DISSERTATION

LIFE BY THE DROP: WATER AS A PHYSIOLOGICAL DRIVER OF THE TALLGRASS PRAIRIE PLANT COMMUNITY

Submitted by
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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JESSE BRIAN NIPPERT ENTITLED LIFE BY THE DROP: WATER AS A PHYSIOLOGICAL DRIVER OF THE TALLGRASS PRAIRIE PLANT COMMUNITY BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

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Competition for water is an important driver of community structure and productivity in many grasslands, including North American tallgrass prairies, with nearly two thirds of annual plant productivity allocated belowground. While the root structure was first described nearly 70 years ago, the functional significance of species-specific differences in rooting patterns has only been the subject of speculation. By comparing species differences in water uptake and how these relate to water availability and plant stress, our ability to predict responses to ecosystem drying from climatic variability will likely be enhanced. Using annually-burned watersheds at the Konza Prairie Biological Station, I measured a suite of C_4 grasses and C_3 woody and forb species to determine how differences in water-use and acquisition may vary among species seasonally and spatially across the landscape. I tested hypotheses related to the extent that inherent physiological differences between photosynthetic pathways (C_3, C_4) explained patterns of C_4 grass dominance in this ecosystem. When carboxylation, electron-transport, and maximum photosynthetic rates were compared between resource-rich and resource limited (ambient) environments, these values declined or remained constant in the C_4 grass and C_3 forb species, while they increased in C_3 legumes. The higher water-use efficiency of

C_4 species did not facilitate increased rates of carbon capture, as the supply of CO_2 remained constant between resource environments, but the demand decreased. These results suggest that resource limitations influenced carbon supply vs. demand of species similarly, regardless of species or photosynthetic pathway. To identify differences in patterns of plant water-uptake, I measured the stable isotope ratio of oxygen in xylem-water, soil water and precipitation to determine the primary depth in the soil profile from which these tallgrass species were acquiring water. Following rainfall events, water was used predominantly from surface soil layers (0-10cm) by all species. However, following 4-6 weeks of drought, C_3 species used water from deeper soil layers (>30cm) compared to the C_4 grasses. These results were date-independent and were consistent over all topographic positions. When water availability and plant water stress were related to water-used, C_3 species relied less on water in surface soil layers (0-10cm) as soils dried and plant water stress increased. Midday water stress of C_3 species increased primarily late in the season when plant leaf area was high, but not from decreases in surface soil layer water availability. Conversely, water-use by C_4 grasses was invariant to water availability in 0-10cm soil layer or the degree of midday water stress experienced. The best predictor of midday water stress in C_4 grasses was surface water availability, suggesting a greater dependence on moisture in shallow soil layers. Water-use, therefore, appears to vary principally between C_4 grasses and C_3 species based on surface soil layer water availability. This pattern suggests mechanisms of drought tolerance are more important for grasses while drought avoidance is more important for C_3 species. These results provide a mechanistic explanation for previously documented responses to experimental changes in precipitation in tallgrass prairie, including increased plant diversity of C_3 species and decreased productivity of C_4 grasses. Drier surface soil layers from increased variability in hydrometeorological processes reduce productivity in the dominant C_4 grass species the most, compared to C_3 species with greater belowground niche diversity and the ability to vary water-use based on water availability.

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TABLE OF CONTENTS

Chapter 1: Introduction

Overview

Plants in grassland ecosystems are characterized by the pursuit for the three resources limiting plant growth: water, nitrogen, and light (Lauenroth et al. 1978, Knapp and Seastedt 1986, Sala et al. 1988, Kucera 1991, Sun et al. 1997, Wilson 1998). Species-specific differences in acquisition and resource partitioning may aid in explaining species co-existence patterns and spatial distribution (Walter 1971, Schulze and Chapin 1987). Compared to forest ecosystems, grasslands experience greater variability in soil moisture during the growing season and year round (James et al. 2003). As a result, higher root:shoot mass ratios, and greater fine root length per soil volume are reported for grasslands relative to forests (Jackson et al. 1997, James et al. 2003). These differences may result in greater belowground competition for water compared to other limiting resources in temperate grasslands (Sala et al. 1997, Weltzin and McPherson 1997), but species responses may differ by the spatial patchiness of water availability (Grime 1994).

Patterns of productivity in the North American Great Plains are best explained in terms of large-scale abiotic parameters reflecting water availability, including mean annual precipitation (MAP), mean annual temperature (MAT), potential evapo-transpiration (PET), soil texture, and soil water holding capacity (Webb et al. 1978, Sala et al. 1988, Epstein et al. 1997). Annual productivity in the tallgrass prairie ecosystem of North America is limited by water availability (Knapp 1984, Abrams et al. 1986, Seastedt et al. 1994, Knapp et al. 2001). While the majority of precipitation falls during the growing season (April-September), rainfall is bimodal; with July and August historically dry. During the growing season, evaporative demand is high leading to increased surface soil layer drying compared to greater soil depths. The explanatory power of precipitation for aboveground productivity depends on plant type, landscape position, fire frequency, and time of year (Knapp 1985, Briggs and Knapp 1995). Grasslands are an ideal ecosystem to study the response of water availability and variability on aboveground net primary productivity (ANPP) because of the high productivity potential and climatic variability present (Knapp and Smith 2001).

Annually-burned watersheds exhibit the strongest relationship between precipitation and ANPP (Briggs and Knapp 1995). In infrequently burned grasslands, cooler soil temperature and reduced soil evaporation lead to greater light rather than water limitations (Knapp and Seastedt 1986). When examined by topography, upland regions have lower ANPP resulting from shallower soils, reduced water availability, and increased soil temperature (Gibson and Hulbert 1987, Briggs and Knapp 1995). Greater variability in resource availability in uplands could lead to greater niche separation and resource partitioning compared to other topographic positions resulting in greater species diversity (Bazzaz and Parish 1982, Tilman 1988). Lowland regions of annually-burned tallgrass prairie have higher ANPP directly resulting from greater C_4 grass cover thereby reducing species diversity in these locations (Abrams et al. 1986, Briggs et al. 1989).

Species differences in root density and depth may be identified through the use of the stable isotopes of water (Ehleringer et al. 2000, Dawson et al. 2002). Shallow soils possess water stable isotopic signatures enriched in D and ^{18}O values from evaporation of the lighter isotope (Dawson 1993, Lajtha and Marshall 1994, Boutton et al. 1999). Isotopic studies using the signature of plant xylem water as a means of detecting the source of water are well suited to address questions of water uptake as influenced by water availability (White et al. 1985, Dawson et al. 1998, Ehleringer et al. 2000, Dawson et al. 2002). Water-use is more conservative when less water is available and this translates into isotopic signatures of plant tissue reflecting varying degrees of discrimination (Smedley et al. 1991). Understanding how water-use varies by species across the landscape may be influential in explaining patterns of biodiversity as influenced by abiotic environmental drivers (Dawson et al. 1998, Briggs and Knapp 2001).

As a mechanistic driver of plant success, water-use will likely vary in the tallgrass prairie from competition and acquisition at the individual, community, and ecosystem scales. Understanding differences and similarities among species with different morphologies, photosynthetic pathways, and functional types will aid in our ability to predict the response of this community to both natural and anthropocentric change in water availability. This dissertation is organized to examine the effects of water as a limiting resource influencing individual plant physiology and species composition within the tallgrass prairie.

Study System

The Konza Prairie Biological Station (KPBS), located in the Flint Hills of northeastern Kansas, USA (39°05' N, 96°35' W), maintains an interdisciplinary research program evaluating the influences of fire, grazing, and climate on the faunal and floral ecology of the mesic prairie. KPBS was established in 1971 with land donated to *The*

Nature Conservancy and is managed by the Division of Biology at Kansas State University. In 1981, KPBS joined the Long-Term Ecological Research (LTER) program funded by the National Science Foundation, as one of the original six field sites designated to document temporal and spatial trends with key ecological patterns and processes within and across biomes.

KPBS is unplowed tallgrass prairie dominated by relatively few perennial warm-season grasses (*Andropogon gerardii, Sorghastrum nutans, Schizachyrium scoparium, Panicum virgatum*), yet supports a species-rich flora community of over 500 plant species including herbaceous forbs, shrubs and trees, and both cool and warm-season grasses (Freeman 1998). KPBS experiences a temperate mid-continental climate characterized by periodic droughts and large seasonal and interannual variability in rainfall. This climate type results in cold, dry winters and warm wet summers with the majority of the annual precipitation occurring between April and September (835mm mean annual precipitation)

Historically, the Flint Hills were not cultivated because the steep hillsides and the rocky, shallow soils of the region prevented conventional tillage practices. For this reason, the region contains the largest remaining area of unplowed tallgrass prairie in North America. Another extraordinary aspect of the KPBS is its experimental design. KPBS is divided into 60 watersheds used to study three factors critical for maintaining the tallgrass prairie ecosystem: periodic fire, ungulate grazing, and a variable climate. KPBS is one of the oldest LTER sites, and experiments with fire and grazing have been carried out over long intervals (e.g. 20 year burns).

Objectives and Hypotheses

Chapter 2: Species responses

In the tallgrass prairie, C_4 grass species dominate in terms of cover and productivity (Knapp et al. 1998), most likely from lower water and nitrogen requirements compared to C_3 species (Pearcy and Ehleringer 1984). While morphological and physiological characteristics of C_4 photosynthesis contribute to increased efficiency of water-use (Black 1973, Pearcy and Ehleringer 1984), other characteristics influence resource acquisition and the subsequent dominance hierarchy of this ecosystem

Inter-specific differences in photosynthetic performance can be attributed to site characteristics (burn frequency, topographical position), morphology, phenology, and initiation of spring growth (Knapp and Seastedt 1986, Briggs and Knapp 1995). Generally, species success depends on the ability to take advantage of the resources present, tolerate the local climatic conditions, and to compete with the other biological organisms present. Despite the success of C_4 grasses, C_3 forbs persist and contribute to the high biodiversity in tallgrass prairies. Forbs persist by existing within the canopy of the grasses, inhabiting areas where grass abundance is low (Briggs and Knapp 2001) and taking advantage of shifts in resource availability arising from both temporal and spatial variability (Turner and Knapp 1996). Determining the role those inherent physiological differences among grasses and forbs play in their relative success remains unclear because studies have been conducted when some level of resource limitation exists in the field (Turner et al. 1995, McAllister et al. 1998) or in the laboratory when resource deficiencies are alleviated and competition between individuals is absent.

In this study, my objectives were to quantify multiple photosynthetic parameters including maximum capacity, substrate regeneration rates, actual photosynthetic rates, and water stress and availability. These parameters were measured on several common grass and forb species growing together under two varying regimes of resource availability. For one treatment, species were measured in aboveground microcosms and provided adequate water, N, and light. For comparison, the same species were measured in ambient upland prairie conditions which commonly have water and N deficiencies. Using this comparative approach, I tested two hypotheses:

1. The success of C_4 grasses in tallgrass prairie results from inherently higher photosynthetic capabilities and assumed traits that lead to high resource capture and use-efficiency evident under both ideal conditions and in the field
2. Photosynthetic variables of C_3 non-leguminous forbs will be more negatively influenced by the resource-limited field environments compared to C_4 grasses and C_3 legumes.

Chapters 3 & 4: Community responses

Species coexistence among plant communities can result from spatial and temporal differences in water uptake (Walter 1971). Shallow-rooted species may be more dependent on recent precipitation events and moisture acquired from the upper portions of the soil profile. These species have been shown to have greater WUE and water stress compared to co-occurring deeper-rooted species (Flanagan et al. 1992). Comparisons of the stable isotopic signature of water in the plant xylem to the soil water signature provide an indirect technique for assessing the water used and the active plant rooting depth (White et al. 1985, Dawson et al. 1998, Ehleringer et al. 2000, Dawson et al. 2002). This is possible because the isotopic signature of ground water does not change throughout the course of a year, but represents the long-term average of precipitation inputs in a given area (Flanagan and Ehleringer 1991). Non-woody grassland species store very little water within the plant tissue, thus xylem-water within herbaceous plants can exhibit isotopic signatures indicative of a precipitation or an irrigation event only 1-8 hours later (Dawson 1993). By measuring the reliance of plant species on shallow or deep soil moisture, we can advance our understanding of species response to abiotic environmental drivers and the potential response to changes in precipitation pattern may be more reliably predicted (Dawson et al. 1998, Briggs and Knapp 2001).

Shallower soil layers have heavier δD and $\delta^{18}O$ signatures from evaporation of the lighter isotope (Dawson 1993, Lajtha and Marshall 1994, Boutton et al. 1999). However, plants using water deeper in the soil profile have signatures reflecting use of seasonally lighter $\delta^{18}O$ and will be able to transpire from and equilibrate with a greater portion of the soil profile (Gat 1998, Ehleringer et al. 2000). Lighter isotopic signatures in plants occur from access to permanent ground or stream water (Dawson and Ehleringer 1998). Water-use is more conservative when less water is available and this translates into isotopic signatures of plant tissue reflecting varying degrees of discrimination (Smedley et al. 1991).

Most grassland species are shallow-rooted, and have the greatest concentration of roots in the upper soil surfaces and acquire the majority of their water from the upper 20cm of the soil profile (Sun et al. 1997, Rice et al. 1998). Shrub species also concentrate their roots in the upper soil surface, but have a greater proportion of roots penetrating deep into the soil compared to grasses (Sun et al. 1997). This allows shrubs and woody species to utilize water from deeper soils (> 1m) during dry periods and switch to surface soils following rain events (Boutton et al. 1999). The root concentration and distribution of forb species differs from both grasses and shrubs and oftentimes, between forb species. Generally, forbs have lower root densities in the soil profile and are more deeply rooted than grass species (Sun et al. 1997). Using the stable isotopes of water, these interspecific differences in belowground rooting patterns may be more completely evaluated.

Understanding plant species differences in the use of various soil water pools is the first step in describing how water is partitioned in the soil profile. Water availability influences plant uptake and water stress. Midday water potential measurements reflect plant water status associated with the seasonal dry-down of the soils or increased transpiration and conductance due to water availability. The combination of measuring soil moisture, available plant water, and species differences in water availability can help evaluate the various patterns in the tallgrass prairie ecosystem and allow us to make more reliable predictions about altered precipitation regimes.

While species-specific differences in rooting depth and density have been known for the last ½ century, these descriptive characteristics do not always translate into functional patterns of plant water use by depth. Based on this need for a greater understanding of the belowground characteristics influencing aboveground patterns, two separate studies were developed. First, I wanted to identify differences in reliance on water from varying depths using the isotopic composition of plant and soil water. This technique can distinguish between the various pools of water used by the C_4 grasses, C_3 forbs and shrubs and assess how species-use might vary by topography and time. The hypotheses tested were:

1. C_3 species will have greater reliance on deeper (>30cm) soil water than C_4 species regardless of time period.
2. Relationships between and among C_3 and C_4 species will be synchronous over the growing season. Generalizable species patterns will exist within photosynthetic or morphological types (C_3:C_4; herbaceous:woody).
3. Species responses to water uptake and use will vary by landscape position (e.g., individuals in uplands locations will be more dependent on shallow water).

The objective of the second study was to decouple water-stress and water availability from water use by topographic position and species. Such an approach should aid in mechanistic interpretation of spatial patterns of mixed plant assemblages and niche differentiation which permits coexistence of multiple plant types. The specific hypotheses tested were:

1. Water-use will vary by as a function of seasonal wetting and drying patterns by depth, based on available soil moisture. These patterns will vary by landscape position with the greatest variability in upland positions.
2. Species changes in water potential will vary as a function of water availability. C_4 species will exhibit greater variability in water potentials, characteristic of comparatively increased dependence on surface water.

3. C_4 species will have similar patterns and relationships among available water, water stress, and water used. Conversely, C_3 species will differ from each other and from C_4 species in patterns and effects suggesting niche differentiation as a key to survival for these species.

Chapter 5: Ecosystem Differences

Our ability to predict ecosystem responses to changes in precipitation regime caused by an altered global climate is perhaps best examined by both experimental approaches and the use of long-term ecological data. Such an approach facilitates comparisons based on the recorded responses to ambient abiotic variability (e.g. changes in temperature or precipitation) as well as predicted variability which may exceed the range of documented observations.

Since the great drought of the 1930's, the role of water availability in maintaining the mesic-prairie plant community has been documented (Weaver and Albertson 1943). Prolonged drought stress influenced tallgrass prairie structure, as only the more drought tolerant species were able to reproduce and survive. Climate change predictions differ with regard to changes in annual precipitation amounts in the central U.S., but they are in agreement with predictions that seasonal dynamics of event distribution will become more variable. General circulation models predict precipitation events of a greater magnitude, but with a longer intervening dry periods and reduced frequency. Experimental results from Konza Prairie suggest greater precipitation variability, without alterations in quantity, can increase plant water stress and decrease productivity (Knapp et al. 2002). Thus, both precipitation amount and pattern can be important in determining productivity within this grassland. Therefore, whether surface soil dries from prolonged drought similar to Weaver's reports or by increased variability in precipitation pattern, species and community differences in response to altered precipitation pattern will likely reflect the importance of soil moisture in this system and facilitate ecosystem predictions of climate change.

Both rainfall amount and variability (seasonal timing and event size) have been shown to influence grassland aboveground net primary productivity (ANPP) in controlled field experiments. Using long-term data sets from the Konza Prairie LTER program, I analyzed patterns of natural variability in precipitation (event size, timing, event variability and length of intra-event dry periods) to assess their influence on ANPP in a tallgrass prairie ecosystem. Data were analyzed from upland sites within an annually burned watershed. Specifically, I wanted to determine if the patterns of variability present in long term Konza datasets mimicked the results found in short term experimental manipulations (Knapp et al. 2002, Fay et al. 2002). The specific hypotheses tested were:

1. Consistent with experimental manipulations of rainfall pattern, the natural variability in precipitation pattern will be a significant predictor of ANPP in upland species using long-term data.
2. Precipitation variability will have a stronger correlation with grass ANPP than forbs and will be independent of precipitation amount.

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Chapter 2: Photosynthetic traits in C_3 and C_4 grassland species: responses in microcosm vs. field environments

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Abstract

The North American tallgrass prairie is composed of a diverse mix of C_3 and C_4 plant species that are subject to multiple resource limitations. C_4 grasses dominate this ecosystem, purportedly due to greater photosynthetic capacity and resource use-efficiency associated with C_4 photosynthesis. I tested the hypothesis that intrinsic physiological differences between C_3 and C_4 species are consistent with C_4 grass dominance by comparing leaf gas exchange and chlorophyll fluorescence variables for 7 C_4 and C_3 herbaceous species in two different settings: experimental microcosms with abundant resources and natural grassland sites with more limited resources. In the microcosms, C_4 grasses had higher photosynthetic rates (A_{max} at ambient C_a), water potentials and water use efficiency than the C_3 species. These differences were absent in the field, where several variables suggested that N limitation reduced photosynthetic rates in all non-leguminous species. Thus, intrinsic photosynthetic advantages for C_4 species measured in resource-rich microcosms could not explain the dominance of C_4 species in the field. Instead, C_4 dominance in this ecosystem may depend more on the ability of the grasses to grow rapidly when resources are plentiful and to tolerate multiple limitations when resources are scarce.

Introduction

Central US grasslands are typically dominated by a few highly productive C_4 grass species (Epstein et al., 1998). These grasses are accompanied by numerous C_3 forb species (Freeman and Hulbert, 1985; Knapp and Seastedt, 1986; Freeman, 1998), which must compete with the dominant grasses for multiple potentially limiting resources, including nitrogen, water, and light (Schulze and Chapin, 1987; Knapp et al., 1998). Thus, in a grassland environment of high-light, warm temperatures, periodic drought, and low nitrogen availability, C_4 dominance over the C_3 species may result from a combination of mechanisms, including intrinsically higher capacity and efficiency of C_4 photosynthesis (Knapp, 1993; Tezara et al., 1998; Long, 1999) and key adaptations to major biotic and abiotic drivers in grasslands including fire, grazing by large herbivores, and climatic variability (Seastedt and Knapp, 1993; Ojima et al., 1994; Knapp and Medina, 1999).

The role of intrinsic physiological differences among C_4 and C_3 species in their relative success in tallgrass prairie assemblages remains unclear. In general, C_4 species have lower water and nitrogen requirements than C_3 species and reduced stomatal conductance and enzyme requirement per mol of CO_2 fixed when measured under controlled laboratory conditions (Pearcy and Ehleringer, 1984; Long, 1999; Sage, 2004). The performance of C_3 and C_4 species in the field should reflect these inherent C_4 advantages (Knapp, 1993). However, the expected physiological advantages of C_4 species are often not realized in the field. This discrepancy may result because laboratory studies often poorly reproduce field growth conditions of water, nutrient, and light limitation, or the plant material studied is unrepresentative of field populations (Evans and Seemann, 1989; Wohlfahrt et al., 1999; Gibson et al., 1999). Further, most field studies of photosynthesis have been conducted when some level of interspecific competition exists, rendering comparisons with laboratory studies difficult (Tieszen et al., 1997; Knee and Thomas, 2002; Vitale and Manes, 2005).

I compared photosynthetic traits in several common tallgrass prairie grass and forb species in environments that differed in resource availability. Species traits were measured in experimental outdoor microcosms and in upland field sites in an undisturbed annually burned prairie. The microcosms provided adequate light, nitrogen, and water and minimal variation in these resources, thus enabling us to estimate *potential* photosynthetic traits in a controlled setting. The field sites represented typical levels of resource limitation common in mid-summer in tallgrass prairie and served as an indicator of *realized* photosynthetic traits. Photosynthetic and water relations traits were measured in mid-July, a time that is typically dry, hot, and when the spring pulse in soil N availability has been depleted; conditions favoring species with higher resource-use efficiency. I tested two hypotheses related to the success of C_4 grasses in this system: (1) C_4 grasses have higher photosynthetic capacity and resource use efficiency than C_3 species under both microcosm and field conditions and (2) because N can be particularly limiting in annually burned sites in this grassland (Seastedt et al., 1991), photosynthetic capacity and resource use efficiency of C_3 non-leguminous forbs will be more negatively influenced in the resource-limited field environment compared to C_4 grasses and C_3 legumes.

Materials and Methods

Study site

Research was conducted on the Konza Prairie Biological Station (KPBS), a 3487 ha unplowed native tallgrass prairie preserve located in northeast Kansas, USA (39°05' N, 96°35' W). KPBS experiences a mid-continental climate of cool dry winters (- 3.0 °C average) and hot summers (27 °C), with the majority of the annual precipitation (835mm) falling between April-September. The vegetation at KPBS consists of approximately 540 species of C_3 herbaceous, woody, and grass species and 31 C_4 species, dominated by the grasses *Andropogon gerardii* Vitman, *Sorghastrum nutans* (L.) Nash, and *Panicum virgatum* L. (Freeman, 1998; Towne, 2002). The species used in this study were from three functional groups: C_4 grasses *A. gerardii* and *S. nutans*, C_3 non-legume forbs *Aster ericoides* L., *Echinacea angustifolia* DC. var. *angustifolia*, and C_3 leguminous forbs *Amorpha canescens* Pursh, *Lespedeza capitata* Michx., and *Psoralea tenuiflora* Pursh var. *floribunda* (Nutt.) Rydb. All are common and widely distributed species in the central North American grasslands.

Comparative growth environments

Microcosm facility

Plants were measured in eight 2.6 m^3 (1.2 m x 1.2 m x 1.8 m deep) microcosms containing newly established assemblages of the study species. The microcosms provided high water, light, and nutrient availability, and favorable soil conditions for root growth. Individuals were planted in late May 2003 by broadcast seeding the grasses and planting greenhouse-grown seedlings for the forbs. All seeds were from commercial sources. The legumes were not inoculated with *Rhizobium* prior to planting. Planting densities mimicked natural stem densities and species relative abundances, and forbs were planted in an identical spatial arrangement in each microcosm to avoid variation in performance from differing species associations. Microcosms were frequently weeded to maintain the desired species composition and were watered approximately 3 times per week to minimize water stress. The soil profile within each microcosm contained well-mixed A-horizon topsoil in the top 30 cm overlying B-horizon subsoil collected on site. The microcosms were free-draining to allow for natural soil moisture profiles. Average extractable inorganic soil nitrogen measured in September 2003 was 2.80 ± 0.33 (SE) μgN-g soil^{-1}.

Field sites

I used two native upland prairie watersheds judged to provide typical field soil moisture, nutrient availability, and competitive interactions. Within each watershed, two separate sites were established where all seven species occurred within a 20m-diameter circle. Watersheds were burned each April since 1982, including the study year, a typical management practice that maintains C_4 grass dominance and results in saturating sunlight intensities for most individual leaves. Extractable inorganic soil nitrogen from these sites has been reported previously (0.93 ± 0.07 (SE) μgN -g soil^{-1}; Blair, 1997) from samples collected in September, the same time of year as that measured in the microcosms.

Sampling procedure

Measurements conducted in the microcosms were performed during July 12-19, 2003. Mid-July is typically the time period for maximum rates of growth in developmentally mature plants prior to late summer senescence. For gas exchange measurements, one individual from each species was randomly selected within each microcosm. For chlorophyll fluorescence and water potential measurements, 5-7 individuals per species/microcosm combination were measured and the average value per microcosm was used in the analysis.

Measurements conducted in the field were performed July 12-19, 2004. This time period had a similar microclimate to measurements conducted in the microcosms in 2003 (air temperature, relative humidity (RH), solar radiation, and windspeed; Table 2.1). Cumulative monthly precipitation in 2004 was 29% above average (Table 2.1), making comparisons with the microcosms conservative. For gas exchange measurements, 2 individuals from each species were randomly selected from each site, and these site measurements were averaged prior to the overall *field* analysis. This resulted in a species sample size of four for measurements of each variable. Similar to measurements in the microcosms, 5-7 individuals per species/site were measured for chlorophyll fluorescence and water potential measurements, and the average value was used in the analysis.

Physiological variables

The physiological parameters measured in this study assessed both the photosynthetic light reactions and carbon fixation biochemistry. These variables included V_{cmax}, the maximum rate of carboxylation by the enzyme Rubisco (ribulose 1,5 bisphosphate carboxylase-oxygenase), J_{max}, the maximum rate of electron transport, A_{max}, the maximum rate of photosynthesis at saturating light and ambient CO_2 (C_a, 370 μmol CO_2 -mol air $^{-1}$) concentrations, F_v/F_m, the maximal quantum yield of photosystem II (PSII), $\Delta F/F_{m'}$, the apparent quantum yield of PSII, and g_s, the stomatal conductance to water vapor at A_{max}. Additionally, predawn and midday water potentials (Ψ), were measured on multiple nearby individuals on the same day as gas exchange measurements were performed. Water-use efficiency (WUE) was calculated using A_{max} at C_a divided by the corresponding transpiration rate.

Gas exchange measurements were conducted using a LI-COR 6400 IRGA with an artificial red/blue LED light source (LI-COR Inc., Lincoln, Nebraska, USA). Plants were placed inside the cuvette and allowed to reach steady-state photosynthesis at ambient C_a (370 µmol-mol^{-1}) and at a saturating light intensity (1500 µmol-m^{-2}-s^{-1}). Leaf temperature was allowed to increase with ambient daily air temperature. To compare changes in the photosynthetic rate (A), as internal CO_2 (C_i) concentration increased, A/C_i curves were constructed by progressively increasing CO_2 concentrations inside the IRGA cuvette from 40 to 1500 µmol-mol^{-1} in twelve steps for the C_3 species and from 360 to 0 then 360 to 1500 µmol-mol^{-1} in eleven steps for the C_4 species. Using this measurement protocol, similar photosynthetic rates at ambient C_a (370 µmol-mol^{-1}) before and during curve construction suggest steady-state activation of Rubisco was maintained across the range of CO_2 concentrations measured (Long and Bernacchi, 2003). For both photosynthetic types, the majority of steps were measured in the linear (sub-atmospheric CO_2) region of the curve. Between each CO_2 concentration change, A, g_s, and flow rate were allowed to stabilize. Species order for A/C_i construction was random between consecutive days of measurement, and all measurements were conducted between 0900 and 1500 daily. Fluorescence measurements of $\Delta F/F_m$' were performed with a modulated fluorometer (OS1-FL, OptiSciences, Tynsboro, Massachusetts, USA) concurrent with the gas exchange measurements. For measurements of F_v/F_m, leaves of each species were selected and placed in dark-adaptation cuvettes pre-dawn, allowing at least 30 minutes prior to measurement. Plant water status was assessed on the measurement dates by measuring predawn and midday leaf water potentials (Ψ) with a pressure chamber (PMS-1000, PMS Instruments, Corvallis, OR, USA).

Model derivation of photosynthetic traits

Rates of leaf-level photosynthesis are determined by the minimum of two co-limiting reaction velocities: CO_2 availability limits Rubisco activity, or ATP synthesis in the light reaction of photosynthesis limits the rate of regeneration of RuBP (ribulose-1,5-bisphosphate) (Farquhar et al., 1980; von Caemmerer and Farquhar, 1981). To estimate the rate of the limiting reaction, I modeled V_{cmax} and J_{max} using photosynthetic pathway-specific models because in C_4 species one must account for the spatial separation of initial CO_2 fixation by PEP-C (phosphoenolpyruvate-carboxylase) in the mesophyll cells from the bundle sheath chloroplasts (Berry and Farquhar, 1978). Additionally, C_4 models must account for leakage of CO_2 out of the bundle sheath that occurs because of the concentration gradient between bundle sheath-mesophyll cells.

C_3 model

To estimate the parameters V_{cmax} and J_{max} for C_3 plants, I used the derivation of the Farquhar et al. (1980) model reported by Medlyn et al. (2002). Temperature-dependent parameter estimates include the Michaelis-Menten coefficients of Rubisco for CO_2, O_2, and the CO_2 compensation point in the absence of mitochondrial respiration. For these parameters, I used the equations developed by Bernacchi et al. (2001) and modified the estimates according to measured leaf temperature at the time of gas exchange measurement. By fitting the photosynthetic model to the measured $A{:}C_i$, estimates of V_{cmax}, J_{max} were produced. Models were fit using the procedure NLMIXED in SAS 9.1 (SAS Institute Inc., Cary, North Carolina, USA).

C_4 model

I used the photosynthesis model for C_4 grasses of Chen et al. (1994) because this model was parameterized using *A. gerardii*. For the mesophyll component of the model, the rate of CO_2 fixation by PEP-C was modeled according to Michealis-Menten kinetics incorporating measured C_i at incident leaf irradiance and the maximum rate of carboxylation by PEP-C. The flux of CO_2 between mesophyll and bundle sheath was a function of the difference between CO_2 concentration at each location and the internal resistance to this flux. I were able to omit the estimates describing the outward diffusion of O_2 from the bundle sheath as both of the C_4 species I measured possessed the NADP-ME subtype, in which oxygen evolution inside the bundle sheath is negligible (Chen et al., 1994; von Caemmerer and Furbank, 1999; Sage et al., 1999).

I incorporated the temperature dependence of the Michaelis-Menten coefficients (K_c, K_o) calculated in the same manner as for the C_3 model into the C_4 model of Chen et al. (1994). The kinetic constants of Rubisco in C_4 plants can range up to 3 times greater than values typically found in C_3 plants (von Caemmerer and Furbank, 1999). When developing the temperature dependence of the kinetic constants, Bernacchi et al. (2001) used transgenic tobacco (a C_3 species). Additionally, von Caemmerer and Furbank (1999) reported that a multiplicative factor of 2.5 would appropriately scale the kinetic constants calculated *in vivo* on tobacco to comparable values for C_4 plants. Therefore, I incorporated the temperature dependence of the Michaelis-Menten coefficients presented by Bernacchi et al. (2001), scaled up by a factor of 2.5 to make them appropriate for use in this C_4 model (Chen et al., 1994).

Statistical procedures

Comparisons between the dependent variables were performed using mixed-effects models (Proc Mixed, SAS V9.1). For species analyses in the microcosms, the random effect was the specific microcosm cell from which species measurements were performed. In the analysis of the field data, the effects of watershed, and site nested within watershed, were incorporated as random effects within the model. Multiple comparison tests between functional groups or species were done using Tukey's HSD.

In order to make direct species comparisons of variables between the field and microcosms, tests of equivalence were performed to test differences in the dependent variables between locations. Equivalence tests were performed using an additive model of two sample means (Proc Power, SAS V9.1. The resulting power of each paired test signified whether the locations (microcosm or field) differed (0.05 significance level).

Results

Microcosms

In the relatively resource rich microcosms, A:C_i curves had higher initial slopes (and greater CO_2 demand per supply) for C_4 compared to C_3 species (Fig. 2.1). The average maximum difference in the photosynthetic rate between species was less than 10 μmol-m^{-2}-s^{-1} and species within the same functional group responded similarly (Fig. 2.1). ANOVA results show significant differences were present among species in the microcosms for each of the variables measured except J_{max} (Table 2.2). For V_{cmax}, no difference between C_3 species was evident in the microcosms, but rates for C_4 species were significantly lower reflecting the lower N per unit area for C_4 species (Fig. 2.2a; Lambers et al., 1998). Species estimates of J_{max} were similar in the microcosms (Fig.

2.2b). A_{max} was significantly higher for the C_4 grasses, than most C_3 species, with the exception of *A. ericoides* (Fig. 2.2c). Measurements of leaf fluorescence (F_v/F_m and $\Delta F/F_m$') were lower for C_4 species than C_3 (Table 2.3). While C_3 species had significantly higher values, species differences between C_3 legumes and C_3 forbs were not present for either fluorescence variable.

Species within a functional group responded similarly for predawn and midday water potentials in the microcosms (Fig. 2.3). C_4 grasses had the highest predawn and midday water potentials, while C_3 forbs and legumes did not differ for either water potential measurement (Fig. 2.3). Species patterns of stomatal conductance to water (g_s) were similar; except for *A. ericoides* which had significantly higher g_s (Fig. 2.4a). C_4 grasses had significantly higher water-use efficiency (WUE = *A*/E; Fig 2.4b), resulting from comparatively higher *A* (Fig. 2.2c) and lower g_s than C_3 species (Fig. 2.4a). WUE did not vary significantly between C_3 species (Fig. 2.4b).

Field

In the more resource limited field sites, A/C_i curves had greater variation among species, with nearly a 20 μmol m^{-2} s^{-1} difference in photosynthetic rates between *L. capitata* and the C_4 grasses at $C_i > 500$ μmol-mol^{-1} (Fig. 2.1). The initial linear slope of the A/C_i curves for C_4 grasses was similar to C_3 species (Fig. 2.1). For the variables measured, only V_{cmax}, J_{max}, F_v/F_m, predawn water potential, and g_s varied significantly by species according to the ANOVA results (Table 2.2). C_4 grasses had the lowest rates for V_{cmax}, J_{max} and A_{max} compared to C_3 forbs and legumes (Fig. 2.2a,b,c). For estimates of V_{cmax} and J_{max}, the C_3 legumes *L. capitata* and *P. tenuiflora*, were significantly higher than other species (Fig. 2.2a,b). For field measurements, A_{max} in C_4 grasses was the lowest of the three functional groups (Fig. 2.2c). Among C_3 species, the legumes had a collectively higher A_{max} than forbs, but did not vary significantly. Similar to measurements in the microcosms, field measurements of leaf fluorescence varied by functional groupings, but not between species within those groupings. Dark-adapted leaf fluorescence (F_v/F_m) was significantly higher for C_3 species than C_4 grasses (Table 2.3). C_3 species had similarly higher values of light-adapted leaf fluorescence ($\Delta F/F_m'$), but C_3 forbs did not vary significantly from C_4 grasses (Table 2.3).

Water potential measurements in the field differed by functional type. C_4 grasses had the lowest predawn, but highest midday leaf water potential (Fig. 2.3). Conversely, C_3 legumes exhibited less than 0.2 MPa average change between predawn and midday measurements. C_3 forbs had intermediary values and were not statistically different from either C_4 grasses or C_3 legumes (Fig. 2.3). Both C_4 grasses had similarly low g_s, while C_3 species varied widely irrespective of functional type (Fig. 2.4a). Averaged across forbs and legumes, C_3 species had g_s rates twice that of the C_4 grasses. Grass WUE was highest for the C_4 grasses, with similar rates among C_3 species (Fig. 2.4b).

Comparisons between locations

Species responses differed for many of the variables measured between the microcosms and field environments. Rates of A_{max} were higher in the microcosms compared to the field for C_4 grasses and C_3 forbs (Fig. 2.2c). C_3 legumes showed no difference between locations. C_3 legumes did have significantly higher J_{max} in the field compared to the microcosms (Fig. 2.2b). Similarly, V_{cmax} was higher for the legumes *L. capitata* and *P. tenuiflora* in the field compared to the microcosms (Fig. 2.2a). As expected, predawn water potentials were lower for all three functional types in the field compared to the microcosms (Fig. 2.3). Midday C_4 grass water potentials were lower in the field, but C_3 functional types did not vary between locations. For C_4 grasses, WUE was significantly lower in the field compared to the microcosms, while C_3 functional types did not vary (Fig. 2.4b).

To determine if changes in photosynthetic rates for microcosm and field measurements were influenced more by the supply or demand of CO_2, supply functions were calculated (Fig. 2.5). While the 'demand' of CO_2 is indicated by the photosynthetic rate at a given C_i, the 'supply' is the slope of a line starting from the intercept at ambient atmospheric CO_2 concentration ($C_a = 370$ µmol-mol^{-1}) (von Caemmerer and Farquhar, 1984; Lambers et al., 1998). The supply function is affected either by changes in CO_2 assimilation rate, or changes in C_i. For each of the species I measured, C_i either remained the same or increased when supply slopes were compared (Table 2.4, Fig. 2.5). For the grass and forb species I measured, decreases in CO_2 demand reduced the CO_2 assimilation rate (Table 2.4, Fig. 2.5). Conversely, for each of the legume species I measured, minor differences were evident in either supply or demand of CO_2 at C_a between locations (Table 2.4, Fig. 2.5). For each of the non-legume forb and grass species, A was always higher in the microcosms for a given C_i when compared to the field (Fig. 2.5). However, while legumes also had higher A at lower C_i in the microcosms, these species exhibit a crossover at the supply function intercept with higher A in the field at high values of C_i (Fig. 2.5).

Discussion

In the fire-prone tallgrass prairie, the success (productivity and cover) of C_4 grasses has been suggested to stem from alterations in post-fire resource availability

(Knapp et al., 1998; Knapp and Medina, 1999). The higher resource capture and use efficiencies (water, N) of these grasses may facilitate success in an environment of high light, high soil temperature, low available N, and periodic water-stress. In this study, I find that when resources were relatively more plentiful in the microcosms, C_4 species had higher rates of photosynthesis and resource use-efficiency than C_3 species. However, when resources were more likely limiting in annually burned sites, C_4 species did not display the expected physiological advantages compared to C_3 species. This result is contrary to my initial hypotheses that C_4 grasses would have inherently higher photosynthetic capacity and resource use efficiency under both microcosm and field conditions. Indeed, photosynthetic traits for C_3 and C_4 herbaceous species were quite similar when measured in the field. Marked decreases in photosynthesis in the field in the relatively more shallow-rooted C_4 grasses could be due to greater water stress, but measurements of stomatal conductance and midday water potentials were not consistent with this explanation (Figs. 2.3 and 2.4; see discussion below). Instead, these data are consistent with N being the most likely resource limiting non-leguminous species, and this limitation affected C_4 and C_3 species similarly. Therefore, contrary to my second hypotheses, C_4 grass species were affected by the N- limitations of the field locations similar to non-legume C_3 species, despite higher N-use efficiencies. Only leguminous species maintained consistent physiological attributes between the contrasting resource environments.

Measurements of V_{cmax}, J_{max} and chlorophyll fluorescence followed expected patterns for their respective functional groups. Estimates of forb and legume V_{cmax} were at least 3 times higher than those of the C_4 grass species and this range is similar to previous reports from similar species (C_3 herbaceous dicot: 29-194 μmol-m^{-2}-s^{-1} (Wullschleger, 1993); C_4 grass: 17-39 μmol-m^{-2}-s^{-1} (Collatz et al., 1992; Chen et al., 1994)). Similarly, the estimates of J_{max} for the three functional groups reported here are comparable to published studies (Wullschleger, 1993; Chen et al., 1994; von Caemmerer and Furbank, 1999). Patterns in chlorophyll fluorescence parameters F_v/F_m and $\Delta F/F_m$' did not vary between the field and microcosms with both C_3 functional groups having slightly higher average values than the grasses (Table 2.3). Similar to J_{max}, F_v/F_m and $\Delta F/F_m$' are generally higher in C_3 species from a lower energy requirement per CO_2 fixed (Pearcy and Ehleringer, 1984; Lambers et al.,1998; Pfündel 1998). The lack of a noticeable decline in J_{max}, F_v/F_m or $\Delta F/F_m$' suggests that the interaction of high air temperature with N and water-stress was insufficient to induce photoinhibition or reduce the rate of electron transport in either measurement location.

The largest reduction in A_{max} between measurement locations was found in C_4 grass species (Fig. 2.2). This reduction in photosynthetic rate in the C_4 grasses occurred despite similar rates of stomatal conductance in the microcosms and field (Fig. 2.4). This suggests that the demand of CO_2 for grass species was lower in the field. The measurements of CO_2 supply functions support this observation (Fig. 2.5, Table 2.4). For each of the grass and forb species measured, reductions in the photosynthetic rate (Table 2.4) did not correspond with reductions in C_i or g_s. The C_3 legumes in Fig. 2.5 show no response, or even a minor increase in g_s, when the microcosm and field locations were compared.

Reductions in the normal rate of A have been attributed to down-regulation and while the exact mechanisms of this process remain unknown, drought, high light, and high temperature are likely contributing factors (Lambers et al., 1998) Down-regulation following drought among C_4 grass species in the tallgrass prairie has been reported previously (Heckathorn et al., 1997). These authors reported a linear decrease in photosynthetic rate in response to decreases in leaf N content associated with the major photosynthetic enzymes (Rubisco, PEPC, PPDK) (Heckathorn et al., 1997). Similar to their results, I found significant reductions in A for a given C_i when species between locations were compared (Table 2.4). This may occur from protective down-regulation of the photosynthetic enzymes among non-leguminous species without concurrent changes in the light harvesting mechanisms within the chloroplast (Table 2.3) optimizing the use of the two-limiting substrates (CO_2, RuBP) at the ambient C_a (von Caemmerer and Farquhar, 1984). Therefore, for species growing within the upland tallgrass prairies, the combination of frequent drought-stress and fire-induced reductions in available soil N may result in greater decreases in photosynthesis from reduced leaf N, rather than the direct effects of fire or drought alone. The data reported here suggest these resource deficiencies affect non-leguminous species similarly (Fig. 2.5).

Of the physiological parameters measured relating to water-use, C_4 species were more conservative than C_3, but differences between resource environments were minimal. Stomatal conductance is the main regulator of plant responses to water-stress (Farquhar and Sharkey, 1982), yet there was little difference in g_s between locations. While WUE declined significantly from the microcosms to the field (Fig. 2.4b), patterns of change in g_s (Fig. 2.4a) suggest that water limitation was not driving photosynthetic responses, a result that is corroborated by the above-average precipitation during June and July in the summer 2004 (Table 2.1). Measurements of leaf water potential suggest that differences existed between sampling locations, but the magnitude of the water potentials measured were not sufficient to invoke water-related stresses as a mechanism (Fig. 2.3).

Previous studies on KPBS have explored the relationship between leaf-level physiology and C_4 species dominance. Turner et al. (1995) reported similar photosynthetic rates in mid-summer between the dominant C_4 grasses and other C_3 forbs despite the lower tissue N concentration and much higher water-use efficiencies for the grasses. In an experiment relating the photosynthetic rates of 27 species to relative abundance, McAllister et al. (1998), found significantly higher photosynthetic rates for C_4 grasses compared to C_3 plants on sites where C_4 species were most abundant. In general, my results support facets of both studies. In the microcosms, photosynthetic rates and WUE by the C_4 species was higher (Fig. 2.2c, 2.4b) suggesting a potential mechanism for C_4 dominance: greater competitive ability and growth when resources are available. However, photosynthetic parameters were similar between all species when resources were more limiting (field). The lack of a difference between the C_3 and C_4 species in the field, despite a lower N use-efficiency by C_3 forbs, suggests C_3 species may have greater uptake of N when available, or a greater ability to retranslocate N to storage organs following pulses in availability or the onset of water stress (Jaramillo and Detling, 1992; Heckathorn and DeLucia, 1994; Heckathorn and DeLucia, 1996).

The lack of physiological differences between C_3 and C_4 species in the field site may reflect the importance of the seasonal patterns of resource availability in this ecosystem. I specifically chose mid-July for my comparisons as resource availability would be lowest and temperatures highest, ecosystem traits that should highlight the higher use-efficiencies of C_4 photosynthesis. Previous work at this site and others has shown the maximum photosynthetic rate of the C_4 grasses occurs early in the growing season when resource availability is also highest (Knapp, 1985; 1993). In burned prairie, water and available soil N are highest during April-June and then decline for the remainder of the growing season (July-September). The greatest potential for temperature to differentially effect photosynthetic types would occur in the spring, when temperatures are likely below optimum for C_4 photosynthesis. Yet early spring fires allow for direct light to the darkened soil surface raising the soil and adjacent air temperatures (Knapp, 1984). These results suggest that the greatest advantage of C_4 photosynthesis may not arise from higher use-efficiencies when resources are low, but by maximizing growth when resources are high (Briggs and Knapp, 2001). The greater use-efficiencies of C_4 species would allow them to remain active over a longer period of the growing season, facilitating increases in activity during pulses of resource availability late in the season when C_3 species may have senesced.

Conclusions

C_4 grasses dominate the annually-burned tallgrass prairie, but the expected photosynthetic advantages of C_4 grasses identified under the more optimal conditions in the microcosms were not manifest in the field. The higher resource use-efficiencies of C_4 photosynthesis have previously been posited as keys to C_4 grass dominance, especially in the water and N-limited annually-burned prairie (Knapp, 1985; Knapp and Seastedt, 1986; Seastedt et al., 1991; Ojima et al., 1994; Blair, 1997). But my research suggests that C_4 dominance may result more from greater acquisition of resources when they are plentiful, and less on the higher use-efficiencies of the C_4 photosynthetic pathway when water and N are limiting. Therefore, in an ecosystem with multiple limiting resources, C_4

grass dominance may depend less on individual physiological responses to any particular environment, and more on the ability to maximize resource capture when available, to grow rapidly, and to persist through periods of resource limitations that may rapidly change within and among years.

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Table 2.1: Microclimate differences between measurement locations. Measurements were performed 07/12-19/03 in the microcosms and 07/16-23/04 in the field. Standard errors are expressed in parentheses.

Measurement location	Avg. temperature (˚C) at 15:00	Max-Min. temp (˚C)	Avg. daily RH (%)	Total daily solar radiation (Joules cm^{-3})	Avg. daily windspeed ($m\ s^{-1}$)
Microcosms (2003)	37.4 (1.0)	40.5 – 34.0	70.64 (4.75)	2430.3 (182.9)	2.93 (0.24)
Field (2004)	34.3 (1.8)	37.5 – 30.1	66.73 (3.27)	2138.1 (185.7)	2.57 (0.26)

Cumulative Precipitation (mm)	April	May	June	July	Total
1982-2003 (μ ± (SE))	74.1 (10.8)	116.5 (17.3)	118.6 (13.0)	101.3 (19.9)	410.4 (29.9)
1982-2003 (Max.- Min.)	233.8 - 8.5	241.7 - 15.3	255.6 - 51.0	385.8 - 5.7	831.7- 166.5
2004	81.5	67.6	213.9	165.1	528.1

Table 2.2: ANOVA results for species effects among the nine variables measured. Results are presented for both locations. For the microcosms, df = 6, 48; for the field, df = 6, 18. Instrument malfunction in the field resulted in the loss of $\Delta F_v/F_m$' values for 1 of the 4 sites measured resulting in df = 6, 12.

	Microcosm		Field	
Response	F value	*P*	F value	*P*
A_{max}	5.16	0.0004	1.02	0.444
V_{cmax}	6.06	<0.0001	16.04	<0.0001
J_{max}	1.97	0.0884	5.79	<0.0001
F_v/F_m	7.96	<0.0001	11.03	<0.0001
$\Delta F_v/F_m$'	5.15	0.0004	1.6	0.2150
predawn	6.61	<0.0001	3.3	0.0158
midday	3.56	0.0054	1.54	0.2093
g_s	7.5	<0.0001	7.86	<0.0001
WUE	15.08	<0.0001	1.55	0.2056

Table 2.3: Average values of dark (F_v/F_m) and light ($\Delta F/F_m'$) adapted fluorescence for both measurement locations. Results are presented as average functional group response because species within each group had similar responses. Varying letters following estimates indicate a significant difference between functional groups within locations ($P < 0.05$, Tukey's HSD). Functional groups did not vary significantly between the microcosm and field locations.

Fluorescence variables		Microcosm		Field	
	Group	Estimate	SE	Estimate	SE
F_v/F_m	C_4 grass	0.759 **a**	0.006	0.747 **a**	0.004
	C_3 forb	0.814 **b**	0.007	0.801 **b**	0.004
	C_3 legume	0.801 **b**	0.007	0.804 **b**	0.004
$\Delta F/F_m'$	C_4 grass	0.438 **a**	0.024	0.431 **a**	0.112
	C_3 forb	0.511 **b**	0.009	0.494 **a,b**	0.111
	C_3 legume	0.514 **b**	0.018	0.503 **b**	0.111

Table 2.4: Species-specific changes in supply slope for measurement locations. Values for supply function slopes correspond to relationships depicted in Fig. 5. Changes in photosynthetic rate (*A*) and mesophyll CO_2 concentration (C_i) between locations are reported and correspond to end member values of the supply slopes in Fig. 5. Values are given as increased (+) or decreased (-) changes when comparing the low (field) to high (microcosms) locations of resource availability.

		Slope of Supply Function				Change between locations	
Group	Species	Microcosm	(SE)	Field	(SE)	*A*	C_i
C_4 grass	*A. gerardii*	0.104	(0.01)	0.033	(0.01)	+14.820	-53
	S. nutans	0.090	(0.01)	0.055	(0.01)	+12.945	-84
C_3 forbs	*A. ericoides*	0.167	(0.02)	0.114	(0.02)	+7.228	-19
	E. angustifolia	0.099	(0.01)	0.133	(0.02)	+2.339	-52
C_3 legume	*A. canescens*	0.086	(0.02)	0.073	(0.02)	+0.621	+2
	L. capitata	0.106	(0.01)	0.112	(0.02)	-0.629	-2
	P. tenuiflora	0.010	(0.01)	0.154	(0.02)	-0.949	-45

Figure 2.1: Representative *A*:C_i curves fit for each species measured in both locations. Each curve was fit to all of the data points for each species (n=8 curves per species). Line types differ according to the three functional groups present (C_4 grass, C_3 forb, C_3 legume).

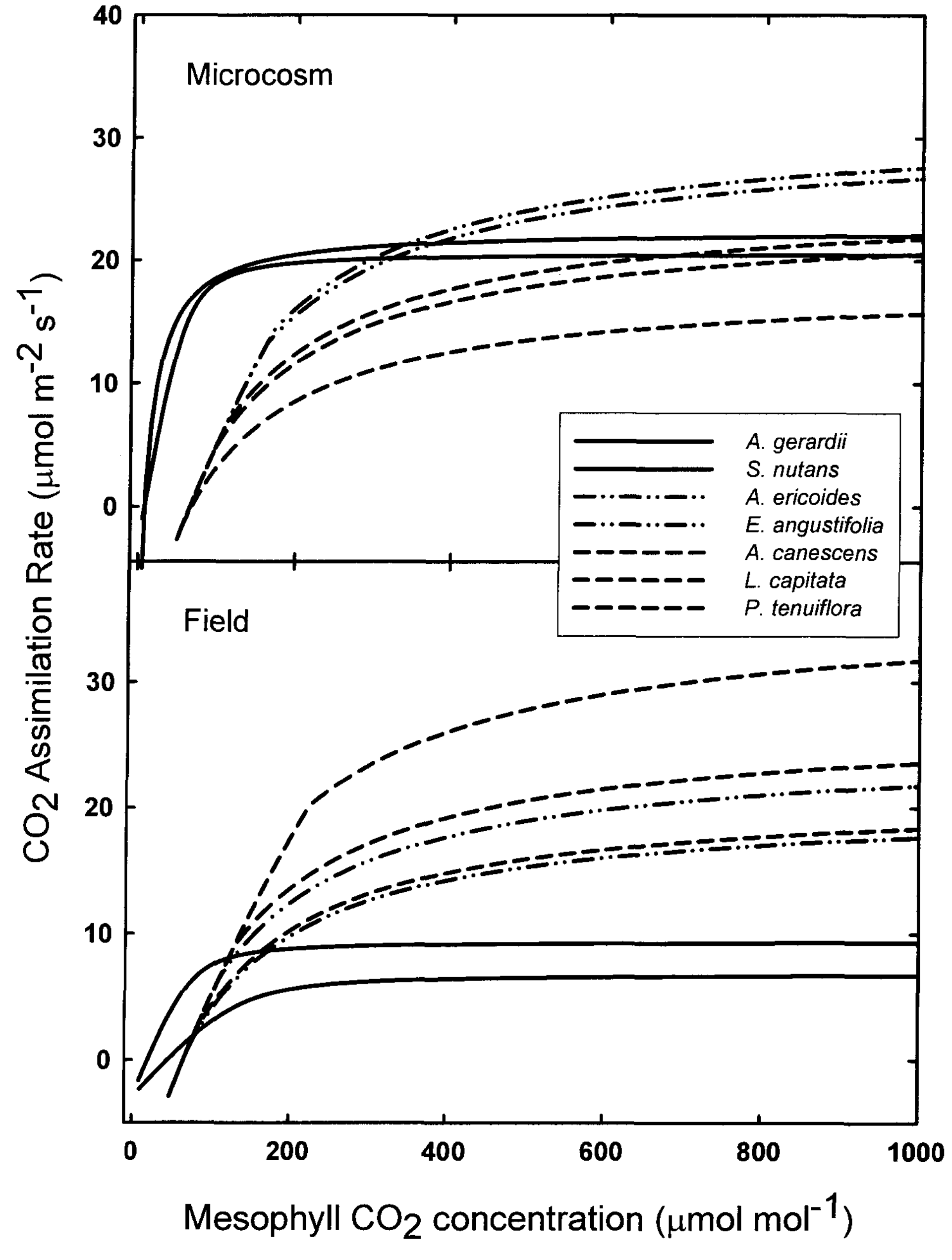


Figure 2.2: Mean species response (±1 SE) for (a) maximum Rubisco carboxylation rate (V_{cmax}), (b) maximum electron transport rate (J_{max}), and (c) CO_2 assimilation rate (A_{max}) at the ambient atmospheric CO_2 concentration (~370 μmol-mol^{-1}). Species are arranged by their respective functional group for both the microcosm and field locations. Points with varying letters indicate a significant difference ($P < 0.05$) using Tukey's HSD. Shaded areas represent a significant difference ($P < 0.05$) within that functional group between locations based on tests of equivalence.

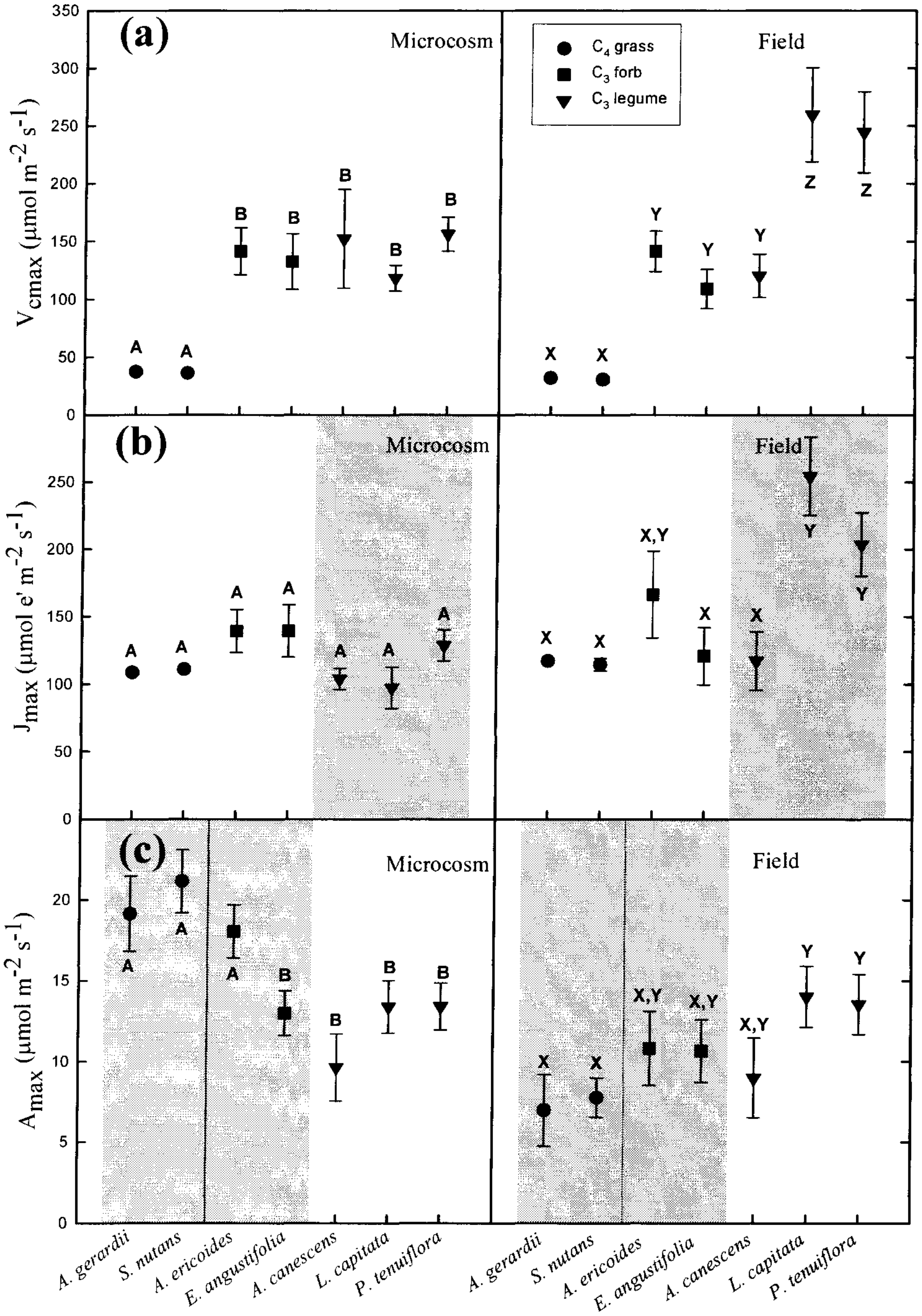


Figure 2.3: Mean functional group response (±1 SE) for plant water potentials. Predawn and midday leaf water potentials are expressed for both the microcosm and field locations. Points with varying letters indicate a significant difference (*P* < 0.05) using Tukey's HSD. Predawn values varied significantly (*P* < 0.05) by functional group between locations, but no difference between midday values occurred between locations.

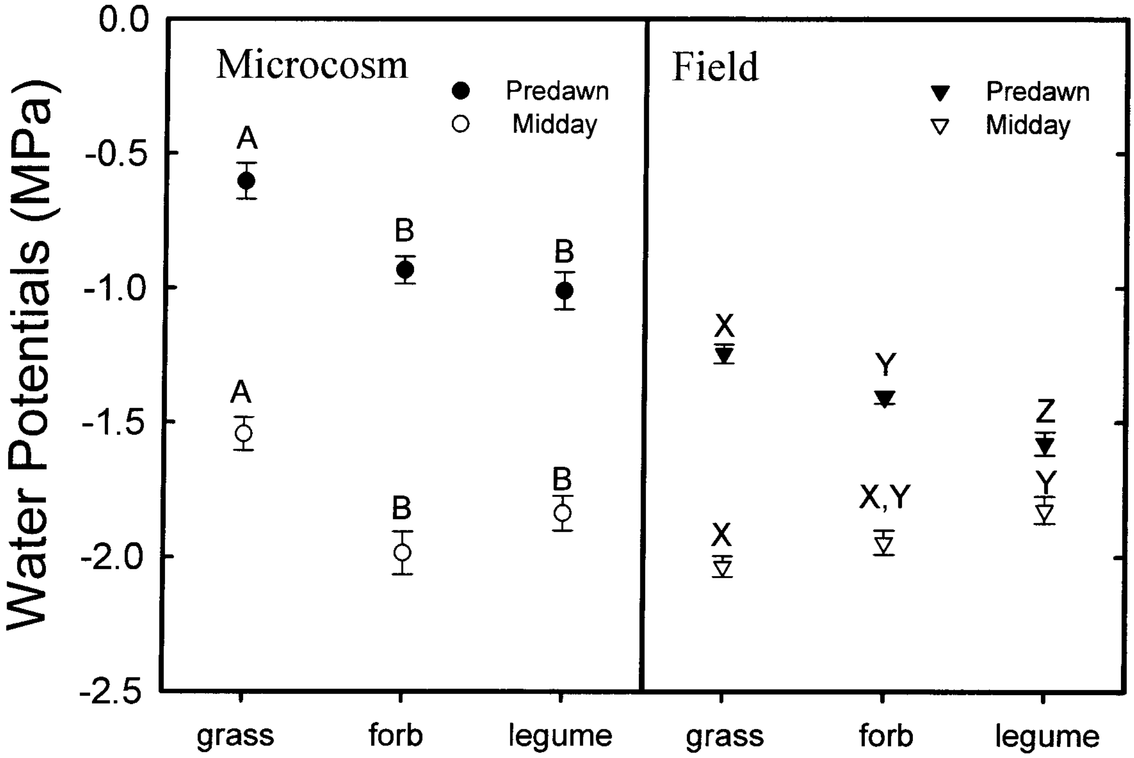


Figure 2.4: Mean species response (±1 SE) for (a) stomatal conductance to water (g_s) and (b) water-use efficiency (WUE). Species are arranged by their respective functional group for both the microcosm and field locations. Points with varying letters indicate a significant difference ($P < 0.05$) using Tukey's HSD. Shaded areas represent a significant difference ($P < 0.05$) within that functional group between locations based on tests of equivalence.

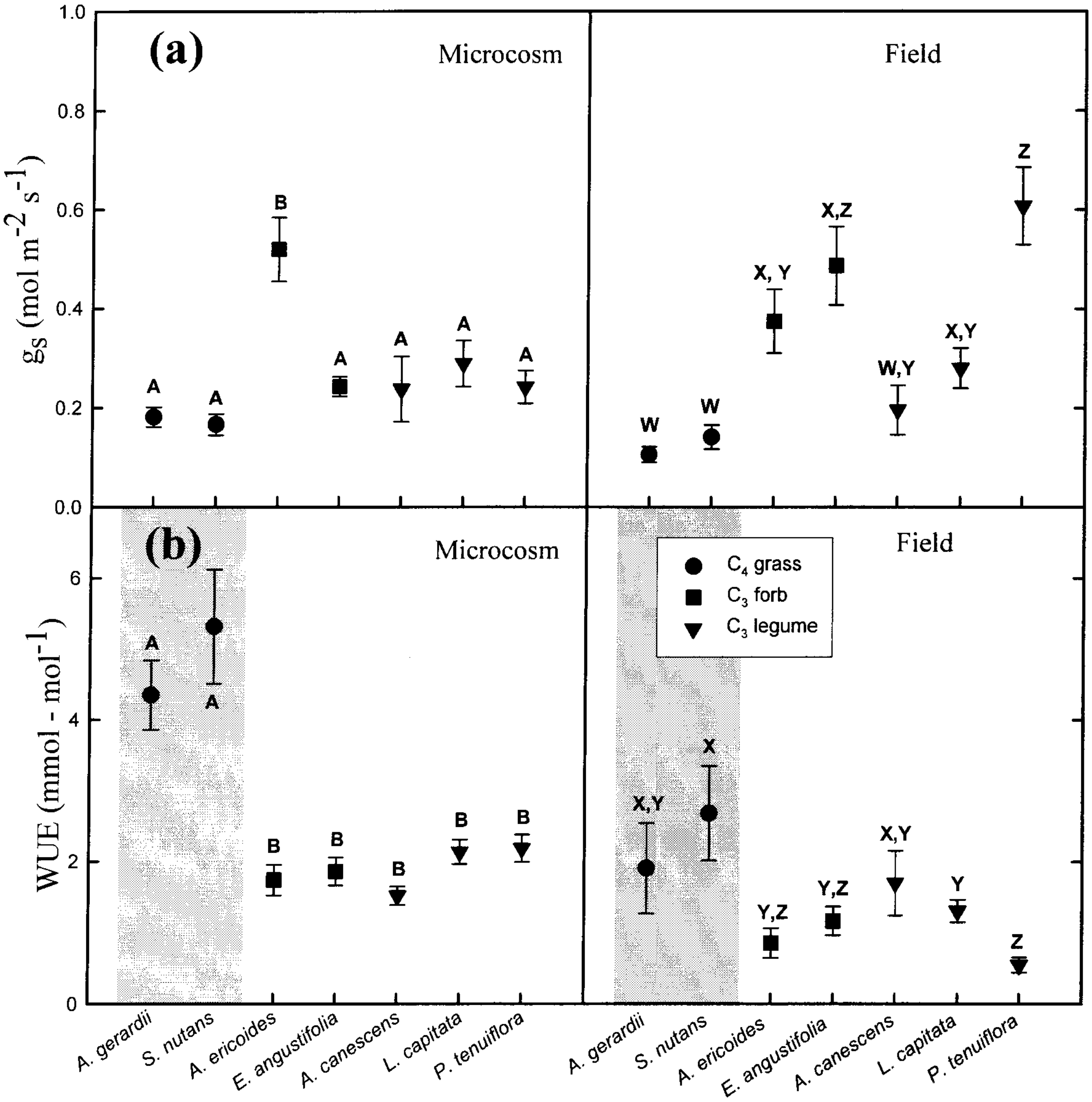
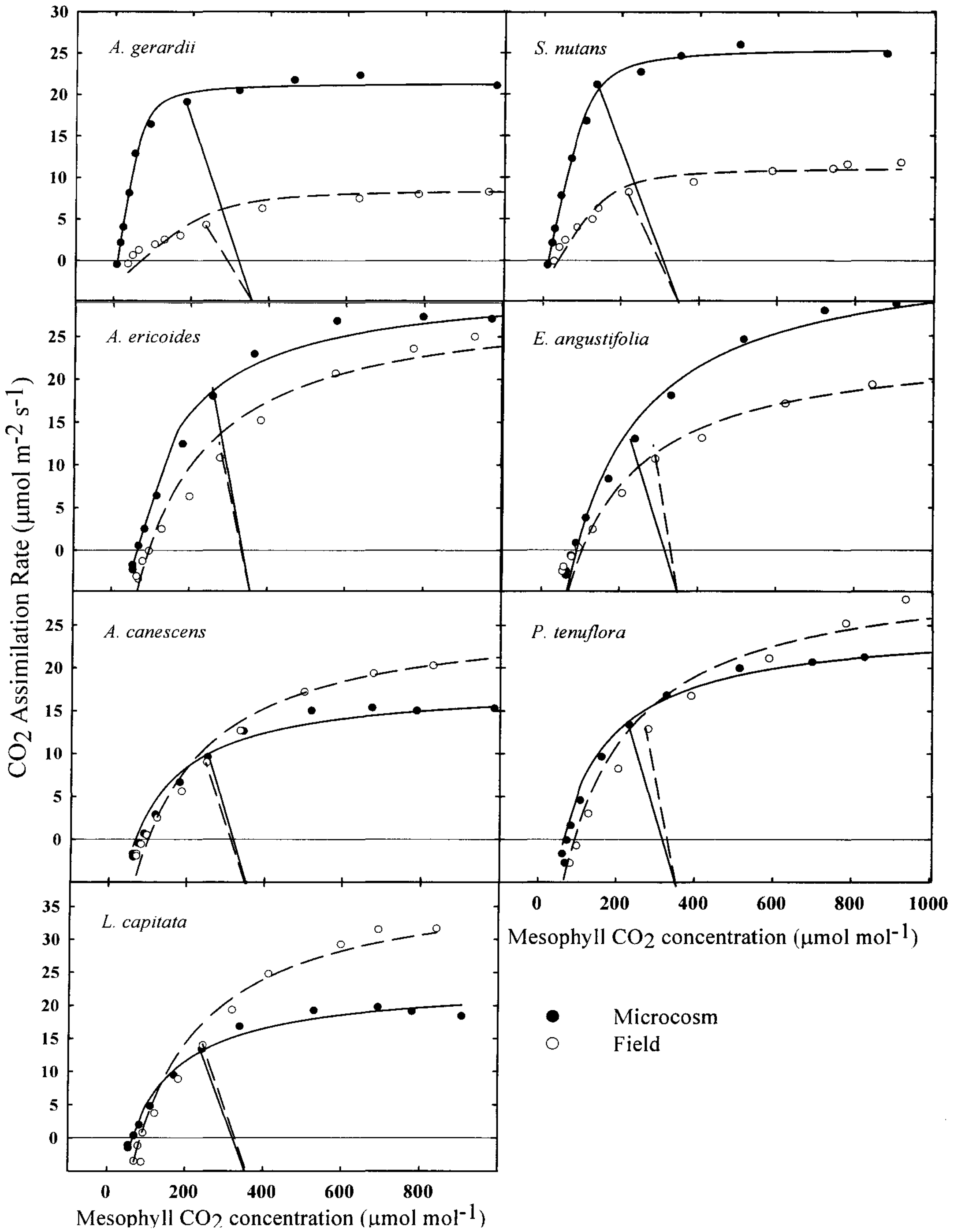


Figure 2.5: Representative A:C_i fit for each species separately. A significant (P = 0.0072, nDF = 6 dDF = 89) species*location relationship exists for the supply function. Each panel illustrates the CO_2 supply function from C_a (370 μmol-mol^{-1}) to the particular C_i associated with that measurement. Species are displayed separately with the solid line expressing the supply function for the microcosm, and the dashed line for the field.



Chapter 3: Linking function to structure belowground with grassland species

Abstract

Water availability strongly governs grassland primary productivity, yet this resource varies dramatically in time (season) and space (soil depth and topography). Moreover, it has been proposed that species differ in their partitioning of water use by depth, but evidence is lacking. I report data from two growing seasons (2004-5) in which I measured the isotopic signature of plant xylem-water from eight species growing along a topographic gradient within annually burned watersheds of the Konza Prairie Biological Station. Plant xylem $\delta^{18}O$ values were compared to soil water $\delta^{18}O$ profiles, as well as from recent rainfall events and groundwater. Species responses varied in time in both patterns of water-use and water-stress following seasonal droughts in both years. During wet periods, species differences in water use were minimal, with common dependence on recent rainfall events stored in the upper soil depths. However during dry periods, several C_3 woody and forb species used water preferentially from deeper portions of the soil profile relative to the C_4 grasses. Plants in uplands used more shallow soil water compared to those in lowlands, but the greatest differences across the topographic gradient occurred during dry periods. In both years, upland soils were drier than in other locations and exhibited a greater amount of soil water variability. These results identify the importance for assessing patterns of soil water use by plants at different landscape positions and times during the growing season. Additionally, my results suggest that C_4

grass species, with greater dependence on surface soil layer moisture, will be most susceptible to greater aridity in shallow soils depths as predicted to occur with increased temperatures and greater variability in precipitation patterns.

Introduction

Much of our present-day ecological understanding of grassland root structure and function is based on the seminal research contributed by Prof. John Weaver and his students. From the 1930's to the 1950's, Weaver et al. mapped root distribution by depth of grassland plant assemblages from Iowa to Kansas (Weaver and Albertson 1943). Weaver determined that 65% of 'true prairie' species (tallgrass) had rooting depths greater than 1.5m (up to 7m deep), and of these deep-rooted species, only 20% relied on shallower (< 1.5m) soil for water and nutrients (Weaver 1966). After decades of research, Weaver concluded that the layering of resource-use by roots within the soil profile permitted the co-occurrence of many species in the tallgrass prairie (Weaver 1966).

Weaver also documented the response of grassland species to the droughts of the first half of the last century and made inferences about the drought resistance and drought avoidance of prairie species in relation to precipitation patterns (Weaver 1966). Despite widespread changes in the grassland community in response to prolonged drought, forb populations withstood change and persisted though the drought (Weaver 1966). This response reinforced Weaver's speculation that forb and shrub species rely to a greater degree on deeper soil water than co-occurring grass species. After drought ended, previously abundant grass species eventually returned to dominance in relic prairie but full recovery took decades of resumed normal precipitation (Weaver 1966).

Could proposed future climate changes (IPCC 2001) affect patterns of soil moisture, and plant stress such that community change occurs to the extent reported by Weaver et al. under extreme drought? Atmospheric warming and greater variability in precipitation patterns are the dominating effects of anthropogenic climate change predicted to occur in the Great Plains in the next century (Easterling 2000, IPCC 2001). Previous work has shown seasonal changes in moisture availability influence C_3/C_4 biomass distributions globally (Winslow et al. 2003). The effects of increased precipitation variability will be primarily related to the distribution of soil moisture occurring by depth and time (Weltzin et al. 2003), with precipitation variability influencing productivity similar to drought (Knapp et al. 2002). The sensitivity to water availability is evident in temperate grasslands, where belowground competition for water is stronger compared to other limiting resources (Sala et al. 1997, Weltzin and McPherson 1997). Despite the recognized importance of water and its influence on patterns of ANPP in grasslands (Knapp and Smith 2001), ecological studies addressing belowground plant activity and functional rooting depth are rare, primarily because of the difficulty in assessing root growth and turnover (Polley et al. 1992, Craine et al. 2002).

An alternate approach to soil excavation and root mapping for determining functional rooting depth and resource use has been to measure stable isotopic signatures of water. Differences among individuals or species in the stable isotopic signature of aboveground tissue or gas flux can be used to infer differences in the patterns of acquisition of this limiting resource. The ratios of $^{18}O/^{16}O$ and D/H serve as conservative water mass tracers in soils and conductive plant tissue (White 1988). Changes in the stable isotopic signature of xylem water provide one such tool for quantifying species differences in the source of water used (Brunel et al. 1990). Natural fractionation affects the stable isotopic composition of water as a function of season, location, rainfall event size, and evaporative demand (see: Ehleringer and Dawson 1992). These effects lead to an isotopic signature of plant xylem water that is unique to the source from which the water was acquired (White et al. 1985). For example, soil $\delta^{18}O$ values within surface soil layers respond rapidly to evaporation and precipitation. However, deeper soil layers have water with a greater mix of year-round precipitation inputs, and a soil water signature more similar to winter precipitation.

Stable isotopic studies of plant water-use have several advantages over descriptive analyses of rooting depth. Studies have shown that the occurrence of live roots at a particular depth does not equate to uptake of water by those roots, regardless of whether the water supply is deep (Thorburn and Ehleringer 1995) or shallow (Dawson and Ehleringer 1991). Similarly, controlled comparisons of the uptake of water often fail to incorporate competitive ecological interactions that are common in plant communities (Chapin 1991, Craine et al. 2002). The use of the stable isotope ratios of water (D, ^{18}O) allows for interpretation of water uptake in relation to the precipitation history in field studies and species assemblages and provides a direct assessment of functional water uptake among co-existing plant assemblages.

My overall goal was to link belowground structure, as documented by Weaver nearly a century ago (Table 3.1), with function (water uptake). Specifically, I hypothesized that if species differences in water uptake exist within the tallgrass prairie, these would vary according to temporal patterns in precipitation and with topographic patterns of soil wetting and drying. Additionally, because grass species have a greater proportion of their belowground biomass in the upper 30cm of the soil profile compared to other grassland species (Canadell et al. 1996), I proposed that grasses would rely more on these soil water sources compared to deeper-rooted C_3 forbs and shrubs. I also predicted that C_3 species will have greater reliance on deeper (>30cm) soil water than the C_4 grasses regardless of time period. This strategy would support Weaver's observations during drought and may reduce belowground competition, permitting C_3 species to persist over time within an ecosystem dominated by C_4 species (Weaver 1966). Finally, upland sites at KPBS have shallower soils, yet experience greater evaporational demands. Therefore, I predicted that species growing on these sites would be more reliant on shallow soil water compared to lowland and hillside locations with deeper surface soil depths.

Materials and Methods

Research was conducted at the Konza Prairie Biological Station (KPBS), a 3487 ha unplowed native tallgrass prairie managed within the framework of the Long-Term Ecological Research Network (LTER). KPBS is located in the Flint Hills of eastern Kansas, USA (39°05'N, 96°35'W), a region characterized by a mid-continental climate consisting of cool, dry winters and warm, wet summers. The site is divided into drainage basins (watersheds) with varying strata of Permian chert-bearing shales and limestones. The effect of long-term weathering and erosion has created a non-uniform topography, with relief of 20-50m within watersheds, consisting of flat upland ridges, steep intermediate hillsides, and lowlands with deep soils (Figure 3.1) (Oviatt 1998). Soil depth varies by location with thin, rocky upland soils characteristic of the surficial

Florence limestone bedrock (<0.5m), while lowland soils are silty-clay loams (Tully soils) and are relatively deep (>2m) (Schimel et al. 1991, Ransom et al. 1998).

In April, 2003, transects were established in east-west directions on replicate, annually-burned, ungrazed watersheds. Watersheds had been burned in late April of each year for the past 24 years. Within each watershed, two transects > 100m long, that spanned the topographic gradient from upland to lowland were permanently marked. Samples were collected monthly from June to August of 2004 and 2005, respectively from upland, hillside, and lowland locations. Therefore, samples were collected from four replicate locales at the three predominant topographic positions over these two watersheds.

Plant Sampling

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

For the collection of plant xylem water, approximately 20-30g of plant tissue was collected from the crown region of each species. The crown region was non-photosynthetic and is located immediately below ground level, but above the rhizomes. Due to the destructive nature of the sampling, different individuals for each species were collected for subsequent sampling periods. Samples were cut into 1-3cm lengths and placed into sealed exetainer vials (Labco Ltd., UK) and immediately stored on ice until transferred to a 1-2°C refrigerator. For each grass species, each vial was a composite sample of 5-15 co-located tillers in order to provide enough water for the CO_2 equilibration process. For the forb and shrub species, one individual provided enough water per sample vial. At each sampling period, three replicates for each species were collected for each replicate location on the landscape and stored in separate exetainers. These replicates constitute the sampling unit. Replicate individuals for each species were collected at least 1m apart in order to capture more of the variability present per site.

Xylem water was extracted from plant samples using cryogenic vacuum distillation (Ehleringer and Osmond 1989, Webb and Longstaffe 2003). To ensure that water samples did not fractionate during the extraction process, water standards were also run through the line and processed. Variation in these standards was not higher than the known precision of the instrument (~0.1‰). Additionally, samples of plant tissue were routinely weighed and oven dried to ensure the time period of extraction was sufficient to vaporize all xylem water within the plant stem. The $\delta^{18}O$ of the collected xylem water was measured using direct equilibration with CO_2 (Epstein and Mayeda 1953). Stable isotopic analyses were performed using a Finnigan Delta-plus (Bremen, Germany) and Micromass VG Optima (Manchester, UK) isotope ratio mass spectrometer (IRMS) in the stable isotopic laboratories at Kansas State and Colorado State Universities, respectively. Analyses were cross-calibrated between locations using a subset of identical samples and variation was minimal (0.18‰). Both instruments operate in continuous-flow with peripheral gas bench microgas injectors. Isotopic abundance is expressed in 'delta notation' as parts per mil (‰) according to:

$$\delta^{18}O\ (‰) = [(R_{sample}/\ R_{standard}) - 1] * 1000 \qquad (1)$$

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

Soil Sampling

Soil samples were collected concurrent to plant tissues using a 5cm diameter sliding hammer corer (AMS Samplers, USA). One soil core was taken per location/sampling date to 35cm deep, or the maximum depth allowed by location. Because most of the sampling locations were inaccessible by vehicle, frequent soil coring to deep depths with a hydraulic corer was not possible, and frequent digging of large soil pits was not consistent with site management plans. In order to determine the $\delta^{18}O$ profile of soil water at greater depth, I used a *Geoprobe* hydraulic direct push corer (Model 6600, Geoprobe Systems, KS, USA) on the north transect of one of the sampled watersheds concurrent to the final sampling period in August, 2005. This instrument extracted soil samples to 2m deep (or bedrock). Cores were immediately separated into

5cm increments, stored in two-layer plastic bags and placed on ice. Until they were processed for analysis, cores were stored in a freezer at – 4 °C. The stable isotopic signature of soil water was quantified in 5cm increments for the first 30cm and in 10cm increments for the remainder of the core (Figure 3.2). Additionally, groundwater $\delta^{18}O$ was determined from water samples collected from five wells on site (personal communication: Gwen Macpherson). Samples were collected from May-September and the average value (-6.5‰) is reported for comparison to soil water values (Figures 3.2, 3.3).

Isotopic analyses of soil water were performed using direct equilibration techniques (Epstein and Mayeda 1953, Scrimgeour 1995). Briefly, 5g of homogenized, root-free sample was swept with a known CO_2/He mix and incubated at 92°F for 14 hours before direct injection into the IRMS. This period of time has been shown to be adequate for complete equilibration with CO_2 using similar soils (Hsieh 1998). The degree of equilibration between soil water and the headspace CO_2 can vary based on the gravimetric soil moisture of the original sample, as well as by soil type. For example, the isotopic signature of water in wetter soil samples deviates from the actual value by a greater degree than drier samples and soil texture may influence the speed at which this reaction occurs. To correct for this, bulk soil was collected from upland and lowland locations and dried. Dry soil from both locations was re-wetted with water of a known isotopic signature and analyzed. Based on the difference between the actual and measured water signatures, a multiple linear regression was used to correct the soil water signature based on the gravimetric water content from the time of collection. Comparison of soils from uplands and lowlands did not show a soil type difference in the isotopic water signature at varying degrees of wetness. Therefore, soil water values were corrected uniformly based on initial soil moisture alone, and not by the topographic location of collection.

Analyses

Statistical analyses of the data were based on a split strip-plot design, where watershed was the blocking variable and species within topographic position were replicated by transects. This design allowed for factor levels within a plot (e.g. all species in uplands) to overlay the levels of a separate factor level (all species in lowlands). The effects of sampling date, topographic position, species, and their interactions were used in the model to predict the isotopic signature of plant xylem water using mixed-effects models (SAS V9.1.3, NC, USA). The Satterthwaite approximation was used in the estimate of denominator degrees of freedom. The random effects structure of the model included three separate error terms: block, block*treatment individually (either transect or species within a given topographic position), and block*treatment*treatment. Sample date was not used as a repeated measure since different individuals per species were randomly selected and sampled at successive intervals. The assumptions of homogeneity of variance and the residual normality were tested and met in all analyses.

To determine the percent contribution of soil water at depth to the signature of water within the plant's xylem, mass balance equations were used (Phillips and Gregg 2003). This technique provides the range of feasible solutions for a system containing more sources than can be solved with a single isotopic signature. In order to reduce the number of contributing sources, the 5cm soil sections were combined a priori (Phillips et al. 2005). Based on a mixed-effects model ANOVA, the $\delta^{18}O$ values of soil water was statistically different by depth, topographic location, sampling date, and the interactions of sampling date with depth and sampling date with topography ($P < 0.0001$). Based on profile plots of ^{18}O content as a function of depth, topographic position, and time (Figure 3.3), I assumed that the high significance by depth was influenced by the large sample size compared (total observations = 345), but the greatest practical significance by depth across topographies and sample date was between 0-10 cm and 11-30cm (referred to hereafter as shallow and intermediate soil depths, respectively, Figure 3.3). Soil samples were averaged into these two depth categories and the associated variability with the aggregation of signature by depth is reported in Table 3.2. The stable isotopic signature of soil at common depths did not differ significantly based on watershed or transect within watershed ($P > 0.05$). The third water source used in the mass balance equations was the average of winter precipitation (October to March; -10.3‰ and -10.1‰ in winter 2003-4 and 2004-5, respectively, Figure 3.4). This signature serves as a proxy of soil water at depth (Figure 3.2) as winter precipitation infiltrates to deeper soil layers without the evapo-transpirational demand common in shallow soil layers during the growing season.

Results

Analysis of $\delta^{18}O$ values of soil water to the maximum achievable soil depth sampled (Figure 3.2) showed a progressively lighter isotopic signature of water with increasing depth. However, the majority of change in this signature occurred in the top 50cm for all topographic positions (approx. 5‰ variation across this depth for hillside and lowland positions; Figure 3.2). Of the soil cores collected to the maximum achievable depth, the profile in the top 30cm had trends similar to samples collected over all four topographic transects at this sampling date (August 2005; Figure 3.3). For shallower soil profiles sampled next to the study plants, variation in the $\delta^{18}O$ values of soil water was greater in time and by topographic position than soil depth (Figure 3.3). Exceptions to this trend occurred in June 2004, and July 2005, the two driest periods sampled. Collectively, sample periods in 2005 had greater variance than 2004 due to the dry period in July, and the comparatively heavier precipitation event preceding the August sample period (rainfall $\delta^{18}O = -2.19‰$, Figure 3.4). For all topographic positions, surface soil layers had generally heavier water signatures. Signatures of soil water during the aforementioned dry periods corresponded to low gravimetric soil water content in the soil.

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

The average xylem $\delta^{18}O$ values varied by nearly 5‰ over both growing seasons (Figure 3.5). This variation in the xylem signature was predictable based on precipitation history and differences between topographic positions over time (Figure 3.5). Precipitation was collected regularly on KPBS and analyzed for $\delta^{18}O$ (Figure 3.4). The $\delta^{18}O$ of the rainfall exhibited clear seasonal variations that reflect the influence of temperature and storm size as ambient fractionation factors in the hydrologic cycle (Gat 1996). Plant samples collected soon after rainfall events had similar xylem-water signatures across all topographic positions (Figure 3.5). Following several weeks with minimal rainfall, uplands had collectively heavier isotopic signatures than hillside and lowlands, respectively. In general, heavier signatures of uplands reflect increased evaporative enrichment compared to the other topographic positions from both reduced plant cover and shallower soils present in upland regions. Plants growing in the hillside and lowland positions had similar signatures indicative of precipitation inputs and deeper water-use (Figure 3.5).

When patterns of xylem $\delta^{18}O$ values were analyzed by species over time, the trends of heavier and lighter isotopic signatures resulting from precipitation history were similar to those seen in the topographic analysis, but differences in the magnitude of $\delta^{18}O$ were greatest between C_3 and C_4 species (Figure 3.5). C_4 grass species had a collective xylem signature that was nearly 2‰ heavier than the C_3 species following dry periods (June 2004, July 2005). Following major precipitation events, species of both photosynthetic pathways showed a convergence in $\delta^{18}O$, suggesting similar reliance on the recent rainfall (July/August 2004, June 2005; Figure 3.5).

Soil water and winter precipitation $\delta^{18}O$ values were used in mass balance equations to determine water sources contributing to the $\delta^{18}O$ value of xylem water. Based on this analysis, C_4 species had a greater average reliance on shallow and intermediate (0-10, and 11-30cm) soil water and were the least dependent on deep water compared to C_3 species regardless of time period (Figure 3.6). During some sampling periods (July-August 2004, July 2005) common soil $\delta^{18}O$ values through the top 30cm made discerning accurate contributions from the shallow and intermediate soil regions difficult due to a high number of potential solutions that achieved mass balance (see inset panel, Figure 3.6, Table 3.3). Proportional contributions from deeper water (>30cm) that satisfied mass balance consistently had more discrete ranges of variability than surface soil water. The C_3 species *V. baldwinii* showed more consistent reliance on deep water compared to all other species (Figure 3.6), as indicated by its lighter stemwater $\delta^{18}O$ value. This was most evident during June of both years, when *V. baldwinii* had 98% dependence on deep water (2004), and was the only species to not obtain more than 80% of its water from the shallow soil region (2005). The opposite trend was noted for the C_4 grass *S. scoparium* which exhibited complete dependence on shallow soil water across most sample periods, most notably in June 2005 (99%) and August 2005 (97%). Trends between photosynthetic pathways were also most pronounced in June/August 2005 when C_4 species obtained less than 3% of their water from deeper than 30cm.

Discussion

The use of $\delta^{18}O$ values as a natural tracer to assess water-use differences among species on disparate water sources has been reported widely in ecological studies. To date, most of these studies have focused on morphologically or developmentally distinct species i.e., exotic vs. native species (Ewe and Sternberg 2002), dimorphic, phreatophytic and dune species (Brunel et al. 1990, Adar et al. 1995, Brunel et al. 1995, Zencich et al. 2002), and coastal plants with varying fresh/saltwater dependence (Sternberg and Swart 1987). Alternatively, other studies have used a contrasting gradient in known water signatures to simplify the interpretation of varying water source dependence (e.g. irrigated vs. desiccated treatments (Plamboeck et al. 1999, Grieu et al. 2001, Schwinning et al. 2002). In each of these cases, either the plants or the water sources being compared were very different. This study extends the utility of these natural tracers by measuring similar co-occurring species reliant upon overlapping portions of the soil for ambient moisture. I asked the question, can differences in water-use be detected among similar and co-occurring prairie species in response to naturally-variable spatial and temporal patterns in soil moisture?

My results show range of species-dependence on soil water pools with the largest differences between grasses and forbs/shrubs. Across all time periods sampled, the C_4 grasses showed greater collective dependence on water in the top 30cm of the soil profile while C_3 species relied proportionally more on deep soil water sources (Figure 3.6). The greatest separation by species and photosynthetic pathways occurred during dry periods (June 2003, July 2004), with overall greater reliance on soil water deeper than 30cm as reflected by lighter stemwater $\delta^{18}O$ values (Figures 3.5, 3.6). This suggests that the greatest degree of niche separation between species and pathways occurred when surface soils were driest and the C_3 species preferentially-used more water from deeper soil water sources. This trend was most noticeable in my data for *V. baldwinii* in June 2005, and all C_3 species during August 2005 when shallow soil water (0-10cm) was available, but under-used compared to C_4 species.

Similar to Weaver's original contention of root depth partitioning among species to minimize competition (Weaver 1966) and Walter's 2-layer niche hypothesis for savannas (1971), I predicted that the C_3 forbs and shrubs would consistently depend on deeper water than grasses to avoid overlap for a common limiting resource. However, my results show that following major precipitation events, all species used common water pools near the soil surface (July, August 2004, June 2005; Figure 3.5).

The varying temporal dependence on soil water seen in my results suggests varying strategies of resource competition by C_3 and C_4 species. Inter-specific competition for water largely results from species differences in water demand and flux, root density, and root depth (Fitter and Hay 2002). As competition for water depletes this resource from a given portion of the soil, species must either tolerate low availability or rely upon other unexploited regions of the soil (Grime 1994). I found that when water was abundant (July, Aug. 2004, June 2005) all species were potentially competing for a similar resource: moisture in surface soil layers. However, when water was more limiting following prolonged dry periods (June 2004, July 2005), competition was reduced as the C_3 species used deeper soil moisture while C_4 grasses continued to rely predominantly on shallow and intermediate soil moisture. These results suggest that the potential for belowground competition for water between C_3 and C_4 species is highest when water is most abundant, but when water becomes limiting, C_3 forb/shrub species avoided direct competition with the C_4 grasses by using deeper sources. This difference in reliance on soil moisture by depth may be key to the stable coexistence between grasses and forbs in this grassland (Weaver and Fitzpatrick 1934, Tilman 1987)

For four of the six sampling periods in this study (July/August 2004, June/August 2005), average gravimetric water content in the shallow and intermediate soils (0-10 and 11-30cm) ranged between 25-50% across all topographic positions. Thus, it is surprising that plant water-use was not more reliant on this available source during these periods. Trends among the C_3 species measured were not consistent across all time periods, however. For example, *V. baldwinii* consistently used a greater proportion of deep water compared to other species regardless of period or precipitation history. This was most evident in June 2005, when only 60% of *V. baldwinii* water-use originated from soil in the top 10cm whereas most other species used 90%. Consistent reliance on deeper soil water suggests *V. baldwinii* has rooting strategies compatible with low drought tolerance, an inability to compete with the high rooting densities in surface soil, or higher transpiration rates and greater water demand than co-occurring species.

Species dependence on water-use also varied by topographic position throughout the season and this pattern can be interpreted best by understanding patterns of soil depth and the precipitation record. Plants growing in upland positions had heavier mean plant $\delta^{18}O$ signatures than other positions following periodic drought, but convergence on similar water pools following rainfall (Figure 3.5). Soil water $\delta^{18}O$ signatures were nearly always heavier and had lower gravimetric soil water contents in the 0-10cm portion of uplands, especially during dry periods (see June 2004, July 2005; Table 3.2). In general, the distribution of feasible solutions from source contributions to stemwater signatures was less constrained when divided into topographic positions than for the species-specific analysis, and this makes predictions of dependence on soil water pools less reliable (Table 3.3 vs. Figure 3.5). Regardless, the patterns of isotopically-heavier water in uplands following dry periods suggest greater dependence on evaporatively-enriched surface soil. The shallow soils and increased dependence on all available water in annually-burned upland prairie may contribute to the well-documented strong correlation between ANPP and precipitation amount (Knapp et al. 1993, Briggs and Knapp 1995, Briggs and Knapp 2001, Nippert et al. 2006). Furthermore, species persistence and increased upland diversity may result from reduced water availability in the upper 30cm soil profile having greater negative impacts on C_4 grasses than C_3 species (Collins 1992).

Other studies of community water-use over time have reported strong temporal trends in soil water use. Indeed, my original predictions were made based on the assumption that plants would show greater dependence on surface soil water early in the growing season when rainfall events are more frequent, but I expected greater reliance on deep water as soils dried through July-August. However the unpredictability of precipitation patterns and periodic drought in this ecosystem prevented absolute 'day of year' predictions of water-use. Other studies reporting seasonal patterns in water-use have been conducted in semi-arid systems (Schwinning et al. 2002) or environments with distinct wet/dry seasons (Zencich et al. 2002). Two years of water-use patterns in the tallgrass prairie show seasonal variation in the signature of the water-used, but this variation had distinct seasonal trends with plant samples earlier in the season having lighter signatures which became progressively heavier as the growing season progressed (Figure 3.5). These differences appear to be regulated predominantly by prevailing isotopic precipitation signatures and the preceding drought conditions prior to rainfall (Figures 3.5, 3.6). If other factors had influenced plant water-use to a greater degree (e.g. morphological development), I would expect greater temporal symmetry between years.

Patterns of plant reliance on similar water sources within and across communities have been previously reported. Attempting to correlate rooting depth to decreased precipitation inputs along an aridity gradient in Patagonia, Schulze et al. (1996) reported similar convergence from surface soils across communities irrespective of precipitation amount. Thorburn and Ehleringer (1995) showed that trees and shrubs generally use water from the wettest portions of the soil profile irrespective of habitat. Le Roux et al. (1995) reported similar patterns of community water uptake between grasses and shrubs when water was available in wet African savannas. I also report convergence on water-use by species and landscape position during periods of water availability. However, this mesic grassland ecosystem differs because species responses to soil drying varied, resulting in water-use from multiple depths that were altered by time and topographic location. This provides greater understanding of how precipitation events and landscape position can interact with species to influence their dependence on soil water pools, and provides insight into the functioning of previously documented patterns of root deployment in this grassland (Weaver 1966).

Common reliance on surface soil water is noted following rainfall events, but the greatest potential niche partitioning of soil water among species occurred during dry periods. Thus, C_4 grass species exhibit greater reliance on moisture in shallow and intermediate (0-10, 11-30cm) layers than C_3 species, which supplement water-use comparatively more with soil moisture at depth (Fay et al. 2002, Weltzin et al. 2003). However, my results contradict Weaver's original contention that structural differences in root depth between species provide functional separation of soil water use. When water in the 0-30cm soil depth was plentiful, each of the species I measured uses proportionally more of this available resource. Because increases in precipitation variability or air temperature, as predicted by climate change models, may dry surface soils more than deeper layers (Schimel et al. 1991, Burke et al. 1991, Knapp et al. 2002), the effects will be most pronounced on C_4 grass species which have a greater comparative reliance on surface moisture.

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Table 3.1: Hypothesized water-use based on Weaver's published reports of maximum root depth for the seven species measured in this study.

Plant Type	Species	Max. Root Depth (m)	Citation	Predicted water use?
C_4 grass	*A. gerardii*	2.13	Weaver & Darland 1949	Water-use will be concentrated in shallow and intermediate soil depths (top 30cm)
	S. scoparium	1.22		
	S. nutans	1.83		
C_3 forb	*L. capitata*	2.74	Weaver 1926	Soil water-use more reliant on intermediate water (10- 30cm)
	V. baldwinii	3.35		
C_3 shrub	*A. canescens*	4.87	Weaver 1919	Consistent reliance on deep water (> 30cm)
	Ceanothus spp.	4.57	Weaver & Fitzpatrick 1934	

Table 3.2: Averaged soil moisture $\delta^{18}O$ used in the mixing model analysis arranged by topographic position.

Topographic Position	Depth combined	Soil Water $\delta^{18}O$ ($\mu \pm 1SE$)					
		2004			2005		
		June	July	August	June	July	August
Uplands	0-10	-0.91 (1.09)	-4.35 (0.68)	-4.43 (0.27)	-3.80 (0.73)	1.37 (0.74)	-1.88 (0.44)
	11-30	-3.95 (0.65)	-5.99 (0.24)	-5.40 (0.36)	-5.44 (0.7)	-2.91 (0.89)	-2.36 (0.21)
Hillside	0-10	-3.58 (0.54)	-4.93 (0.44)	-4.69 (0.29)	-5.92 (0.51)	-2.09 (0.73)	-2.13 (0.43)
	11-30	-5.29 (0.38)	-5.91 (0.21)	-5.57 (0.14)	-6.89 (0.42)	-6.28 (0.4)	-3.90 (0.3)
Lowlands	0-10	-3.99 (0.67)	-4.79 (0.39)	-5.91 (0.29)	-5.50 (0.64)	-3.02 (0.73)	-2.31 (0.49)
	11-30	-5.87 (0.49)	-5.68 (0.16)	-6.07 (0.19)	-8.45 (0.44)	-7.41 (0.51)	-4.26 (0.2)
All Positions Combined	0-10	-2.83 (0.53)	-4.68 (0.3)	-5.01 (0.2)	-5.26 (0.41)	-1.25 (0.56)	-2.11 (0.25)
	11-30	-5.47 (0.3)	-5.85 (0.11)	-5.77 (0.13)	-7.05 (0.36)	-6.01 (0.42)	-3.64 (0.19)

Table 3.3: Results of the mixing-model analysis estimating the proportional contribution of multiple sources to the mixed water signature in the plant stem when analyzed by topographic position. Values represent the 50th percentile and the distribution of feasible solutions (1st and 99th percentile, respectively).

		Proportional water contribution from source (50%ile ± 99,1%iles)					
Topographic Position	Depth combined	2004			2005		
		June	July	August	June	July	August
Uplands	0-10	0.17 (0.17)	0.36 (0.35)	0.35 (0.35)	0.46 (0.25)	0.28 (0.28)	0.45 (0.11)
	11-30	0.14 (0.12)	0.47 (0.47)	0.44 (0.42)	0.41 (0.39)	0.38 (0.34)	0.36 (0.32)
	Deep	0.66 (0.08)	0.17 (0.13)	0.2 (0.07)	0.09 (0.09)	0.28 (0.06)	0.19 (0.18)
Hillside	0-10	0.08 (0.07)	0.4 (0.4)	0.32 (0.32)	0.89 (0.07)	0.3 (0.22)	0.32 (0.32)
	11-30	0.19 (0.19)	0.5 (0.49)	0.42 (0.38)	0.08 (0.08)	0.46 (0.46)	0.42 (0.38)
	Deep	0.73 (0.02)	0.09 (0.09)	0.25 (0.06)	0.02 (0.02)	0.24 (0.24)	0.25 (0.06)
Lowlands	0-10	0.14 (0.14)	0.41 (0.41)	0.49 (0.49)	0.99 (0)	0.45 (0.11)	0.7 (0.15)
	11-30	0.21 (0.2)	0.43 (0.43)	0.4 (0.35)	0.01(0)	0.36 (0.32)	0.21 (0.2)
	deep	0.62 (0.06)	0.15 (0.08)	0.1 (0.02)	0 (0)	0.19 (0.18)	0.05 (0.05)

Figure 3.1: Redrawn from Schimel et al. 1991, drawing depicts a generalized Konza watershed profile. Uplands commonly have loess caps, and soil depth increases down-slope to the lowlands. Topographic designations used throughout the paper refer to positions identified on this drawing.

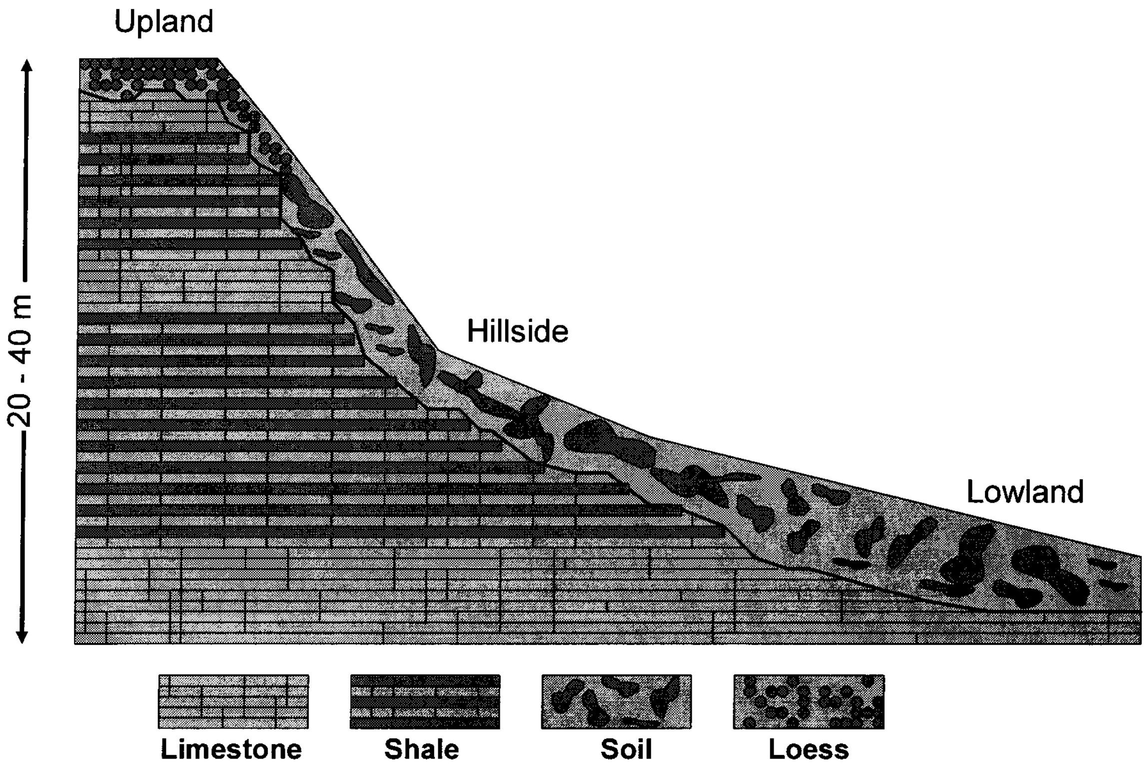


Figure 3.2: Soil water $\delta^{18}O$ profile plots to maximum achievable depth using a hydraulic soil corer (Geoprobe). Samples were collected on the north transect of watershed 1B on August 30, 2005 concurrent to the final plant stem/soil sampling period. Vertical dashed line indicates the groundwater $\delta^{18}O$ of samples collected on site.

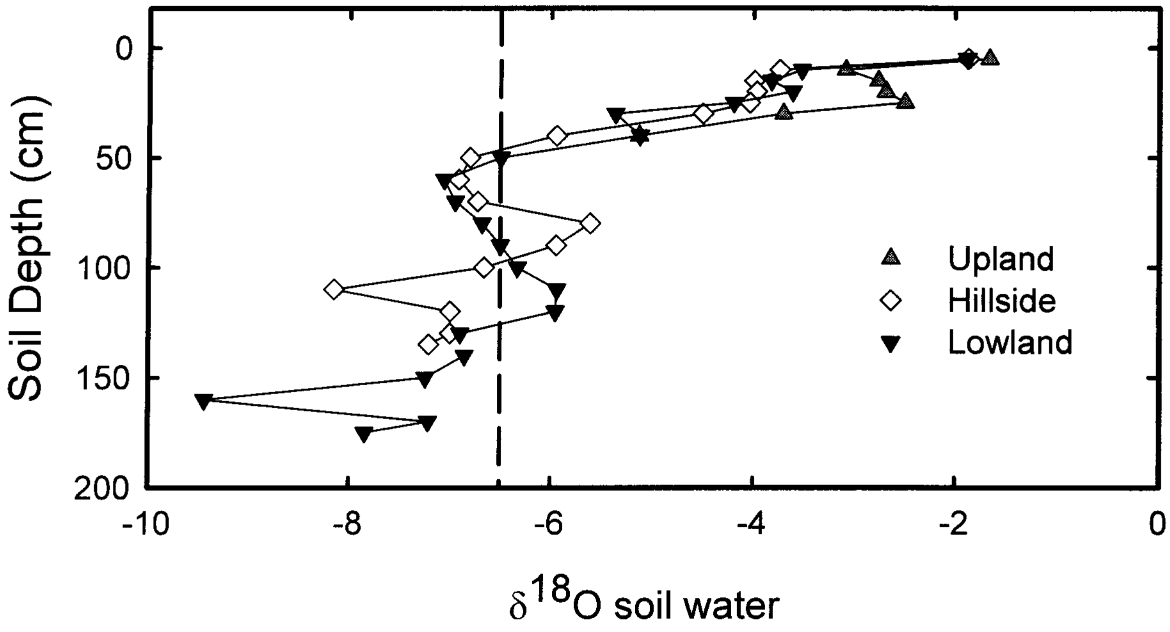


Figure 3.3: Change in the $\delta^{18}O$ values of soil water by depth for each topographic position over all sampling dates. Each point is an average value for the four transects with corresponding variance (± 1SE). Vertical dashed lines indicate the summer groundwater signature ($\delta^{18}O$) collected from wells on site.

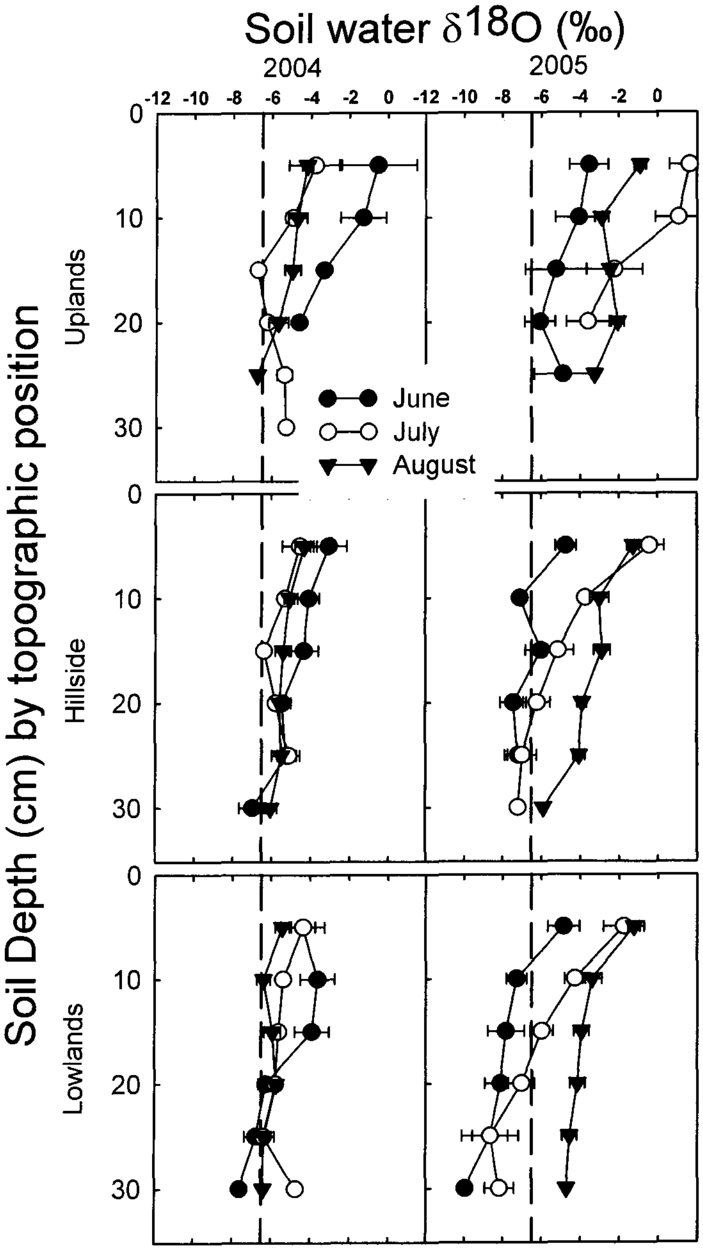


Figure 3.4: Isotopic signature of ambient precipitation events from rainfall collected on KPBS between 12/17/02 to 08/25/05. Arrows indicate sampling dates for plant stem and soil water collections. Bar height on the second x-axis correspond to precipitation amount over time on the right y-axis.

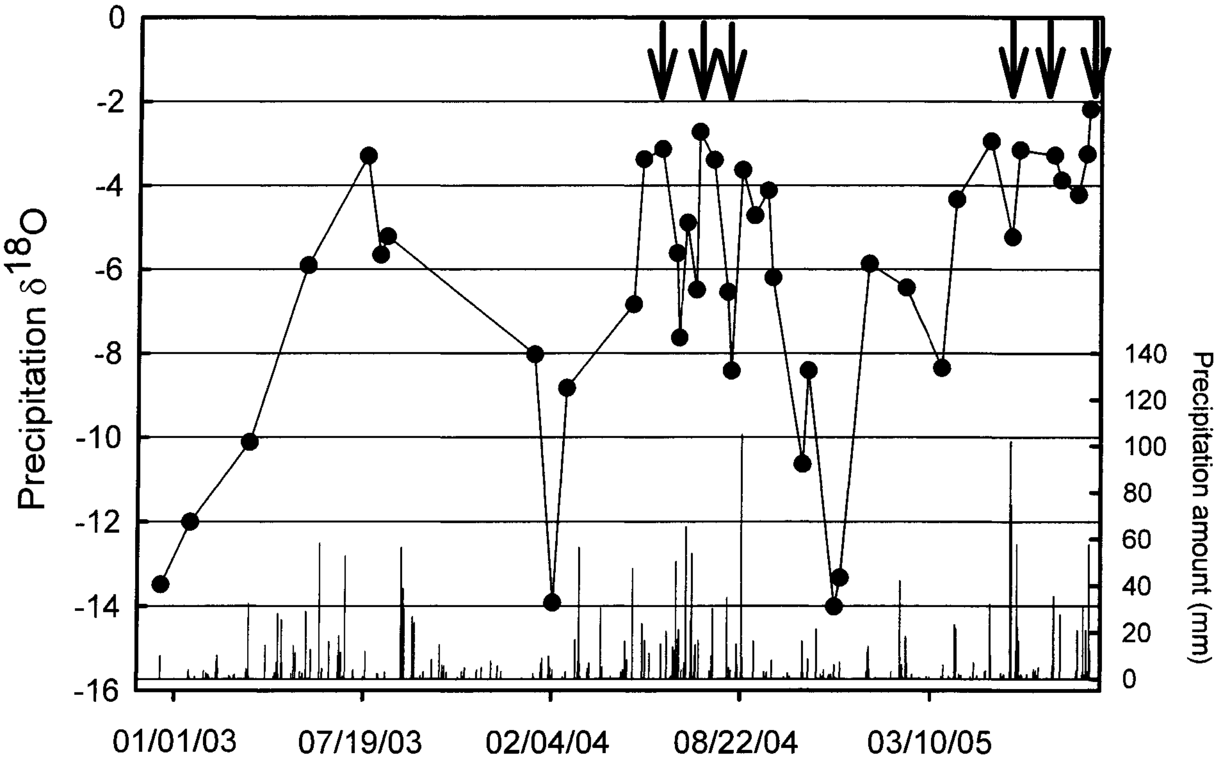


Figure 3.5: Change in the stable isotopic signature of plant xylem over time. The top panel reflects the average $\delta^{18}O$ value of all seven species plus error (± 1SE) by topographic position and the bottom panel is arranged by C_3 and C_4 functional types. Vertical bar height is indicative of precipitation amount across day of year 2004 and 2005. Asterisks indicate a significant difference ($P < 0.05$) in the xylem $\delta^{18}O$ for a given month.

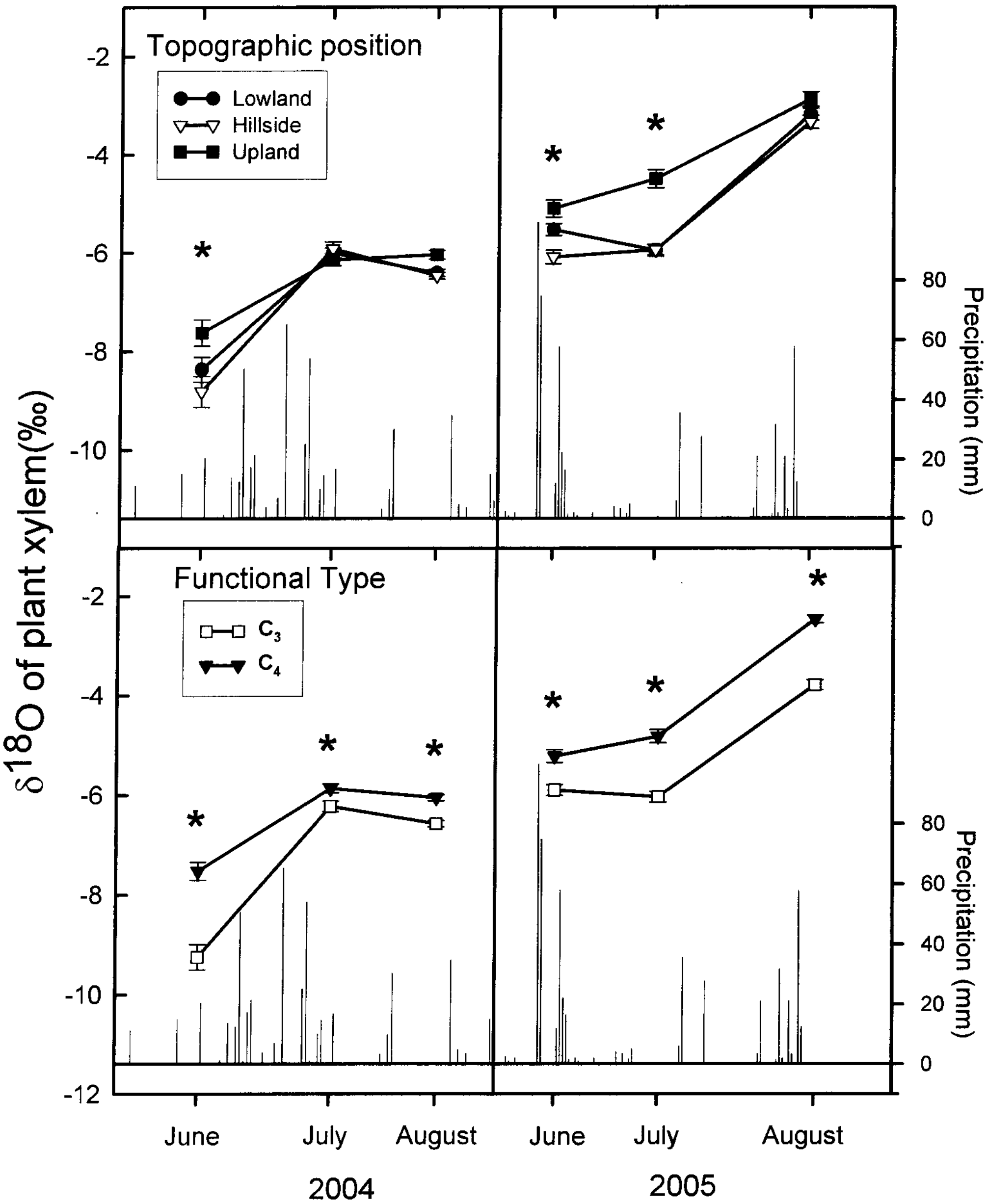
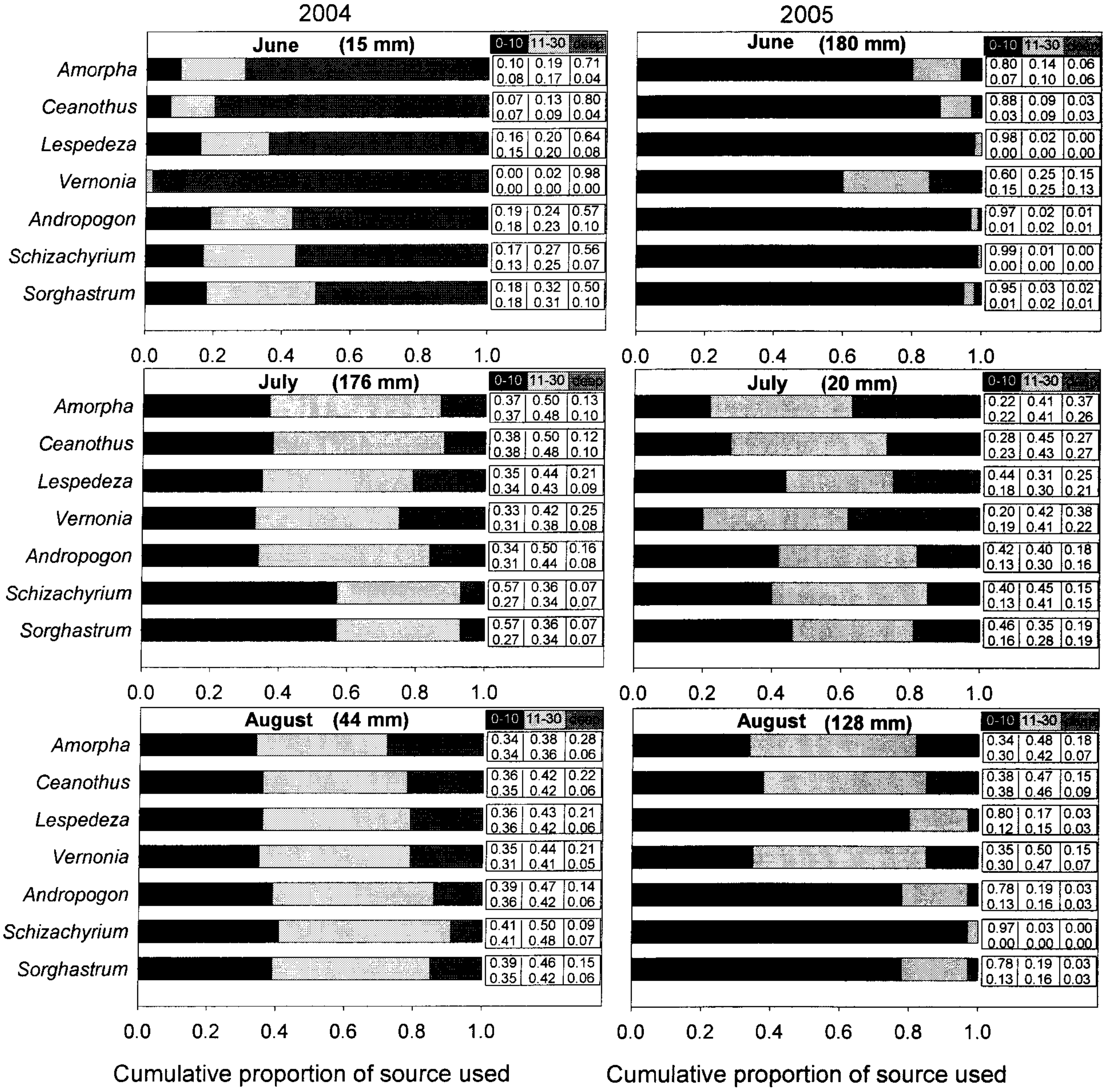


Figure 3.6: Results of the mixing-model analysis estimating the proportional contribution of multiple sources to the mixed water signature in the plant stem. Each panel depicts species differences on source reliance for a given sampling date. The parenthetical value next to each sampling date indicates the cumulative precipitation amount (mm) occurring over the 14-day period prior to sampling. Bar color corresponds to source location with bar length reflecting the magnitude of the average contribution to achieve isotopic mass balance in the mixing model. Inset table within each panel depicts the mean proportion of each source used for a given species (top) and the distribution of feasible solutions for this prediction (bottom: value added/subtracted to the mean gives the 99th and 1st percentile, respectively).



Chapter 4: Water availability and water stress determines species water-use

Abstract

The majority of tallgrass prairie root biomass is located in the surface layer of soil (0-30cm), but species differences in belowground root density and depth exist. These differences may translate into varying plant water stress that furthers our understanding of how the diverse forb community persists among a few highly dominant grasses. I collected data from 8 species representing C_4 grasses, C_3 forbs and C_3 shrubs along a replicated topographic gradient in annually burned tallgrass prairie from May to August 2003-2005. I used structural equation modeling (SEM) to relate direct and indirect effects among the isotopic signature of xylem water, leaf water potentials, and soil moisture (vol.) by depth. In general, plants with the ability to use water from multiple sources exhibited reduced variability in water potential and interspecific differences in plant water potentials were highest during dry periods. Changes in volumetric soil moisture at 5cm had smaller effects on $\delta^{18}O$ values in C_4 than C_3 species suggesting continued dependence on this portion of the soil profile by C_4 species, irrespective of changes in overall soil moisture. Similarly, water-stress in C_4 species was highly correlated to 5cm soil moisture reflecting greater C_4 dependence on surface soil moisture. Model parameters varied minimally among C_4 species, while C_3 species had varying models and relationships among the parameters therein. These results reinforce documented water-use strategies by tallgrass species, suggesting increased drought

tolerance among C_4 grasses, while C_3 species avoid drought with decreased dependence on surface soil layer moisture.

Introduction

Prairies have been referred to as 'inverted forests' (Salinger 1947), because a defining characteristic of grassland ecosystems is the high proportion of biomass and annual productivity belowground (Kucera 1991). Within the tallgrass prairie, 2/3 of total plant biomass is belowground, yet species differences in belowground biomass and proportional distributions by depth are poorly documented (Rice et al. 1998). In general, as much as 80% of total belowground biomass is located in the surface 25cm, with up to 44% of the total located in 0-10cm profile (Kucera and Dahlman 1968, Sims and Singh 1978, Rice et al. 1998). This surface concentration of roots suggests that grassland species rely heavily on shallow portions of the soil profile for the majority of their water (Sun et al. 1997, Knapp et al. 2002). Grass species in the tallgrass prairie concentrate roots in these shallow soil layers, but can have roots to depths > 2m (Albertson 1937). Generally, shrub and forb species have reduced root densities in the soil profile but have greater root diameters and a greater proportion of roots at depth (up to 3m) than grass species (Albertson 1937, Weaver 1954, Turner et al. 1995, Sun et al. 1997). This may permit C_3 herbaceous and woody species to supplement total water used with deeper soil water during dry periods and yet use shallow soil water after rain events (Boutton et al. 1999).

Total root biomass varies seasonally as root growth, length, and turnover respond phenologically to plant carbon balance and changing soil moisture and precipitation patterns (Hays and Seastedt 1987, Rice et al. 1998). This response is a direct product of the competition for soil water as well as nutrient availability (Weaver 1966). Root dynamics in the surface 10cm are most closely associated with soil water content and patterns of root turnover reflect patterns in wetting and drying. Drought effects vary between and within growing seasons in response to temporal and spatial variation in water availability (Kucera et al. 1967, Rice et al. 1998). Drought influences total root biomass negatively, with increased root branching, maximum depth obtained, and surface root death (Weaver 1966, Hays and Seastedt 1987).

Understanding landscape patterns in diversity requires information on the inherent responses of species to environmental drivers (Turner and Knapp 1996). Competition for available soil moisture and other limiting resources likely influences patterns of tallgrass species coexistence and persistence (Weaver 1966). High uptake rates and transport of water are mechanisms enabling success in pulse-driven ecosystems like the tallgrass prairie (Schulze and Chapin 1987). Temporal variability in soil water may influence other physiological variables such as plant water potential and ANPP (Knapp et al. 1993). In general, plants primarily use the most available water source, driven by water potential gradients from the surrounding soils (Thorburn and Ehleringer 1995). Differences in water-use between species or functional types may allow for indirect examination of drought-avoidance or drought-tolerance within the community assemblage. Grasses may possess an advantage for uptake in surface soils from more fibrous roots and a greater ability to acquire pulsed availability of water (Caldwell and Richards 1986). The combination of measuring soil moisture, individual plant stress, and spatial differences in water availability may mechanistically illustrate reliance on soil moisture at specific depths providing valuable information on patterns of diversity and species occurrence within the tallgrass prairie.

In this study I address species and topographic differences investigating: (1) changes in water-use (using xylem ^{18}O values) as a function of seasonal soil moisture wetting and drying patterns at depths in the soil, (2) changes in plant water potential resulting from changes in seasonal soil moisture patterns at soil depth, and (3) the relationships between soil moisture, seasonality, and plant water potential to predict water-use and make inferences about rooting distribution and biomass. These objectives allow us to interpret patterns of species coexistence which may vary according to the inherent abiotic variability present in this ecosystem.

Materials and Methods

This research was conducted on the Konza Prairie Biological Station (KPBS), a mesic grassland located in northeastern Kansas, USA (39°05'N, 96°35'W). This region experiences high climatic variability in both temperature and precipitation within and between years (Borchert 1950, Hayden 1998). KPBS receives 835mm average annual precipitation, 75% of which falls during the summer growing season (May-September). The average annual air temperature is 13°C with mean January and July ranges between -9 to 3°C and 20 to 33°C, respectively. Aboveground productivity on KPBS is dominated by a relatively few perennial C_4 species, but a diverse community of C_3 species (> 500 species) exists within this grassland (Freeman and Hulbert 1985, Towne 2004).

Two permanent east-west transects of 100m were established in April, 2003 within replicate, annually-burned, ungrazed watersheds. These watersheds are burned each year in April but regrow photosynthetically-mature aboveground biomass by late

May. Each of the four transects spanned a replicated topographical gradient from upland to lowland (ca. 50 m) common within KPBS. Within each transect, one permanent location was established for three distinct topographical positions outlined by Ransom et al. (1998): upland, hillside, and lowland. At each position, replicate sets of 30cm TDR (time-domain reflectometry) probes were inserted horizontally into an intact soil profile at 5, 20, 40, and 90cm in the hillside (n= 32) and lowlands (n=32) and at 5 and 20cm in the uplands (n=16). The extreme rockiness of upland soil prevented the placement of TDR probes at comparable depths to hillsides and lowlands. Volumetric soil water content (θ_v, %) was recorded mid-morning from all probes on a weekly basis during the summer sampling seasons of 2003-2005 (late May to late August).

Plant Sampling

The seven most abundant perennial species over all locations were identified and included three C_4 grasses (*Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium*), a leguminous and non-leguminous C_3 forb (*Lespedeza capitata* and *Vernonia baldwinii*, respectively), a leguminous C_3 sub-shrub (*Amorpha canescens*) and a woody C_3 shrub (*Ceanothus americanus*). *A. gerardii* and *S. nutans* are the co-dominant species of KPBS (Siletti and Knapp 2001) while *S. scoparium* is a very common bunchgrass. *C. americanus* is most common in upland, rocky locations and *V. baldwinii* responds favorably in disturbed systems (Drew 1947). Only the grasses and *A. canescens* were uniformly present at each measurement location. *C. americanus* had the lowest frequency (n= 6 sites) being largely confined to uplands and hillsides. *L. capitata* and *V. baldwinii* were present and available for study at $\geq$ 9 of the 12 locations.

Plant tissue for stable isotopic analysis of water was collected in August 2003, and then monthly from June to August of 2004 and 2005, respectively from all transects/topographical positions. Fractionation of the source water signature used does not occur upon uptake by roots, but isotopic enrichment through transpiration is common within actively photosynthesizing tissue (Wershaw et al. 1966, Allison et al. 1983, Flanagan et al. 1991). Thus, I collected tissue samples from the non-photosynthetic crown region. This tissue position lies immediately belowground, but above the rhizomes if present. Three replicates per species were collected for each sampling location and date. These replicates were collected from spatially non-adjacent regions (>3m apart) within each topographic position in order to capture more of the inherent site variability. For the C_3 species, one individual provided enough stem tissue for water extraction. However, multiple co-located tillers of each C_4 species were required to provide sufficient water during extraction. For each sampling date, different individuals per position were collected, cut into 1-3cm lengths and sealed inside exetainer vials (Labco Ltd., UK) and placed on ice until the samples were returned to the laboratory and stored at 1 °C.

Xylem water was extracted from plant samples using cryogenic vacuum distillation (Ehleringer and Osmond 1989, Webb and Longstaffe 2003). The extraction line was tested to determine the appropriate amount of time required to extract all water from each species and to ensure that water samples did not fractionate during the extraction process. For the 20-30g of plant tissue sample collected, 45 minutes was an adequate amount of time to vaporize all xylem water from within the plant stems. This was determined by recording the weight of plant tissue used for xylem extraction before and after oven drying. To confirm the robustness of the technique itself, working laboratory standards were vaporized and collected through the line. No fractionation of the standards occurred during the process. The $\delta^{18}O$ of the collected xylem water was measured using direct equilibration with CO_2 (Epstein and Mayeda 1953). Stable isotopic analyses were cross-validated by analyses performed at the stable isotopic laboratories at Kansas State and Colorado State Universities. IRMS at both locations operate in continuous-flow with peripheral gas bench microgas injectors. Raw values were corrected based on known gas and water standards and resulting values are in reference to the VSMOW (Vienna standard mean ocean water) international standard. All values are expressed using delta notation (δ):

$$\delta^{18}O\ (‰) = [(R_{sample}/\ R_{standard}) - 1] * 1000 \qquad (1)$$

where R_{sample} and $R_{standard}$ are the molar abundance ratios, ^{18}O:^{16}O of the sample and standard (VSMOW), respectively. Raw values were corrected based on calibration curves developed using internal laboratory standards. The working laboratory standard (deionized water) used throughout the study (actual value δ= -7.64) had an average value (±1SD) of -7.61(0.24), and -7.62(0.26) ‰ within and across runs, respectively. Arbitrarily selected blind replicates were sent to the Idaho Stable Isotope Laboratory (Moscow, ID), and on average differed by < 0.15‰ compared to the values obtained in my analyses.

Plant xylem water potential was measured to assess the gradient existing between soil and plant. Measurements were conducted at all locations over the growing season for all three years both pre-dawn (Ψ_p) and midday (Ψ_m) to estimate soil water potential in the zones of total root distribution and active uptake, respectively. At each location, 5 to 10 replicates leaves from separate individuals per species were collected. Measurements were made as soon as possible following excision using a Scholander-type pressure chamber (PMS Instruments, OR, USA).

Statistical analysis of the water potentials, and volumetric soil moisture were conducted using mixed-effects model in Proc Mixed (SAS 9.1.3, NC, USA). The Satterthwaite approximation was used to calculate the denominator degrees of freedom. The design of the analysis was a split strip-plot where watershed was the blocking variable and species within topographic position were replicated by transects. This design allows for comparisons of all species at one topographic location to be compared to all species at a separate location. Fixed-effects included date, topographic position, species, and their interactions. The random effects structure included three separate error terms. The error associated with watershed, watershed by transect, watershed by species within a given topographic position, and the 3-way interaction of watershed* transect* species within topography. In this analysis, sampling date was not used as a repeated measure since different individuals were randomly selected and sampled at successive dates. The analysis of soil moisture was similar to the water potential analysis, except soil depth was nested within topographical position rather than species, and sampling date was used a repeated measure, and not as a predictor variable. Assumptions of residual normality and homogeneity of variance were met in all analyses.

Structural Equation Modeling

Structural equation modeling (SEM), as an alternative to traditional multivariate statistical methodology, uses a hypotheses-testing framework to examine relationships among interacting variables based on prior information about the system (Byrne 1998, Shipley 2000). Once the predicted relationships amongst the suite of variables are specified, a series of structured equations describing the system are tested simultaneously to assess the applicability of the entire model as an appropriate hypothesis that is consistent with the data (Grace and Bollen 2005). Because the relationships tested among variables are established *a priori* and are not exploratory, SEM allows for inferential confirmation of processes operating within the system, and is not limited to descriptive relationships similar to general multivariate analysis (Byrne 1998, Shipley 2000).

The initial hypotheses used in SEM development included a comprehensive suite of causal relationships anticipated among the parameters compared (Table 4.1; Mitchell 2001, Stoner and Joern 2004). The parameters identified within the model included soil moisture at 4 depths (θ_v at 5, 20, 40, 90cm), both water potential measurements (Ψ_p and Ψ_m), $\delta^{18}O$ of xylem water, and a 'seasonality' variable. Season was a simple categorical variable representing the progressive sampling order across the summer for the three years of the study (1= June, 2= July, 3= August). This variable was included because this analysis was conducted on annually-burned watersheds and the regrowth of a new aboveground canopy each spring results in predictable phenological effects, independent of abiotic processes. Many of these expected relationships were not realized synonymously across all species, and subsequent derivations of the original 'photosynthetic type' hypothesis (Table 4.1) differed by species. Therefore, each of the final 'best-fit' models for each species reflects simplified species derivations constrained by the relationships outlined *a priori* in Table 4.1. Relationships between parameters used in SEM development were multivariate normal. Covariance matrices between parameters were developed using maximum-likelihood estimation in LISREL 8.72 (Jöreskog and Sörbom, SSI Scientific Software Inc. 2005).

Identification of the best-fit model was based on several criteria describing the fit of the model to the data as well as model parsimony (Table 4.2). The sample size of each model was dependent on the species investigated as well as topographic position due to aforementioned differences in species occurrence by location. Species estimates from each location*time used in the model reflect an average of multiple measurements (n = 3 ($\delta^{18}O$), 7-10 ($\Psi_{p,m}$), and 2 (θ_v per depth). As sample size varied based on species and parameters compared, I included goodness of fit statistics that account for small sample sizes (Table 4.2).

Results

Water potentials and volumetric soil moisture

θ_v varied significantly by topographic position ($P = 0.0025$) and soil depth (0.0186). Differences between watersheds, and all interactions were not significant ($P > 0.05$). For the 5 and 20cm depths, upland soils dried more rapidly, had more variable θ_v, and the absolute maximum θ_v by depth was lower in uplands than in the other topographic positions (Figure 4.1). When analyzed by depth for all positions, variability in θ_v over time decreased with increasing depth. In general, θ_v responded quickly to soil drying and wetting at 5cm depths for all locations, while 0_v varied minimally at the 90cm depth for both the hillside and lowland positions. However, after multiple weeks without rain (see 7/08/05), θ_v at 90cm decreased with only minor recovery evident after rainfall compared to more shallow soil depths (Figure 4.1).

Both predawn and midday leaf water potential (Ψ_p and Ψ_m) varied significantly by sampling date, species, topographic position, and date*species ($P < 0.0001$). The interaction of date*topographic position was significant for Ψ_p and Ψ_m ($P < 0.0001$ and $P = 0.0073$, respectively). The three-way interaction of predictor effects was also significant for Ψ_p ($P < 0.0001$). Although differences between species were present, trends among photosynthetic types were similar for both Ψ_p and Ψ_m measurements (Figure 4.2). As expected, trends in water potential were consistent with precipitation history with decreases following periodic drought and consistently higher values following rainfall. Ψ_p values were lowest for all species during the late summer of 2003, following 50 days with only 1 rainfall event over 10cm. In general C_4 species had lower Ψ_m following dry periods (late 2003, mid 2005), but the general magnitude and pattern was similar for both photosynthetic types. 2004 was a wet year, and had markedly little change over time for either variable of water potential for any of the species measured. Ψ_p and Ψ_m were generally synchronous over time, but Ψ_m responded faster to dry periods with proportionally greater change than Ψ_p (Figure 4.2). The pattern of change across the growing season was similar by topographic position (Figure 4.3), to patterns by species (Figure 4.2). When all species were averaged by topographic position, plant water potentials had similar trends and magnitude over time. The largest differences were noted after several weeks of drying, when upland Ψ_p and Ψ_m had lower values than either other topographic positions measured (Figure 4.3).

Structural equation modeling

C_3 species

Differences between species and photosynthetic pathways were evident among parameters compared in the best-fit models constructed (Table 4.3: C_3, Table 4.4: C_4). Path models with relationships and covariances among parameters are depicted for a representative species from each photosynthetic pathway (C_3: *A. canescens*, Figure 4.4; C_4: *A. gerardii*, Figure 4.5). Of the four C_3 species compared, each had different combinations and relationships between variables for predicting $\delta^{18}O$ and Ψ_m in the best-fit models developed (Table 4.3). For *A. canescens*, *L. capitata*, and *V. baldwinii*, θ_v at 5cm had significant positive direct effects of similar magnitude with $\delta^{18}O$. Therefore, as the surface soil water increased (θ_v), the isotopic signature of water ($\delta^{18}O$) in the plant stem increased, indicative of increased reliance on water in the shallow soil layers (isotopically- heavier). Similarly, the same species showed direct positive effects of Ψ_p on $\delta^{18}O$. For example, a high Ψ_p likely corresponds to dependence on surface soil water (isotopically-heavier) while decreased Ψ_p would reflect greater dependence on water from deeper, isotopically-lighter soils. Ψ_m had a significant direct effect on $\delta^{18}O$ for these species, but the effect was negative. Therefore, as midday water stress increased (noted by decreases in Ψ_m), these species were using proportionally more water from shallow soil layers (heavier $\delta^{18}O$ water signature). Significant indirect effects on $\delta^{18}O$ were present for each of these species, but they varied by parameter. Contrary to my initial hypotheses, θ_v at 20, 40, or 90cm did not have a significant direct effect on $\delta^{18}O$ for any of the C_3 species measured. *C. americanus*, a shrub, differed from the other C_3 herbaceous species compared. The relationship between θ_v and Ψ_p on $\delta^{18}O$ was negative for *C. americanus*. This species was also the only C_3 species to show a significant effect of season on stem water $\delta^{18}O$.

For the C_3 species, predictions of Ψ_m had fewer parameters and a lower amount of explained variance compared to predictions of $\delta^{18}O$. In my original hypotheses, I expected that C_3 species would be dependent on water throughout the soil profile, and not just surface water. *L. capitata* and *C. americanus* were the only species in which θ_v (5 and 20cm, respectively) had a significant effect on Ψ_m. In general, the strongest predictor of Ψ_m was season. This variable was included to reflect phenological species differences occurring independent of average seasonal climatic patterns (e.g. wet spring, dry summer). This effect was negative, suggesting Ψ_m declines as the summer progressed, potentially from greater whole-plant transpiration. For each species, θ_v of upper soils had a significant direct effect on θ_v at the next depth measured. This effect illustrates the influence of superficial soil moisture defining subsequent depths of soil moisture if other model parameters are held constant.

C_4 species

In contrast to the C_3 species, the models developed for the C_4 grasses had very similar combinations of parameters and directional effects for the response variables tested (Table 4.4). Most of the C_3 species had a strong positive relationship between available soil water and xylem water signature. However, θ_v, at any depth, had little effect on $\delta^{18}O$ for any of the C_4 species. Only θ_v at 5cm had a significantly positive effect on $\delta^{18}O$ for *A. gerardii*. Ψ_p and season were better predictors of $\delta^{18}O$ for the C_4 species. Similar to C_3 species, high Ψ_p correspond to heavier $\delta^{18}O$ values suggesting decreased leaf water stress correlates to water-use from surface soil layers. As the summer season advanced, stemwater $\delta^{18}O$ increased for all three species to a much high magnitude than reported for the C_3 species. Unlike the C_3 response, Ψ_m did not significantly influence C_4 $\delta^{18}O$.

Almost half of the variance associated with predictions of Ψ_m for C_4 species was explained by θ_v at 5cm, Ψ_p, and season (Table 4.4). Increases in available surface water (5cm) corresponded to increases in Ψ_m. This effect was common for all C_4 species, but the magnitude of the effect was greater for *S. nutans*. While θ_v at 20 and 40cm did not have significant relationships with Ψ_m in the models developed for *A. gerardii* and *S. nutans*, these predictions were absent in the best-fit model for *S. scoparium*, suggesting reduced effects of deeper soil moisture on *S. scoparium* compared to *A. gerardii* and *S. nutans*. Ψ_m in *A. gerardii* and *S. scoparium* decreased across the summer, but no change in *S. nutans* was present. Like path models developed for the C_3 species, θ_v of soils had a strong direct effect on subsequent depths of measured soil moisture. Few indirect effects were noted for any of the parameters for these C_4 species suggesting response variables change from direct causal association and effects are not mediated through other model variables.

Discussion

Belowground plant traits such as root distribution, water-use by depth, and plant water stress may exhibit similar patterns between species, photosynthetic types, or landscape position and these characteristic may be strongly correlated with water availability (Dawson 1993, Dawson et al. 1998, Jackson et al. 2000, Meinzer et al. 2001). Combining information on water used with patterns in soil moisture availability may serve as a foundation for understanding and explaining patterns of productivity, diversity and success within the tallgrass prairie (Fay et al. 2002).

Compared to grasses, forb productivity has not been shown to be strongly correlated with precipitation history within or between years (Briggs and Knapp 2001). Yet, forb dependence on water for nutrient uptake and CO_2 assimilation is commensurate or greater than grasses (Black 1973, Pearcy and Ehleringer 1984). The lack of a correlation between rainfall amount and productivity for forbs and shrubs suggests that demand rarely exceeds supply. This may occur if these species have proportionally greater access than C_4 species to water that is relatively unaffected from the wetting and drying of surface soil. I have previously reported that forbs and shrubs rely on subsurface soil (deeper than 30cm) water proportionally more than C_4 grasses (Chapter 3, this dissertation). This tenet of greater dependence on deep water by forbs has been periodically assumed in tallgrass prairie research over the past 70 years (Albertson 1937, Weaver and Albertson 1943, Sims and Singh 1978, Knapp 1985, Abrams et al. 1986, Kucera 1991, Knapp et al. 1998, Fay et al. 2002). Understanding plant stress to changes in soil moisture, in both amount and variability, has broad implications for tallgrass ecosystem functioning including annual ANPP regulation, and net CO_2 exchange of the prairie (Suyker et al. 2003).

In the path models developed, the drivers of Ψ_m and their relationships varied by photosynthetic type (Table 4.3, 4.4). For C_3 species, the best predictor of Ψ_m was the class variable 'season'. This response is not surprising as Ψ_m reflects changes in transpiration and conductance in relation to the available water supply (Jackson et al. 2000b). In general, seasonal responses to changes in water availability are generally higher for C_3 species than C_4 (McAllister et al. 1998). C_3 species may shift reliance from soil moisture in surface layers to deeper sources without changes in Ψ_p or Ψ_m (Smith et al. 1991). The best predictor of Ψ_m for C_4 species was 5cm θ_v (Table 4.4). Thus, for all three C_4 species (and *L. capitata*), as surface soil moisture increased, midday plant water stress decreased. This result corroborates C_4 dependence on surface moisture as reports suggest that nearly half of the root biomass for these C_4 species occurs in the surface 10cm (Rice et al. 1998).

Multiple variables had significant direct effects on the xylem $\delta^{18}O$, but these effects varied by photosynthetic type (Table 4.3, 4.4). For the C_3 species except *C. americanus*, 5cm θ_v, Ψ_p, and Ψ_m all had strong significant effects on $\delta^{18}O$. As surface soil moisture and Ψ_p increased, these species were using water of a collectively heavier signature. While C_4 species exhibited a positive correlation between surface soil moisture and xylem $\delta^{18}O$, the relationship was weaker than that reported for C_3 species, and only significant for *A. gerardii*. Therefore, the water used by C_3 species varied by availability in the surface soil; when θ_v increases, C_3 species used more surface water. When 5cm θ_v dries, the C_3 species have a lighter xylem $\delta^{18}O$ signature that indicates water-use from deeper soils. This relationship is considerably weaker for C_4 species suggesting C_4 plant dependence on surface soil was more invariant, and water-use depended less on patterns of water availability.

The primary drivers of xylem $\delta^{18}O$ for the C_4 species were season and Ψ_p. The effect of season was especially large on *A. gerardii* and *S. scoparium* with heavier xylem $\delta^{18}O$ signature of 1‰ per month as the growing season advanced (Table 4.4). Similar to C_3 species, Ψ_p was positively correlated with C_4 species; suggesting increased plant water stress corresponds to heavier xylem $\delta^{18}O$. The reduced, non-significant effect of midday water stress on water used by C_4 species ($\Psi_m \sim \delta^{18}O$), may be indicative of the higher water-use efficiency of C_4 species compared to C_3 species (Pearcy and Ehleringer 1984), which do show strong, direct relationships between water stress and water-use.

In general, C_3 species had distinctly dissimilar models. The response of *L. capitata* was more similar to the C_4 grasses than the other C_3 species for both absolute values of Ψ_m and $\delta^{18}O$ and the amount of variance explained in the path models. *L. capitata* was the only C_3 species in which Ψ_p and 5cm θ_v positively affected Ψ_m (Table 4.3), periodic drought decreased Ψ_p and Ψ_m similar to C_4 species, and xylem water signatures were consistently heavier than co-occurring C_3 species. This response may be related to shallower rooting depths compared to the other C_3 species measured. In a previous study, maximum root depth of *L. capitata* was shallow (60cm) compared to 15 other common forbs in this system (Zajicek et al. 1986). The path model developed for the shrub *C. americanus* was dissimilar to all other species and had the smallest amount of overall variance explained and was the only species measured in which soil moisture deeper than 5cm was a significant predictor for either $\delta^{18}O$ or Ψ_m (Table 4.3). Tallgrass prairie shrubs have previously been shown to exhibit variable responses to water availability both within this functional grouping and compared to other species (McCarron and Knapp 2001). For predictions of xylem $\delta^{18}O$ in *C. americanus*, season and Ψ_p were the only significant predictors (Table 4.3). Additionally, the relationships between 5cm θ_v and Ψ_p on $\delta^{18}O$ were negative, suggesting that when the surface soil moisture or water potential increases, *C. americanus* is using proportionally more water from depth, and hence the depleted water signature. *C. americanus* had coarse woody roots in the surface soil and may be an inferior competitor for surface soil moisture (personal observation).

Competition for water may be a particularly strong determinant of vegetative community structure in temperate grasslands (Fowler 1986, Weltzin and McPherson 1997, Köchy and Wilson 2000, Tsialtas et al. 2001). The ability of species to acquire water from sources different than their competitors may aid in explaining species co-existence patterns, spatial distribution at varying landscape positions, or productivity-diversity relationships common in the tallgrass plant community (Walter 1971, Knapp et al. 1993, Turner et al. 1995). In the path models presented, the relationships among parameters varied by species and photosynthetic type. The most interesting response was the similarity of effects and models for the C_4 species. The best-fit models for *A. gerardii* and *S. nutans* were identical, and *S. scoparium* only differed by the lack of a θ_v relationship with Ψ_p at 20 and 40cm (Table 4.4). Conversely, each C_3 species had distinctly different best-fit models that varied from the other C_3 species, even those with similar functional characteristics (shrubs or legumes). These results suggest niche separation among photosynthetic types in relation to competition for water (Sun et al. 1997). C_4 grasses which dominate prairie productivity all have very similar path models showing dependence on surface soil layer despite changes in plant water potentials or soil water availability. C_3 forbs and shrubs each had unique models with relationships among parameters showing increased dependence on deeper soil water during plant water stress.

Are the mechanisms responsible for tallgrass community assemblages influenced more by drought stress or drought avoidance as inferred by species differences in water stress and water acquisition? Dominant species generally have the capability to persist the longest at the lowest concentration of an available resource (Tilman and Wedin 1991a, 1991b). Presumably, the C_4 species I measured are drought tolerant, an adaptation contributing to their success within this ecosystem (Turner et al. 1995, Sun et al. 1997). Water-use by C_4 species were less responsive to changes in surface soil moisture compared to C_3 species. However, changes in the plant water stress of C_4 species were primarily influenced by chances in surface soil moisture. This result suggests C_4 species are actively using water from this region of the soil, even when the amount of water present is low. As a group, C_3 species had varied relationships between water use, availability, and stress. In general, these species relied on surface soil moisture when available, but overall water stress depended less on the amount of water in surface soil layers. Their ability to vary water-use based on availability and stress, suggests drought avoidance as the mechanism for C_3 persistence. Greater water-use plasticity among C_3 species is a beneficial adaptation for persistence through seasonal and periodic drought and this response may attribute to their persistence and the diversity reported within this ecosystem.

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Table 4.1: Hypothesized causal relationships between variables used in SEM construction separated for C_3 and C_4 species. Relationships predicted are irrespective of topography or time and are photosynthetic pathway specific. Subsequent species-specific derivations are revisions of these initial hypotheses, but with fewer overall effects.

Directional relationships used for construction of SEM's

Hypothesized

C_3	C_4
i. xylem $\delta^{18}O \sim$ all θ_v depths (5, 20, 40, 90cm)	i. xylem $\delta^{18}O \sim$ 5, 20 cm θ_v
ii. xylem $\delta^{18}O \sim \Psi_p$ and Ψ_m	ii. xylem $\delta^{18}O \sim \Psi_p$ and Ψ_m
iii. xylem $\delta^{18}O \sim$ season	iii. xylem $\delta^{18}O \sim$ season
iv. Ψ_m will not mirror Ψ_p	iv. Ψ_m will mirror Ψ_p
v. $\Psi_m \sim$ all θ_v depths (5, 20, 40, 90cm)	v. $\Psi_m \sim \theta_v$ at 5, 20cm
vi. $\Psi_m \sim$ season	vi. $\Psi_m \sim$ season
vii. soil $\theta_v \sim$ soil θ_v at overlaying depths	vii. soil $\theta_v \sim$ soil θ_v at overlaying depths

Table 4.2: Best-fit model statistics by species within both photosynthetic pathways. Low X^2 and $P > 0.05$ suggest no evidence to reject the model based on a lack of fit with the data. Values of root mean square error of approximation (RMSEA) and standardized root mean square residual (RMR) < 0.05 indicates a good fit between model and data. Comparative fit index (CFI) accounts for sample size and values near 1.0 indicate a good fit. Once a candidate set of acceptable models that fit the data were characterized for each species, AICc was used to select the most parsimonious model (Burnham and Anderson 2002). Models presented here had the lowest AICc value of the candidate set per species.

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

Table 4.3: Unstandardized coefficients (covariance) associated with the prediction of each multivariate hypotheses for the C_3 species. Values are direct and (indirect) effects of the suite of predictors on the dependent variable. Significant relationships ($P < 0.05$) are in bold. The complete model is the aggregation of each structural equation and the associated error variance for a given species. %water at 90cm depth did not have additional explanatory power for the SEM of any species.

Response Variable		**Predictor Variables**							
	C_3 Species	**5cm**	**Ψ_p**	**season**	**Ψ_m**	**20cm**	**40cm**	**Error**	**R^2**
$\delta^{18}O$	*A. canescens*	**0.076** (0.01)	**2.05** (0.08)	**(0.44)**	**-1.08**	0.016 (0.014)	0.012	1.11	0.74
	L. capitata	**0.09 (-0.06)**	**2.63** (-0.08)	0.34 (0.57)	**-1.60**	(-0.06)	-0.075	3.38	0.51
	C. americanus	-1.70	**-0.70** (0.01)	**0.25** (0.01)	-2.07	(-0.04)		6.19	0.31
	V. baldwinii	**0.092 (-0.02)**	**1.69** (0.22)	0.40 **(0.55)**	**-2.08**			2.07	0.55
Ψ_m	*A. canescens*		-0.077	**-0.41**				0.20	0.35
	L. capitata	**0.025**	0.049	**-0.36**				0.21	0.54
	C. americanus		-0.004	-0.003		**.019**		0.08	0.30
	V. baldwinii	0.010	**-0.110**	**-0.27**				0.086	0.44
20cm	*A. canescens*	**0.47**						32.58	0.52
	L. capitata	**0.41**						37.08	0.44
	V. baldwinii	**0.48**						29.55	0.55
40cm	*A. canescens*	**(0.34)**				**0.73**		28.94	0.62
	L. capitata	**(0.33)**				**0.80**		29.87	0.59
	V. baldwinii	**(0.41)**				**0.86 (-0.13)**		26.67	0.65

Table 4.4: Unstandardized coefficients (covariance) associated with the prediction of each multivariate hypotheses for the C_4 species. Values are direct and (indirect) effects of the suite of predictors on the dependent variable. Significant relationships ($P < 0.05$) are in bold. The complete model is the aggregation of each structural equation and the associated error variance for a given species. %water at 90cm depth did not have additional explanatory power for the SEM of any species.

Response Variable		**Predictor Variables**							
	C_4 Species	**5cm**	**Ψ_p**	**season**	**Ψ_m**	**20cm**	**40cm**	**Error**	**R^2**
$\delta^{18}O$	*A. gerardii*	**0.056** (-0.01)	**2.38** (-0.06)	**1.12** (0.08)	-0.50			2.35	0.49
	S. nutans	0.042 (-0.01)	**2.46** (-0.75)	**0.63**	-0.84			2.03	0.44
	S. scoparium	0.014 (0.01)	1.03 (0.34)	**1.05** (-0.11)	0.67			3.45	0.27
Ψ_m	*A. gerardii*	**0.022** (0.001)	0.13	**-0.17**		0.001	-0.006	0.16	0.42
	S. nutans	**0.19** (-0.01)	**0.89**	0.004		-0.026	0.0088	0.20	0.58
	S. scoparium	**0.016**	**0.50**	**-0.17**				0.16	0.57
20cm	*A. gerardii*	**0.48**						29.84	0.55
	S. nutans	**0.48**						30.50	0.53
	S. scoparium	**0.53**						32.43	0.54
40cm	*A. gerardii*	**(0.40)**				**0.83**		29.39	0.61
	S. nutans	**(0.40)**				**0.82**		29.95	0.59

Figure 4.1: Average changes (±1SE) in volumetric soil moisture (θ_v) over depth and time. Rows depict different topographic positions and columns express change over the growing season (late May-Aug.) from 2003-2005. The right y-axis depicts the ambient precipitation record over the same time period. θ_v was measured at 5 and 20cm deep in upland topographic positions, but hillside and lowland positions had measured θ_v at 5, 20, 40, and 90cm.

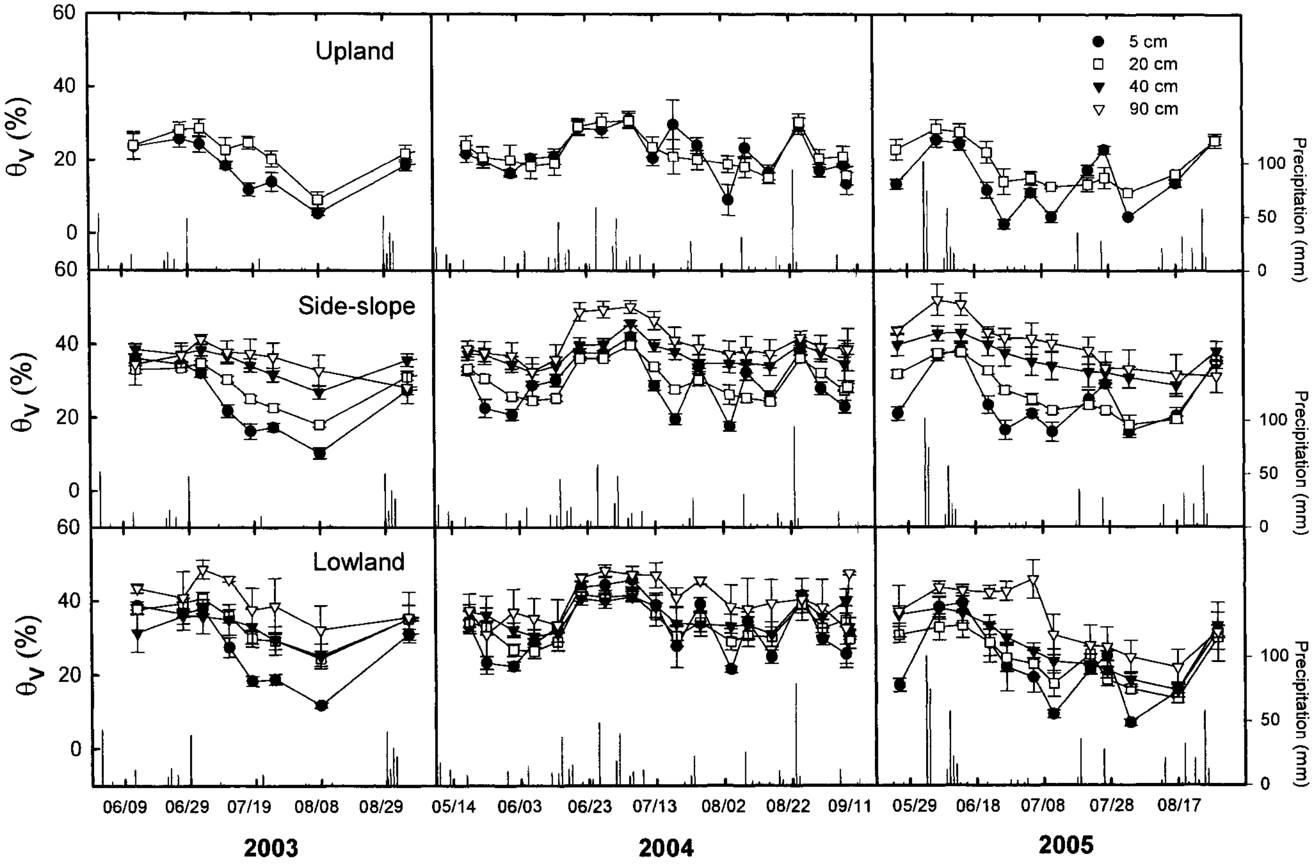


Figure 4.2: Average change (±1SE) in water potential (Ψ_p and Ψ_m) during the summer months from 2003-2005. Species grouped in the top panel are C_3, and the bottom panel contains the C_4 species. The lower right y-axis of the C_3 and C_4 species groupings depicts the ambient precipitation record over the same time period.

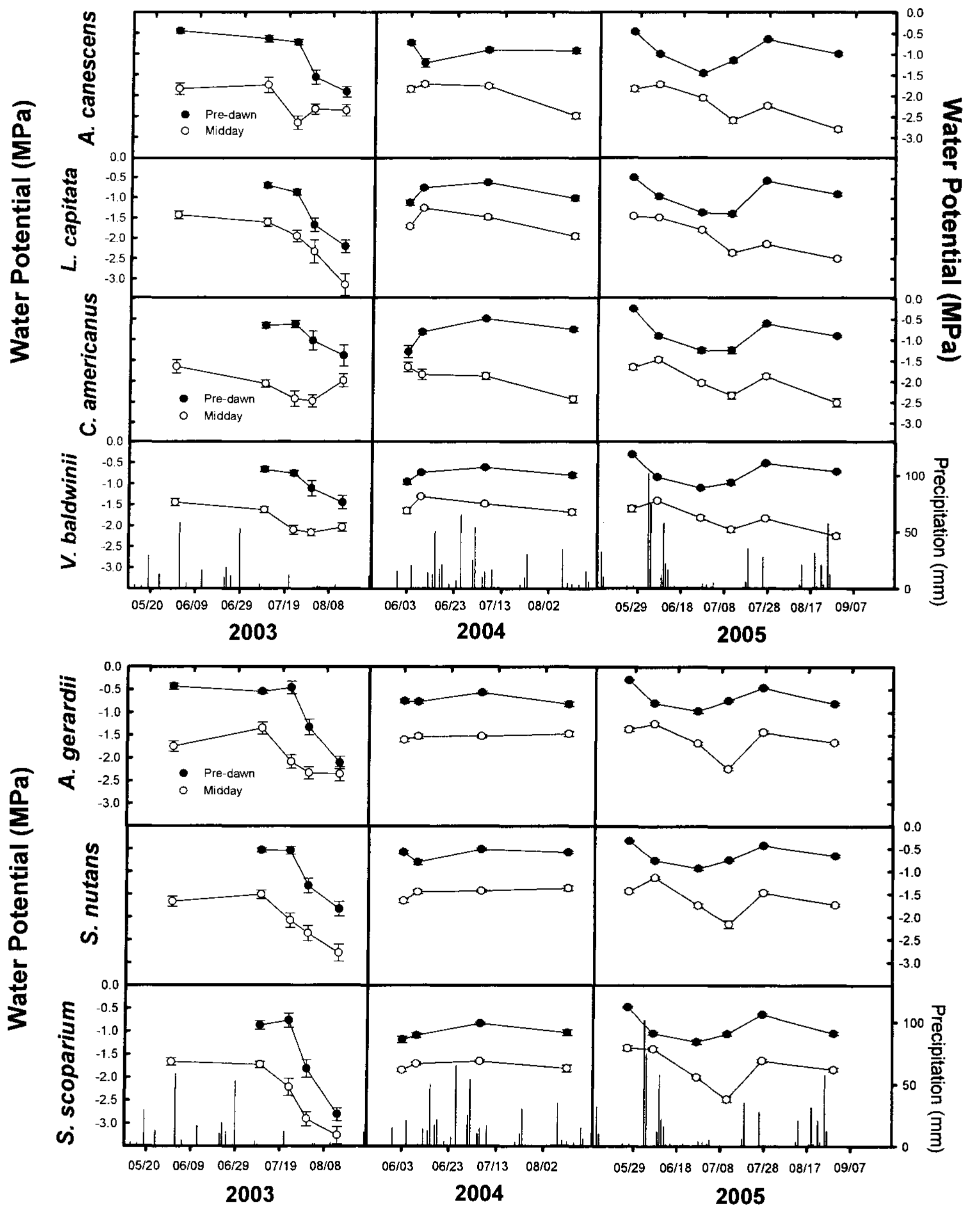


Figure 4.3: Average change (±1SE) in water potential (Ψ_p and Ψ_m) across the summer from 2003-2005. Rows are arranged by topographic position. The lower right y-axis depicts the ambient precipitation record over the same time period.

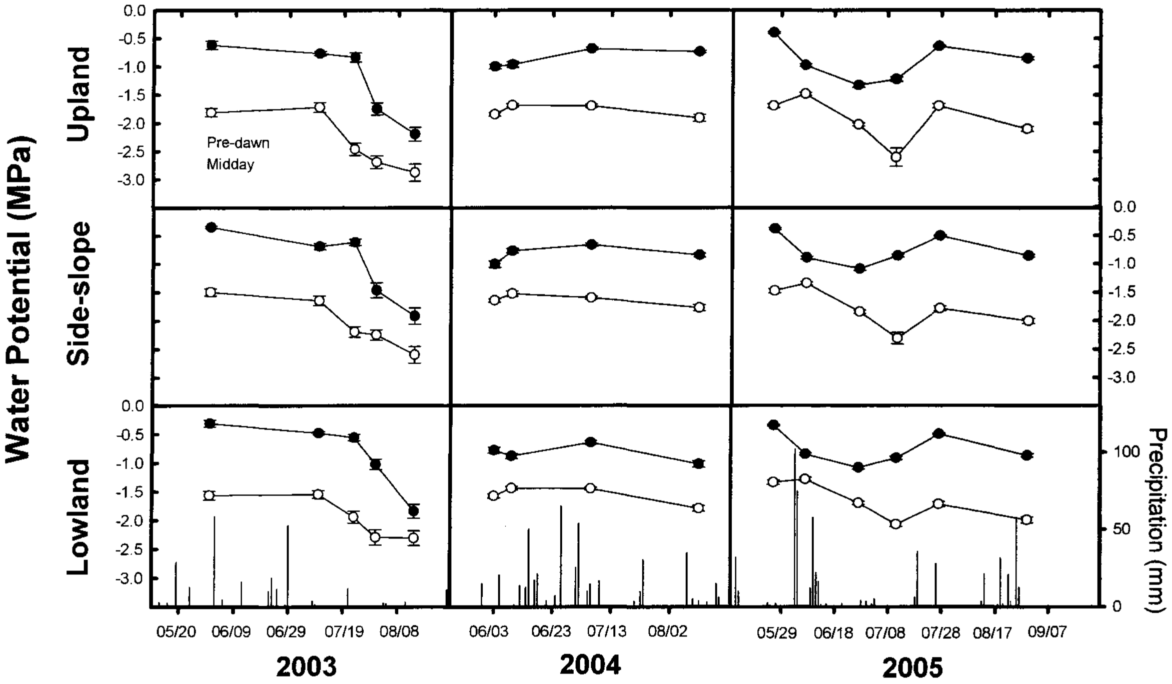


Figure 4.4: Fully parameterized path model for *A. canescens* (representative diagram for the C_3 species). Boxes depict variables comprising the overall model. '5, 20, and 40cm' reflect soil moisture by depth (θ_v), '$\delta^{18}O$' is plant xylem water, 'season' is a categorical variable reflecting phenological development, and 'Ψ_p and Ψ_m' are predawn and midday water potentials, respectively. Path values on directional arrows are unstandardized coefficients estimated using maximum likelihood. Solid arrows indicate a significant relationship ($P < 0.05$) while dashed arrows indicate overall importance in the model developed, but a non-significant ($P > 0.05$) directed relationship among the specific variables compared. θ_v at 5cm, Ψ_p, and season are exogenous variables in the model and are assumed unit normal with mean 0, and a standard deviation of 1. The residual error variance (ε) for the other variables was free and estimated using maximum likelihood.

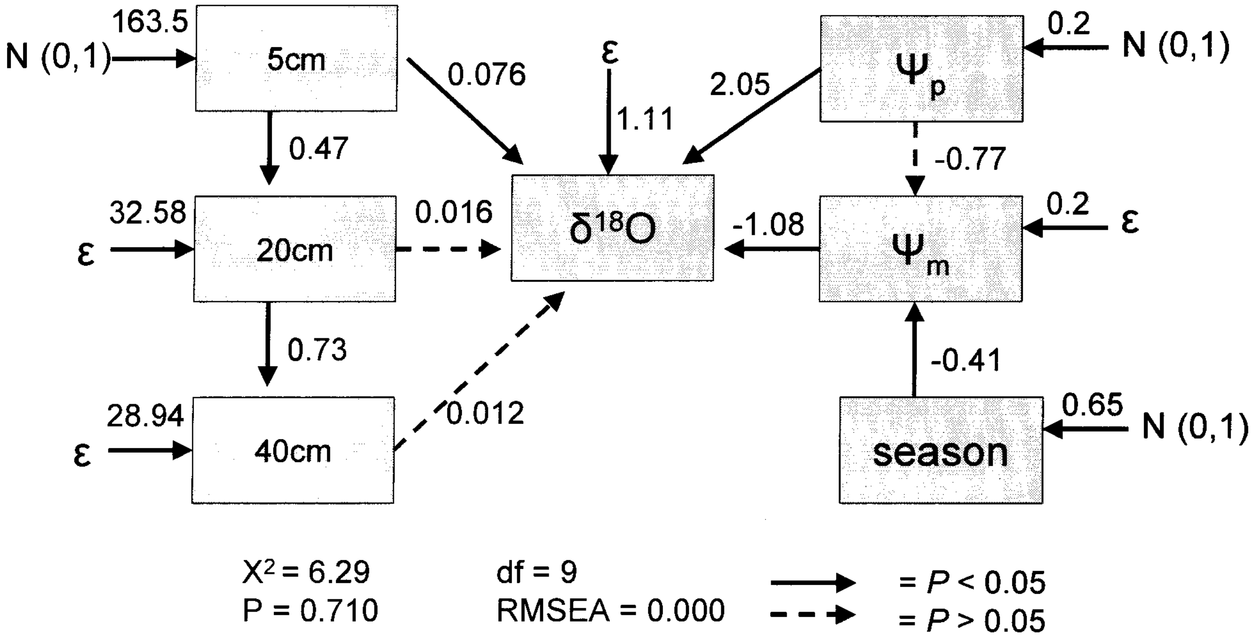
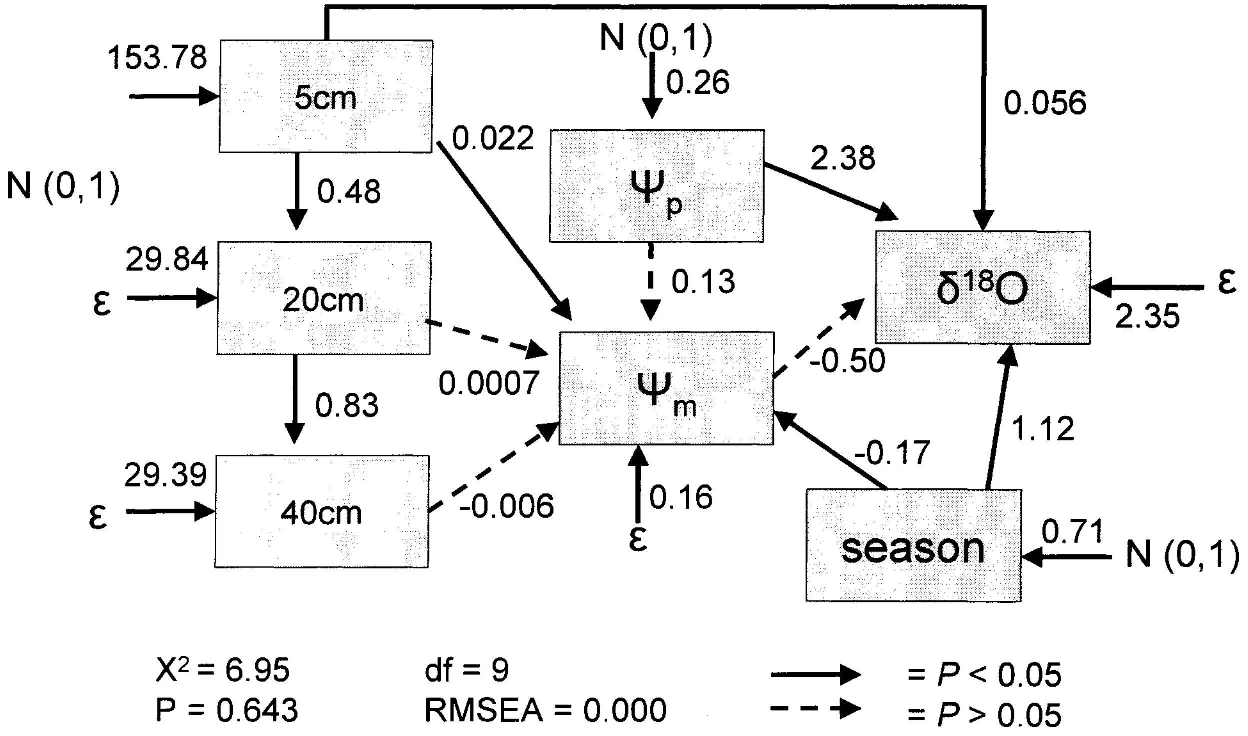


Figure 4.5: Fully parameterized path model for *A. gerardii* (representative diagram for the C_4 species). Boxes depict variables comprising the overall model. '5, 20, and 40cm' are soil moisture by depth (θ_v), '$\delta^{18}O$' is the isotopic signature of plant xylem, 'season' is a categorical variable reflecting phenological development, and 'Ψ_p and Ψ_m' are predawn and midday water potentials, respectively. Path values on directional arrows are unstandardized coefficients estimated using maximum likelihood. Solid arrows indicate a significant relationship ($P < 0.05$) while dashed arrows indicate overall importance in the model developed, but a non-significant ($P > 0.05$) directed relationship among the specific variables compared. θ_v at 5cm, Ψ_p, and season are exogenous variables in the model and are assumed unit normal with mean 0, and a standard deviation of 1. The residual error variance (ε) for the other variables was free and estimated using maximum likelihood. Path values are directly interpretable in the units of the variables compared. For example, a 1% increase in soil moisture at the 5cm depth would have a corresponding enrichment of 0.056‰ in the $\delta^{18}O$ for *A. gerardii*.



Chapter 5: Intra-annual rainfall variability and grassland productivity: can the past predict the future?

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Abstract

Precipitation quantity has been shown to influence grassland aboveground net primary productivity (ANPP) positively whereas experimental increments of temporal variability in water availability commonly exhibit a negative relationship with ANPP. I evaluated long term ANPP datasets from the Konza Prairie Long Term Ecological Research (LTER) program (1984-1999) to determine if similar relationships could be identified based on patterns of natural variability (magnitude and timing) in precipitation. ANPP data were analyzed from annually burned sites in native mesic grassland and productivity was partitioned into graminoid (principally C_4 grasses) and forb (C_3 herbaceous) components. Although growing season precipitation amount was the best single predictor of total and grass ANPP ($r^2 = 0.62$), several measures of precipitation variability were also significantly and positively correlated with productivity, independent of precipitation amount. These included soil moisture variability, expressed as CV, for June ($r^2 = 0.45$) and the mean change in soil moisture between weekly sampling periods in June and August (%wv) ($r^2 = 0.27$ and 0.32). In contrast, no significant relationships were found between forb productivity and any of the precipitation variables ($P > 0.05$). A multiple regression model combining precipitation amount and both measures of soil moisture variability substantially increased the fit with

productivity ($r^2 = 0.82$). These results were not entirely consistent with those of short-term manipulative experiments in the same grassland however, because soil moisture variability was often positively, not negatively related to ANPP. Differences in results between long and short term experiments may be due to low variability in the historic precipitation record compared to that imposed experimentally as experimental levels of variability exceeded the natural variability of this dataset by a factor of two. Thus, forecasts of ecosystem responses to climate change (i.e. increased climatic variability), based on data constrained by natural and recent historical rainfall patterns may be inadequate for assessing climate change scenarios if precipitation variability in the future is expected to exceed current levels.

Introduction

Climate change models differ with regard to projected changes in annual precipitation amounts in the central U.S., but they are in agreement with predictions that the dynamics of event distribution will become more variable (Groisman *et al.* 1999; Easterling *et al.* 2000; Houghton *et al.* 2001). General circulation models predict precipitation events of a greater magnitude, but with longer intervening dry periods and reduced frequency. The longer dry periods between storms will generally lead to reduced soil moisture levels (Knapp *et al.* 2002). Predictions by the Canadian Model Scenario (VEMAP) suggest that the Great Plains region of North America will experience an approximate 30% decrease in annual precipitation over the next century (USGCRP 2003). Perhaps more importantly, similar model predictions for soil moisture forecast a 50% decline during June, July, and August over the next century (USGCRP 2003). Substantial changes in moisture availability and temporal variability will undoubtedly impact ecosystems in which productivity is limited by water availability (Sala *et al.* 1988; Weltzin *et al.* 2003). The mesic grasslands (tallgrass prairie) ecosystem of the Central Great Plains is one such region sensitive to dynamic changes in precipitation timing (Fay *et al* 2003). Thus, a better understanding of the relationship between productivity and precipitation amount and variability is warranted.

The importance of precipitation amount versus precipitation pattern on grassland productivity has been assessed using experimental rainfall manipulation plots (RaMPs) at the Konza Prairie Biological Station (KPBS) (Fay *et al.* 2000). Results of this research indicate that when temporal variability in soil moisture was increased independent of rainfall quantity, carbon cycling processes and plant community composition were altered (Knapp *et al.* 2002; Fay *et al.* 2003). Specifically, greater precipitation variability (changes in rainfall pattern, independent of seasonal amount), increased soil moisture variability and reduced mean soil water content, which resulted in increased plant water stress and decreased productivity (Fay *et al.* 2002; Knapp *et al.* 2002; Fay *et al.* 2003). Thus, based on experimental approaches, both precipitation amount (Knapp *et al.* 2001) and temporal pattern have been shown to be important in determining productivity within this grassland.

An alternative approach to assessing potential changes in climate on grassland ecosystems is to use long term ecological data and climate records to identify those aspects of climate to which ecological processes are most likely to be sensitive (Sala *et al.* 1988; Burke *et al.* 1991; Lauenroth and Sala 1992; Sala *et al.* 1992; Paruelo *et al.* 1999; Jobbágy and Sala 2000). For example, Briggs and Knapp (1995; 2001) used regression analysis to assess the responsiveness of aboveground net primary productivity

(ANPP) in tallgrass prairie to interannual variation in precipitation based on long term data. Subsequent experimental manipulation of precipitation events confirmed and further defined this relationship (Knapp *et al.* 2001).

The objective of this research was to compare the results of manipulative experiments, which have focused primarily on intra-annual precipitation alterations, to those derived from analyses of long term natural precipitation variability recorded at the Konza Prairie LTER site. The Konza LTER site has archived biological and climatological data since its inception in 1981, and this dataset was used as a proxy for assessing decadal-scale changes in this grassland. The overarching question that guided this analysis was: "do the patterns of variability present in long term Konza datasets mimic the results found in short term experimental manipulations?" To answer this question, I used sixteen years of precipitation and ANPP data from an annually burned watershed on site. Annually-burned sites are both the most productive and water limited of all burn frequencies in the tallgrass prairie (Knapp *et al.* 2001). I analyzed patterns of natural precipitation and soil moisture variability (inter- and intra-annually) to assess their influence on ANPP of both common growth forms (C_4 grasses and C_3 forbs) in this grassland. Specifically, I sought evidence for the importance of intra-annual variability on ANPP independent of precipitation amount using these long term datasets. I predicted that the productivity response to precipitation and/or soil moisture variability would be consistent with patterns identified through experimental manipulations in annually burned prairie.

Materials and Methods

Analyses were based on long term ecological data collected at KPBS, in northeastern Kansas, USA (39°05' N, 96°35' W). KPBS is a 3487 ha unplowed tallgrass prairie dominated by a few warm-season C_4 grasses, yet supporting a species-rich pool of herbaceous C_3 forbs (Freeman 1998). KPBS experiences a temperate mid-continental climate of cold, dry winters and warm wet summers with the majority of the annual precipitation occurring between April and September (835mm mean annual precipitation).

Total aboveground productivity is estimated by quantifying the current years' biomass in the annually burned watersheds (Briggs and Knapp 1995). Plant biomass is harvested during late August/early September, the time of peak biomass. Total ANPP is measured using four transects with five 0.1 m^2 subplots therein. This protocol is repeated for each soil type –watershed combination. The clipped subplots are marked so as to avoid subsequent re-sampling for at least four years. This method ensures independence in productivity data between consecutive years. For comparisons in this study, measurements of ANPP come from a single annually-burned watershed on KPBS which has historically been the most representative of all the annually-burned watersheds on site. For the data I compared, each transect in this watershed was located on the same soil type. Biomass was separated into multiple components that included graminoid and forb biomass, current year's dead, and a minor woody plant component (if present). Following sorting, biomass was oven-dried at 60 °C for 48 hours and weighed to the nearest 0.01g (Abrams et al. 1986). Total ANPP can vary widely across years, but this response is largely driven by the grass component (Fig. 5.1).

As part of the LTER program, soil moisture is measured at bi-weekly intervals across many sites on KPBS. Because these estimates are too coarse temporally to quantify variability, I estimated daily values in soil moisture. These estimates were derived using a soil hydrology model (WaterMod 2.0.9, Greenhat Software, 1998). This mechanistic model is described in detail in Johnson *et al.* (2003), but briefly, the model is driven by the relationship between biomass productivity and agents of soil moisture change, particularly soil water infiltration and drainage, runoff, soil characteristics, precipitation amount, and estimates of potential evapotranspiration (PET) (calculated using the Penman-Monteith equation). Soil water infiltration is calculated using a capacitance model, which is parameterized using saturated water content, drainage point, and saturated hydraulic conductivity of the soil (Johnson *et al.* 2002; 2003). Measured input variables included end of season ANPP, daily precipitation, and daily PET, and they were used to derive daily model estimates of soil moisture for each year. The model was sensitive to annual biomass changes, and was parameterized with dates for emergence (5/1), maximum growth (7/15), date of harvest (9/30) and water use efficiency (209 mm precipitation per kg dry weight, which equates to the average total biomass divided by the growing season precipitation, Briggs and Knapp 1995). To assess the accuracy of the model, estimates of soil moisture from the 20-30cm soil depth were compared to bi-weekly neutron probe measurements available from the site at a 25cm depth. I calculated the percent difference between the measured soil moisture value and the modeled estimate and then noted the average monthly difference across the entire dataset for each month of the growing season. The largest difference between measured and modeled values occurred in the month of April ($\mu = 16.7\%$, SE = 1.4%). However, as the growing season advanced, predictions of soil moisture were ≥ 90% similar to measured values for July, August, and September ($\mu = 8.6$, 10.5, and 10.8%, and SE = 1.2, 1.6, 1.9%, respectively). Model estimates were consistently lower than measured values in April, but for the subsequent five months, no consistent bias between measured and modeled predictions occurred, and the model followed the temporal dynamics of soil moisture following wetting and drying events. The linear relationship between measured and modeled soil moisture is portrayed graphically in the inset panel of Fig. 5.2.

Statistical analyses were focused on several abiotic parameters that could potentially influence productivity. Variables analyzed during the growing season included timing of precipitation events, length of dry-periods, the magnitude of the precipitation-event, mean monthly pan evaporation, indices of rainfall evenness (Bronikowski and Webb 1996), and consecutive differences in precipitation amount between events, months, and years (Oesterheld *et al.* 2001). Simple and multiple linear regression (SLR, MLR) comparisons were made between ANPP and these abiotic parameters using the GLM functions of SAS (SAS 2001). Multiple linear regression procedures were performed using a stepwise model selection method to identify significant reduced models containing non-correlated variables. The appropriate model to use was identified from the pool of candidate models by Akaike's Information Criterion.

Analyses of colinearity were performed to ensure independence among the predictor variables used. Yearly measurements of ANPP were independent from consecutive years due to the aforementioned biomass harvesting protocol. Point estimates in the analyses refer to an average growing season value for each year, unless otherwise specified. Due to the time-series nature of the data, a test of autocorrelation among residuals was performed to identify any first-order serial correlation between year-to-year ANPP or precipitation data. Based on the Durbin-Watson test statistic, errors between years were uncorrelated for either variable (DW = 1.728 and 1.719 for precipitation and ANPP, respectively).

Results

The majority of predictor variables I used exhibited no relationship with grass productivity, and of those that did, many lacked independence from precipitation amount. However, two parameters describing soil moisture variability were significantly related to ANPP independent of precipitation amount. The first variable was an absolute difference index expressing the mean change in soil moisture between weekly sample periods. This index has been previously used as an indicator of soil moisture variability (Knapp *et al.* 2002). The second index of variability was the coefficient of variation (CV) of mean monthly soil moisture. CV has also been used as a representative index of variability (Le Houérou *et al.* 1988, Fay *et al.* 2003). Both parameters were calculated for each of the growing season months (April-September) for all 16 years.

Precipitation and soil moisture amount were significantly and positively related to grass ANPP in this annually burned grassland (Fig. 5.2). Growing season precipitation amount best explained the variation of grass ANPP ($r^2 = 0.62$). However, none of the abiotic predictor variables analyzed were significantly related to forb ANPP during this 16-year period. Neither index of soil moisture variability was significantly related to productivity across the entire growing season, but when analyses were conducted with monthly timesteps, relationships were significant for portions of the growing season

(Table 1). For the absolute difference index, variability and productivity were significantly correlated for the months of June and August, but the nature of the relationship differed. For this index, variability and productivity were positively correlated in June, but negatively correlated in August (Table 5.1). The remaining months had positive trends, albeit extremely weak correlations. The CV index had similar seasonal trends to the absolute difference index with significant positive trends in June, and subsequent negative trends for the remainder of the season (Table 5.1). Although non-significant, the CV index exhibited a negative trend across the entire growing season.

The magnitude of the natural variability noted in the two soil moisture indices using long term datasets was considerably lower than that imposed experimentally in the rainfall manipulation plots (RaMPs) study (Fig. 5.3). The maximum CV of soil water content in July for the long term data (16 years) was only 54% of the variability reported in the RaMPs experiment (3 years) (CV = 21 vs. 39%; Fig. 5.3a). Similarly, the maximum variability in soil water content (absolute difference index) reported in the long term datasets was only 33% of the maximum variability imposed in the RaMPs experiment (variability = 3.5 vs. 10.5; Fig. 5.3b).

A multiple linear regression (MLR) model was used to determine if multiple factors could explain more variation than the analysis of precipitation amount alone (Fig. 5.2). Predictor variables included in the full model MLR analysis included five variables added in this order: annual precipitation amount, average annual soil moisture, the mean difference in weekly soil water contents (for May and June only), CV of June soil moisture, and the average length of consecutive dry-periods between rain occurring during the growing season. The analysis identified three variables to be significant for predicting ANPP: precipitation amount and the two soil moisture variability parameters ($r^2 = 0.82$, Fig. 5.4). Each of these variables exhibited a positive correlation with ANPP.

Discussion

Analyses of long term datasets or natural climatic gradients have been used to predict ecosystem responses to future climates (Burke *et al.* 1991; Paustain *et al.* 1995; Alward *et al.* 1999; Rastetter *et al.* 2003; Dunne *et al.* 2004). These methods provide a long term alternative to experimental approaches to climate change research that rely on highly manipulative experiments. Long term data sets are expected to reveal patterns of ecosystem responses to climate variability similar to those identified by short-term manipulation. However, this assumes that predicted changes for future climates are of a similar magnitude to that recorded in the historic data. A key question addressed by this study is: can the past predict the future for this grassland?

Assessing the impact of precipitation variability on ecosystem productivity and function is inherently difficult due to spatial and temporal differences within a site as well as across an entire region. Grasslands exhibit higher inter-annual variation in productivity and may require longer time periods to reveal trends in variability compared to forested biomes (Lauenroth and Sala 1992; Frank and Inouye 1994). Within grassland biomes, the influence of precipitation variability on ANPP depends on the ecosystem structure and whether the constraint is biological or biogeochemical (Paruelo *et al.* 1999). Inter-annual variation in ANPP within the shortgrass steppe of Colorado, USA resulted from both current year precipitation amount and ANPP of the previous year (Oesterheld *et al.* 2001). However, in this tallgrass ecosystem, within-season variability in rainfall patterns are more likely to contribute to the large variation in annual ANPP reported (Frank and Inouye 1994; Knapp and Smith 2001). Tallgrass prairie ANPP can respond quickly to changes in precipitation due to the inherently high RGR of the dominant vegetation resulting in LAI adjustments during the growing season (Paruelo *et al.* 1999). This leads to high temporal plant and soil water dynamics (James *et al.* 2003). Within the tallgrass prairie, the dynamics of rainfall distribution are characterized by the majority of events being small (< 10mm) and not contributing largely to the annual sum, interspersed with a small number of large events (> 25mm) that constitute the majority of the total annual amount of precipitation. Because of the relative contribution and frequency of small vs. large events, the variance of precipitation patterns can potentially be as important as the overall annual amount and serve as a key driver of biomass production (Lauenroth and Sala 1992; Williams *et al.* 1998).

In order to compare the long term data archived at KPBS to short-term manipulative experiments, I required soil moisture data at a finer temporal scale (daily) than available from the long term data (bi-weekly). Modeling soil moisture on a daily timestep allowed for comparisons of identical measures of precipitation variability, as manifested through changes in soil moisture between both datasets (long term and experimental). Without this daily timestep, the central theme of the manuscript comparing experimental manipulations and long term data would be impossible. As an alternate approach to determining the influence of abiotic variability, models can be used heuristically to explore how varying amounts of precipitation translate into different levels of soil moisture by progressively changing the values of other influential abiotic variables used in the correlative analysis. This technique would provide mechanistic support for conclusions derived from studies of correlative patterns between abiotic parameters and ANPP without reliance upon daily estimates of modeled soil moisture.

Analyzing the natural variability in precipitation patterns from the LTER data sets at KPBS, several similarities and differences were evident when compared to the reported findings of the rainfall manipulation plots (RaMPs). Perhaps the most prominent result of the RaMPs experiment was the negative relationship between ANPP and soil moisture variability; a relationship stronger than that between productivity and soil water amount (Knapp *et al.* 2002; Fay *et al.* 2003). While a significant relationship between productivity and soil moisture variability was present in the long term datasets (Table 5.1), the relationship between ANPP and average soil moisture amount was much stronger (Fig. 5.2; $r^2 = 0.58$). The differing results between studies using long vs. short-term data was likely due to differences in the magnitude of variability being compared. Both indices of soil moisture variability calculated from the historical record were of a magnitude that was less than half of that imposed in RaMPs studies (Fig. 5.3; Knapp *et al.* 2002; Fay *et al.* 2003). Indeed, if the results of Knapp *et al.* (2002) or Fay *et al.* (2003) were constrained to the range of values reported in the long term dataset, the patterns, significance, and implications would be altered markedly.

The nature of the relationship between variability and productivity also differed between the RaMPs experiment and the long term datasets. Results from both the site and biome level have shown that precipitation variability and precipitation amount are inversely correlated (Knapp and Smith 2001; Knapp *et al.* 2002; Fay *et al.* 2003). In this study, indices of soil moisture variability were not significantly correlated with productivity when averaged across the entire growing season (Table 5.1). However, the relationship between productivity and variability were significant when analyzed as a monthly response (Table 5.1). The response differed during the growing season, with generally positive trends for April, May, and June, and negative trends during July, August, and September. The change in slope from positive to negative may reflect the seasonal pattern of shifting limitations within the tallgrass prairie community (Seastedt and Knapp 1993; Blair 1997). The positive relationship early in the growing season suggests that productivity was limited by variables other than precipitation and soil moisture (i.e., light and temperature). For example, because soil moisture is high in the spring, extended warm dry periods with high irradiance that would increase soil water variability would almost certainly increase growth in the dominant C_4 grasses. This transition from a positive to negative relationship between ANPP and soil moisture variability illustrates the time periods for which precipitation exerts the greatest control over growth. Little influence of soil moisture is noted during the cool, dry spring season, but variability exerts a greater impact following the summer dry-down of soil moisture. Changing seasonal relationships between productivity and precipitation have been reported for other grasslands. Jobbágy and Sala (2000) found that cumulative precipitation was a nonsignificant predictor of grass productivity in the Patagonian Steppe when expressed annually, but a significant relationship between ANPP and precipitation amount arose when the analysis was divided into seasons. Similarly, Jobbágy *et al.* (2002) have reported that in space and time, temperature, not precipitation, is the primary variable initiating growth.

Using space for time substitutions, Sala *et al.* (1988) explained a significant amount of grassland ANPP ($r^2 = 0.90$) using a single predictor variable: precipitation amount. However, using nineteen years of productivity and meteorological data at a single site (KPBS), the explanatory power of this relationship was reduced substantially (Briggs and Knapp 1995). Because the relationship between productivity and a single variable weakens at the site level as Sala *et al.* (1988) predicted, I included other variables in this analysis in an attempt to improve the model. I found a substantial increase in the amount of variability explained ($r^2 = 0.82$) using a MLR model that incorporated variability indices compared with a single predictor variable ($r^2 = 0.62$ for precipitation amount alone). The substantial increase in explanatory power reported here contradicts the results of Briggs and Knapp (1995), who found that the inclusion of multiple meteorological variables resulted in <10% increase in explanatory power. The increase I report may result from the inclusion of parameters reflecting variability rather than means of additional variables (soil moisture, evaporation, etc.). The increased fit of this MLR model does support the contention of Sala *et al.* (1988), that at an individual site, the inclusion of multiple variables will be required to explain the dynamics of inter-annual ANPP.

Increased precipitation variability in an altered global climate will likely contribute to wider inter-annual ANPP fluctuations in the grassland regions of North America (Knapp *et al.* 2002; Fay *et al.* 2003). Previous results from experimental studies suggest that increased variability will negatively influence productivity in the tallgrass prairie (Knapp *et al.* 2002; Fay *et al.* 2003). The relationship between ANPP and moisture variability in this long term data set differed across the growing season with positive relationships early, and negative relationships later. Despite these differences, results from both short and long term studies illustrate the importance of temporal patterns of precipitation, not just seasonal means, on grassland ANPP. These results also indicate that long term datasets may not capture the range of variability forecast under altered climate scenarios, and thus analyses based solely on these historic data may not be sufficient to predict future ecosystem responses.

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Table 5.1: Correlation coefficient matrix depicting the relationships between grass ANPP (end of season) and two indices of soil moisture variability (an absolute difference index vs. the CV, see text) partitioned by the six growing season months and for the entire season. Both the coefficient of determination and the Pearson correlation coefficient are given to describe the proportional reduction in error and nature (positive or negative) of the linear relation, respectively. Values for significant associations (*P* < 0.05) are in bold.

		Soil moisture variability index vs. Grass ANPP						
Index by Month		Apr.	May	June	July	Aug.	Sept.	Entire Season
Absolute Difference	r^2	0.14	0.02	**0.27**	0.01	**0.32**	0.01	0.05
	Pearson's	0.37	0.13	**0.52**	0.09	**-0.57**	0.12	0.23
CV	r^2	0.15	0.06	**0.45**	0.01	0.15	0.02	0.03
	Pearson's	-0.38	0.26	**0.67**	-0.32	-0.38	-0.14	-0.17

Figure 5.1: Long term record of aboveground net primary production (ANPP) plus SE (n=20) for grass (primarily C_4 species) and forb (C_3 herbaceous plants) with corresponding growing season precipitation amount in annually burned mesic grassland in NE Kansas (Konza Prairie LTER site). Typically, grass productivity accounts for approximately 95% of total ANPP with variations in timing and amount of precipitation shifting this percentage between 90-99% (Briggs and Knapp 1995).

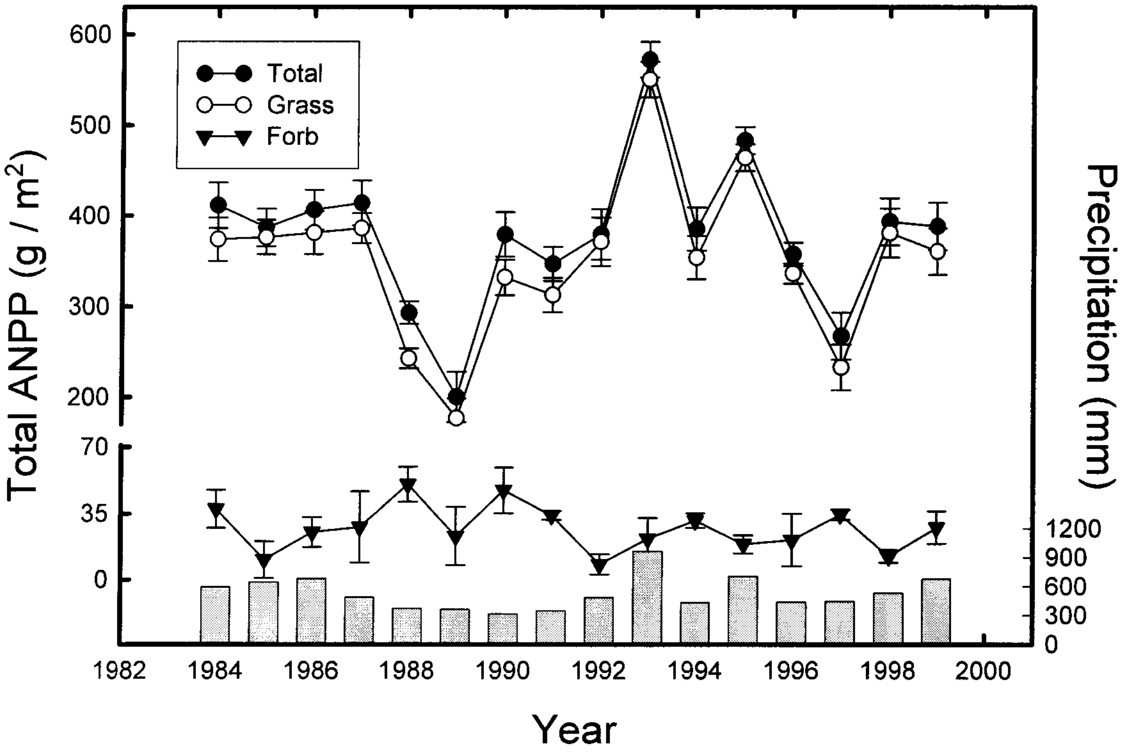


Figure 5.2: Grass aboveground net primary production vs. growing season (April-Sept.) precipitation (mm) and mean growing season soil moisture (modeled) at 30 cm depth. Inset figure shows model predictions vs. measured soil moisture (neutron probes at 25cm) averaged over the entire season for each year of the study. The solid line is a 1:1 line between measured vs. modeled soil moisture.

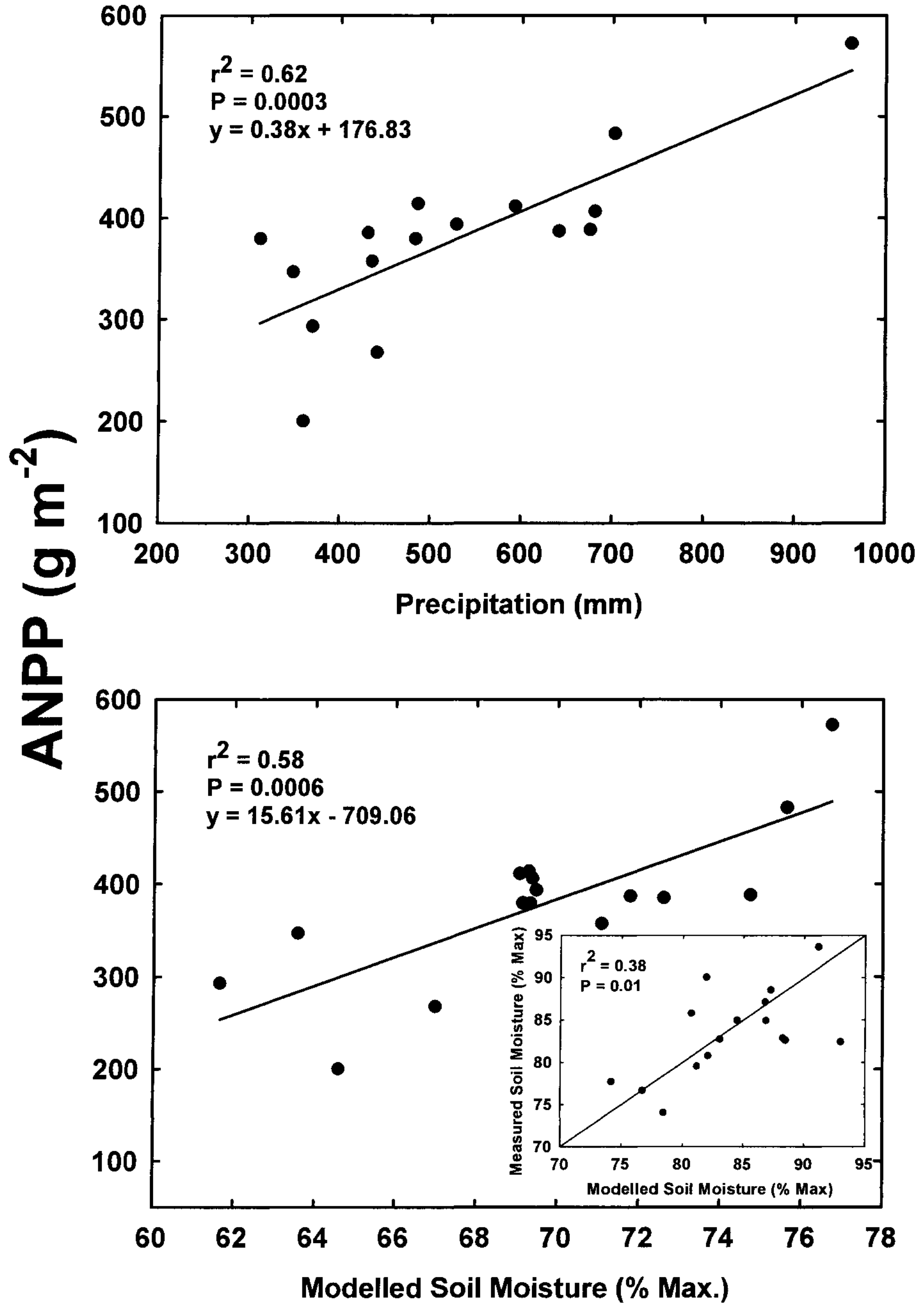


Figure 5.3: Comparison of the magnitude of soil water variability imposed experimentally vs. estimated from the long term record of precipitation variability. Bars correspond to the magnitude of variability experienced for CV of July soil water content (A) and mean variability in soil water content over the growing season (B). The different time periods of variability compared between A and B (July vs. entire season, respectively) were chosen to match those reported from experimental studies (Fay *et al.* 2003, Knapp *et al.* 2002).

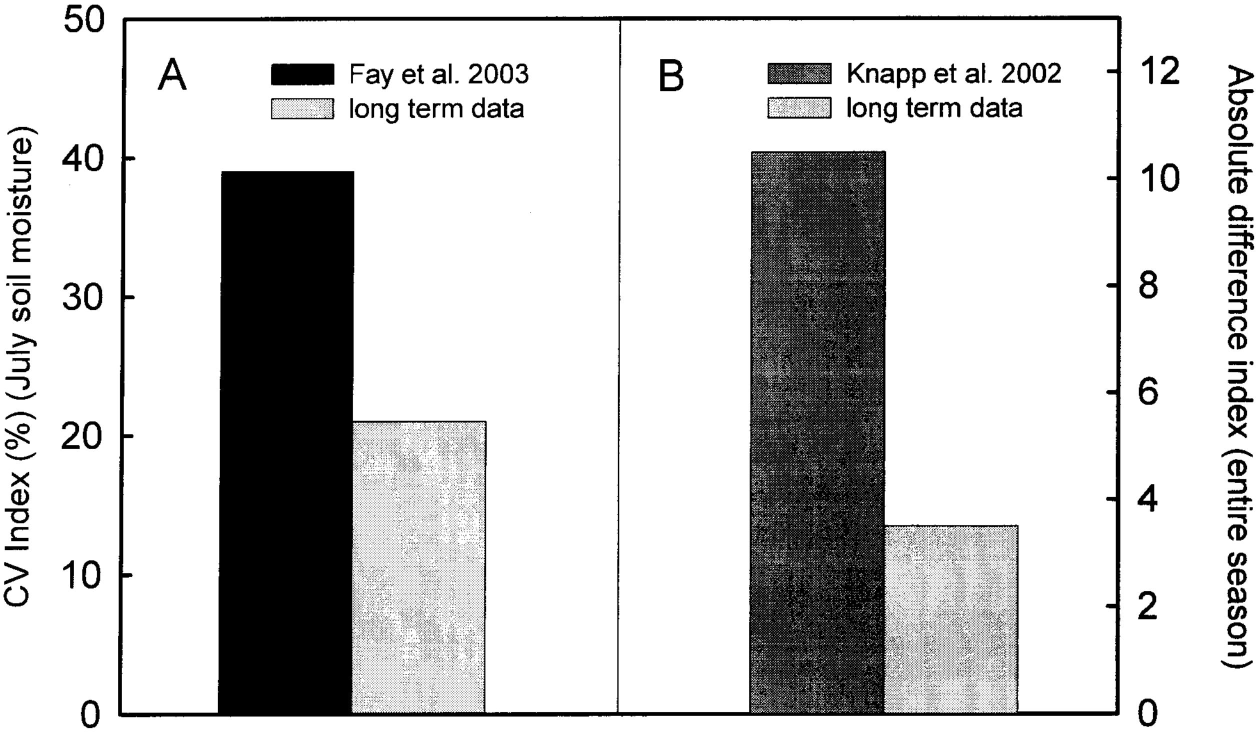
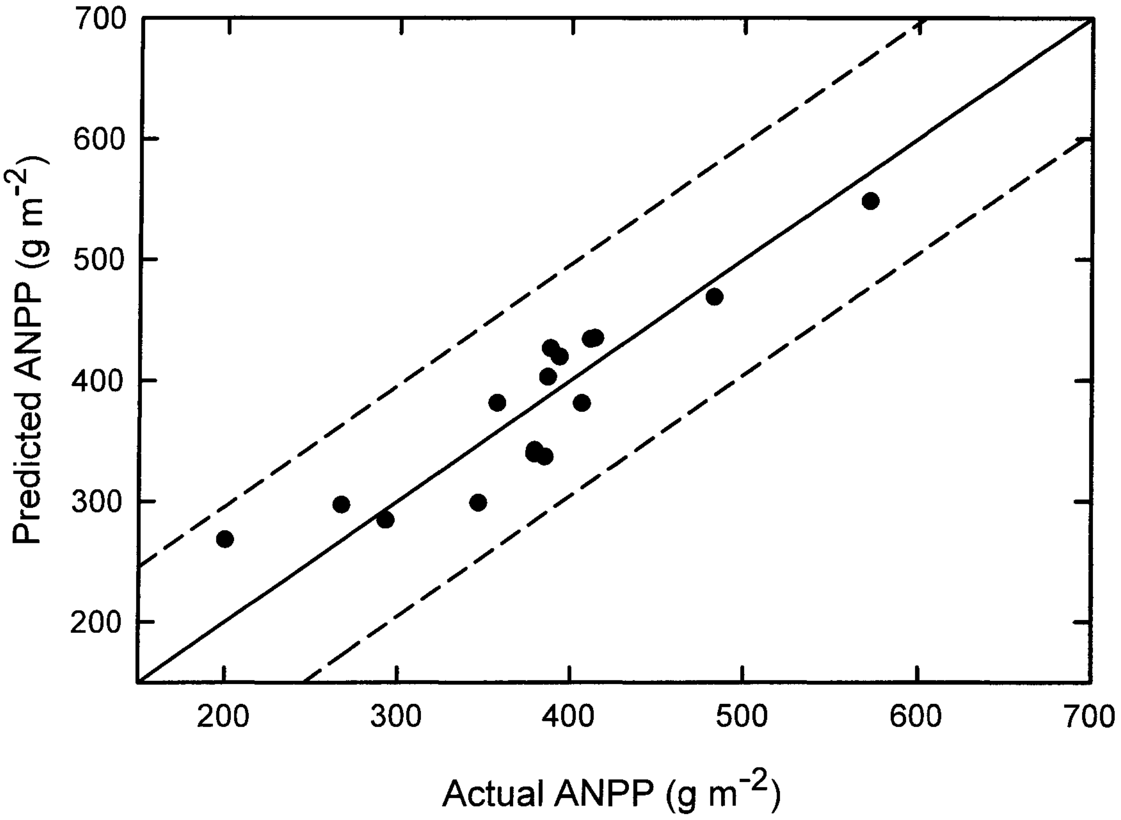


Figure 5.4: Actual vs. predicted ANPP based on a multivariate regression model incorporating three independent variables (precipitation amount, CV of June soil moisture, and mean variability in soil water content for May and June combined, r^2 = 0.82). The proportion of explained variance for the three variables is 0.62, 0.04, and 0.16, respectively. The solid line is a 1:1 relationship and the dashed line represents the 95% C.I. for the multivariate model. Filled circles represent values predicted from the multivariate regression model.



Chapter 6: Summary and Conclusions

In the tallgrass prairie, instantaneous photosynthesis and annual productivity vary by species, functional type, landscape location, and precipitation history. Variation in landscape productivity and diversity may arise from species or photosynthetic pathway differences in resource capture and use or differences in low resource avoidance or tolerance. To understand the interactive effects of resource availability and species responses, I compared the responses of C_3 and C_4 species in the tallgrass prairie to variations in water availability and growth environment as the primary objective of my dissertation. To address these responses, I used long-term data, experimental manipulations and annually burned watersheds at the Konza Prairie Biological Station.

In order to determine if the success of C_4 species in the tallgrass prairie results from inherently higher photosynthetic capabilities or higher resource capture and use-efficiency, I compared multiple photosynthetic traits of C_3 and C_4 species under contrasting resource environments. Photosynthetic traits were measured in outdoor microcosms with high light, available soil N, as well as upland prairie field sites, with ambient light, soil N and water. The C_4 grasses had the highest photochemical values in the microcosms, but the lowest in the field. C_3 leguminous species showed little change between contrasting resource environments. My conclusions suggest C_4 dominance in this prairie results from greater capture of resources when abundant and higher use-efficiency when scarce. I did not find a C_4 photochemical advantage when plants are grown in competition and resources are constrained.

To test where differences in resource-capture exist between C_3 and C_4 species in this grassland, I quantified patterns of water-use among species of both photosynthetic types, by growing season, and across a topographic gradient. I hypothesized that differences in water-use by C_3 and C_4 species may help explain our understanding of species dominance and persistence. My results show a greater common dependence on moisture in surface soil layers by C_4 species and greater flexibility of water-use by C_3 species. In general, all species used proportionally more water from deeper in the profile during dry periods, but there was common use of recent precipitation from shallow soil layers following major rainfall events. C_3 species used less surface water than C_4 species regardless of the time of measurement or the precipitation history. The flexibility by C_3 species to vary the source of their water used based on precipitation history suggests that the greater rooting depths of these species does not always reflect greater water use from depth, especially when surface soils are wet. Water-use varied by topographic position, with greater use of surface soil layer moisture following rainfall in hillside and lowland positions, while uplands had greater proportional reliance on all soil layers.

In order to uncouple the effects of water-stress and water availability on water-use by species, I used structural equation models (SEM) to identify the variance-covariance relationships among predictor variables. The results of the SEM analysis suggest that C_3 species using water from multiple sources (per $\delta^{18}O$ in xylem water) had reduced midday water stress. Conversely, midday water stress in C_4 species was governed by surface (5cm) soil moisture. Therefore, when volumetric soil moisture was high in this region, C_4 grasses had reduced stress and when soil moisture was low, grasses had greater stress. This response reinforces my previous results that C_4 grasses have greater dependence on surface soil moisture regardless of availability. The SEM analyses had relationships therein that varied by photosynthetic types. C_4 grasses had similar models and relationships among parameters. However, models and relationships were divergent for each of the C_3 species measured. My results suggest C_4 grasses are more drought tolerant compared to C_3 species, while C_3 species exhibit drought avoidance with unique species strategies for water capture to minimize water stress and overlap among other coexisting species.

Finally, I investigated whether the relationship between soil moisture variability and ecosystem productivity reported in experimental manipulations on Konza Prairie were present using long-term data of annually-burned watersheds. Using 16 years of aboveground annual productivity, variability in precipitation pattern was still an important predictor of aboveground biomass in the annually-burned prairie, but not of comparable magnitude as reported in the experiment manipulations of precipitation pattern. The magnitude of variability in precipitation pattern experienced long-term was less than half of that induced experimentally, suggesting that predictions of the tallgrass prairie response to future climate scenarios may not be adequately predicted using long-term data based on the constraints of historical variability.

The results of this dissertation highlight the varying responses of experimental versus field measurements in the tallgrass prairie. I found differences in water-use vary by C_3 and C_4 species by location, time, water availability, and plant stress. The success of C_4 grasses in this prairie likely stems from increased drought tolerance and greater uptake of water when available in surface soil layers. Conversely, persistence of C_3 species results from drought avoidance and greater flexibility in the source of water used. The unique relationships in water used in response to water stress and availability among tallgrass species may at least partially contribute to the unique structure of high grass productivity and high forb diversity present in this grassland.

Appendix:

i. Standard operating procedures for the xylem extraction line

SOP’s for running samples on the vacuum extraction line

RHS= right hand side; LN = liquid Nitrogen; vaccutainer = collection vial w/ septum

1. Fill the thin dewar on the upper RHS with LN. This dewar serves as a cold-trap for the line, freezing molecules before they reach the pump, and creating a stronger vacuum.
2. Turn on hotplates to setting ‘10’. Fill beakers with water and heat until they reach a rolling boil.
3. While water is heating, prepare samples for placement on the line. Holding the sample vaccutainers by the cap, place in a dewar of LN to freeze the sample within. The sample is generally frozen when the LN stops boiling (10-15 sec.).
4. Immediately after freezing, place samples on the LHS of the line, making sure the needle tips are unblocked. Evacuate the vaccutainers by opening the top valve and noting the drop in vacuum pressure. When the vacuum gauge < 300, close top valve. If multiple frozen samples are placed on the line simultaneously, it is possible to evacuate multiple samples at once. Be aware that if the samples thaw (even slightly), you will lose water and jeopardize the accuracy of your subsequent measurements.
5. After the water is boiling, slide the big LN dewar up under the center trap and place on the wood blocks. If done correctly, the top lip of the dewar will be 1-2” from the top glass tubing, and the trap will be submerged in ~3” of LN.
6. Raise the jack holding the hotplates and boiling water up under the vaccutainers. Each vaccutainer should have a separate boiling water bath, with the sample completely submerged up to the cap. If the cap becomes submerged, it may leak and fill with water (VERY BAD). Once each vaccutainer is submerged in the water bath, open the bottom valves for each sample, allowing water vapor, CO_2 and noncondensibles to move to the center trap.
7. Until now, all the valves should be open except the top valves on the LHS.
8. Let the samples boil for at least 40 minutes or until you are certain that all the water has moved to the center trap. As the water in the beakers boils away, add water to keep the entire sample submerged.
9. Pour LN into the 5 styrofoam cups and place on the RHS jack. Elevate the jack, sliding the cups under each collection vaccutainer. Only let the bottom tip of the vaccutainer submerge in the LN. As the LN boils away, raise the jack to maintain about ½” of the vaccutainer tip in the LN.
10. Close the valves on the top RHS. Turn off the hotplates and lower the jack and water beakers from the sample vaccutainers. Close the LHS bottom valves and THEN remove the your sample vaccutainers from the line. You can also now remove the LN dewar from the center trap. At this point, only the bottom RHS valves should be open.
11. Turn on the heating tape by turning the power regulator to a setting of ‘7’.
12. Using the heat gun, warm the center trap until the frozen water turns to vapor. The vapor will scatter throughout the line and you will need to use the heat gun to move the vapor droplets to the ports and sample vaccutainers on the RHS of the line.

13. When all the water vapor is collected between the sample vaccutainer, yet above the valve on the bottom RHS, close this valve.
14. Using the heat gun, continue to heat the vapor that is trapped between the top and bottom valves on the RHS. It doesn't matter how hot you head the glass outlet, but pay special attention to the Cajun fitting and the greased O-rings on the valves. If these get too hot, the O-rings will denature and leak. As a general rule of thumb, I don't heat in the same place for > 5 seconds. Also, if you heat the cap on the vaccutainer, it will quickly denature and you will lose your sample.
15. It is very important to get all of the water out of the line, Cajun fitting, needle port, etc. As the heavy and light isotopes of your sample will move at varying speeds, it is critical to collect all of the water. If any is left in the line, the signature of your sample may be imprecise.
16. When you believe all the water has moved into the sample vaccutainer, turn off the heating tape and lower the RHS jack and LN cups. You can now take your sample vaccutainer off the line. While the sample is still frozen, unscrew the cap and place a new 'unpierced' cap on your sample.
17. The sample collection is now finished and you must prepare the line for the next run. Put a new clean, empty vaccutainer on the RHS and open the top and bottom valves on the RHS. The vacuum pump will scrub the line back down to approximately 100 mT. When it reaches this pressure, you can repeat the process with your next 5 samples.
18. If you have enough samples to do several days worth of work, leave the vacuum pump on when you are finished. If however you are finished for at least a week, turn it off.

Extras:

1. Always wear safety glasses when pouring/ handling the LN
2. Never, ever leave residual water in the line. If you have an accident or leak, take the Cajun fittings off or unscrew the bottom valves on both sides and heat the water with the heat gun until you get it out of the line.
3. If you cannot keep or reach a good vacuum in the line, troubleshoot the problem by closing all the valves and opening them one at a time until you find the source of the leak. At this point, you may have to re-grease the O-rings, change needle tips or take the appropriate action to create a good seal and establish a strong vacuum. The water will not move and you will kick yourself if you try to run samples without a strong vacuum.
4. Keep the line clean and ask the lab supervisor for help when it's time to re-grease the line. This should be done every 50 samples or so.