasses, forbs, and small shrubs (*A. canescens* and *R. glabra*) were taken from previous work at this site (Klodd et al. 2016). Due to its large, clonal growth form, leaf area index was measured for *C. drummondii* with a leaf area index meter in 2015 (LiCor, Inc, Lincoln, NE, USA). Due to the small-stemmed and herbaceous nature of our study species, sapwood area index values were approximated as  $\text{m}^2$  stem area per  $\text{m}^2$  ground area for each species at our study location.

The final hierarchical Bayesian model is a joint distribution of sap flow data observations, canopy conductance latent states, and model parameters. When possible, priors for the data model and process model were utilized from Bell et al. (2015). Species-specific priors are described in Appendix 1 (Table D1). See Bell et al. (2015) for a full description of prior distributions and the joint posterior distribution.

A separate model analysis was implemented for each of the eight species. We ran the Gibbs sampler for 20,000 iterations and discarded the first 10,000 iterations as a burn-in. The model produced  $J_t$ ,  $G_t$ ,  $E_L$ , and  $E_t$  values for each 30 min time step and we used this information to evaluate differences in water flux among species and across the growing season. First,

‘diurnal’ and ‘nocturnal’ classifications were assigned to each 30 min time step throughout a day based on daily sunrise and sunset times (e.g., ‘diurnal’ = time steps between sunrise and sunset, ‘nocturnal’ = time steps between sunset and sunrise). We then averaged  $J_t$ ,  $G_t$ ,  $E_L$ , and  $E_t$  values separately across diurnal and nocturnal time steps for each day and for each species. To evaluate the significance and drivers of nocturnal water flux we calculated percent nocturnal  $J_t$  and percent nocturnal  $E_t$  for each day using the daily diurnal and nocturnal averages. We then evaluated the relationship between daily percent nocturnal  $E_t$  and various environmental variables (nocturnal VPD, nocturnal air temperature, soil moisture, diurnal VPD, and diurnal air temperature) using linear regression analyses. Separate linear regression analyses were conducted for each environmental variable. All analyses were performed using the statistical program R V3.1.0 (R Core Team 2012).

## **Results**

### **Environmental Data**

Soil moisture measured at 10 cm depth declined over the growing season (Figure 5.1a) with highest values at DOY 161 and lowest soil moisture values at DOY 242. Air temperature was variable throughout the growing season and increased slightly as the growing season progressed. Maximum air temperature during the day (15:00) was recorded on DOY 196 and the minimum air temperature at night (03:00) was recorded on DOY 236. Vapor pressure deficit was also variable over time but was generally higher during the latter part of the growing season (Figure 5.1c). Maximum VPD values measured during the day (15:00 h) occurred on DOY 236, while minimum VPD values measured at night (03:00) occurred on DOY 219.

## Model Performance

Model performance varied based on plant functional type. Generally, model convergence (Figure D1-8) and model predictions (Figure D9-17) were greatest in woody species, intermediate in forbs, and lowest in grasses. Squared Pearson correlation coefficients ( $r^2$ ) for model predictions of individual sap flow sensors ranged from 0.21 – 0.97 in woody species, 0.03-0.92 in forbs, and 0.00 – 0.22 in grasses. Average  $r^2$  values for the measured versus modeled fit of individual sensors within species were: *C. drummondii* = 0.90, *R. glabra* = 0.84, *A. canescens* = 0.86, *V. baldwinii* = 0.71, *S. canadensis* = 0.48, *S. nutans* = 0.08, *P. virgatum* = 0.08, and *A. gerardii* = 0.05.

## Model Results

Diel sap flow patterns varied among species and across the growing season (Figure 5.2). During the day,  $J_t$  was greatest in woody species, with the highest rates ( $> 30 \text{ g h}^{-1}$ ) recorded in *C. drummondii*. Grasses exhibited the lowest diurnal  $J_t$ , with the lowest rates observed in *A. gerardii* and *P. virgatum* ( $< 5 \text{ g h}^{-1}$ ). Diurnal  $J_t$  declined throughout the growing season, although the extent of this pattern was more pronounced in certain species. For instance, the greatest seasonal declines occurred in *S. nutans* and *C. drummondii*, while the lowest declines occurred in *A. gerardii* and *P. virgatum*. Conversely, nocturnal  $J_t$  was generally consistent among species and across the growing season, with rates under  $3 \text{ g h}^{-1}$ . *S. nutans* was the exception to this trend, as this species had high rates of nocturnal  $J_t$  early in the season ( $> 6 \text{ g h}^{-1}$ ) that declined sharply by DOY 180.

We observed similar trends in modeled  $G_t$ ,  $E_L$ , and  $E_t$  (Figure D18-20). Diurnal  $G_t$ ,  $E_L$ , and  $E_t$  were greatest in woody species and *S. nutans*, and these values declined for most species



throughout the growing season. Patterns of nocturnal  $E_L$  and  $E_t$  were also similar to modeled  $J_t$ , with consistently low values observed among most species. However, nocturnal  $G_t$  was nearly identical to diurnal  $G_t$  in grasses, and exhibited a more pronounced seasonal decline in woody species than nocturnal sap flow or transpiration rates (Figure D18).

### **Percent Nocturnal Water Flux**

Percent nocturnal  $J_t$  was similar among species within functional groups, but varied across different functional groups (Figure 5.3). Percent nocturnal  $J_t$  was highest in grasses (ranging from 20.9 – 89.3%), lower in forbs (ranging from 13.2 – 61.4%), and lowest in woody species (ranging from 8.3 – 46.7%). Percent nocturnal  $J_t$  also varied over time, with a steady increase throughout the latter half of the growing season.

We observed similar trends for percent nocturnal  $E_t$ . When averaged across species within functional groups, diurnal  $E_t$  was greatest in woody species, intermediate in grasses, and lowest in forbs (Figure 5.4a). At night,  $E_t$  was greatest among grasses, intermediate in woody species, and lowest in forbs (Figure 5.4b). These values resulted in highest percent nocturnal  $E_t$  in grasses (26.8 -86.4%), intermediate percent  $E_t$  in forbs (14.3 – 54.8%), and lowest percent nocturnal  $E_t$  in woody species (8.9 – 40.8%). Like percent nocturnal  $J_t$ , percent nocturnal  $E_t$  increased in all functional groups over time (Figure 5.4c).

Variation in percent nocturnal  $E_t$  was best explained by nocturnal VPD (Figure 5.5; Table 5.1). Percent nocturnal  $E_t$  increased with increasing VPD for all species, although the rate of this increase and the strength of this relationship were greatest in the grasses (Figure 5.5a), weaker in forbs (Figure 5.5b) and weakest in woody plants (Figure 5.5c). Species showed similar responses to VPD within functional groups. Although linear regressions between percent nocturnal  $E_t$  and

other environmental drivers were often significant, the strength of the correlation coefficients for these relationships were generally weak (Table 5.1).

## **Discussion**

Although sap flow sensors have been used extensively to assess species-specific patterns of whole-plant water flux across a range of ecosystems, technical limitations associated with the methodology have restricted most sap flow measurements to trees (Barbeta et al. 2012; Brinkman et al. 2016; Catovsky et al. 2002; Dawson et al. 2007; Holscher et al. 2005; Martinez-Vilalta et al. 2003; Zeppel et al. 2008). To date, only a few studies have used sap flow probes in large-stemmed monocots such as corn (Cohen et al. 1990, 1993; Cohen and Li 1996) and bamboo (Kume et al. 2010). Others have used external heat balance sap flow sensors to measure water flux in petioles and fruit pedicels (Clearwater et al. 2009; Roddy and Dawson 2012), wheat (Langensiepen et al. 2014), small shrubs (Skelton et al. 2013), and perennial grasses (Senock and Ham 1995; Owensby et al. 1997). Despite these advances, we still lack a comprehensive understanding of continuous, whole-plant water-use in small stems and herbaceous species, which limits our understanding of community and ecosystem responses to environmental change. Here, we addressed this limitation by using external heat balance sap flow sensors to assess diurnal and nocturnal sap flow patterns within a diverse grassland community.

Diurnal water flux varied across functional groups and throughout the growing season. Woody species had the highest rates of sap flow and transpiration, which is unsurprising considering these species have larger stem diameters and should therefore have greater xylem conductivity than smaller-stemmed forbs or grasses. Due to their smaller stem diameters, grasses had the lowest sap flow and canopy transpiration rates. This pattern differs from the high rates of

transpiration measured in C<sub>4</sub> grasses at the leaf scale (Kalapos et al. 1996; Knapp et al. 1993; Nippert et al. 2007; Turner et al. 1995) and illustrates the importance of measuring whole-plant water flux when determining accurate species-specific contributions to ecosystem hydrological cycling. Additionally, water flux varied through time. Diurnal sap flow declined across the growing season for most species, and this was likely associated with the seasonal decline in soil moisture (Figure 5.1a), as well as seasonal increases in temperature (Figure 5.1b) and VPD (Figure 5.1c). Many previous studies have reported similar responses of water to environmental fluctuations at the stem (Huang et al. 2015; Martinez-Vilalta et al. 2003) and at the leaf level (Knapp 1984, 1985; Nippert et al. 2009).

We also observed interspecific differences in diurnal water flux, which may reflect species-specific strategies to persist through drought. For instance, *S. nutans* and *C. drummondii* both had higher sap flow rates and larger seasonal declines in sap flow than other grasses and woody species, respectively. The temporally dynamic response of *S. nutans* may be explained by its enhanced sensitivity of leaf physiology and growth to water limitation compared to *A. gerardii* (Nippert et al. 2009; Silletti and Knapp 2001). Conversely, the relatively constant water fluxes of *S. canadensis*, *V. baldwinii*, *A. canescens*, and *R. glabra* could be explained not by tolerance to drought, but by greater plasticity in the source of water used. Specifically, these species possess deep roots and shift reliance from shallow to deep water during periods of drought (Nippert et al. 2007a,b; O’Keefe and Nippert 2016, Chapter 2), which may aid in the maintenance of static flux rates. The seasonal decline observed in *C. drummondii* is somewhat surprising considering that this species relies consistently on deep water (Ratajczak et al. 2011). However, *C. drummondii* might exhibit stomatal responses that are more sensitive to temperature or VPD than to water availability. Alternatively, the phenology of this species may promote

greater water flux early in the season during periods of clonal and reproductive growth, compared to later in the growing season after reproduction has ceased (K. O'Keefe, *personal observation*).

Conversely, nocturnal water flux was generally constant among species and through time. When averaged across functional groups, grasses exhibited somewhat greater nocturnal canopy transpiration rates than forbs or woody species. The combination of lower diurnal flux rates and higher nocturnal flux rates consequently resulted in greatest percent nocturnal flux in grasses (Figure 5.4). These results suggest that nocturnal transpiration may contribute substantially to the water budgets of mesic ecosystems that are dominated by C<sub>4</sub> grasses. Similarly, leaf gas exchange measurements show that nocturnal transpiration accounts for a greater proportion of daytime gas exchange rates in grasses than in forbs or shrubs, which may indicate that nocturnal water loss has functional significance for the grasses in this ecosystem (Chapter 3). Although previous studies have shown that nocturnal sap flow can represent nocturnal storage tissue refilling in trees, rather than nocturnal transpiration (Daley and Phillips 2006; Fisher et al. 2007), refilling is not likely an important mechanism here because small stems do not store an appreciable amount of water (Nobel and Jordan 1983). Additionally, the similarity of these results to leaf gas exchange measured at this site suggests that nocturnal sap flow is driven by nocturnal transpiration in these species.

Percent nocturnal  $E_t$  was best explained by nocturnal VPD (Figure 5.5; Table 5.1). Previous studies have also shown that nocturnal water flux is driven primarily by VPD (Dawson et al. 2007; Fisher et al. 2007) and is modulated by other environmental variables such as soil moisture (Howard and Donovan 2006; Cirelli et al. 2015) or wind speed (Benyon 1995; Karpul and West 2016). Interestingly, we did not detect a strong relationship between nocturnal water

flux and soil moisture (Table 5.1). This may have occurred because we measured soil moisture at a central location within the study area, and variation in soil moisture within the immediate rhizosphere may be more important for driving flux patterns among individuals. Of the other potential environmental drivers investigated, nocturnal air temperature explained some variation in nocturnal water flux; however the effects of temperature are difficult to disentangle from the effects of VPD. Diurnal VPD and air temperature measured prior to nocturnal measurements did not impact nocturnal water flux, contrary to observations in previous studies (Resco deDios et al. 2015). Importantly, the relationship between percent nocturnal  $E_t$  and nocturnal VPD varied across plant functional groups, with the strongest relationship and greatest rate of increase observed in grasses (Figure 5.5). This result is similar to previous studies showing interspecific differences in stomatal sensitivity to VPD (Oren et al. 1999), as well as and similar stomatal responses within functional groups (Ocheltree et al. 2013). Given that these functional groups have contrasting responses to changes in VPD at the whole-plant scale, differences in stomatal sensitivity could result in changes in water flux at the watershed level with future changes in climate.

While we believe our sensor data and model output provides reasonable estimates of stem sap flux and canopy transpiration across a range of herbaceous and woody species, there are several important caveats to consider. As with any model, we made several simplifying assumptions when predicting  $J_t$ ,  $G_t$ ,  $E_L$ , and  $E_t$  from sensor data (Box 1978). First, we assumed that water flux scales linearly with stem cross-sectional area, and that stem capacitance is negligible within these small-stemmed species. While previous research has shown that flux rates vary radially (Phillips et al. 1996) and with capacitance dynamics (Phillips et al. 2003) in tree stems, radial patterns of sap flow and water storage are less likely to occur in small stems

and herbaceous plants. Second, we assumed that LAI and SAI were the same across species within functional groups. While delineating LAI and SAI across functional groups may result in missed nuances among species, these plant traits exhibit fairly low variation within functional groups (Klodd et al. 2016). Furthermore, we observed interspecific differences in sap flow within functional groups despite this simplification (Figure 5.2), suggesting that the tradeoff of model complexity for model parsimony still produced useful and informative comparisons across species.

To conclude, we show that whole-plant and canopy flux rates vary among species, plant functional types, and across a growing season within a tallgrass prairie. These results highlight the spatial and temporal variability of local ecohydrological processes observed within tallgrass prairies, and also indicate that other water-use processes (e.g., stomatal regulation, water source use) likely have consequences for water fluxes at larger scales. The interspecific differences observed here also suggest that shifts in the abundances of these species with future climate changes or ecosystem state changes would not only impact tallgrass prairie diversity and productivity (Collins et al. 1998; Knapp et al. 2002; Smith and Knapp 2003), but also ecosystem-level water fluxes (Scott et al. 2008; Wang et al. 2010; Logan and Brunsell 2015). Finally, our results also show that the contribution of nocturnal water fluxes to daily water budgets vary among plant functional types, which indicates a need to incorporate nighttime evapotranspiration in climate models when providing estimates of grassland water balance. Overall, we show high-temporal resolution data on whole-plant water flux within diverse plant communities is essential for assessing ecosystem responses to a changing climate.

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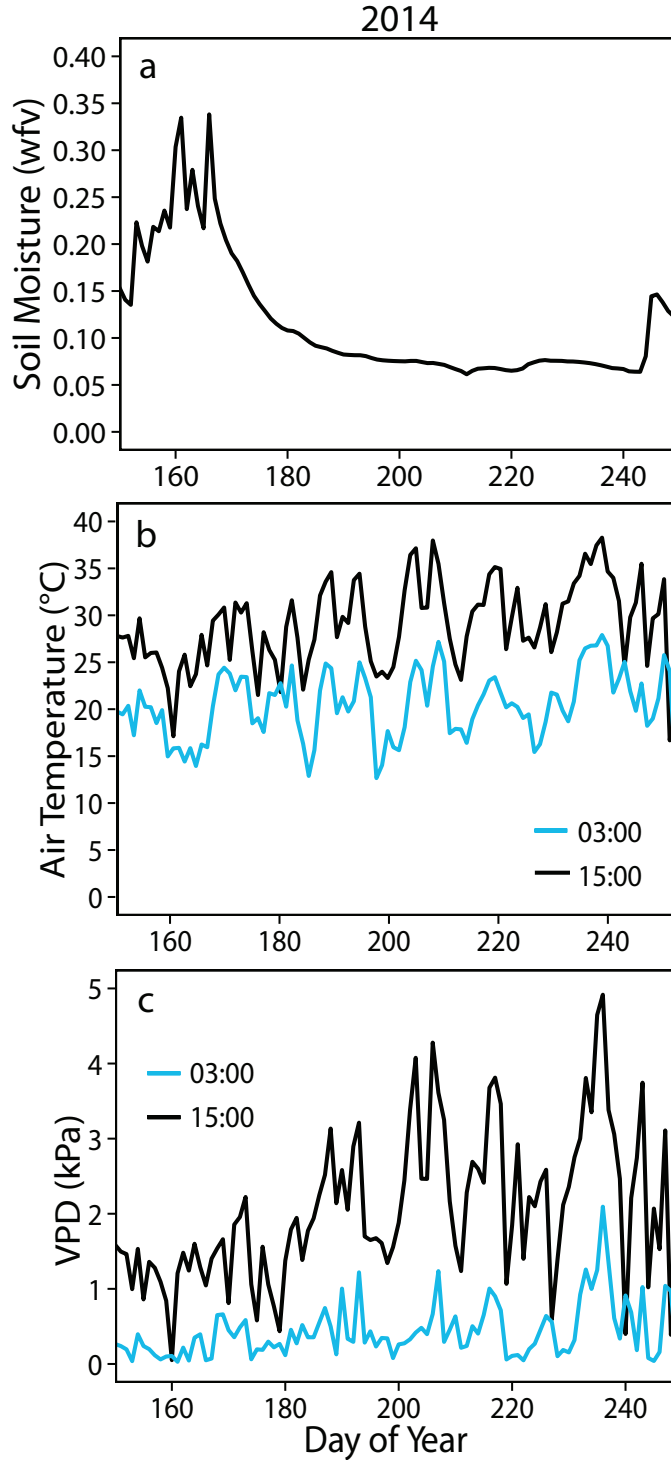
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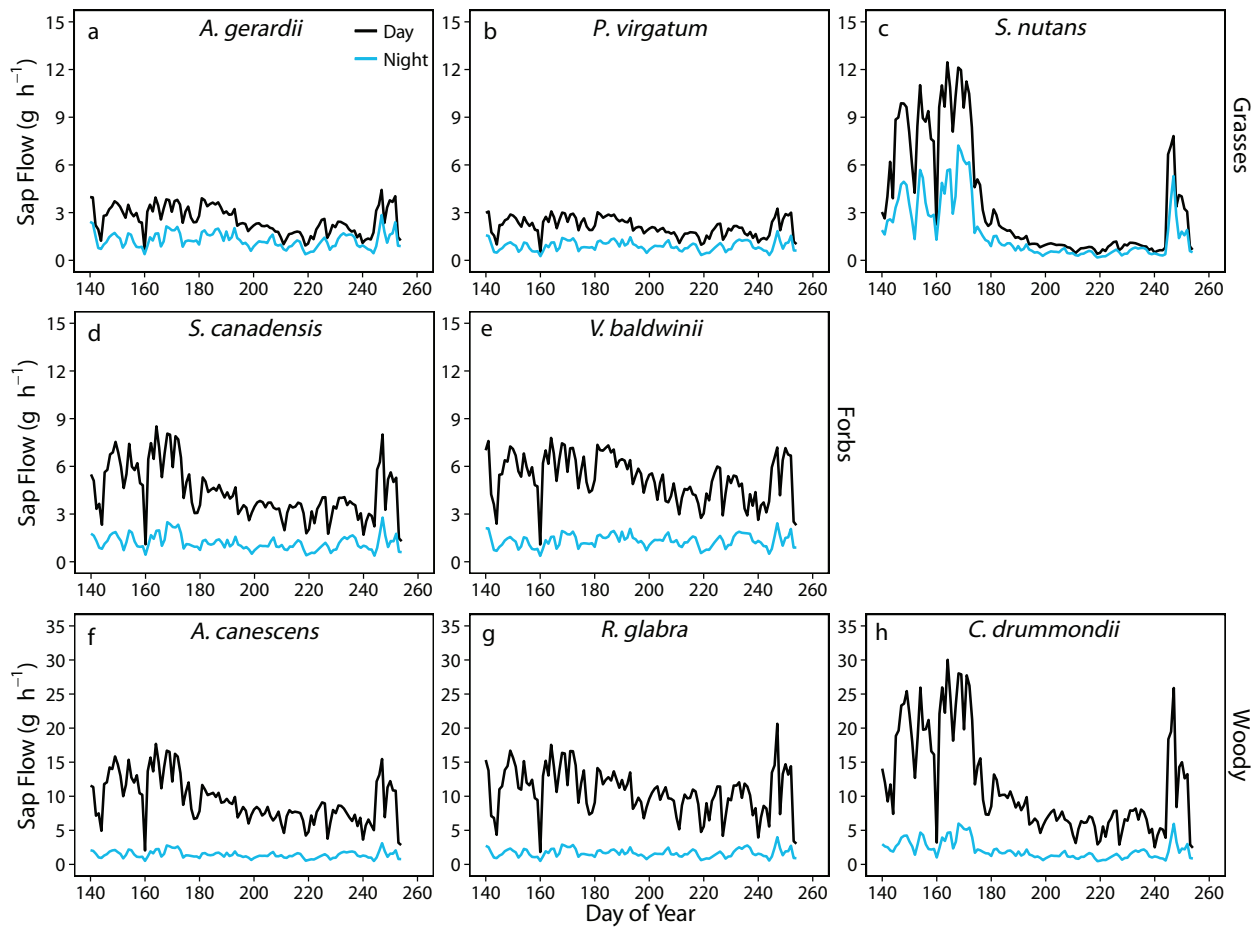
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**Table 5.1 Linear regression statistics for analyses comparing the relationship between percent nocturnal transpiration and individual environmental variables (average soil moisture, diurnal VPD (15:00 h), nocturnal air temperature (03:00 h), and diurnal air temperature (15:00 h). Significance at the  $\alpha=0.05$  level is indicated with bold font.**

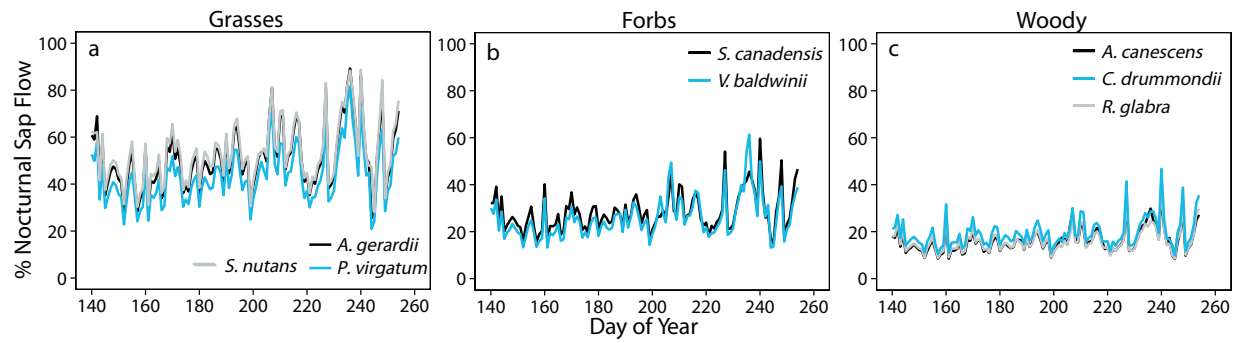
Species	Statistic	Soil Moisture	VPD (15:00 h)	Air Temp (03:00 h)	Air Temp (15:00 h)
<i>A. gerardii</i>	Intercept	60.14 $\pm$ 2.45	41.48 $\pm$ 2.59	6.19 $\pm$ 5.82	29.12 $\pm$ 7.29
	Slope $\pm$ 1SE	-75.97 $\pm$ 17.88	4.83 $\pm$ 1.17	2.22 $\pm$ 0.28	0.77 $\pm$ 0.25
	R <sup>2</sup>	0.14	0.13	0.35	0.08
	<i>p</i>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>
<i>P. virgatum</i>	Intercept	52.03 $\pm$ 2.14	33.79 $\pm$ 2.21	2.75 $\pm$ 5.01	20.31 $\pm$ 6.29
	Slope $\pm$ 1SE	-69.36 $\pm$ 15.63	5.03 $\pm$ 1.00	2.03 $\pm$ 0.24	0.82 $\pm$ 0.22
	R <sup>2</sup>	0.15	0.18	0.38	0.11
	<i>p</i>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>
<i>S. nutans</i>	Intercept	61.92 $\pm$ 2.41	45.16 $\pm$ 2.58	10.28 $\pm$ 5.78	34.91 $\pm$ 7.24
	Slope $\pm$ 1SE	-72.21 $\pm$ 17.63	4.10 $\pm$ 1.17	2.13 $\pm$ 0.28	0.64 $\pm$ 0.25
	R <sup>2</sup>	0.13	0.10	0.34	0.06
	<i>p</i>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>	<b>0.01</b>
<i>S. canadensis</i>	Intercept	32.69 $\pm$ 1.57	26.39 $\pm$ 1.71	5.00 $\pm$ 3.89	25.65 $\pm$ 4.70
	Slope $\pm$ 1SE	-35.77 $\pm$ 11.46	1.01 $\pm$ 0.78	1.16 $\pm$ 0.19	0.10 $\pm$ 0.16
	R <sup>2</sup>	0.08	0.01	0.25	0.003
	<i>p</i>	<b>0.002</b>	0.20	<b>&lt; 0.01</b>	0.56
<i>V. baldwinii</i>	Intercept	32.40 $\pm$ 1.60	18.27 $\pm$ 1.63	-4.62 $\pm$ 3.74	8.12 $\pm$ 4.69
	Slope $\pm$ 1SE	-52.17 $\pm$ 11.69	3.99 $\pm$ 0.74	1.52 $\pm$ 0.18	0.63 $\pm$ 0.16
	R <sup>2</sup>	0.15	0.20	0.38	0.12
	<i>p</i>	< 0.01	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>	<b>&lt; 0.01</b>
<i>A. canescens</i>	Intercept	19.21 $\pm$ 0.97	13.96 $\pm$ 1.05	0.39 $\pm$ 2.37	12.01 $\pm$ 2.93
	Slope $\pm$ 1SE	-25.36 $\pm$ 7.10	1.11 $\pm$ 0.48	0.78 $\pm$ 0.12	0.15 $\pm$ 0.10
	R <sup>2</sup>	0.10	0.05	0.29	0.02
	<i>p</i>	<b>&lt; 0.01</b>	<b>0.02</b>	<b>&lt; 0.01</b>	0.15
<i>R. glabra</i>	Intercept	18.35 $\pm$ 1.04	16.64 $\pm$ 1.11	3.55 $\pm$ 2.63	18.93 $\pm$ 3.04
	Slope $\pm$ 1SE	-17.12 $\pm$ 7.56	-0.18 $\pm$ 0.51	0.63 $\pm$ 0.13	-0.09 $\pm$ 0.11
	R <sup>2</sup>	0.04	0.001	0.18	0.01
	<i>p</i>	<b>0.03</b>	0.72	<b>&lt; 0.01</b>	0.38
<i>C. drummondii</i>	Intercept	21.84 $\pm$ 1.21	19.92 $\pm$ 1.30	4.66 $\pm$ 3.08	22.74 $\pm$ 3.55
	Slope $\pm$ 1SE	-20.22 $\pm$ 8.83	-0.26 $\pm$ 0.59	0.73 $\pm$ 0.15	-0.12 $\pm$ 0.12
	R <sup>2</sup>	0.04	0.002	0.17	0.01
	<i>p</i>	<b>0.02</b>	0.66	<b>&lt; 0.01</b>	0.34



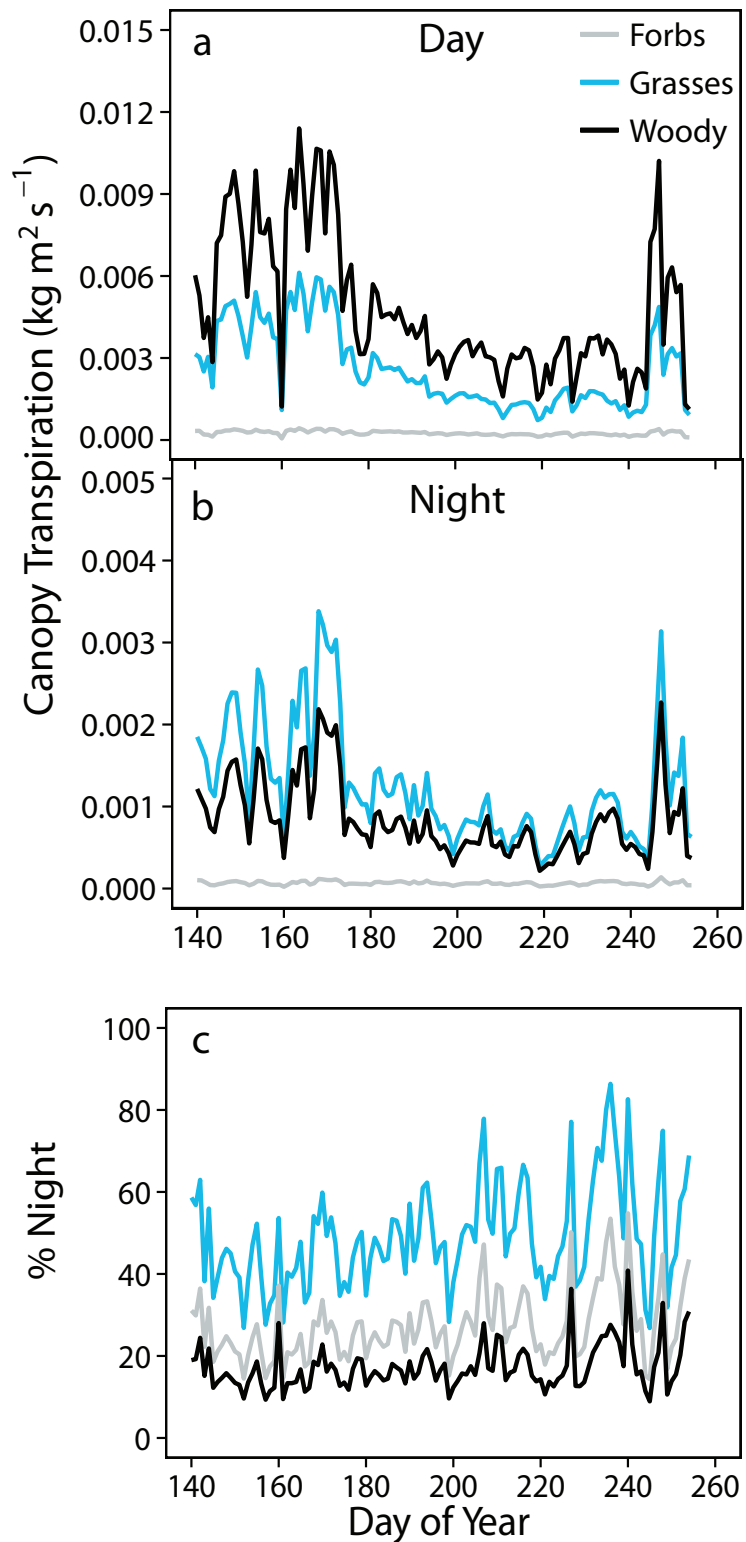
**Figure 5.1** Environmental data measured between day of year 140-254 in 2014. Shown are daily averages of soil moisture at 10 cm measured in units of water fraction by volume, wfv (a), air temperature reported at average daily maximum (15:00 h) and minimum (03:00 h) conditions (b), and vapor pressure deficit (VPD) reported at average daily maximum (15:00 h) and minimum (03:00 h) conditions (c).



**Figure 5.2 Modeled sap flow rates for each species between DOY 140-254. Shown are daily average diurnal (black) and nocturnal rates (blue).**

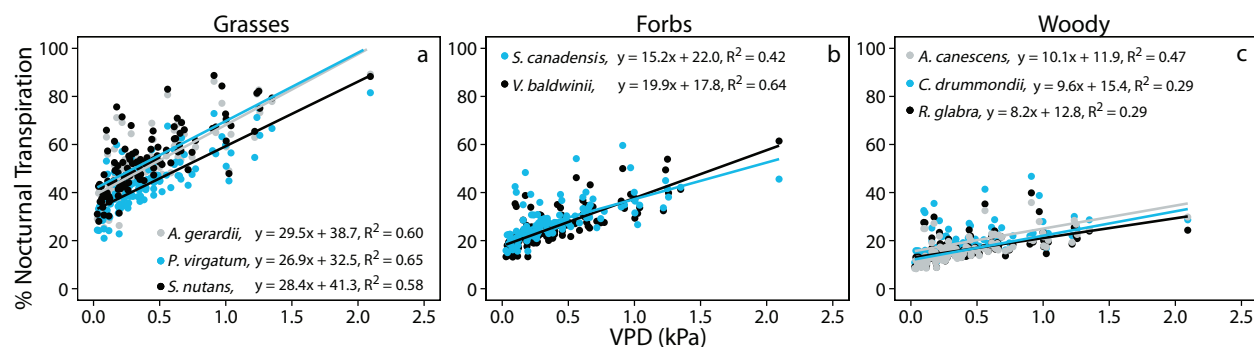


**Figure 5.3 Percent nocturnal of diurnal sap flux rates for grasses (a), forbs (b), and woody plants (c) measured across the growing season.**



**Figure 5.4 Modeled canopy transpiration, averaged across plant functional groups, between day of year 140-254. Shown are average daily diurnal rates (a), average daily nocturnal rates (b), and average daily percent nocturnal of diurnal rates (c).**





**Figure 5.5** Linear regression relationships between average daily percent nocturnal transpiration and vapor pressure deficit (VPD) reported at average daily minimum conditions (03:00 h) for grasses (a), forbs (b), and woody plants (c). For all linear regression models,  $p < 0.01$ .

## Chapter 6 - Conclusion

Water availability is a key driver of many plant and ecosystem processes in tallgrass prairies (Jones et al. 2016; Knapp et al. 2001; Schuster 2016; Zeglin et al. 2013), yet we have a limited understanding of how grassland plants utilize water through space and time. Considering that tallgrass prairies experience tremendous heterogeneity in soil resources (Nippert et al. 2011; Ransom 1998; Williams and Rice 2007), identifying spatial and temporal variation in plant ecohydrology is critical for understanding current drivers of plant responses to water and for predicting ecosystem responses to future changes in climate. To address this knowledge gap, I evaluated the patterns, drivers, ecological consequences of plant water use, (e.g., water uptake, water redistribution, and water loss), in a native tallgrass prairie located in northeastern Kansas, USA.

First, I investigated spatial and temporal variation in water uptake by four common grassland plants (*Andropogon gerardii*, *Vernonia baldwinii*, *Amorpha canescens*, and *Rhus glabra*). Specifically, in Chapter 2, I evaluated the role of fire and bison grazing on vertical source water partitioning within a tallgrass prairie community. Using stable isotope techniques, I showed that grazing increases the depth of water uptake in *A. gerardii* and *R. glabra*, reducing niche overlap with co-occurring species. These results indicate that grassland management practices can impact plant water fluxes at small spatial scales, with potential impacts on community-level dynamics.

Next, I investigated whether spatial and temporal variation exists in water redistribution in these same grassland species. In Chapter 3, I showed that hydraulic lift can occur in *V. baldwinii* and *R. glabra*, but is generally uncommon and is likely limited by nocturnal transpiration. Furthermore, the few instances of hydraulic lift in *V. baldwinii* and *R. glabra* did

not facilitate water uptake by neighboring grasses, as has been observed in other systems. I suggest that, while hydraulic lift can occur in tallgrass prairies, it is not an important ecohydrological process in this ecosystem under current climate conditions.

Finally, I evaluated daily and season variation in water loss in eight prairie grasses, forbs, and shrubs (*A. gerardii*, *Panicum virgatum*, *Sorghastrum nutans*, *Solidago canadensis*, *V. baldwinii*, *A. canescens*, *R. glabra*, and *Cornus drummondii*). In Chapter 4, I used leaf gas exchange measurements to evaluate patterns and drivers of nocturnal transpiration across two growing seasons. Nocturnal transpiration commonly occurred among these species and was greatest in grasses and early in the growing season. Interestingly, nocturnal transpiration was not driven by VPD, as commonly observed in other systems (Dawson et al. 2007; Fisher et al. 2007; Howard and Donovan 2006), but was correlated with nocturnal stomatal conductance in most species. This result suggests that nocturnal transpiration is regulated by some grassland species and may have competitive advantage in diverse plant communities. Chapter 5 builds on these results by evaluating diurnal sap flow patterns in the same eight species. Using micro external heat balance sensors and a Bayesian modeling technique (Bell et al. 2015) I show that daytime sap flow rates were variable among species and functional types, with larger flux rates among woody species. However, nighttime sap flow rates were more consistent across species, which caused nighttime sap flow and transpiration to account for a larger proportion of daily flux rates in grasses than in forbs or shrubs.

The results of this dissertation show that water uptake, redistribution, and loss are indeed spatially and temporally variable in a native tallgrass prairie. Additionally, extensive differences in water flux exist among co-occurring species and plant functional groups, which likely reflect varying strategies to tolerate water limitation. These results suggest that shifts in the abundance

of these species with future climate changes or with ecosystem state changes (e.g., woody encroachment; Rataczak et al. 2011) will likely impact ecosystem-level water balance (Logan and Brunsell 2015). I also showed that water uptake, water redistribution, and water loss are all influenced by different biotic and abiotic drivers. For example, grazing alters source water uptake but does not impact hydraulic lift. However, these water fluxes were all influenced by key environmental factors such as soil moisture, VPD, and air temperature, suggesting that they will likely be very responsive to future changes in climate. Finally, I showed that different fluxes have varying ecological consequences. While some fluxes can potentially impact community interactions (e.g., source water uptake may impact niche partitioning), others do not likely have an important contribution to ecosystem function (e.g., hydraulic lift does not likely impact facilitation). However, the strength of these consequences may also change under future climates. For example, hydraulic lift and facilitation may become increasingly important under warmer, drier conditions predicted for this region (Christensen et al. 2007). Overall, these results highlight the importance of assessing fine-scale spatiotemporal variation in plant water fluxes to better understand ecological processes in a changing climate.

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## **References**

Please see individual chapters for references.



## Appendix A - Appendix for Chapter 2

**Table A.1 Soil  $\delta^{18}\text{O}$  collected from five depths in all grazing treatments, months, and topographic locations, as well as the deep soil  $\delta^{18}\text{O}$  collected from five random lowland locations. Shown are mean  $\pm$  1SE.**

	Soil Depth (cm)	Ungrazed	Grazed
<b>July</b>			
Upland	0-5	-2.717 $\pm$ 0.488	-1.839 $\pm$ 0.393
	5-10	-4.827 $\pm$ 0.516	-4.322 $\pm$ 0.445
	10-15		-5.158 $\pm$ 0.554
	15-20		-5.288 $\pm$ 0.591
	20-25	-5.100 $\pm$ 0.000	-4.550 $\pm$ 0.250
Lowland	0-5	-3.539 $\pm$ 0.446	-2.441 $\pm$ 0.342
	5-10	-5.247 $\pm$ 0.421	-4.344 $\pm$ 0.350
	10-15	-5.782 $\pm$ 0.335	-4.975 $\pm$ 0.322
	15-20	-6.044 $\pm$ 0.251	-5.350 $\pm$ 0.270
	20-25	-6.218 $\pm$ 0.229	-5.458 $\pm$ 0.262
<b>August</b>			
Upland	0-5	-3.782 $\pm$ 0.369	-1.750 $\pm$ 0.412
	5-10	-4.800 $\pm$ 0.228	-3.788 $\pm$ 0.156
	10-15	-5.300 $\pm$ 0.100	-4.175 $\pm$ 0.325
	15-20		-3.500 $\pm$ 0.000
	20-25		
Lowland	0-5	-3.819 $\pm$ 0.230	-2.682 $\pm$ 0.195
	5-10	-4.919 $\pm$ 0.275	-4.472 $\pm$ 0.315
	10-15	-5.477 $\pm$ 0.295	-5.239 $\pm$ 0.396
	15-20	-5.669 $\pm$ 0.315	-4.940 $\pm$ 0.406
	20-25	-5.857 $\pm$ 0.312	-5.564 $\pm$ 0.392
<b>Deep Soil</b>			
Lowland	100-200	-8.080 $\pm$ 0.213	

**Table A.2 Effects of grazing, month, topography, fire, and their interactions for *A. gerardii* xylem  $\delta^{18}\text{O}$ . Shown are summary results after model averaging, including the average model estimates, standard error (SE), adjusted standard error (Adj SE), Z value, P value, 95% confidence interval, and number of models in which the factor occurs (N models).**

Parameter	Estimate	SE	Adj SE	Z value	Confidence Interval	N models
Intercept	-5.335	0.390	0.393	13.572	(-6.106, -4.565)	
Grazing	-0.721	0.167	0.171	4.223	(-1.056, -0.386)	23
Month	-0.661	0.197	0.171	3.872	(-0.996, -0.327)	23
Topography	0.209	0.172	0.173	1.206	(-0.112	21
Fire	-0.219	0.211	0.212	1.033	(-0.678, 0.058)	17
Grazing x Topography	0.551	0.417	0.419	1.313	(0.040, 1.373)	16
Month x Topography	-0.451	0.415	0.417	1.081	(-1.328, 0.004)	13
Grazing x Fire	0.063	0.196	0.197	0.318	(-0.342, 0.991)	6
Topography x Fire	-0.008	0.104	0.104	0.076	(-0.757, 0.577)	3
Month x Fire	-0.007	0.102	0.103	0.065	(-0.743, 0.591)	4
Grazing x month	-0.007	0.120	0.127	0.059	(-0.724, 0.608)	3

**Table A.3 Effects of grazing, month, topography, fire, and their interactions for *V. baldwinii* xylem  $\delta^{18}\text{O}$ . Shown are summary results after model averaging, including the average model estimates, standard error (SE), adjusted standard error (Adj SE), Z value, P value, 95% confidence interval, and number of models in which the factor occurs (N models).**

Parameter	Estimate	SE	Adj SE	Z value	Confidence Interval	N models
Intercept	-11.967	1.018	1.028	11.647	(-13.981, -9.953)	
Grazing	-0.763	0.282	0.284	2.683	(-1.310, -0.206)	33
Month	-2.363	0.282	0.284	8.313	(-2.920, -1.806)	33
Topography	0.062	0.238	0.240	0.256	(-0.466, 0.641)	25
Fire	-0.199	0.295	0.297	0.669	(-0.620, 0.366)	28
Month x Fire	0.394	0.580	0.582	0.676	(-0.208, 2.008)	15
Topography x Fire	-0.822	0.792	0.794	1.035	(-2.461, -0.247)	20
Grazing x Topography	-0.110	0.332	0.334	0.328	(-1.651, 0.556)	9
Grazing x month	0.110	0.336	0.338	0.317	(-0.671, 1.554)	11
Grazing x Fire	0.080	0.294	0.295	0.269	(-0.679, 1.542)	9
Month x Topography	0.040	0.220	0.222	0.178	(-0.771, 1.432)	6

**Table A.4 Effects of grazing, month, topography, fire, and their interactions for *A. canescens* xylem  $\delta^{18}\text{O}$ . Shown are summary results after model averaging, including the average model estimates, standard error (SE), adjusted standard error (Adj SE), Z value, P value, 95% confidence interval, and number of models in which the factor occurs (N models).**

Parameter	Estimate	SE	Adj SE	Z value	Confidence Interval	N models
Intercept	-10.377	0.495	0.499	20.758	(-11.357, -9.397)	
Grazing	-0.537	0.299	0.302	1.778	(-1.128, 0.055)	16
Month	-2.502	0.299	0.302	8.291	(-3.093, -1.910)	16
Topography	0.205	0.379	0.382	0.537	(-0.551, 1.041)	13
Fire	1.142	0.329	0.333	3.435	(0.491, 1.794)	16
Grazing x month	1.694	0.598	0.604	2.806	(0.511, 2.887)	16
Grazing x Topography	1.206	0.797	0.801	1.506	(0.319, 2.674)	12
Grazing x Fire	0.373	0.552	0.556	0.672	(-0.599, 1.751)	9
Month x Fire	0.798	0.662	0.666	1.198	(-0.194, 2.167)	12
Grazing x Month x Fire	-1.592	1.659	1.663	0.957	(-5.167, -0.481)	7
Month x Topography	0.073	0.309	0.311	0.235	(-0.835, 1.504)	5
Topography x Fire	-0.047	0.256	0.258	0.181	(-1.472, 0.863)	3
Month x Topography x Fire	-0.108	0.548	0.549	0.197	(-4.591, 0.005)	1
Grazing x Month x Topography	-0.033	0.279	0.280	0.119	(-3.364, 1.277)	1

**Table A.5 Effects of grazing, month, topography, fire, and their interactions for *R. glabra* xylem  $\delta^{18}\text{O}$ . Shown are summary results after model averaging, including the average model estimates, standard error (SE), adjusted standard error (Adj SE), Z value, P value, 95% confidence interval, and number of models in which the factor occurs (N models).**

Parameter	Estimate	SE	Adj SE	Z value	Confidence Interval	N models
Intercept	-8.108	0.310	0.314	25.858	(-8.723, -7.494)	
Grazing	-0.613	0.238	0.241	2.547	(-1.084, -0.141)	22
Month	0.498	0.242	0.244	2.042	(0.047, 0.971)	21
Topography	-0.209	0.240	0.241	0.867	(-0.762, 0.172)	15
Grazing x month	-0.711	0.582	0.585	1.216	(-1.890, -0.011)	15
Month x Topography	-0.352	0.507	0.509	0.691	(-1.742, 0.126)	9
Grazing x Topography	-0.209	0.408	0.410	0.511	(-1.602, 0.288)	7
Fire	-0.030	0.143	0.144	0.210	(-0.639, 0.404)	10
Grazing x Month x Topography	-0.028	0.232	0.234	0.120	(-2.609, 1.134)	1
Month x Fire	-0.018	0.129	0.130	0.140	(-1.381, 0.470)	2
Grazing x Fire	-0.013	0.111	0.111	0.115	(-1.308, 0.557)	2

**Table A.6 Xylem  $\delta^{18}\text{O}$  collected from each species in all grazing treatments, months, and topographic locations. Shown are mean  $\pm$  1SE.**

		July		August	
		Grazed	Ungrazed	Grazed	Ungrazed
<i>A. gerardii</i>					
Upland		-5.456 $\pm$ 0.458	-6.006 $\pm$ 0.481	-4.611 $\pm$ 0.164	-4.839 $\pm$ 0.212
Lowland		-5.111 $\pm$ 0.297	-6.100 $\pm$ 0.208	-4.781 $\pm$ 0.198	-5.856 $\pm$ 0.202
<i>V. baldwinii</i>					
Upland		-12.561 $\pm$ 0.583	-13.483 $\pm$ 0.533	-10.250 $\pm$ 0.722	-11.400 $\pm$ 0.642
Lowland		-13.194 $\pm$ 0.633	-13.356 $\pm$ 0.583	-10.339 $\pm$ 0.471	-11.156 $\pm$ 0.594
<i>A. canescens</i>					
Upland		-11.828 $\pm$ 0.616	-11.033 $\pm$ 0.592	-8.922 $\pm$ 0.406	-9.289 $\pm$ 0.356
Lowland		-11.761 $\pm$ 0.500	-12.100 $\pm$ 0.359	-8.006 $\pm$ 0.506	-10.389 $\pm$ 0.455
<i>R. glabra</i>					
Upland		-7.253 $\pm$ 0.477	-8.861 $\pm$ 0.485	-8.161 $\pm$ 0.204	-8.422 $\pm$ 0.251
Lowland		-7.178 $\pm$ 0.455	-7.722 $\pm$ 0.370	-8.400 $\pm$ 0.293	-8.406 $\pm$ 0.183

**Table A.7 Xylem  $\delta^{18}\text{O}$  collected from each species in all time since fire (TSF) contrasts. Shown are mean  $\pm$  1SE.**

	TSF	Xylem $\delta^{18}\text{O}$
<i>A. gerardii</i>	0	-4.765 $\pm$ 0.205
	1	-6.150 $\pm$ 0.167
	2	-4.911 $\pm$ 0.161
	3	-6.521 $\pm$ 0.200
<i>V. baldwinii</i>	0	-10.731 $\pm$ 0.336
	1	-14.146 $\pm$ 0.453
	2	-10.952 $\pm$ 0.364
	3	-14.292 $\pm$ 0.367
<i>A. canescens</i>	0	-10.925 $\pm$ 0.458
	1	-10.975 $\pm$ 0.383
	2	-9.211 $\pm$ 0.276
	3	-11.008 $\pm$ 0.277
<i>R. glabra</i>	0	-7.786 $\pm$ 0.251
	1	-8.771 $\pm$ 0.275
	2	-7.681 $\pm$ 0.211
	3	-8.929 $\pm$ 0.224

**Table A.8 SIAR estimates of the proportion of shallow and deep water used by each species within each month, topography, and time since fire (TSF) contrast. Shown are mean estimates for shallow and deep sources, with the 95% credible interval in parentheses.**

<i>A. gerardii</i>	TSF	July		August	
		Surface	Deep	Surface	Deep
Upland	0	0.472 (0.325, 0.613)	0.528 (0.387, 0.675)	0.808 (0.476, 0.683)	0.192 (0.000, 0.297)
	1	0.689 (0.278, 1.027)	0.311 (0.000, 0.722)	0.809 (0.663, 0.959)	0.191 (0.041, 0.337)
	2	0.578 (0.390, 0.779)	0.422 (0.221, 0.61)	0.817 (0.689, 0.960)	0.183 (0.040, 0.311)
	3	0.311 (0.037, 0.548)	0.689 (0.452, 0.963)	0.513 (0.356, 0.679)	0.487 (0.321, 0.644)
Lowland	0	0.605 (0.480, 0.724)	0.395 (0.276, 0.520)	0.601 (0.347, 0.805)	0.399 (0.196, 0.653)
	1	0.842 (0.647, 1.009)	0.158 (0.000, 0.353)	0.570 (0.402, 0.755)	0.430 (0.245, 0.598)
	2	0.559 (0.446, 0.664)	0.441 (0.336, 0.554)	0.744 (0.535, 0.917)	0.256 (0.083, 0.465)
	3	0.651 (0.389, 0.965)	0.349 (0.035, 0.611)	0.609 (0.213, 0.999)	0.391 (0.000, 0.787)
<i>V. baldwinii</i>					
Upland	0	0.033 (0.000, 0.099)	0.967 (0.901, 1.005)	0.037 (0.000, 0.107)	0.963 (0.893, 1.005)
	1	0.466 (0.012, 0.900)	0.534 (0.100, 0.988)	0.292 (0.000, 0.681)	0.708 (0.319, 1.027)
	2	0.050 (0.000, 0.154)	0.950 (0.846, 1.007)	0.029 (0.000, 0.084)	0.971 (0.916, 1.004)
	3	0.384 (0.000, 0.804)	0.616 (0.196, 1.016)	0.297 (0.000, 0.688)	0.703 (0.312, 1.026)
Lowland	0	0.041 (0.000, 0.126)	0.959 (0.874, 1.006)	0.043 (0.000, 0.126)	0.957 (0.874, 1.006)
	1	0.394 (0.000, 0.818)	0.606 (0.183, 1.013)	0.317 (0.000, 0.720)	0.683 (0.280, 1.026)
	2	0.040 (0.000, 0.120)	0.960 (0.881, 1.006)	0.035 (0.000, 0.105)	0.965 (0.895, 1.005)
	3	0.428 (0.000, 0.850)	0.572 (0.150, 1.002)	0.330 (0.000, 0.737)	0.670 (0.263, 1.022)
<i>A. canescens</i>					
Upland	0	0.069 (0.000, 0.211)	0.931 (0.789, 1.010)	0.060 (0.000, 0.185)	0.940 (0.815, 1.009)
	1	0.456 (0.009, 0.891)	0.544 (0.109, 0.991)	0.139 (0.000, 0.445)	0.861 (0.555, 1.022)
	2	0.039 (0.000, 0.118)	0.961 (0.882, 1.006)	0.085 (0.000, 0.199)	0.916 (0.801, 1.006)
	3	0.345 (0.000, 0.756)	0.655 (0.244, 1.023)	0.174 (0.000, 0.513)	0.826 (0.487, 1.025)
Lowland	0	0.055 (0.000, 0.166)	0.945 (0.834, 1.008)	0.111 (0.000, 0.326)	0.889 (0.674, 1.016)
	1	0.350 (0.000, 0.760)	0.650 (0.240, 1.020)	0.217 (0.000, 0.574)	0.783 (0.426, 1.028)
	2	0.073 (0.000, 0.227)	0.927 (0.773, 1.011)	0.055 (0.000, 0.154)	0.945 (0.426, 1.007)
	3	0.405 (0.000, 0.825)	0.595 (0.175, 1.010)	0.301 (0.000, 0.693)	0.699 (0.307, 1.026)
<i>R. glabra</i>					
Upland	0	0.064 (0.000, 0.166)	0.936 (0.834, 1.007)	0.040 (0.000, 0.098)	0.960 (0.902, 1.003)
	1	0.392 (0.000, 0.817)	0.608 (0.183, 1.015)	0.202 (0.000, 0.502)	0.798 (0.498, 1.018)
	2	0.158 (0.011, 0.299)	0.842 (0.701, 0.989)	0.029 (0.000, 0.085)	0.971 (0.915, 1.004)
	3	0.216 (0.000, 0.577)	0.784 (0.423, 1.029)	0.071 (0.000, 0.232)	0.929 (0.768, 1.011)
Lowland	0	0.201 (0.084, 0.320)	0.799 (0.680, 0.916)	0.032 (0.000, 0.091)	0.968 (0.910, 1.004)



1	0.234 (0.000, 0.620)	0.766 (0.380, 1.030)	0.286 (0.000, 0.711)	0.714 (0.289, 1.032)
2	0.122 (0.003, 0.229)	0.878 (0.771, 0.997)	0.043 (0.000, 0.116)	0.957 (0.884, 1.005)
3	0.394 (0.000, 0.811)	0.606 (0.190, 1.011)	0.114 (0.000, 0.370)	0.886 (0.631, 1.017)

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**Table A.9 Effects of grazing, month, topography, fire, and their interactions for *A. gerardii*  $\Psi_{md}$ . Shown are summary results after model averaging, including the average model estimates, standard error (SE), adjusted standard error (Adj SE), Z value, P value, 95% confidence interval, the number of models in which the factor occurs (N models), and the relative importance (Importance).**

Parameter	Estimate	SE	Adj SE	Z value	Confidence Interval	N models	Importance
Intercept	-1.590	0.169	0.171	9.295	(-1.925, -1.254)		
Grazing	0.063	0.076	0.077	0.828	(-0.072, 0.233)	20	0.79
Month	0.671	0.077	0.078	8.587	(0.518, 0.824)	25	1
Topography	-0.296	0.077	0.078	3.784	(-0.449, -0.143)	25	1
Fire	0.211	0.085	0.086	2.447	(0.042, 0.380)	25	1
Grazing x month	0.220	0.199	0.200	1.103	(0.023, 0.632)	16	0.67
Month x Fire	-0.231	0.189	0.189	1.220	(-0.613, -0.0004)	17	0.75
Grazing x Fire	0.032	0.094	0.094	0.337	(-0.451, 0.159)	7	0.22
Topography x Fire	0.033	0.096	0.096	0.340	(-0.172, 0.441)	8	0.24
Grazing x Topography	-0.011	0.061	0.061	0.175	(-0.393, 0.215)	4	0.12
Month x Topography	0.012	0.066	0.066	0.179	(-0.225, 0.386)	5	0.15
Grazing x Month x Fire	0.001	0.044	0.044	0.012	(-0.581, 0.635)	1	0.02

**Table A.10 Effects of grazing, month, topography, fire, and their interactions for *V. baldwinii*  $\Psi_{md}$ . Shown are summary results after model averaging, including the average model estimates, standard error (SE), adjusted standard error (Adj SE), Z value, P value, 95% confidence interval, the number of models in which the factor occurs (N models), and the relative importance (Importance).**

Parameter	Estimate	SE	Adj SE	Z value	Confidence Interval	N models	Importance
Intercept	-1.126	0.329	0.332	3.394	(-1.776, -0.476)		
Grazing	0.092	0.052	0.053	1.751	(-0.011, 0.196)	13	1
Month	0.359	0.052	0.053	6.807	(0.256, 0.462)	13	1
Topography	0.057	0.054	0.055	1.037	(-0.033, 0.173)	11	0.81
Grazing x month	0.268	0.105	0.106	2.541	(0.061, 0.475)	13	1
Month x Topography	-0.125	0.128	0.129	0.971	(-0.410, 0.001)	8	0.61
Fire	0.023	0.046	0.046	0.491	(-0.056, 0.172)	7	0.39
Grazing x Topography	0.022	0.065	0.065	0.344	(-0.110, 0.302)	4	0.23
Month x Fire	0.003	0.025	0.025	0.110	(-0.139, 0.272)	1	0.04
Topography x Fire	-0.0004	0.019	0.019	0.019	(-0.217, 0.195)	1	0.03
Grazing x Fire	0.00003	0.019	0.019	0.001	(-0.205, 0.207)	1	0.03
Grazing x Month x Fire	0.002	0.040	0.040	0.057	(-0.341, 0.480)	1	0.03

**Table A.11 Effects of grazing, month, topography, fire, and their interactions for *A. canescens*  $\Psi_{md}$ . Shown are summary results after model averaging, including the average model estimates, standard error (SE), adjusted standard error (Adj SE), Z value, P value, 95% confidence interval, the number of models in which the factor occurs (N models), and the relative importance (Importance).**

Parameter	Estimate	SE	Adj SE	Z value	Confidence Interval	N models	Importance
Intercept	-1.535	0.215	0.217	7.075	(-1.960, -1.109)		
Month	0.715	0.099	0.100	7.180	(0.520, 0.910)	21	1
Topography	-0.023	0.075	0.076	0.301	(-0.236, 0.152)	14	0.54
Month x Topography	-0.154	0.223	0.223	0.689	(-0.761, 0.013)	10	0.41
Grazing	0.038	0.078	0.078	0.485	(-0.092, 0.298)	10	0.37
Fire	-0.054	0.093	0.095	0.574	(-0.327, 0.102)	13	0.48
Month x Fire	-0.036	0.115	0.115	0.346	(-0.617, 0.162)	5	0.16
Topography x Fire	-0.024	0.095	0.095	0.252	(-0.623, 0.151)	4	0.10
Grazing x month	0.001	0.033	0.034	0.024	(-0.363, 0.422)	1	0.03
Grazing x Topography	0.002	0.033	0.033	0.073	(-0.277, 0.497)	1	0.02

**Table A.12 Effects of grazing, month, topography, fire, and their interactions for *R. glabra*  $\Psi_{md}$ . Shown are summary results after model averaging, including the average model estimates, standard error (SE), adjusted standard error (Adj SE), Z value, P value, 95% confidence interval, the number of models in which the factor occurs (N models), and the relative importance (Importance).**

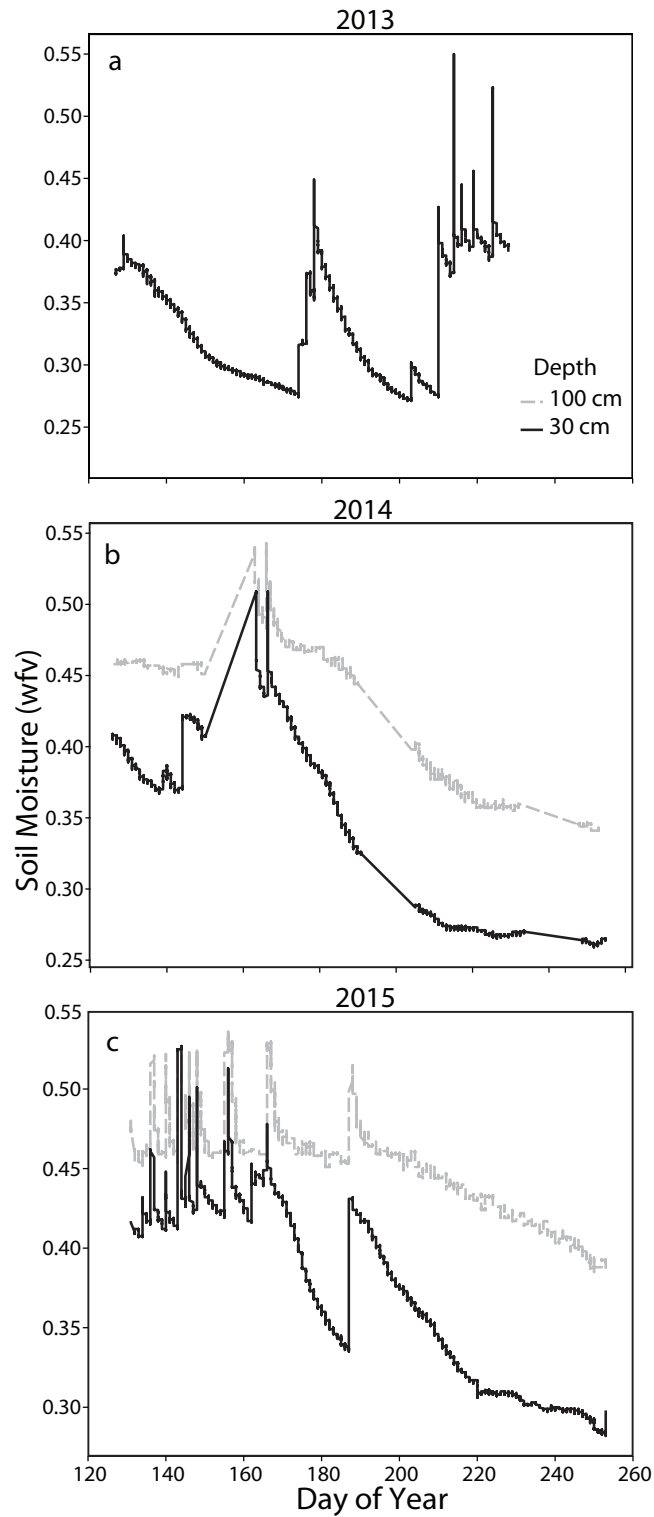
Parameter	Estimate	SE	Adj SE	Z value	Confidence Interval	N models	Importance
Intercept	-1.216	0.357	0.361	3.372	(-1.924, -0.509)		
Grazing	0.188	0.057	0.058	3.245	(0.074, 0.301)	28	1
Month	0.252	0.055	0.056	4.497	(0.142, 0.362)	28	1
Topography	-0.054	0.059	0.059	0.909	(-0.187, 0.038)	22	0.72
Grazing x month	0.377	0.115	0.116	3.254	(0.150, 0.604)	28	1
Month x Topography	-0.096	0.129	0.129	0.743	(-0.430, 0.018)	13	0.47
Fire	-0.048	0.062	0.062	0.770	(-0.194, 0.057)	23	0.70
Month x Fire	0.084	0.121	0.121	0.697	(-0.026, 0.412)	14	0.44
Grazing x Fire	-0.035	0.083	0.083	0.422	(-0.360, 0.085)	10	0.25
Grazing x Topography	0.015	0.058	0.059	0.257	(-0.139, 0.314)	7	0.16
Topography x Fire	-0.004	0.033	0.033	0.113	(-0.276, 0.169)	4	0.07
Grazing x Month x Fire	-0.004	0.044	0.044	0.085	(-0.569, 0.311)	2	0.03

**Table A.13 Effects of grazing, month, topography, fire, and their interactions for soil RWC. Shown are summary results after model averaging, including the average model estimates, standard error (SE), adjusted standard error (Adj SE), Z value, P value, 95% confidence interval, the number of models in which the factor occurs (N models), and the relative importance (Importance).**

Parameter	Estimate	SE	Adj SE	Z value	Confidence Interval	N models	Importance
Intercept	0.156	0.019	0.019	8.383	(0.120, 0.193)		
Grazing	-0.011	0.006	0.006	1.706	(-0.023, 0.003)	13	1
Month	0.072	0.006	0.006	11.242	(0.059, 0.084)	13	1
Topography	-0.067	0.006	0.006	10.503	(-0.079, -0.055)	13	1
Fire	0.0002	0.007	0.007	0.028	(-0.014, 0.014)	13	1
Grazing x Topography	-0.049	0.013	0.013	3.828	(-0.074, -0.024)	13	1
Grazing x Fire	-0.024	0.015	0.015	1.541	(-0.053, -0.003)	10	0.85
Month x Topography	-0.031	0.013	0.013	2.451	(-0.056, -0.006)	13	1
Month x Fire	0.042	0.013	0.013	3.314	(0.017, 0.067)	13	1
Topography x Fire	-0.011	0.013	0.013	0.857	(-0.039, 0.011)	10	0.77
Month x Topography x Fire	-0.038	0.033	0.034	1.121	(-0.106, -0.006)	8	0.67
Grazing x month	-0.007	0.011	0.011	0.580	(-0.040, 0.009)	7	0.42
Grazing x Topography x Fire	0.003	0.013	0.013	0.270	(-0.026, 0.073)	2	0.15
Grazing x Month x Fire	0.001	0.007	0.007	0.133	(-0.030, 0.068)	1	0.05
Grazing x Month x Topography	-0.0002	0.005	0.005	0.046	(-0.056, 0.043)	1	0.04

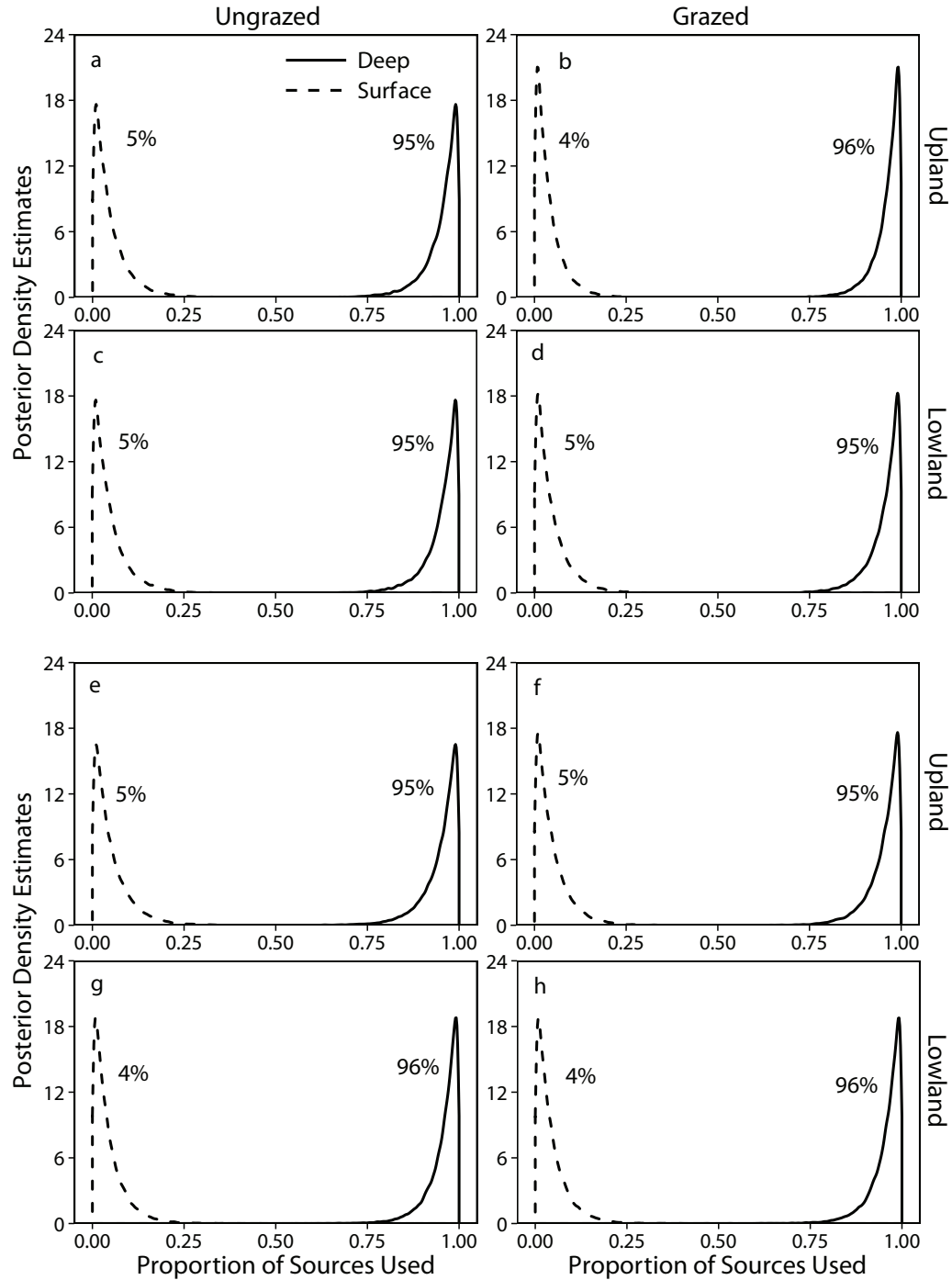
**Table A.14 Soil RWC measure in watersheds of each burn history during July and August. Shown are mean  $\pm$  1SE.**

<b>Time since fire (years)</b>	<b>July</b>	<b>August</b>
0	0.188 $\pm$ 0.009	0.146 $\pm$ 0.014
1	0.186 $\pm$ 0.025	0.083 $\pm$ 0.008
2	0.205 $\pm$ 0.011	0.146 $\pm$ 0.014
3	0.181 $\pm$ 0.019	0.056 $\pm$ 0.009

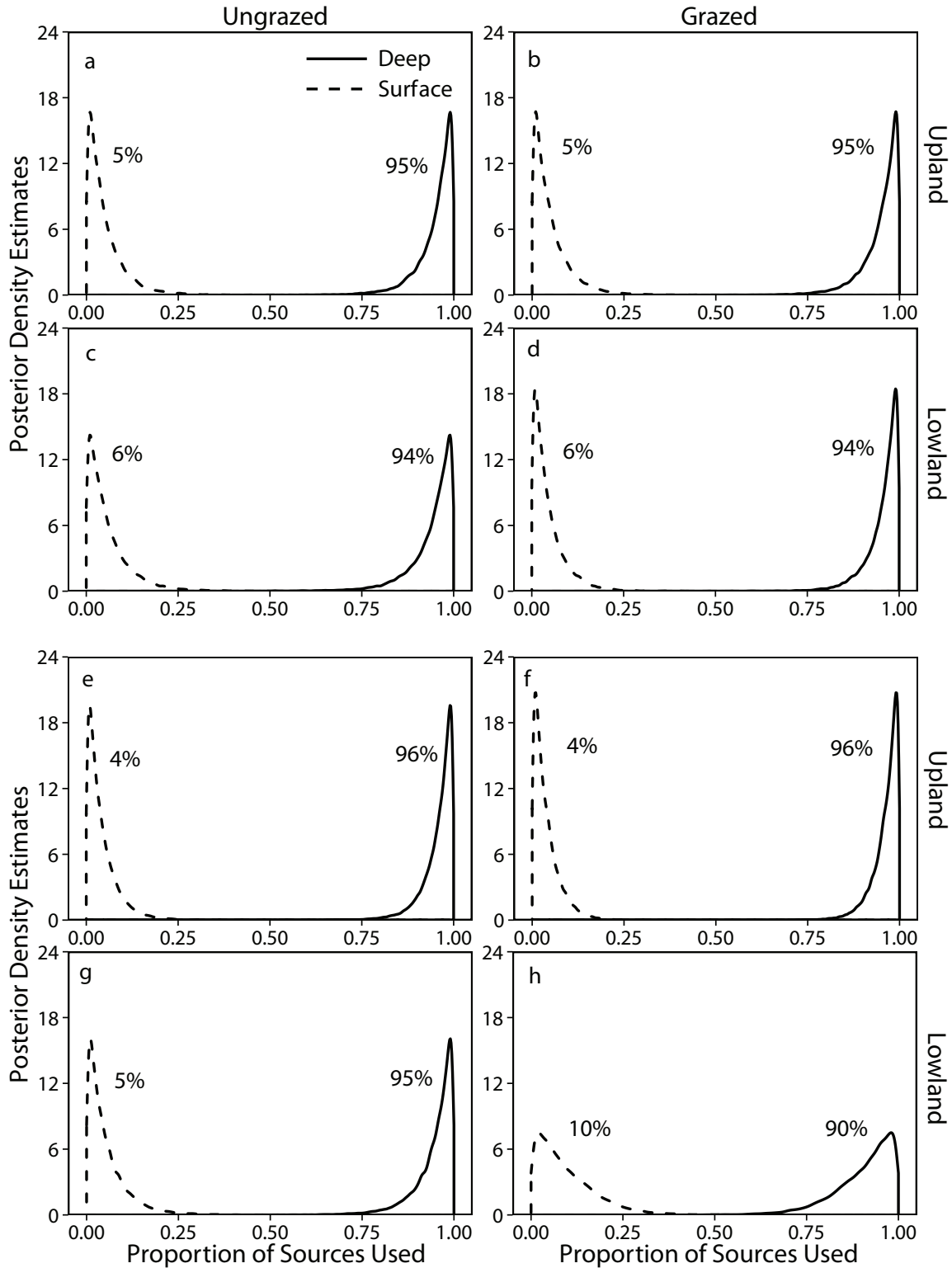


**Figure A.1 Deep soil moisture in units of water fraction by volume (wfv) measured at 30 cm and 100 cm depth for (a) 2013, (b) 2014, and (c) 2015.**





**Figure A.2** Posterior density estimates for the proportion of surface and deep water sources used by *V. baldwinii* in ungrazed (a,c,e,g) and grazed (b,d,f,h) watersheds, upland (a,b,e,f) and lowland (c,d,g,h) topographic positions, and during July (a-d) and August (e-h).



**Figure A.3** Posterior density estimates for the proportion of surface and deep water sources used by *A. canescens* in ungrazed (a,c,e,g) and grazed (b,d,f,h) watersheds, upland (a,b,e,f) and lowland (c,d,g,h) topographic positions, and during July (a-d) and August (e-h).

## Appendix B - Appendix for Chapter 3

**Table B.1 Statistical results of soil moisture analyses. Shown are F and P values for relative water content (RWC) measured in the top 10 cm soil and for gravimetric water content measured at 5 cm increments in 25 cm deep soil cores. Factors within the RWC analysis include year (Y), month (M), grazing treatment (G), sampling time of day (T), species (S), and their interactions. Factors within the GWC analysis include month (M), grazing treatment (G), sampling time of day (T), species (S), depth within soil core (D), and their interactions. Significance at the  $\alpha=0.05$  level is indicated with an asterisk.**

Source	RWC		GWC	
	F	P	F	P
Y	3.89	0.05		
M	43.61	<0.01*	18.80	<0.01*
G	3.34	0.14	0.24	0.64
T	3.38	0.07	1.72	0.19
S	1.44	0.24	1.40	0.24
D			0.94	0.33
Y x M	2.95	0.09		
Y x G	3.83	0.05		
M x G	2.27	0.13	8.49	<0.01*
Y x T	0.55	0.46		
M x T	0.21	0.64	0.09	0.77
G x T	0.26	0.61	0.05	0.82
Y x S	1.74	0.16		
M x S	0.93	0.43	0.16	0.92
G x S	0.17	0.92	3.54	0.02*
T x S	0.83	0.48	0.15	0.93
M x D			0.01	0.92
G x D			0.001	0.98
T x D			0.99	0.32
S x D			0.38	0.77
Y x M x G	11.31	<0.01*		
Y x M x T	1.06	0.31		

Y x G x T	0.06	0.81		
M x G x T	3.09	0.08	4.05	0.05
Y x M x S	1.18	0.32		
Y x G x S	1.32	0.27		
M x G x S	0.45	0.72	1.62	0.18
Y x T x S	0.21	0.89		
M x T x S	0.14	0.94	0.72	0.54
G x T x S	0.27	0.85	0.66	0.58
M x G x D			0.06	0.81
M x T x D			0.05	0.82
G x T x D			0.02	0.90
M x S x D			0.03	0.99
G x S x D			1.77	0.15
T x S x D			0.13	0.94
Y x M x G x T	0.21	0.65		
Y x M x G x S	3.36	0.02*		
Y x M x T x S	1.38	0.25		
Y x G x T x S	0.88	0.45		
M x G x T x S	0.14	0.93	2.35	0.07
M x G x T x D			0.68	0.41
M x G x S x D			0.66	0.58
M x T x S x D			0.63	0.60
G x T x S x D			0.98	0.40
Y x M x G x T	1.09	0.36		
x S				
M x G x T x S			2.00	0.11
x D				

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**Table B.2 Soil relative water content (wfv) measured in the first 10cm soil adjacent to each species in all sampling locations and time points. Shown are mean  $\pm$  1 SEM.**

		2013		2014	
		July	August	July	August
Ungrazed					
<i>A. gerardii</i>	pre-HL	0.15 $\pm$ 0.01	0.15 $\pm$ 0.02	0.14 $\pm$ 0.004	0.10 $\pm$ 0.02
	post-HL	0.13 $\pm$ 0.01	0.13 $\pm$ 0.03	0.15 $\pm$ 0.002	0.08 $\pm$ 0.03
<i>V. baldwinii</i>	pre-HL	0.14 $\pm$ 0.02	0.11 $\pm$ 0.02	0.14 $\pm$ 0.02	0.11 $\pm$ 0.05
	post-HL	0.17 $\pm$ 0.02	0.11 $\pm$ 0.02	0.12 $\pm$ 0.01	0.10 $\pm$ 0.02
<i>A. canescens</i>	pre-HL	0.12 $\pm$ 0.01	0.13 $\pm$ 0.04	0.15 $\pm$ 0.02	0.11 $\pm$ 0.02
	post-HL	0.11 $\pm$ 0.02	0.12 $\pm$ 0.03	0.16 $\pm$ 0.01	0.05 $\pm$ 0.02
<i>R. glabra</i>	pre-HL	0.14 $\pm$ 0.01	0.13 $\pm$ 0.03	0.14 $\pm$ 0.01	0.08 $\pm$ 0.03
	post-HL	0.15 $\pm$ 0.01	0.12 $\pm$ 0.03	0.15 $\pm$ 0.02	0.09 $\pm$ 0.01
Grazed					
<i>A. gerardii</i>	pre-HL	0.16 $\pm$ 0.03	0.15 $\pm$ 0.01	0.14 $\pm$ 0.01	0.15 $\pm$ 0.01
	post-HL	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01	0.14 $\pm$ 0.02	0.12 $\pm$ 0.01
<i>V. baldwinii</i>	pre-HL	0.17 $\pm$ 0.02	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01	0.12 $\pm$ 0.01
	post-HL	0.17 $\pm$ 0.02	0.13 $\pm$ 0.01	0.14 $\pm$ 0.02	0.13 $\pm$ 0.01
<i>A. canescens</i>	pre-HL	0.17 $\pm$ 0.03	0.10 $\pm$ 0.02	0.15 $\pm$ 0.01	0.14 $\pm$ 0.02
	post-HL	0.14 $\pm$ 0.004	0.10 $\pm$ 0.01	0.13 $\pm$ 0.004	0.13 $\pm$ 0.02
<i>R. glabra</i>	pre-HL	0.17 $\pm$ 0.02	0.10 $\pm$ 0.002	0.17 $\pm$ 0.03	0.16 $\pm$ 0.01
	post-HL	0.14 $\pm$ 0.02	0.14 $\pm$ 0.01	0.17 $\pm$ 0.02	0.13 $\pm$ 0.01

**Table B.3 Soil gravimetric water content measured for each soil core depth collected adjacent to all species in every sampling location and sampling time point. Shown are mean  $\pm$  1 SEM.**

		July			August	
		Depth	Ungrazed	Grazed	Ungrazed	Grazed
<i>A. gerardii</i>	pre-HL	5	0.26 ± 0.01	0.22 ± 0.03	0.19 ± 0.01	0.16 ± 0.01
		10	0.21 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.18 ± 0.02
		15	0.26 ± 0.02	0.20 ± 0.03	0.19 ± 0.01	0.17 ± 0.02
		20	0.25 ± 0.02	0.22 ± 0.02	0.18 ± 0.01	0.16 ± 0.01
		25	0.21 ± 0.02	0.21 ± 0.02	0.19 ± 0.02	0.15 ± 0.00
	post-HL	5	0.22 ± 0.02	0.21 ± 0.04	0.19 ± 0.06	0.16 ± 0.02
		10	0.23 ± 0.01	0.21 ± 0.03	0.19 ± 0.05	0.19 ± 0.03
		15	0.24 ± 0.03	0.19 ± 0.02	0.17 ± 0.04	0.17 ± 0.02
		20	0.26 ± 0.05	0.21 ± 0.01	0.17 ± 0.04	0.17 ± 0.02
		25	0.23 ± 0.03		0.17 ± 0.04	0.15 ± 0.00
<i>V. baldwinii</i>	pre-HL	5	0.22 ± 0.04	0.19 ± 0.3	0.16 ± 0.01	0.15 ± 0.02
		10	0.30 ± 0.10	0.20 ± 0.01	0.18 ± 0.01	0.18 ± 0.02
		15	0.20 ± 0.02	0.21 ± 0.02	0.15 ± 0.01	0.17 ± 0.02
		20	0.19 ± 0.04	0.23 ± 0.01	0.15 ± 0.01	0.18 ± 0.03
		25		0.20 ± 0.00	0.14 ± 0.004	0.18 ± 0.00
	post-HL	5	0.23 ± 0.02	0.17 ± 0.02	0.18 ± 0.01	0.18 ± 0.02
		10	0.22 ± 0.02	0.17 ± 0.03	0.16 ± 0.01	0.20 ± 0.02
		15	0.21 ± 0.001	0.17 ± 0.02	0.16 ± 0.01	0.20 ± 0.02
		20	0.20 ± 0.01	0.20 ± 0.03	0.16 ± 0.01	0.20 ± 0.03
		25	0.21 ± 0.03	0.19 ± 0.00	0.17 ± 0.003	0.21 ± 0.01
<i>A. canescens</i>	pre-HL	5	0.23 ± 0.02	0.19 ± 0.02	0.19 ± 0.03	0.18 ± 0.03
		10	0.21 ± 0.02	0.20 ± 0.01	0.19 ± 0.03	0.21 ± 0.01
		15	0.21 ± 0.02	0.21 ± 0.01	0.18 ± 0.01	0.19 ± 0.003
		20	0.24 ± 0.04	0.19 ± 0.03	0.18 ± 0.01	0.19 ± 0.001
		25	0.19 ± 0.02		0.18 ± 0.02	0.19 ± 0.00
	post-HL	5	0.25 ± 0.01	0.16 ± 0.02	0.15 ± 0.03	0.19 ± 0.01

<i>R. glabra</i>	pre-HL	10	$0.25 \pm 0.01$	$0.17 \pm 0.03$	$0.15 \pm 0.02$	$0.20 \pm 0.02$
		15	$0.24 \pm 0.02$	$0.18 \pm 0.03$	$0.14 \pm 0.01$	$0.18 \pm 0.02$
		20	$0.23 \pm 0.02$	$0.19 \pm 0.01$	$0.16 \pm 0.02$	$0.19 \pm 0.02$
		25	$0.22 \pm 0.00$	$0.17 \pm 0.00$	$0.21 \pm 0.03$	$0.16 \pm 0.02$
		5	$0.22 \pm 0.02$	$0.26 \pm 0.03$	$0.18 \pm 0.04$	$0.19 \pm 0.01$
		10	$0.22 \pm 0.01$	$0.22 \pm 0.01$	$0.18 \pm 0.03$	$0.23 \pm 0.01$
		15	$0.22 \pm 0.02$	$0.21 \pm 0.01$	$0.17 \pm 0.02$	$0.20 \pm 0.01$
		20	$0.23 \pm 0.01$	$0.20 \pm 0.02$	$0.18 \pm 0.02$	$0.19 \pm 0.002$
	post-HL	25	$0.23 \pm 0.02$	$0.19 \pm 0.00$	$0.17 \pm 0.02$	$0.17 \pm 0.00$
		5	$0.23 \pm 0.01$	$0.22 \pm 0.02$	$0.16 \pm 0.03$	$0.21 \pm 0.01$
		10	$0.23 \pm 0.01$	$0.20 \pm 0.02$	$0.16 \pm 0.02$	$0.21 \pm 0.01$
		15	$0.22 \pm 0.01$	$0.21 \pm 0.01$	$0.16 \pm 0.02$	$0.21 \pm 0.003$
		20	$0.22 \pm 0.01$	$0.21 \pm 0.01$	$0.16 \pm 0.02$	$0.19 \pm 0.003$
		25	$0.22 \pm 0.02$		$0.14 \pm 0.01$	$0.19 \pm 0.00$

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**Table B.4 Statistical results of soil water  $\delta^{18}\text{O}$  analyses. Shown are F and P values for the  $\delta^{18}\text{O}$  signature of soil water measured at 5 cm increments in 25 cm deep soil cores. Factors within the analysis include year (Y), month (M), grazing treatment (G), sampling time of day (T), species (S), depth within soil core (D), and their interactions. Significance at the  $\alpha=0.05$  level is indicated with an asterisk.**

Source	F	P
Y	720.95	<0.01*
M	0.02	0.88
G	11.40	0.03*
T	7.21	0.01*
S	4.45	<0.01*
D	582.20	<0.01*
Y x M	482.73	<0.01*
Y x G	0.01	0.92
M x G	20.23	<0.01*
Y x T	0.48	0.49
M x T	0.54	0.46
G x T	4.48	0.03*
Y x S	2.52	0.06
M x S	0.05	0.98
G x S	8.17	<0.01*
T x S	1.24	0.29
Y x D	10.84	<0.01*
M x D	1.86	0.14
G x D	28.63	<0.01*
T x D	0.22	0.88
S x D	0.45	0.91
Y x M x G	1.97	0.16
Y x M x T	0.03	0.87
Y x G x T	1.35	0.25
M x G x T	0.34	0.56
Y x M x S	2.26	0.08



Y x G x S	3.02	0.03*
M x G x S	0.36	0.78
Y x T x S	0.28	0.84
M x T x S	1.15	0.33
G x T x S	2.08	0.10
Y x M x D	23.89	<0.01*
Y x G x D	0.91	0.44
M x G x D	0.32	0.81
Y x T x D	0.45	0.72
M x T x D	0.04	0.99
G x T x D	0.21	0.89
Y x S x D	0.62	0.78
M x S x D	0.47	0.90
G x S x D	0.66	0.75
T x S x D	0.29	0.98
Y x M x G x T	0.30	0.58
Y x M x G x S	3.64	0.01*
Y x M x T x S	1.46	0.23
Y x G x T x S	0.65	0.59
M x G x T x S	0.94	0.42
Y x M x G x D	4.31	0.01*
Y x M x T x D	0.11	0.95
Y x G x T x D	0.78	0.50
M x G x T x D	0.32	0.81
Y x M x S x D	0.33	0.97
Y x G x S x D	0.30	0.97
M x G x S x D	0.26	0.98
Y x T x S x D	0.21	0.99
M x T x S x D	0.16	0.10
G x T x S x D	0.48	0.88
Y x M x G x T x S	3.71	0.01*

Y x M x G x T x D	0.56	0.64
Y x M x G x S x D	0.27	0.98
Y x M x T x S x D	0.63	0.77
Y x G x T x S x D	0.92	0.51
M x G x T x S x D	0.13	0.10
Y x M x G x T x S x D	0.99	0.45

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**Table B.5 Change in the proportion of deep water in plant xylem from night to morning time points in isolated *A. gerardii*, as well as *A. gerardii* (AG) adjacent to *V. baldwinii*, *A. canescens*, and *R. glabra*. Samples were collection during July and August in 2013 and 2014. Negative values indicate a decrease in deep water contribution to xylem water from night to morning.**

	2013		2014	
	July	August	July	August
Ungrazed				
<i>A. gerardii</i>	-0.0217	-0.1515	-0.1204	-0.1350
<i>AG-V. baldwinii</i>	-0.1687	-0.1311	-0.1193	-0.1576
<i>AG-A. canescens</i>	-0.1053	-0.1687	-0.0515	-0.1370
<i>AG-R. glabra</i>	-0.2242	-0.2215	-0.0343	-0.2204
Grazed				
<i>A. gerardii</i>	-0.238	-0.2385	-0.1217	-0.2139
<i>AG-V. baldwinii</i>	-0.1406	-0.0956	-0.0779	-0.1034
<i>AG-A. canescens</i>	-0.2197	-0.2202	-0.2373	-0.0683
<i>AG-R. glabra</i>	-0.0737	-0.1787	-0.2086	-0.2383

**Table B.6 Nocturnal transpiration (mmol H<sub>2</sub>O m<sup>2</sup>s<sup>-1</sup>) measured on *A. gerardii* (AG) during July and August 2014. Shown are mean  $\pm$  1 SEM for isolated *A. gerardii* and *A. gerardii* adjacent to *V. baldwinii*, *A. canescens*, and *R. glabra* in both grazing locations.**

	July 2014		August 2014	
	Ungrazed	Grazed	Ungrazed	Grazed
<i>A. gerardii</i>	0.63 $\pm$ 0.25	0.58 $\pm$ 0.22	0.57 $\pm$ 0.33	0.45 $\pm$ 0.09
<i>AG-V. baldwinii</i>	0.48 $\pm$ 0.23	0.21 $\pm$ 0.07	0.84 $\pm$ 0.41	0.80 $\pm$ 0.49
<i>AG-A. canescens</i>	0.60 $\pm$ 0.09	0.31 $\pm$ 0.26	0.56 $\pm$ 0.21	0.72 $\pm$ 0.35
<i>AG-R. glabra</i>	0.39 $\pm$ 0.15	0.27 $\pm$ 0.26	0.50 $\pm$ 0.18	0.56 $\pm$ 0.27

**Table B.7 Statistical results of nocturnal transpiration analyses. Shown are F and P values for the analysis comparing all species (isolated *A. gerardii*, *V. baldwinii*, *A. canescens*, and *R. glabra*) and for the analysis containing only *A. gerardii* (isolated *A. gerardii* and *A. gerardii* adjacent to *V. baldwinii*, *A. canescens*, and *R. glabra*). Factors within the analysis include month (M), grazing treatment (G), species (S), and their interactions.**

Source	All species		<i>A. gerardii</i> only	
	F	P	F	P
M	0.24	0.63	2.19	0.15
G	0.55	0.46	0.42	0.55
S	2.41	0.08	0.27	0.84
M x G	0.13	0.72	0.43	0.52
M x S	0.09	0.97	0.81	0.50
G x S	0.16	0.92	0.17	0.92
M x G x S	0.22	0.87	0.04	0.99

**Table B.8 Statistical results of predawn ( $\Psi_{pd}$ ) and midday ( $\Psi_{md}$ ) leaf water potential analyses. Shown are F and P values for the analysis comparing all species (isolated *A. gerardii*, *V. baldwinii*, *A. canescens*, and *R. glabra*) and for the analysis containing only *A. gerardii* (isolated *A. gerardii* and *A. gerardii* adjacent to *V. baldwinii*, *A. canescens*, and *R. glabra*). Factors within the analysis include year (Y), month (M), species (S), grazing treatment (G), and their interactions. Significance at the  $\alpha=0.05$  level is indicated with an asterisk.**

Source	All species				<i>A. gerardii</i> only			
	$\Psi_{pd}$		$\Psi_{md}$		$\Psi_{pd}$		$\Psi_{md}$	
	F	P	F	P	F	P	F	P
Y	149.75	<0.01*	260.99	<0.01*	76.12	<0.01*	72.33	<0.01*
M	20.90	<0.01*	84.09	<0.01*	11.09	<0.01*	47.71	<0.01*
S	11.86	<0.01*	20.33	<0.01*	1.03	0.39	0.38	0.77
G	0.025	0.88	0.02	0.90	1.06	0.36	0.07	0.80
Y x M	0.29	0.59	2.22	0.14	0.82	0.37	0.75	0.39
Y x S	0.13	0.94	12.99	<0.01*	0.94	0.43	0.49	0.69
M x S	0.42	0.74	10.98	<0.01*	2.15	0.10	0.95	0.43
Y x G	0.07	0.79	0.004	0.95	3.27	0.08	0.1	0.92
M x G	0.04	0.84	1.19	0.28	0.03	0.86	5.54	0.02
S x G	7.69	<0.01*	0.40	0.75	2.31	0.09	1.07	0.37
Y x M x S	0.25	0.86	7.51	<0.01*	0.74	0.53	2.19	0.10
Y x M x G	2.39	0.13	0.05	0.82	0.01	0.94	0.84	0.36
Y x S x G	0.98	0.41	1.16	0.33	0.88	0.46	0.06	0.98
M x S x G	0.90	0.45	0.47	0.71	0.36	0.78	1.67	0.18
Y x M x S x G	3.59	0.02*	0.58	0.63	1.55	0.21	0.19	0.90

**Table B.9** Predawn leaf water potential ( $\Psi_{pd}$ , MPa) measured on isolated *A. gerardii*, *V. baldwinii*, *A. canescens*, and *R. glabra*. Shown are mean  $\pm$  1 SEM for both grazing treatments in all months and years. Significant differences between species within a sampling period are indicated with letters.

	2013		2014	
	July	August	July	August
Ungrazed				
<i>A. gerardii</i>	-0.62 $\pm$ 0.31	-1.27 $\pm$ 0.21 <sup>A</sup>	-1.60 $\pm$ 0.05	-1.67 $\pm$ 0.06
<i>V. baldwinii</i>	-0.15 $\pm$ 0.03	-0.32 $\pm$ 0.03 <sup>B</sup>	-0.55 $\pm$ 0.15	-1.02 $\pm$ 0.19
<i>A. canescens</i>	-0.42 $\pm$ 0.08	-0.37 $\pm$ 0.09 <sup>B</sup>	-1.05 $\pm$ 0.33	-1.45 $\pm$ 0.24
<i>R. glabra</i>	-0.13 $\pm$ 0.04	-0.20 $\pm$ 0.03 <sup>B</sup>	-0.83 $\pm$ 0.07	-1.23 $\pm$ 0.02
Grazed				
<i>A. gerardii</i>	-0.35 $\pm$ 0.10	-0.55 $\pm$ 0.13	-0.90 $\pm$ 0.20	-1.43 $\pm$ 0.07
<i>V. baldwinii</i>	-0.20 $\pm$ 0.06	-0.35 $\pm$ 0.13	-1.22 $\pm$ 0.25	-1.20 $\pm$ 0.10
<i>A. canescens</i>	-0.25 $\pm$ 0.05	-1.18 $\pm$ 0.53	-1.40 $\pm$ 0.38	-1.47 $\pm$ 0.55
<i>R. glabra</i>	-0.12 $\pm$ 0.04	-0.53 $\pm$ 0.03	-1.02 $\pm$ 0.02	-1.10 $\pm$ 0.15

**Table B.10** Midday leaf water potential ( $\Psi_{\text{md}}$ , MPa) measured on isolated *A. gerardii*, *V. baldwinii*, *A. canescens*, and *R. glabra*. Shown are mean  $\pm$  1 SEM for both grazing treatments in all months and years. Significant differences between species within a sampling period are indicated with letters.

	2013		2014	
	July	August	July	August
Ungrazed				
<i>A. gerardii</i>	-0.63 $\pm$ 0.09	-1.57 $\pm$ 0.04 <sup>A</sup>	-1.57 $\pm$ 0.12	-1.90 $\pm$ 0.23
<i>V. baldwinii</i>	-0.33 $\pm$ 0.10	-0.32 $\pm$ 0.02 <sup>B</sup>	-1.25 $\pm$ 0.20	-2.03 $\pm$ 0.11
<i>A. canescens</i>	-0.60 $\pm$ 0.10	-2.13 $\pm$ 0.43 <sup>A</sup>	-1.55 $\pm$ 0.18	-2.18 $\pm$ 0.11
<i>R. glabra</i>	-0.40 $\pm$ 0.15	-0.30 $\pm$ 0.06 <sup>B</sup>	-2.00 $\pm$ 0.31	-1.97 $\pm$ 0.12
Grazed				
<i>A. gerardii</i>	-0.65 $\pm$ 0.28	-1.68 $\pm$ 0.09 <sup>A</sup>	-1.48 $\pm$ 0.06	-1.90 $\pm$ 0.15
<i>V. baldwinii</i>	-0.32 $\pm$ 0.03	-0.60 $\pm$ 0.15 <sup>B</sup>	-1.40 $\pm$ 0.10	-1.93 $\pm$ 0.09
<i>A. canescens</i>	-0.42 $\pm$ 0.06	-2.02 $\pm$ 0.14 <sup>A</sup>	-1.78 $\pm$ 0.34	-2.43 $\pm$ 0.29
<i>R. glabra</i>	-0.30 $\pm$ 0.05	-0.40 $\pm$ 0.03 <sup>B</sup>	-1.50 $\pm$ 0.15	-2.05 $\pm$ 0.30

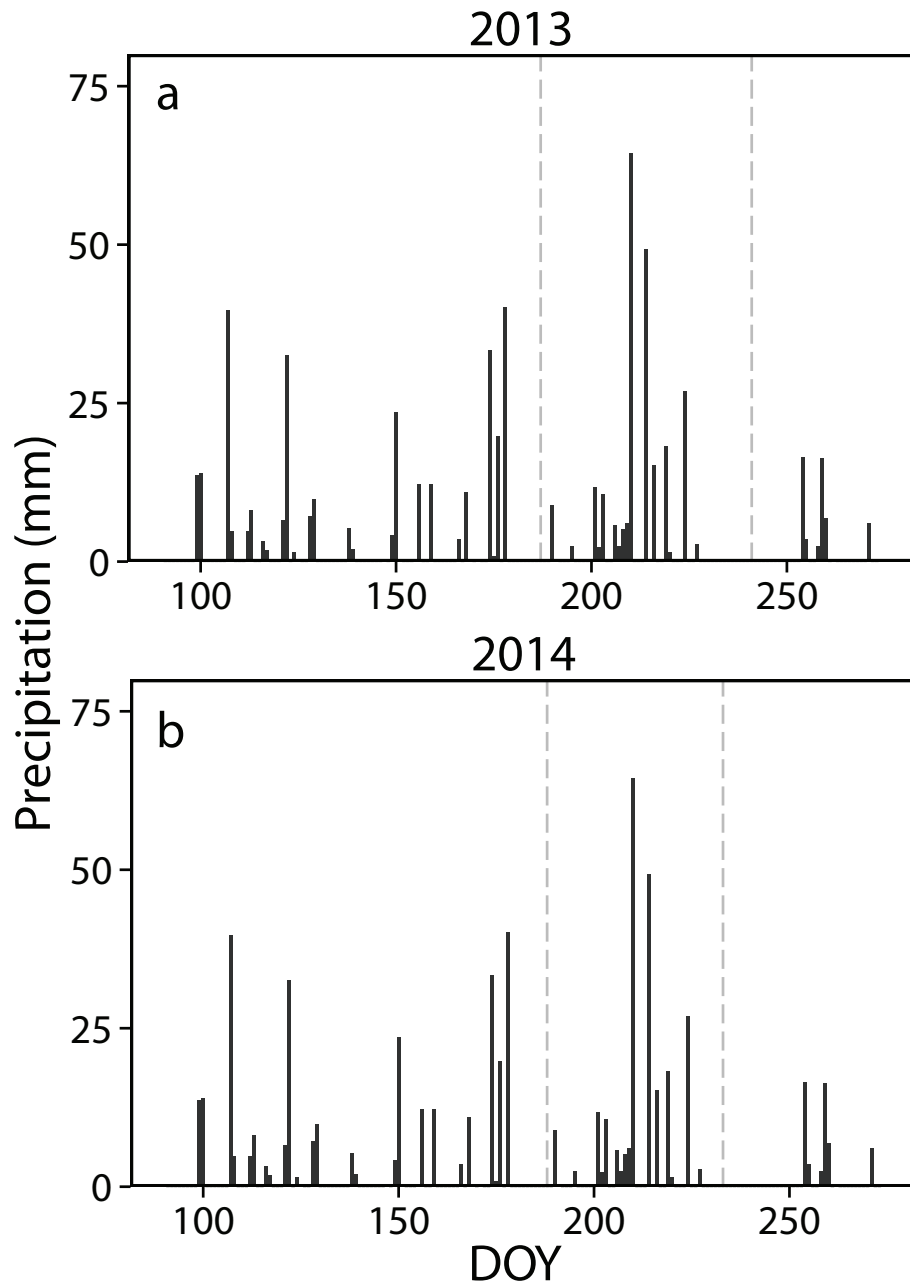


**Table B.11** Predawn leaf water potential ( $\Psi_{pd}$ , MPa) measured on isolated *A. gerardii* and *A. gerardii* (AG) adjacent to *V. baldwinii*, *A. canescens*, and *R. glabra*. Shown are mean  $\pm$  1 SEM for both grazing treatments in all months and years.

	2013		2014	
	July	August	July	August
Ungrazed				
<i>A. gerardii</i>	-0.62 $\pm$ 0.10	-1.27 $\pm$ 0.21	-1.60 $\pm$ 0.05	-1.67 $\pm$ 0.06
<i>AG-V. baldwinii</i>	-0.70 $\pm$ 0.14	-1.05 $\pm$ 0.28	-1.18 $\pm$ 0.19	-1.73 $\pm$ 0.15
<i>AG-A. canescens</i>	-0.55 $\pm$ 0.09	-1.07 $\pm$ 0.27	-1.08 $\pm$ 0.04	-1.42 $\pm$ 0.18
<i>AG-R. glabra</i>	-0.78 $\pm$ 0.35	-0.72 $\pm$ 0.27	-1.33 $\pm$ 0.06	-1.20 $\pm$ 0.20
Grazed				
<i>A. gerardii</i>	-0.35 $\pm$ 0.10	-0.55 $\pm$ 0.13	-0.90 $\pm$ 0.20	-1.43 $\pm$ 0.07
<i>AG-V. baldwinii</i>	-0.32 $\pm$ 0.14	-0.68 $\pm$ 0.18	-1.80 $\pm$ 0.40	-1.72 $\pm$ 0.39
<i>AG-A. canescens</i>	-0.22 $\pm$ 0.09	-1.18 $\pm$ 0.56	-1.47 $\pm$ 0.12	-1.60 $\pm$ 0.08
<i>AG-R. glabra</i>	-0.65 $\pm$ 0.35	-0.47 $\pm$ 0.18	-1.10 $\pm$ 0.08	-1.27 $\pm$ 0.36

**Table B.12** Midday leaf water potential ( $\Psi_{md}$ , MPa) measured on isolated *A. gerardii* and *A. gerardii* (AG) adjacent to *V. baldwinii*, *A. canescens*, and *R. glabra*. Shown are mean  $\pm$  1 SEM for both grazing treatments in all months and years.

	2013		2014	
	July	August	July	August
Ungrazed				
<i>A. gerardii</i>	-0.63 $\pm$ 0.09	-1.57 $\pm$ 0.04	-1.57 $\pm$ 0.12	-1.90 $\pm$ 0.23
AG- <i>V. baldwinii</i>	-0.58 $\pm$ 0.31	-1.72 $\pm$ 0.387	-1.50 $\pm$ 0.10	-2.43 $\pm$ 0.21
AG- <i>A. canescens</i>	-1.285 $\pm$ 0.15	-1.35 $\pm$ 0.08	-1.50 $\pm$ 0.13	-2.37 $\pm$ 0.11
AG- <i>R. glabra</i>	-0.78 $\pm$ 0.34	-1.48 $\pm$ 0.10	-1.35 $\pm$ 0.09	-2.08 $\pm$ 0.34
Grazed				
<i>A. gerardii</i>	-0.65 $\pm$ 0.28	-1.68 $\pm$ 0.09	-1.48 $\pm$ 0.06	-1.90 $\pm$ 0.15
AG- <i>V. baldwinii</i>	-0.92 $\pm$ 0.28	-1.27 $\pm$ 0.19	-1.92 $\pm$ 0.11	-1.89 $\pm$ 0.20
AG- <i>A. canescens</i>	-1.05 $\pm$ 0.32	-1.17 $\pm$ 0.19	-1.57 $\pm$ 0.09	-1.83 $\pm$ 0.07
AG- <i>R. glabra</i>	-1.03 $\pm$ 0.42	-1.48 $\pm$ 0.44	-1.82 $\pm$ 0.17	-2.02 $\pm$ 0.27



**Figure B.1 Daily precipitation (mm) measured throughout the (a) 2013 and (b) 2014 growing seasons (April – September). Dotted gray lines indicate the day of year (DOY) of each sampling campaign.**

## Appendix C - Appendix for Chapter 4

**Table C.1 Statistical results from 3-way ANOVA comparing nocturnal gas exchange traits among years, growing season date, species, and their interactions. Shown are *F* and *P* values for nocturnal stomatal conductance of water vapor ( $g_{\text{night}}$ ), nocturnal transpiration rate ( $E_{\text{night}}$ ), and the percent nocturnal of daytime transpiration rates ( $\% E_{\text{night}}$ ).**

Source	$G_{\text{night}}$		$E_{\text{night}}$		$\% E_{\text{night}}$	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Year (Y)	10.23	0.002	8.80	0.003	1.16	0.282
Date (D)	8.56	<0.001	6.83	<0.001	7.68	<0.001
Species (S)	4.14	<0.001	6.13	<0.001	4.83	<0.001
Y x D	23.10	<0.001	16.13	<0.001	10.90	<0.001
Y x S	1.22	0.295	2.16	0.04	3.18	0.003
S x D	1.72	0.012	3.06	<0.001	2.31	<0.001
Y x S x D	1.12	0.310	1.35	0.103	1.66	0.018

**Table C.2 Statistical results from 3-way ANOVA comparing daytime gas exchange traits among years, growing season date, species, and their interactions. Shown are *F* and *P* values for maximum CO<sub>2</sub> assimilation at ambient C<sub>a</sub> (*A*<sub>max</sub>), daytime stomatal conductance of water vapor (*g*<sub>sday</sub>), and daytime transpiration rate (*E*<sub>day</sub>).**

Source	<i>A</i> <sub>max</sub>		<i>G</i> <sub>sday</sub>		<i>E</i> <sub>day</sub>	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Year (Y)	139.85	<0.001	318.80	<0.001	49.31	<0.001
Date (D)	4.58	<0.001	5.43	<0.001	54.75	<0.001
Species (S)	106.10	<0.001	60.54	<0.001	28.26	<0.001
Y x D	47.20	<0.001	52.45	<0.001	76.19	<0.001
Y x S	16.97	<0.001	23.45	<0.001	9.45	<0.001
S x D	1.91	0.002	1.78	0.005	2.43	<0.001
Y x S x D	3.53	<0.001	4.19	<0.001	4.82	<0.001

**Table C.3 Statistical results from 3-way ANOVA comparing leaf water potential traits among years, growing season date, species, and their interactions. Shown are F and *P* values for predawn ( $\Psi_{pd}$ ) and midday ( $\Psi_{md}$ ) leaf water potential.**

Source	$\Psi_{pd}$		$\Psi_{md}$	
	F	<i>P</i>	F	<i>P</i>
Year (Y)	565.11	<0.001	474.99	<0.001
Date (D)	174.01	<0.001	127.89	<0.001
Species (S)	25.46	<0.001	20.11	<0.001
Y x D	161.68	<0.001	49.99	<0.001
Y x S	9.48	<0.001	6.64	<0.001
S x D	5.92	<0.001	4.07	<0.001
Y x S x D	5.85	<0.001	5.57	<0.001

## Appendix D - Appendix for Chapter 5

**Table D.1** Species specific input data and priors.

	<b>LAI</b>	<b>SAI</b>	<b>G<sub>ref</sub></b>	<b>G<sub>night</sub></b>
<i>A. gerardii</i>	1.50	9.42	30	0.72
<i>P. virgatum</i>	1.50	9.42	10	0.78
<i>S. nutans</i>	1.50	9.42	40	0.79
<i>S. canadensis</i>	0.25	0.53	10	0.87
<i>V. baldwinii</i>	0.25	0.53	20	0.80
<i>A. canescens</i>	0.50	2.00	40	0.89
<i>R. glabra</i>	0.50	2.00	40	0.91
<i>C. drummondii</i>	4.50	9.04	40	0.80

**Table D.2 Parameter descriptions.**

Symbol	Description
$\lambda$	VPD sensitivity ( $\text{mmol m}^{-2}\text{s}^{-1} \ln[\text{kPa}]^{-1}$ )
$G_{\text{ref}}$	Canopy conductance at VPD = 1 ( $\text{mmol m}^{-2}\text{s}^{-1}$ )
$\lambda/G_{\text{ref}}$	Ratio of $\lambda$ to $G_{\text{ref}}$
$\alpha_1$	Nighttime conductance
$\alpha_2$	Sensitivity to light
$\alpha_3$	Threshold soil moisture
$\alpha_4$	Sensitivity to soil moisture
$\sigma^2$	Process error variance
S	Observation error variance
$v_a$	Random effects variance



**Table D.3 Mean parameter estimates and 95% credible intervals for woody species.**

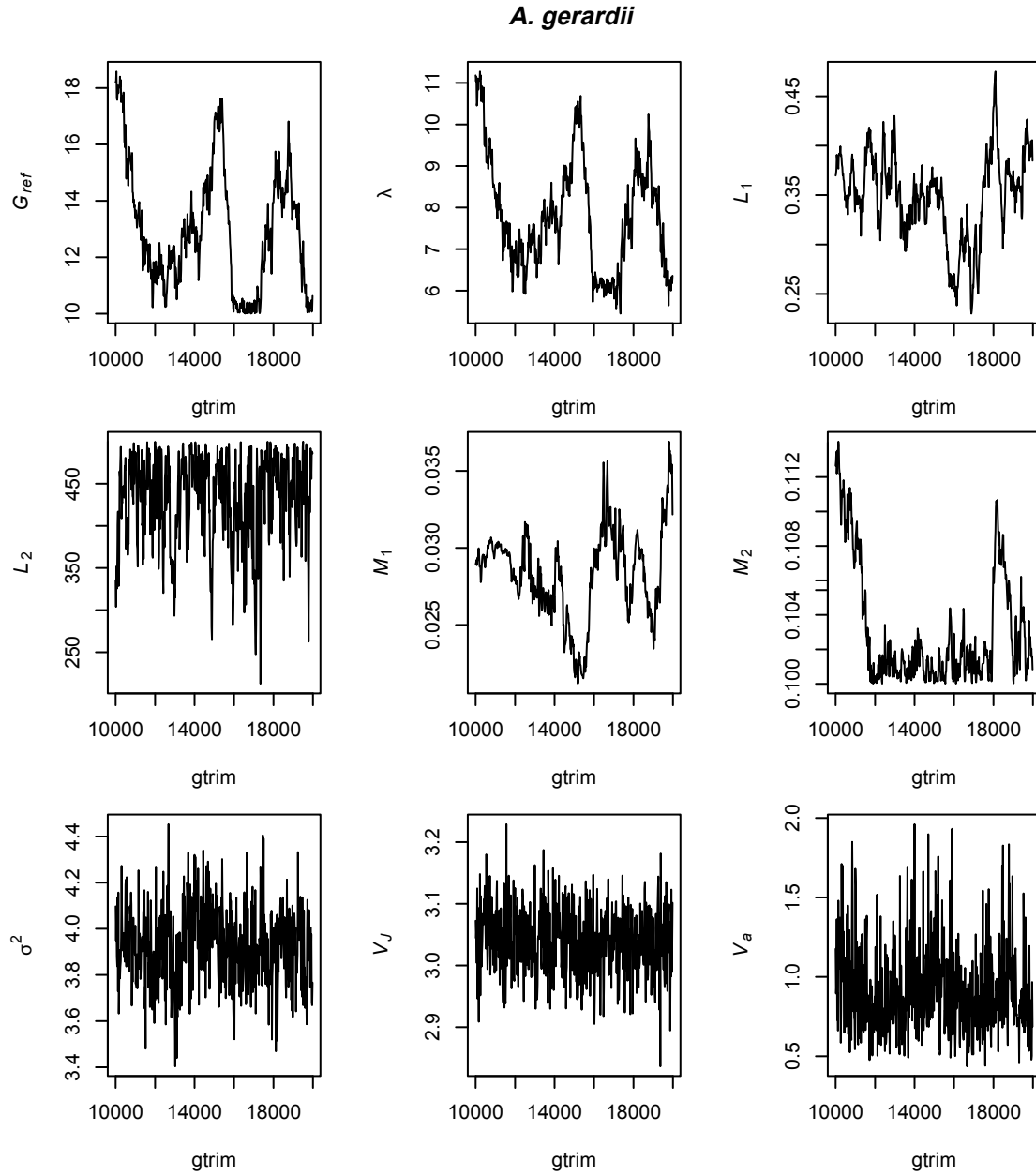
Parameter	<i>C. drummondii</i>	<i>A. canescens</i>	<i>R. glabra</i>
$\lambda$	15.445 (13.417, 19.290)	24.370 (20.169, 27.038)	19.076 (16.222, 23.742)
$G_{\text{ref}}$	34.368 (32.440, 36.312)	42.999 (41.167, 45.114)	40.582 (39.007, 42.13)
$\lambda/G_{\text{ref}}$	0.449 (0.402, 0.549)	0.567 (0.480, 0.614)	0.470 (0.404, 0.584)
$\alpha_1$	0.774 (0.753, 0.795)	0.841 (0.827, 0.854)	0.817 (0.798, 0.835)
$\alpha_2$	498.017 (493.65, 499.943)	497.748 (492.248, 499.936)	496.582 (487.862, 499.894)
$\alpha_3$	0.073 (0.071, 0.075)	0.104 (0.097, 0.111)	0.079 (0.069, 0.089)
$\alpha_4$	0.1997 (0.1989, 0.1999)	0.197 (0.191, 0.199)	0.156 (0.145, 0.168)
$\sigma^2$	3.838 (3.577, 3.894)	7.733 (6.480, 8.689)	10.687 (9.618, 11.870)
S	3.205 (3.114, 3.299)	3.443 (3.344, 3.546)	3.612 (3.502, 3.730)
$v_a$	0.730 (0.436, 1.224)	0.699 (0.407, 1.193)	0.704 (0.389, 1.274)

**Table D.4 Mean parameter estimates and 95% credible intervals for forbs.**

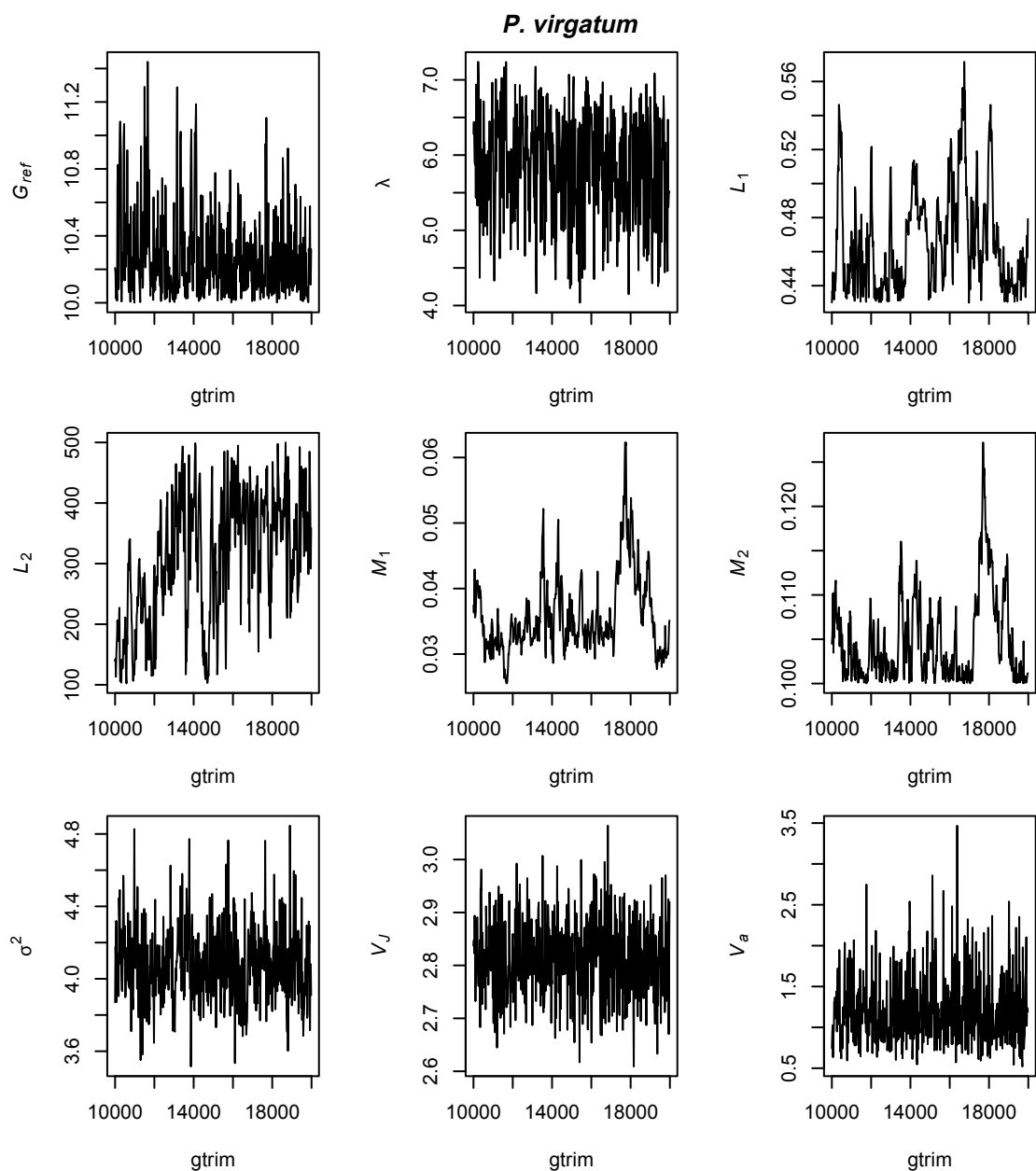
Parameter	<i>S. canadensis</i>	<i>V. baldwinii</i>
$\lambda$	5.078 (4.095, 6.547)	6.092 (5.242, 6.663)
$G_{\text{ref}}$	10.183 (10.006, 10.674)	10.025 (10.001, 10.082)
$\lambda/G_{\text{ref}}$	0.499 (0.405, 0.643)	0.606 (0.523, 0.664)
$\alpha_1$	0.673 (0.628, 0.714)	0.751 (0.717, 0.781)
$\alpha_2$	496.211 (485.986, 499.905)	496.747 (488.851, 499.89)
$\alpha_3$	0.098 (0.092, 0.104)	0.039 (0.033, 0.048)
$\alpha_4$	0.1996 (0.1987, 0.1999)	0.102 (0.100, 0.106)
$\sigma^2$	3.790 (3.436, 4.165)	3.266 (2.955, 3.610)
S	2.895 (2.804, 2.986)	3.060 (2.961, 3.163)
$v_a$	0.941 (0.546, 1.602)	0.738 (0.408, 1.306)

**Table D.5 Mean parameter estimates and 95% credible intervals for grass species.**

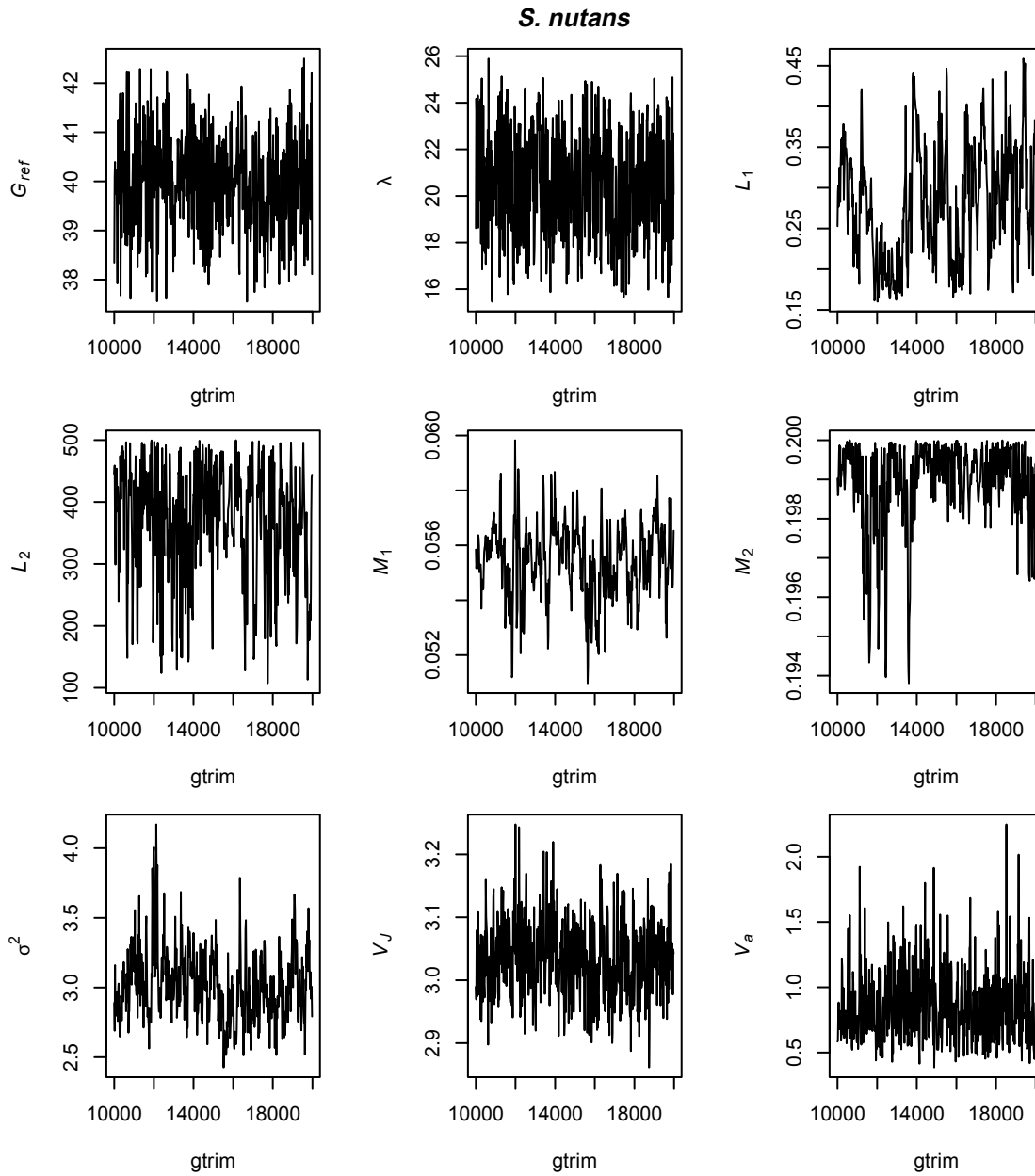
Parameter	<i>A. gerardii</i>	<i>P. virgatum</i>	<i>S. nutans</i>
$\lambda$	7.789 (5.939, 10.848)	5.877 (4.397, 6.935)	20.445 (16.226, 24.678)
$G_{\text{ref}}$	12.953 (10.061, 17.781)	10.292 (10.010, 11.029)	39.995 (38.066, 41.897)
$\lambda/G_{\text{ref}}$	0.601 (0.560, 0.616)	0.571 (0.432, 0.664)	0.511 (0.409, 0.610)
$\alpha_1$	0.349 (0.254, 0.424)	0.466 (0.431, 0.536)	0.282 (0.170, 0.420)
$\alpha_2$	431.675 (306.676, 496.732)	310.897 (116.607, 489.098)	366.219 (159.814, 493.152)
$\alpha_3$	0.028 (0.022, 0.035)	0.036 (0.028, 0.051)	0.055 (0.053, 0.058)
$\alpha_4$	0.103 (0.100, 0.112)	0.105 (0.100, 0.117)	0.198 (0.195, 0.199)
$\sigma^2$	3.921 (3.595, 4.281)	4.081 (3.673, 4.539)	3.011 (2.590, 3.593)
S	3.038 (2.935, 3.147)	2.812 (2.674, 2.959)	3.037 (2.918, 3.161)
$v_a$	0.920 (0.509, 1.645)	1.206 (0.604, 2.349)	0.843 (0.452, 1.580)



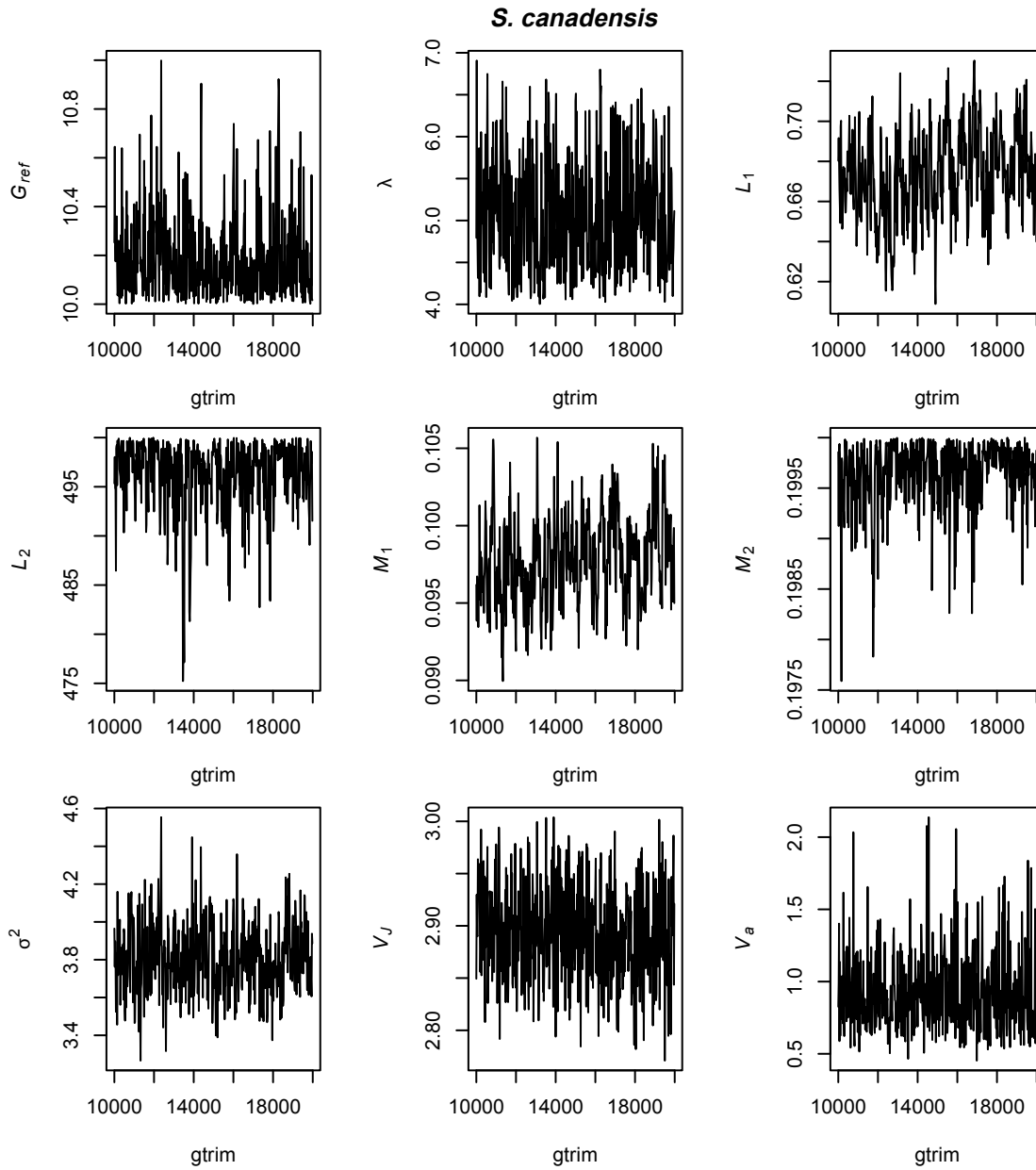
**Figure D.1 Model convergence for *A. gerardii* parameter estimates. Shown are model iterations following the burn-in period (Gibbs sampler iterations 10,000 – 20,000).**



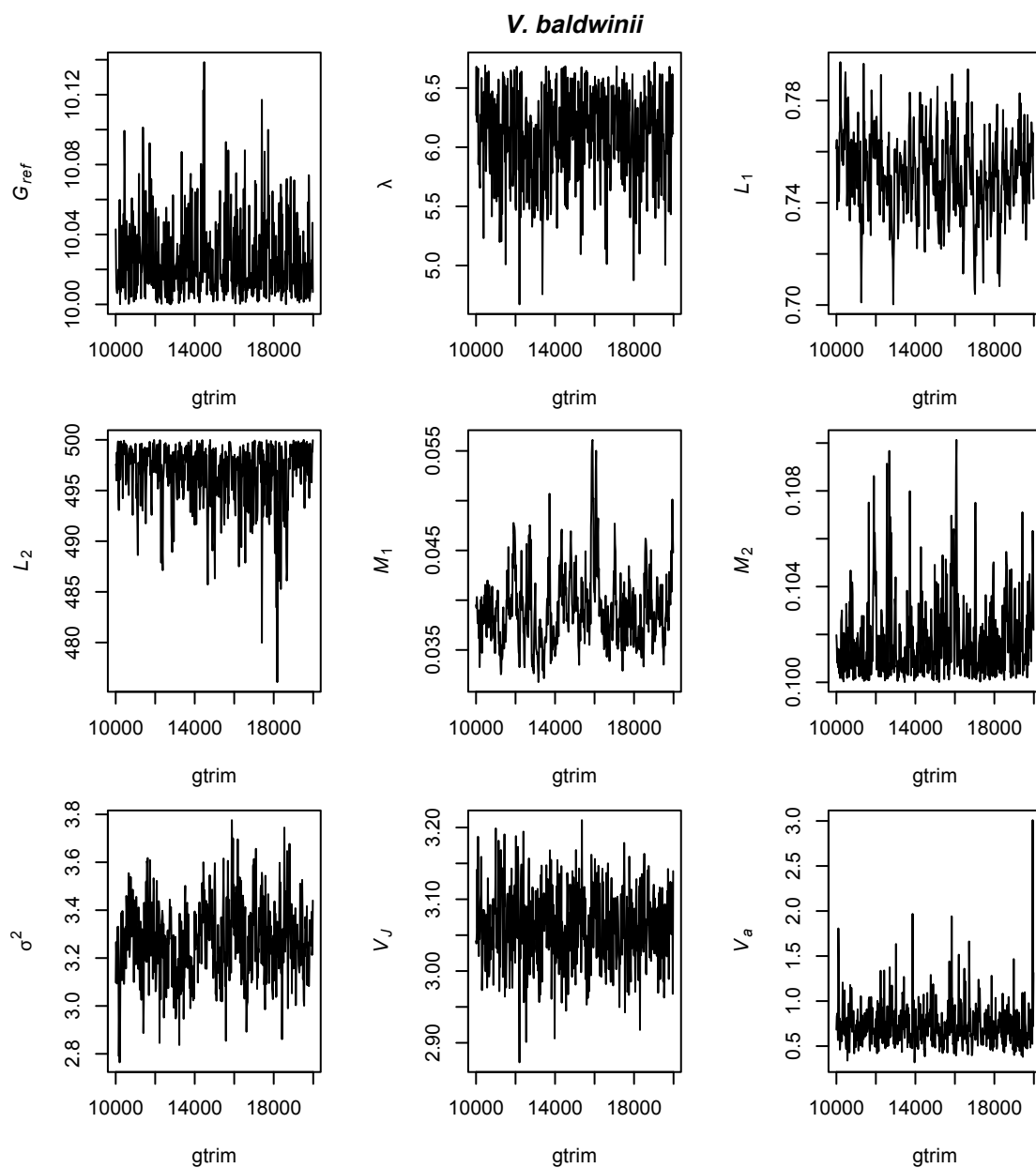
**Figure D.2 Model convergence for *P. virgatum* parameter estimates. Shown are model iterations following the burn-in period (Gibbs sampler iterations 10,000 – 20,000).**



**Figure D.3 Model convergence for *S. nutans* parameter estimates. Shown are model iterations following the burn-in period (Gibbs sampler iterations 10,000 – 20,000).**

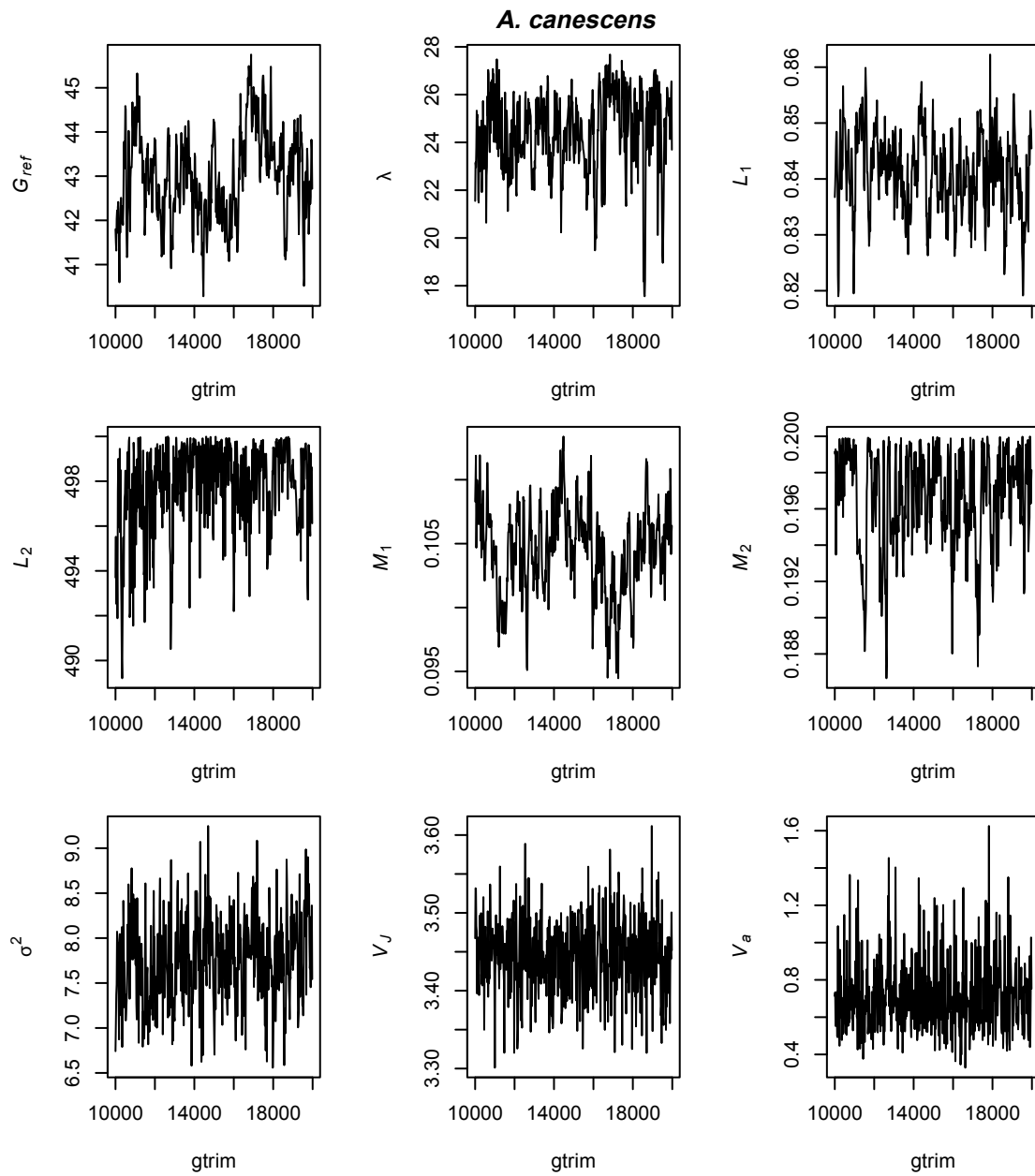


**Figure D.4 Model convergence for *S. canadensis* parameter estimates. Shown are model iterations following the burn-in period (Gibbs sampler iterations 10,000 – 20,000).**

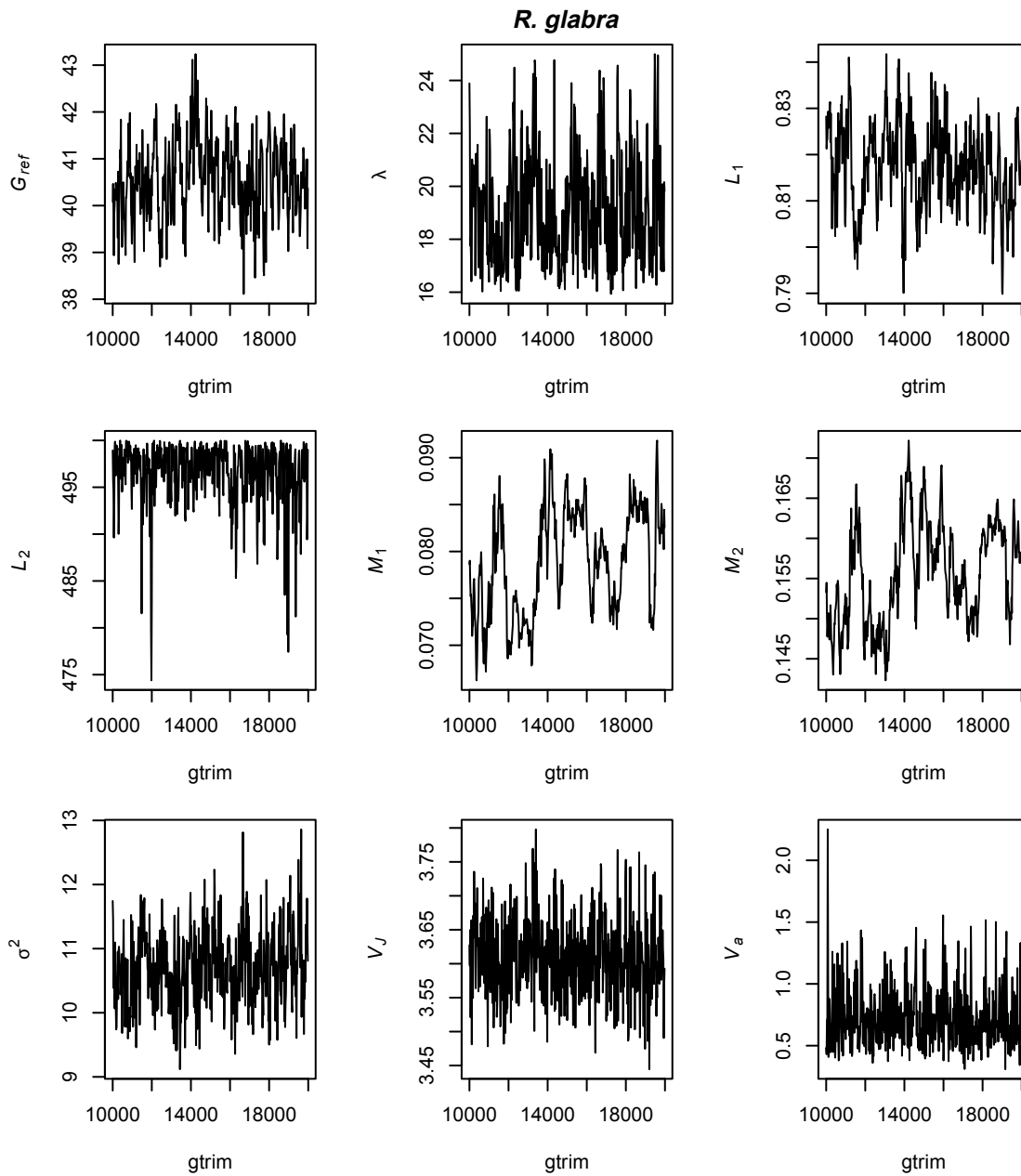


**Figure D.5 Model convergence for *V. baldwinii* parameter estimates. Shown are model iterations following the burn-in period (Gibbs sampler iterations 10,000 – 20,000).**

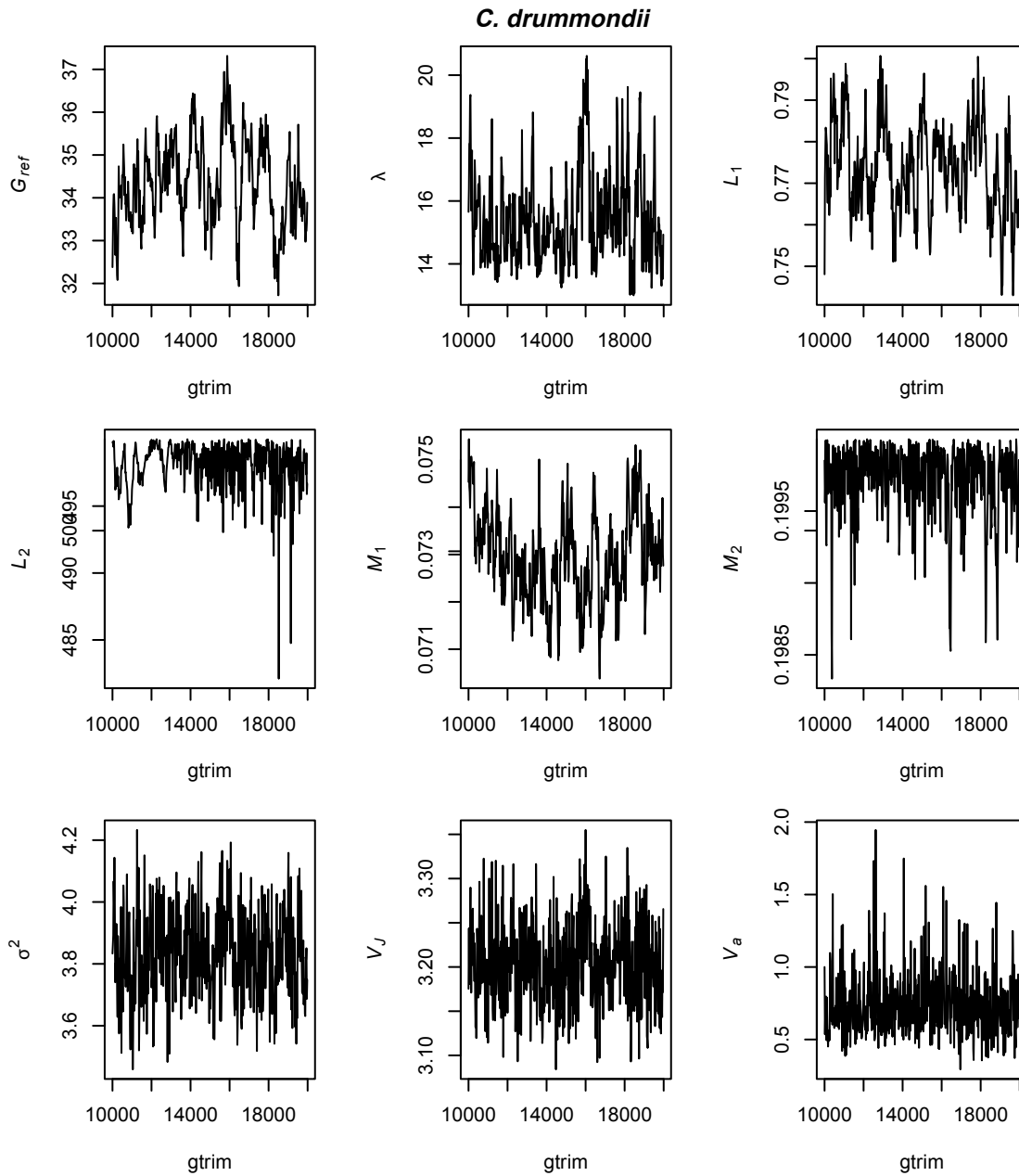




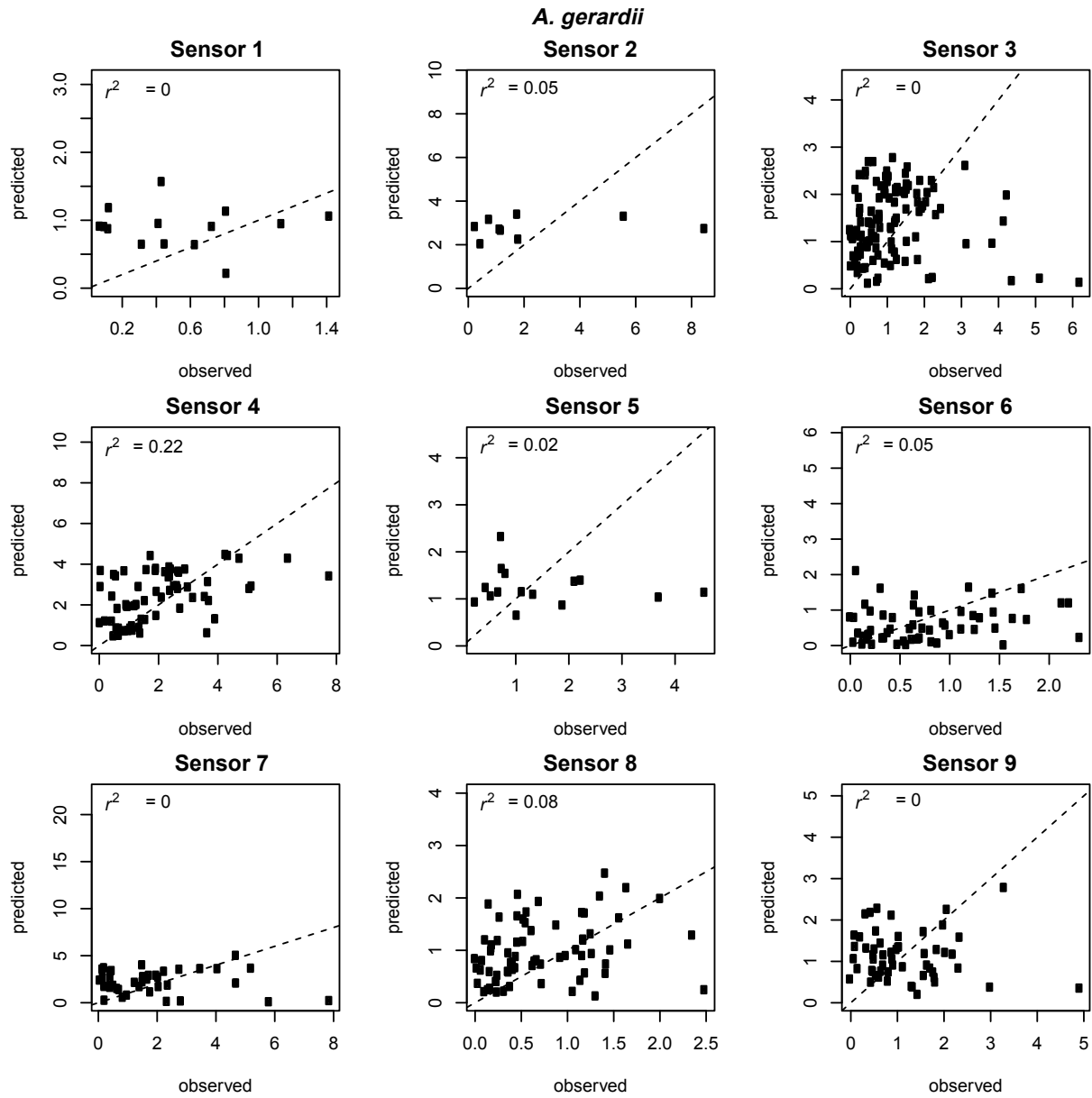
**Figure D.6 Model convergence for *A. canescens* parameter estimates. Shown are model iterations following the burn-in period (Gibbs sampler iterations 10,000 – 20,000).**



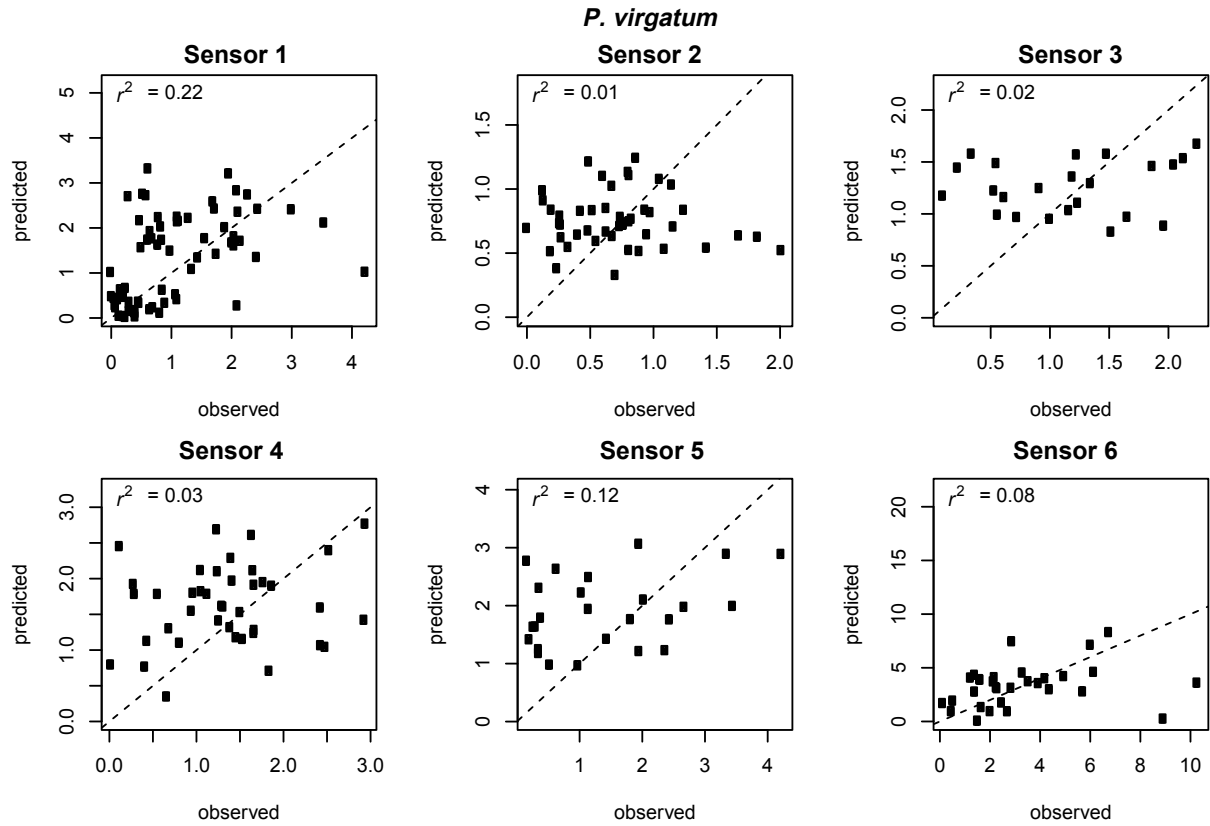
**Figure D.7 Model convergence for *R. glabra* parameter estimates. Shown are model iterations following the burn-in period (Gibbs sampler iterations 10,000 – 20,000).**



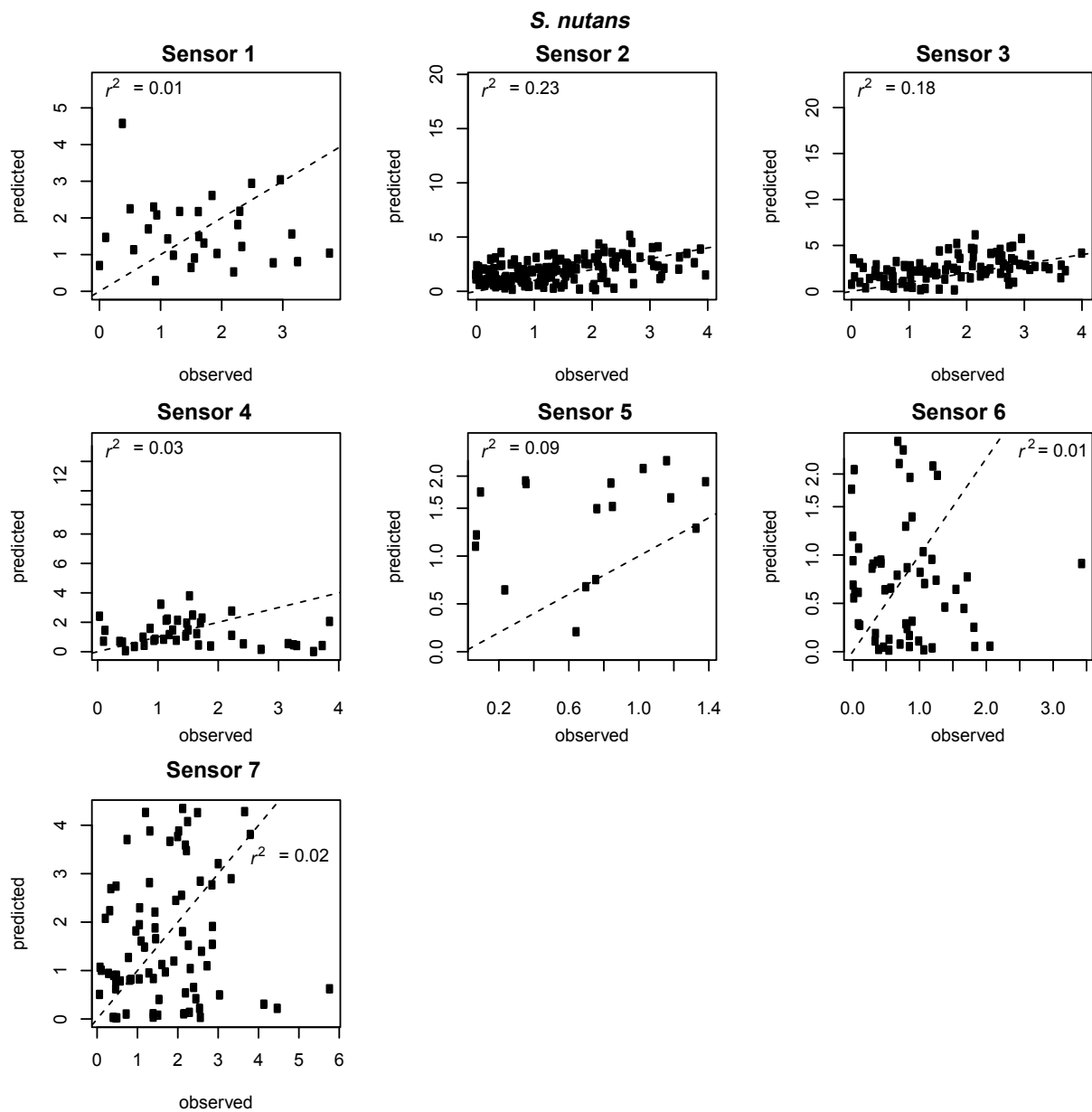
**Figure D.8 Model convergence for *C. drummondii* parameter estimates. Shown are model iterations following the burn-in period (Gibbs sampler iterations 10,000 – 20,000).**



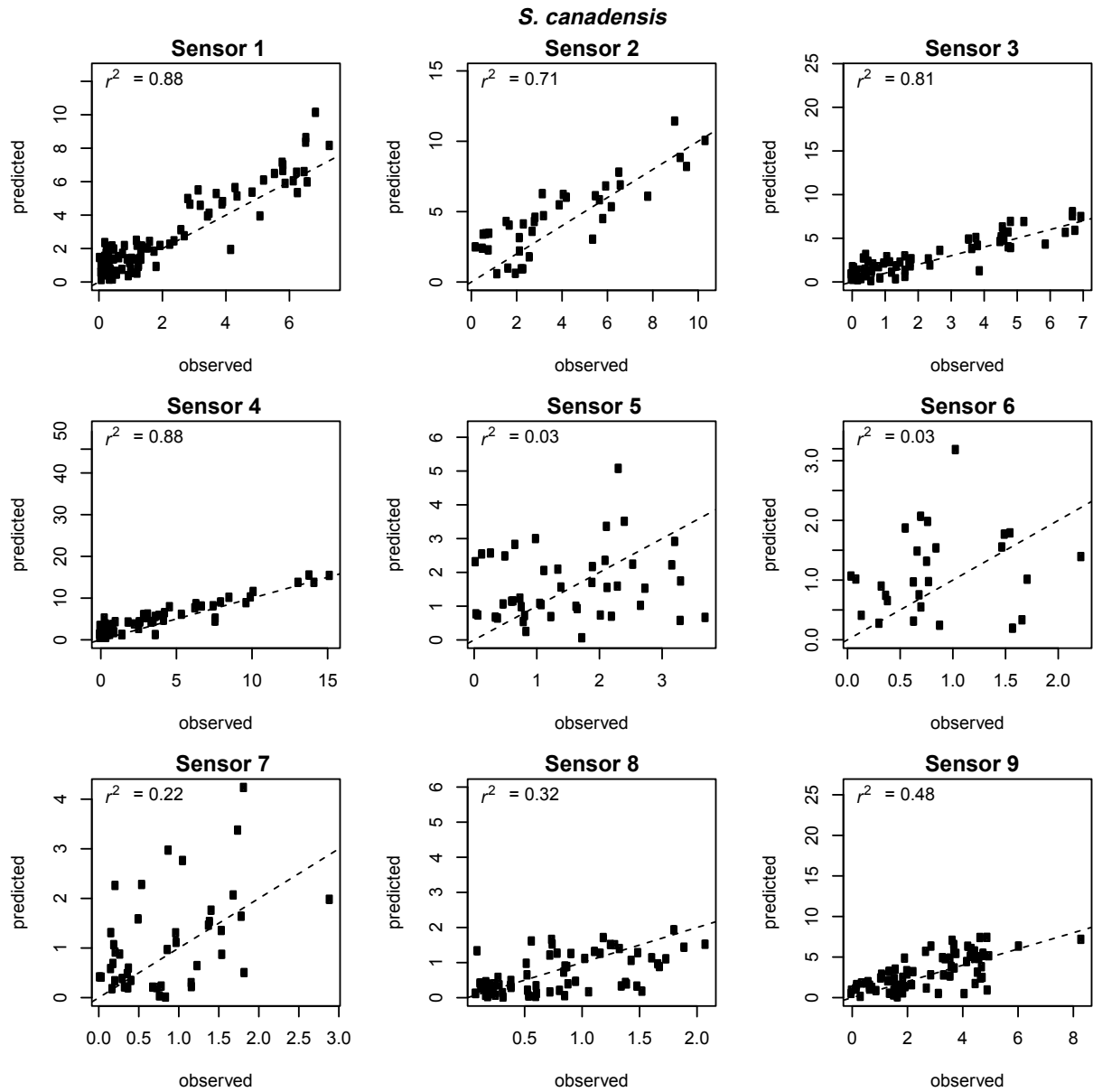
**Figure D.9 Predicted versus observed sap flow for each sensor used to measure *A. gerardii* sap flow. Also shown are squared Pearson correlation coefficients ( $r^2$ ) for each sensor.**



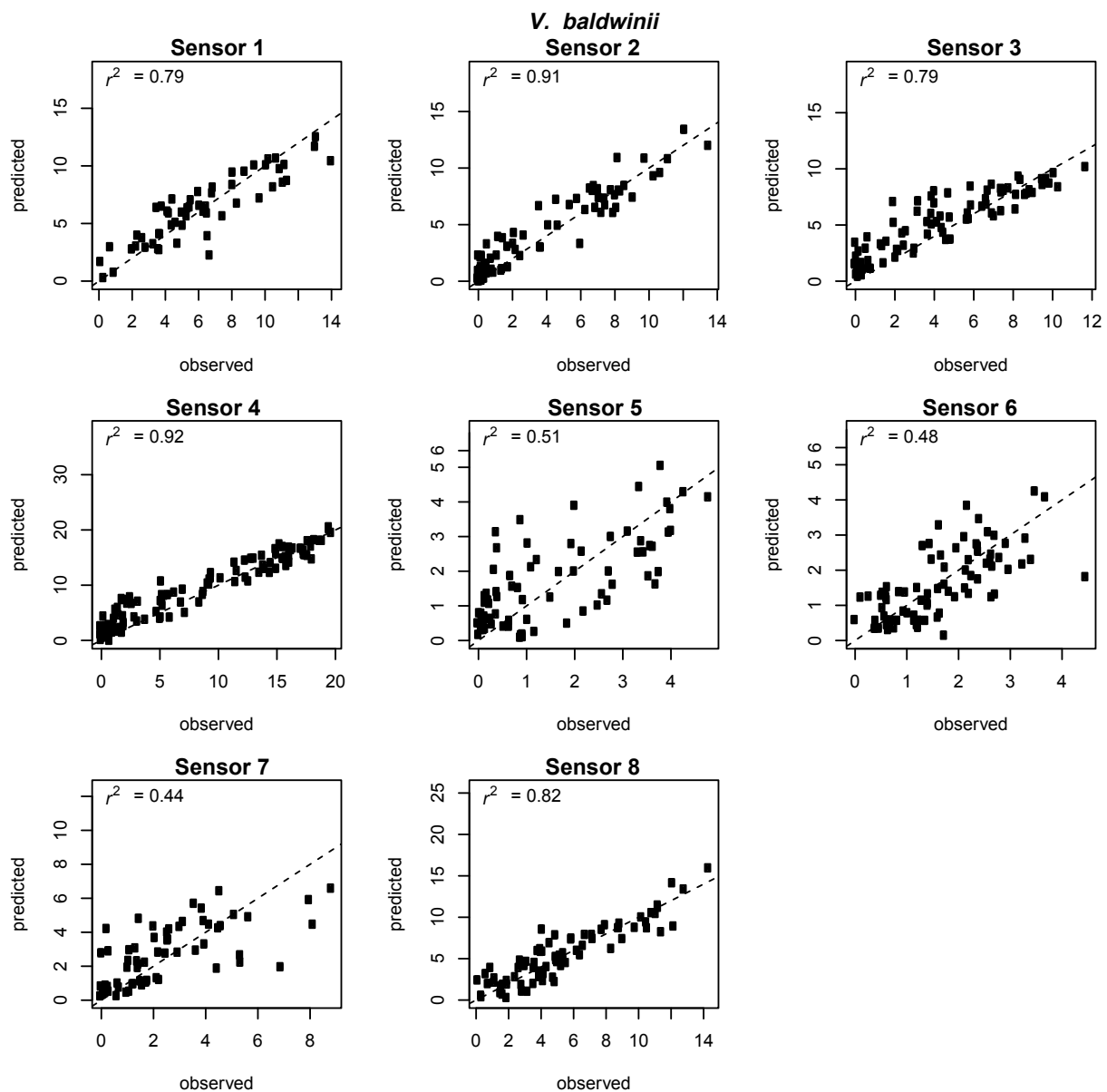
**Figure D.10 Predicted versus observed sap flow for each sensor used to measure *P. virgatum* sap flow. Also shown are squared Pearson correlation coefficients ( $r^2$ ) for each sensor.**



**Figure D.11 Predicted versus observed sap flow for each sensor used to measure *S. nutans* sap flow. Also shown are squared Pearson correlation coefficients ( $r^2$ ) for each sensor.**

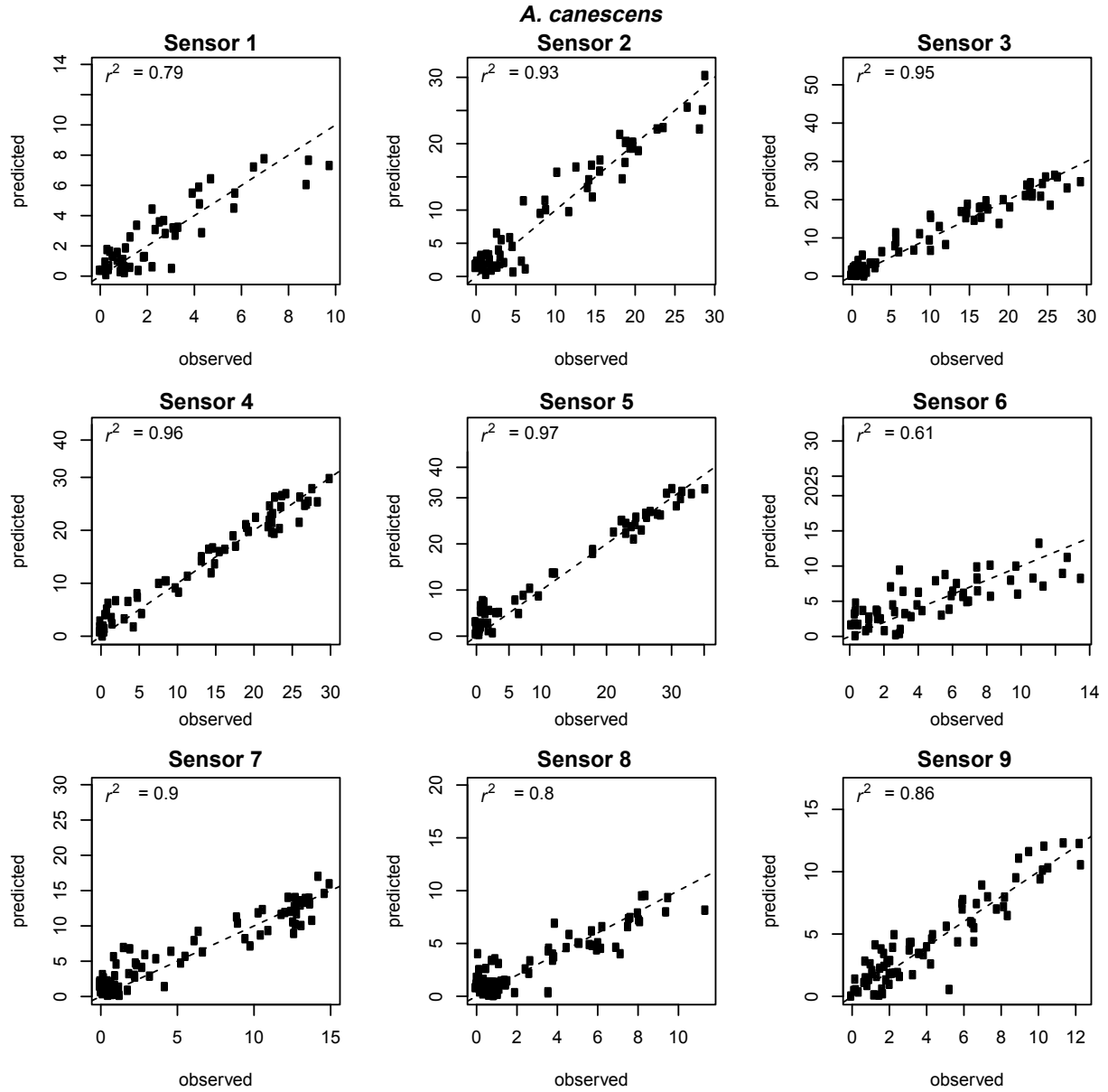


**Figure D.12 Predicted versus observed sap flow for each sensor used to measure *S. canadensis* sap flow. Also shown are squared Pearson correlation coefficients ( $r^2$ ) for each sensor.**

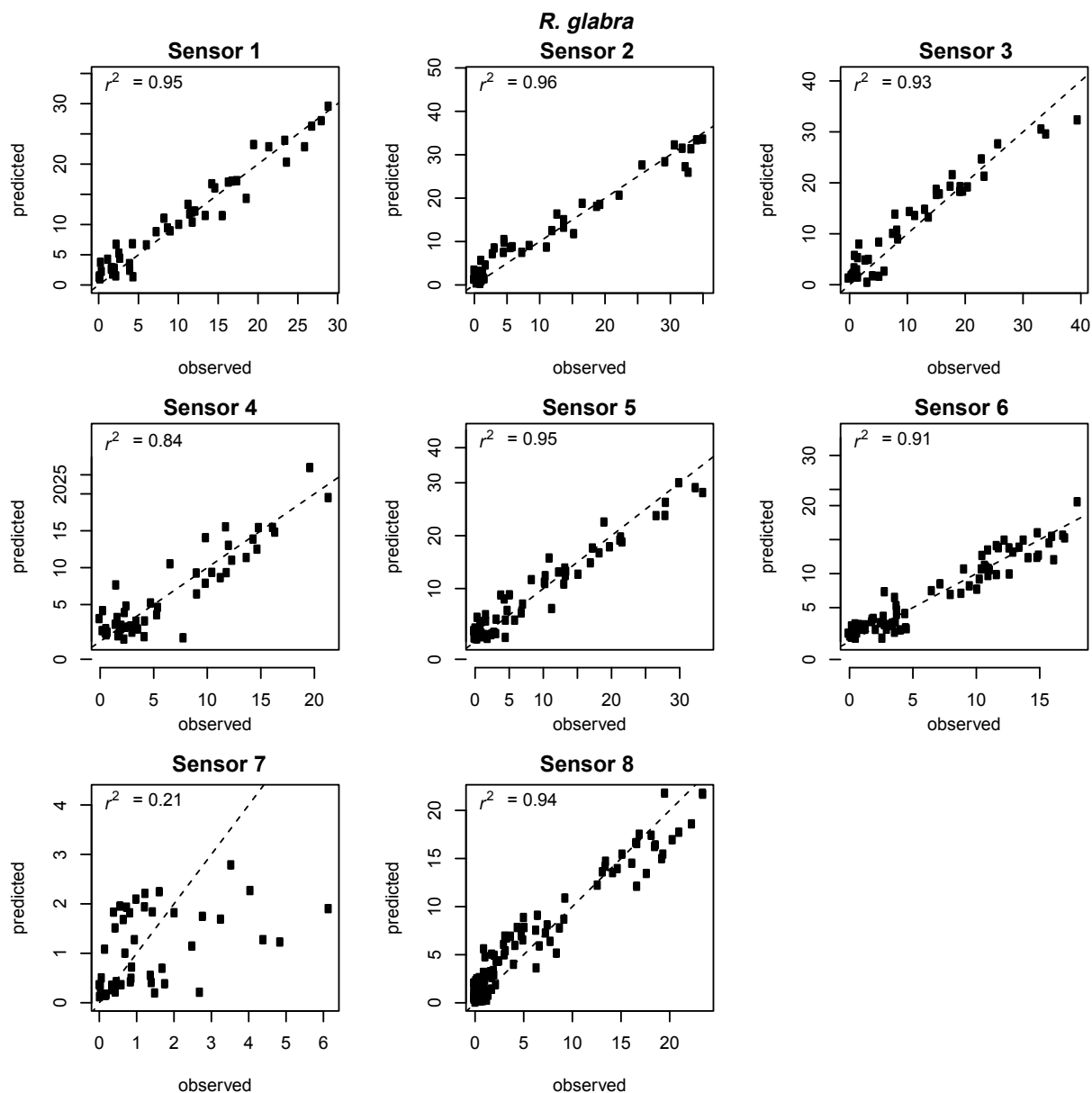


**Figure D.13** Predicted versus observed sap flow for each sensor used to measure *V. baldwinii* sap flow. Also shown are squared Pearson correlation coefficients ( $r^2$ ) for each sensor.

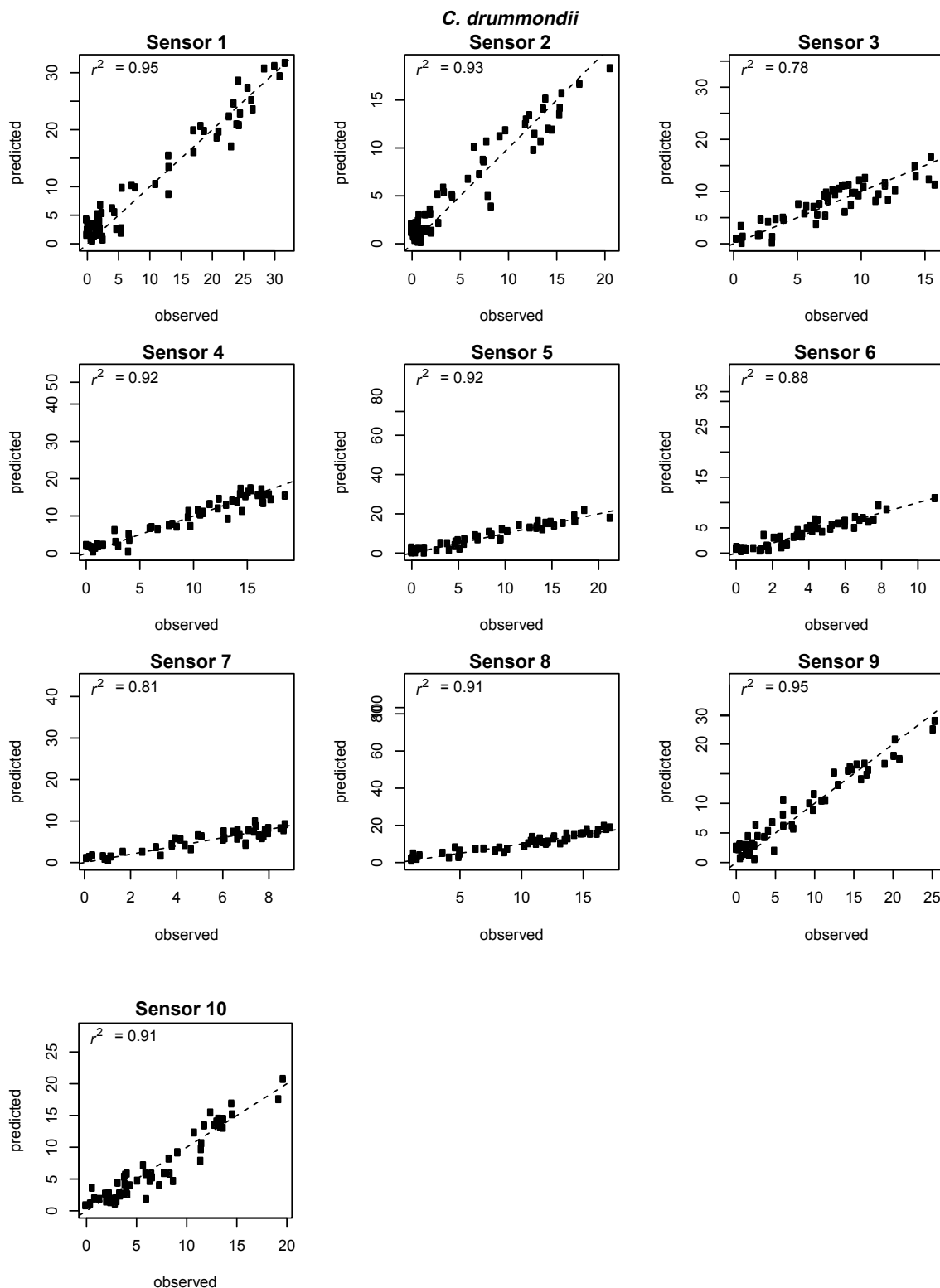




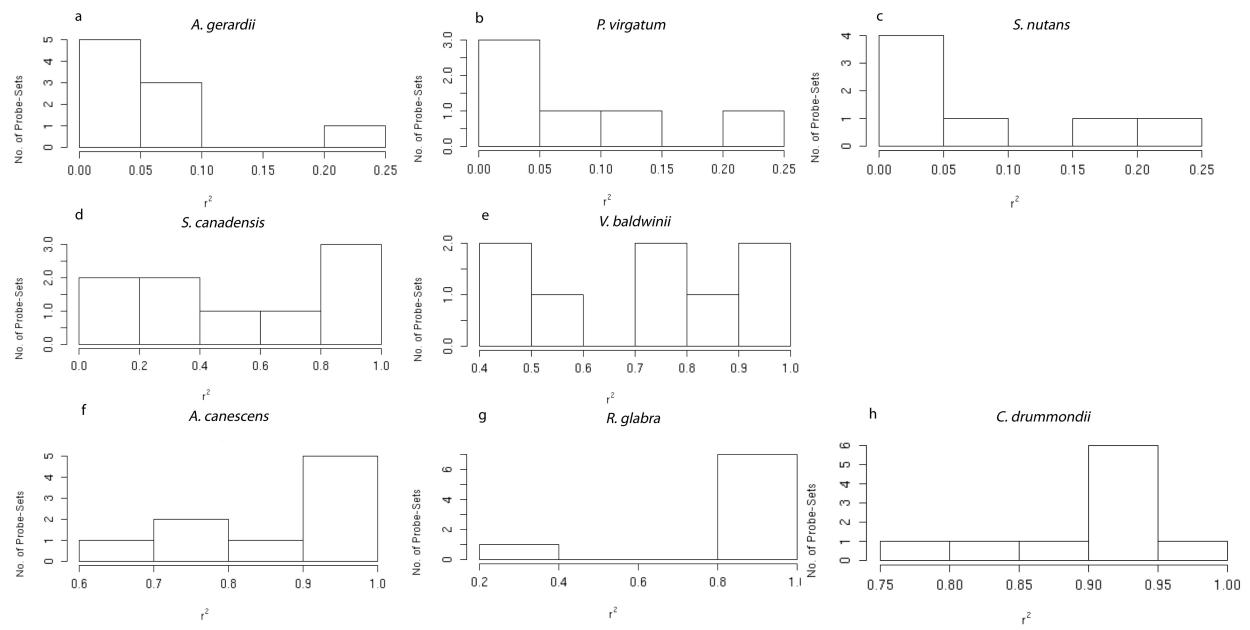
**Figure D.14 Predicted versus observed sap flow for each sensor used to measure *A. canescens* sap flow. Also shown are squared Pearson correlation coefficients ( $r^2$ ) for each sensor.**



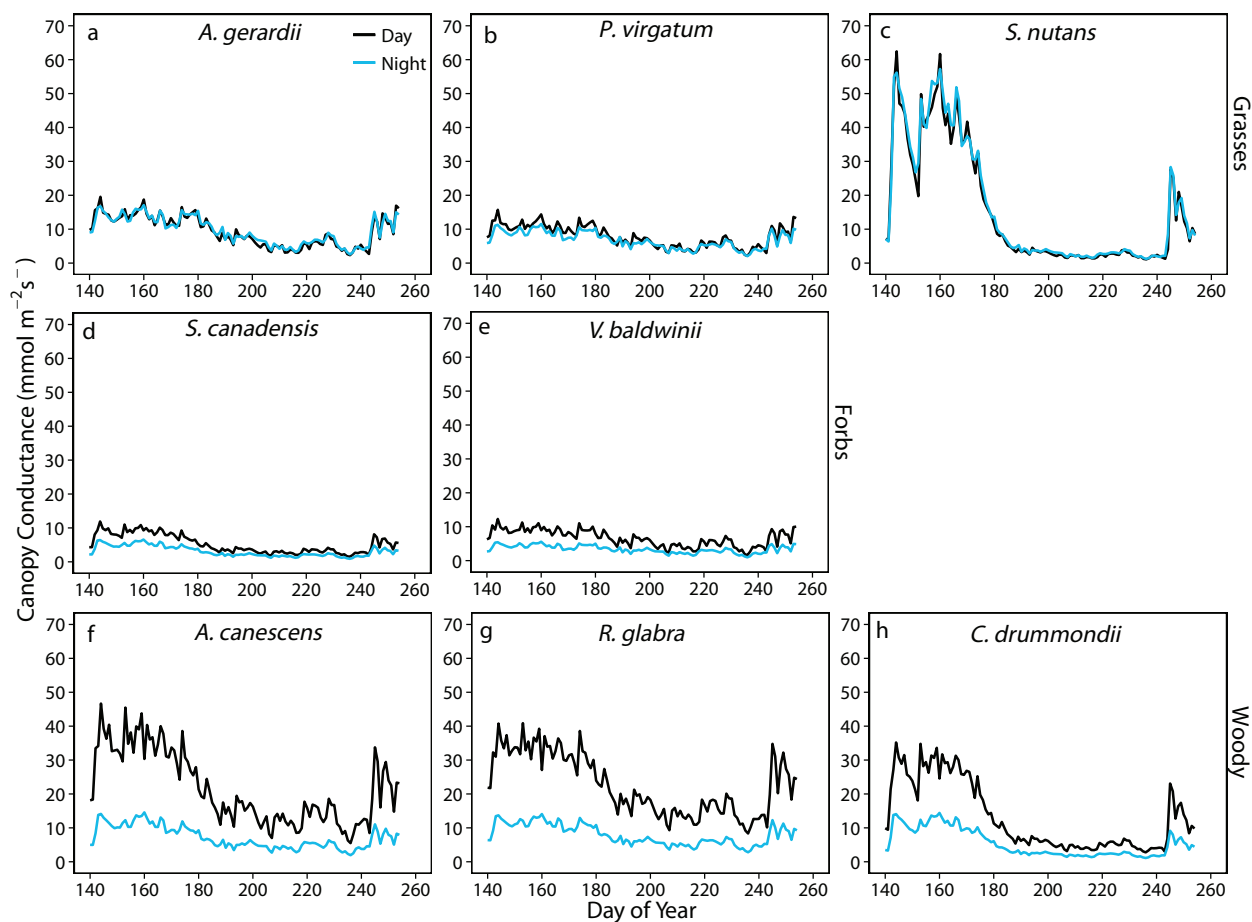
**Figure D.15 Predicted versus observed sap flow for each sensor used to measure *R. glabra* sap flow. Also shown are squared Pearson correlation coefficients ( $r^2$ ) for each sensor.**



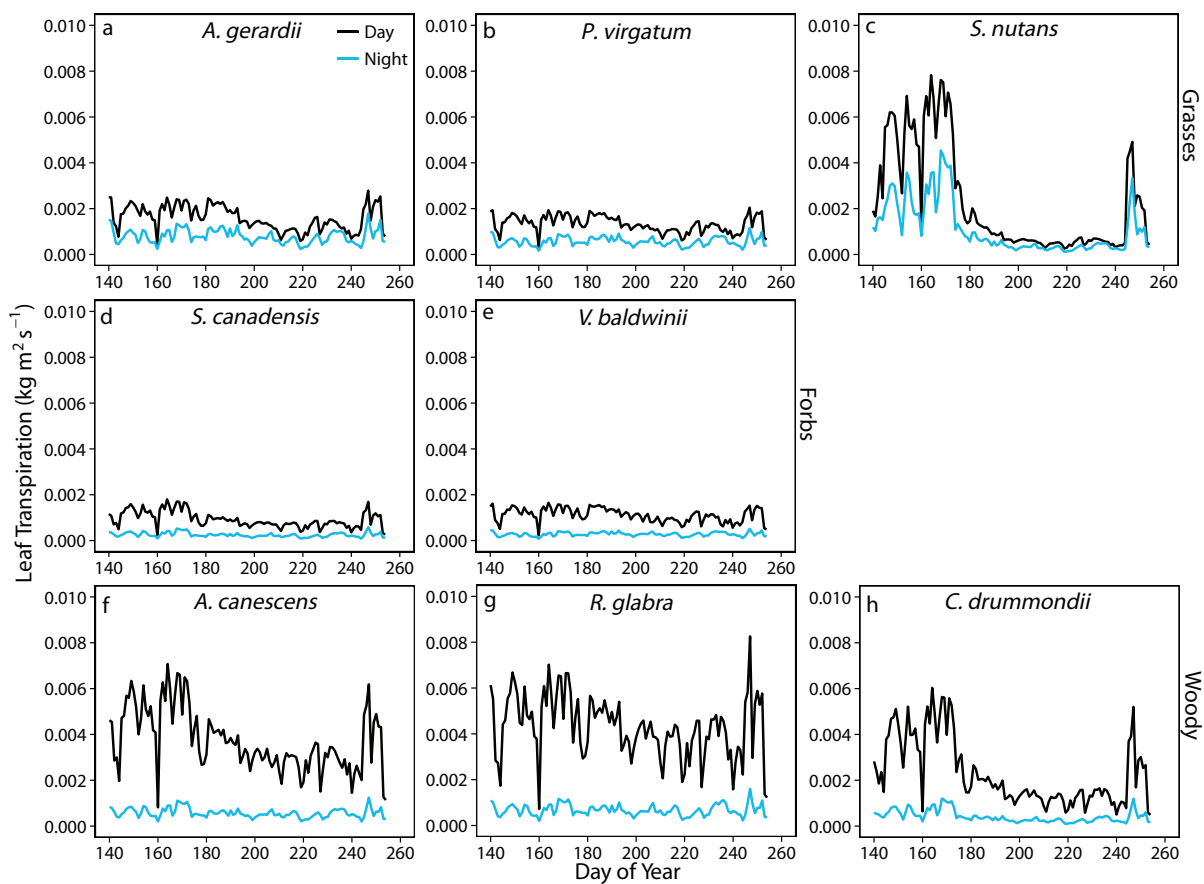
**Figure D.16 Predicted versus observed sap flow for each sensor used to measure *C. drummondii* sap flow. Also shown are squared Pearson correlation coefficients ( $r^2$ ) for each sensor.**



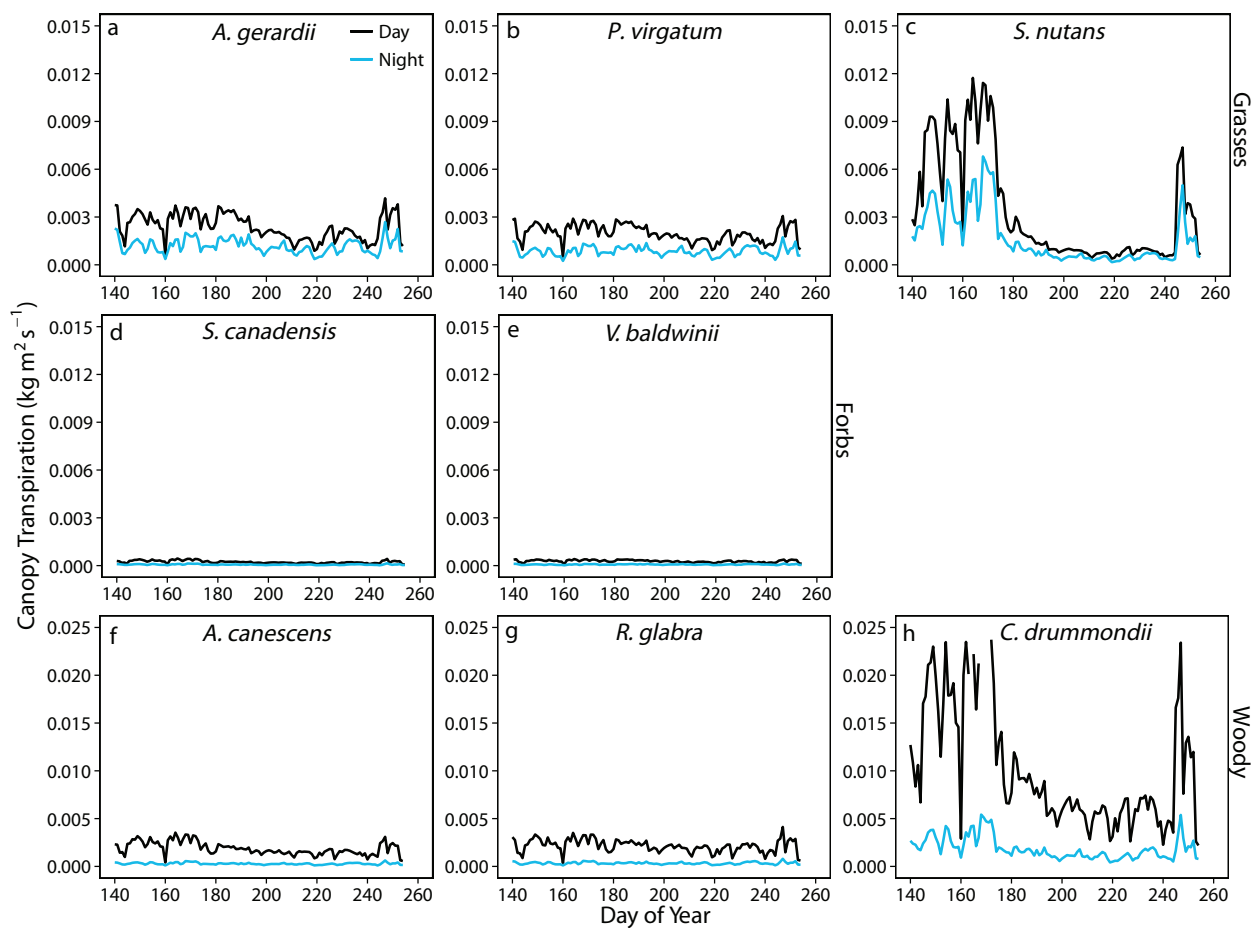
**Figure D.17** Histograms of Pearson correlation coefficients ( $r^2$ ) for the sensor set of each species.



**Figure D.18 Modeled canopy conductance for each species between DOY 140-254. Shown are daily average diurnal (black) and nocturnal rates (blue).**



**Figure D.19 Modeled leaf transpiration rates for each species between DOY 140-254. Shown are daily average diurnal (black) and nocturnal rates (blue).**



**Figure D.20** Modeled canopy transpiration rates for each species between DOY 140-254. Shown are daily average diurnal (black) and nocturnal rates (blue).