Role of the Leguminous Shrub *Amorpha canescens* (Leadplant) in the Nebraska Sandhills Grasslands: Water Relations and Patterns of Water Uptake

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ROLE OF THE LEGUMINOUS SHRUB AMORPHA CANESCENS (LEADPLANT) IN THE NEBRASKA SANDHILLS GRASSLANDS: WATER RELATIONS AND PATTERNS OF WATER UPTAKE

by

Adam J. Yarina

A THESIS

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This study investigated the ecophysiological role of *Amorpha canescens*, a leguminous shrub native to the Nebraska Sandhills. Although *A. canescens* is an important ecological player in the Sandhills, its impacts on the surrounding plant community are poorly understood. To remedy this, two sites were selected for study at the Gudmundsen Sandhills Laboratory near Whitman, Nebraska – one with *A. canescens* (G-L) and one without *A. canescens* (G-NL). Both sites contained five representative herbaceous species: two C₃ grasses (*Hesperostipa comata* and *Koeleria macrantha*), two C₄ grasses (*Andropogon hallii* and *Calamovilfa longifolia*), and one forb (*Helianthus pauciflorus*). Plant canopy cover and aboveground biomass were characterized on both sites in June and August 2010, along with soil composition, organic matter, carbon, and nitrogen content. Seasonal trends in transpiration (E), water use efficiency (WUE), and predawn (Ψ_{pre}) and midday (Ψ_{mid}) water potentials were determined for all species in both plots at 2-week intervals from June through September. Precipitation, groundwater, plant root crown, and soil water samples were collected to determine sources of plant water uptake via stable isotope analysis and the IsoSource mixing model. The results indicate that the presence of *A. canescens* is favorable to C₃ grasses when water is plentiful.
However, under water limited conditions, the additional demands on shallow soil water coupled with increased rainfall interception from shrub canopy and litter were disadvantageous to $C_3$ grasses. $A. \text{canescens}$ also appeared to enhance the amount of water available deeper in the soil profile, resulting in greater overall moisture in the upper 1 m of soil. Water resource partitioning was not observed during the wetter periods in the first half of the study period, with all species predominantly using shallow soil water. However, $H. \text{pauciflorus}$ and $A. \text{canescens}$ switched to deeper water sources as water became limited, while $C_3$ and $C_4$ grasses senesced or reduced stomatal conductance to limit water loss. The ecological implications of these results are discussed.
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1 Introduction

Grassland ecosystems comprise a quarter of all potential natural terrestrial vegetation across the globe, occurring across a wide range of regions and climates (Krebs 2001). They are highly productive ecosystems, producing as much as 1400 g m\(^{-2}\) yr\(^{-1}\) of dry plant matter in wetter regions and as much as 500 g m\(^{-2}\) yr\(^{-1}\) in North America (Lauenroth 1979). In the United States, grasslands cover approximately 125 million hectares of land and span all or part of 13 states, including Nebraska (Stubbendieck et al. 2003, Stubbendieck and Kottas 2005). Nebraskan grassland is managed chiefly for domestic grazing and forage production, covering more than 10 million hectares of land and contributing over $1 billion to the state’s economy each year (Stubbendieck and Kottas 2007). The Nebraska Sandhills form an important part of this expanse.

The Sandhills are a formation of vegetation-stabilized sand dunes located in north central Nebraska and southern South Dakota. They are the largest sand dune formation in the Western Hemisphere and one of the largest worldwide, spanning approximately 50,000 km\(^2\) and ranging nearly 430 km from end to end (Bleed and Flowerday 1998). Comprised of mixed grass prairie, the Sandhills form a transitional zone between the short-grass prairie to the west and the tall-grass prairie to the east (Stubbendieck and Kottas 2005). This zone is primarily the result of changes in water availability as the climate becomes progressively drier from east to west across the region (Kaul 1998, Wilhite and Hubbard 1998). Predominantly sandy soils allow for rapid rainfall infiltration and minimize the amount of moisture lost to surface runoff (Stubbendieck and Kottas 2005).
Sandhills vegetation is a diverse mix of C\textsubscript{3} and C\textsubscript{4} grasses, herbaceous plants, shrubs, and succulents. Similar to most of the Great Plains, C\textsubscript{4} grasses are the dominant plant group, producing more biomass each year than their C\textsubscript{3} counterparts (Epstein et al. 1997, Kaul 1998). Shrubs are an important component of many grassland ecosystems; their presence has been shown to boost soil nutrients (West 1989, Cable et al. 2009), facilitate vegetation recovery (King and Stanton 2008), mitigate microclimatic extremes (Moro et al. 1997), and increase biodiversity (Pihlgren and Lennartsson 2008). Shrubs can also redistribute water within the soil profile via hydraulic lift, often to the benefit of nearby plants (Richards and Caldwell 1987, West 1989). On the other hand, shrubs can negatively affect the surrounding plant community through increased competition for resources (Lett and Knapp 2003, McCarron et al. 2003, Lett and Knapp 2005).

Leadplant (\textit{Amorpha canescens} Pursh) is a native leguminous shrub found throughout the Sandhills (Weaver and Fitzpatrick 1934b, Kaul 1998). The shrub has distinct ecological and economic benefits; its presence improves soil fertility, provides nutrient-rich forage for wildlife and livestock, and serves as an indicator of good range condition (Stubbendieck and Kottas 2007). Despite its importance, \textit{A. canescens}’ exact role within the Sandhills and its effects on the surrounding plant community are not well-characterized. This study aimed to shed light on these areas, particularly with respect to the impact of \textit{A. canescens} on the water uptake patterns of dominant C\textsubscript{3} and C\textsubscript{4} plants. To accomplish this, we selected two similar Sandhills plant communities for study. Both communities featured dominant C\textsubscript{3} and C\textsubscript{4} grasses and forbs, but only one contained \textit{A. canescens}. Data on local weather, soil characteristics, and plant physiology were collected at regular intervals over the course of the growing season, and water samples
were taken from plants, soil, groundwater, and precipitation to determine plant water uptake patterns.

Given the initial benefits seen in *A. canescens* and the benefits of shrubs found in other grassland ecosystems, I hypothesized that the presence of *A. canescens* would have a positive effect on the water uptake patterns of the surrounding plant community. This would manifest as lower measures of water stress (e.g., less negative water potential), greater water availability (e.g., higher transpiration and lower water-use efficiency rates), and potentially greater plant biomass in the community with *A. canescens* compared to the community without it.
2 Literature Review

2.1 Grassland Ecology

Grasslands cover approximately 125 million hectares of land in the United States. The Great Plains are home to much of this expanse, covering all or part of 13 states (IA, MN, MO, ND, SD, NE, KS, OK, TX, NM, CO, WY and MT) including the Nebraska Sandhills (Burke et al. 1991, Luaenroth et al. 1999, Stubbendieck et al. 2003). North American grassland ecosystems are characterized by high variability, largely due to their intercontinental location and vast expanse. Grasslands found near the Rocky Mountains in the west have starkly different characteristics compared to those found in the Central Lowlands in the east (Weaver and Fitzpatrick 1934a, Weaver and Albertson 1956a, Luaenroth et al. 1999). Seasons are characterized by hot, dry summers and cold winters with windy conditions year-round, and blizzards in late winter or early spring are not uncommon (Weaver and Fitzpatrick 1934a, Weaver and Albertson 1956a, Burke et al. 1991, Bleed and Flowerday 1998, Luaenroth et al. 1999).

Precipitation is best described as sporadic. Although between 70 and 80 % of annual precipitation falls during the growing season – April to September – it tends to occur either in light showers or heavy downpours rather than slow and steady rain, often resulting in moisture that is unavailable to vegetation. Light rains can be lost to canopy interception and evaporation while heavy rains can percolate rapidly into the soil profile, resulting in water that is accessible only to deep-rooted vegetation, if at all (Weaver and Fitzpatrick 1934a, Weaver and Albertson 1956a, Burke et al. 1991, Luaenroth et al. 1999). Drought is a constant threat to grassland plants and typically occurs at least once a year, especially after midsummer. Even during periods of adequate rainfall, moisture can be
poorly distributed within the soil profile and negatively impact vegetation; grassland species must have adaptations to avoid or at least tolerate drought (Weaver and Fitzpatrick 1934a, Weaver and Albertson 1956a, Knapp 1984, Barnes 1985, Letts et al. 2010). The unpredictability of available soil moisture coupled with the presence of periodic fire plays a significant role in limiting the distribution of trees and other large woody species (Axelrod 1985, Abrams and Hulbert 1987), although their occurrence in some grassland ecosystems is increasing in response to changes in these and other environmental factors (Auken 2000, Heisler et al. 2003, Eggemeyer et al. 2006, Knapp et al. 2008).

Wind is omnipresent in North American grasslands and magnifies the impact of seasonal climate. Hot southerly winds accelerate water loss from soil and vegetation during the summer, while winter winds from the northwest bring sub-zero temperatures and heavy snowfall. Recorded wind speeds are higher than anywhere else in the country with the exception of some coastal shores (Weaver and Fitzpatrick 1934a, Weaver and Albertson 1956a).

The Great Plains region is home to multiple grassland types, including tallgrass, shortgrass, and mixed grass prairies (Luaenroth et al. 1999). Tallgrass prairies are characterized by tall-grass species (> 1 m tall) such as big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans* (L.) Nash), and switchgrass (*Panicum virgatum* L.) and historically occupied the eastern parts of the Great Plains, although only remnants of their original expanse remain. Shortgrass prairies are found in the western Great Plains, and are home to short grass species (< 0.3 m tall) such as buffalograss (*Buchloe dactyloides* (Nutt.) J.T. Columbus), blue grama (*Bouteloua gracilis* (Willd. ex
Kunth) Lag. ex Griffiths), and western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve).

Mixed-grass prairies, including the Nebraska Sandhills, fall between the shortgrass and tallgrass types, and have a mix of short, tall, and mid-height grasses (0.3 to 1.0 m tall) such as little bluestem (*Schizachyrium scoparium* (Michx.) Nash) and prairie junegrass (*Koeleria macrantha* (Ledeb.) Schult) (Stubbendieck et al. 2003).

### 2.2 The Sandhills

The Sandhills comprise nearly 50,000 km$^2$ of vegetation-stabilized sand dunes in central and western Nebraska and southern South Dakota. They are the largest sand dune formation in the Western Hemisphere and one of the largest worldwide, ranging nearly 430 km from end to end. In Nebraska, their extent ranges from Morrill and Box Butte counties in the west to Antelope and Boone counties in the east, and from Dawson county in the south to Cherry county in the north and into South Dakota (Bleed and Flowerday 1998). The Sandhills formed geologically in the last 8,000 years and their topography remains variable, with dunes as high as 120 m, as long as 30 km, and with gradients as steep as 25% (Bleed and Flowerday 1998).

#### 2.2.1 Environment

Three major processes govern life in the Sandhills: precipitation, topography, and wind (Weaver and Albertson 1956b, Wilhite and Hubbard 1998). Overall climate in the Sandhills ranges from sub-humid in the east to semi-arid in the west, a pattern fairly typical of the Central Great Plains. Mean annual precipitation in the Sandhills varies from 584 mm yr$^{-1}$ in the east to less than 432 mm yr$^{-1}$ in the west, with approximately 75%
falling during the growing season – April to September – and 50 % falling during May, June and July. Winter precipitation – October to March – is predominantly snow, ranging from 560 to 710 mm in the south to 1140 mm in the north, and plays an important role in determining available soil moisture for new plant growth early in the growing season (Wilhite and Hubbard 1998).

Temperatures in the Sandhills follow a similar west-to-east gradient; mean annual temperatures are approximately 9.4 °C in the east and 8.9 °C in the west. Mean maximum daily temperatures in July are 32.2 °C in the southwest and 31.1 °C in the northeast, and mean minimum daily temperatures in January are -11.67 °C and -13.89 °C in the same regions, respectively. The eastern Sandhills are frost-free 150 days out of the year, whereas western areas are frost-free 120 days, owing to differences in elevation; the western Sandhills are approximately 61 m higher than their eastern counterparts (Wilhite and Hubbard 1998). Eolian erosion, transport and deposition have shaped the formation of the Sandhills for the last 65 million years (Swinehart and R. F. Diffendal 1998) and continue to do so in areas not stabilized by vegetation. Mean annual wind speed ranges from 6 to 8 m s⁻¹ (Bleed and Flowerday 1998, NEO 2005).

The diverse topography of the Sandhills produces substantial differences in species composition over relatively small distances. Dry prairie ecosystems situated on top of dunes are often found adjacent to low-lying wetlands, lakes and meadows. The Sandhills’ highly permeable sandy soil is partly responsible for this variation, facilitating high recharge rates from precipitation and producing significant variation in the depth and availability of soil moisture (Bleed 1998, Kaul 1998).
2.2.2 Plant Life

The Sandhills’ variable topography and seasonal moisture produce a wide variety of vegetation types, with nearly 700 native species documented across the $C_3$, $C_4$, and CAM (Crassulacean Acid Metabolism) functional groups. (Kaul 1998). Tall grasses such as prairie sandreed (*Calamovilfa longifolia* (Hook.) Scribn.) and sand bluestem (*Andropogon hallii* Hack.) coexist with mid grasses such as needleandthread (*Hesperostipa comata* (Trin. and Rupr.) Barkworth) and sand dropseed (*Sporobolus cryptandrus* (Torr.) A. Gray), and short grasses such as Scribner’s panicum (*Dichanthelium oligosanthes* (Schult.)), blue grama (*Bouteloua gracilis*), hairy grama (*Bouteloua hirsute* Lag.) and sedges (*Carex* spp.) (Kaul 1998).

Topography plays a major role in species composition due to its influence on soil moisture. For example, interdunal valleys that have finer-textured soils result in slower infiltration rates and higher moisture contents at shallow depths until mid-summer, when accessible water is exhausted by dense stands of early-growing shallow-rooted plants (e.g., blue grama (*B. gracilis*), Kentucky bluegrass (*Poa pratensis* L.) and *Carex* spp.) (Barnes and Harrison 1982, Barnes et al. 1984). Upland soils have a coarser texture and a different species makeup; dune tops and south-facing slopes tend to be dominated by tall $C_4$ grasses, whereas north-facing slopes are favored by $C_3$ grasses, forbs, and certain shrubs (*A. canescens* Pursh), although some $C_4$ grasses are also present (*S. scoparium*) (Schacht et al. 2000). Sandier upland soils allow for faster infiltration of precipitation and minimize runoff, providing favorable growing conditions for tall grasses (Burzlaff 1962). Mid and short grasses are often found in the understory and dominate in areas unfavorable to tall grasses. Forbs are common but secondary to grasses in terms of
biomass production and cover, while shrubs contribute even less to these two parameters outside of their immediate surroundings (Tolstead 1942, Kaul 1998).

2.2.3 Root Depth and Distribution

The distribution of root systems is a major factor in determining the species composition of a given region. Root characteristics of several biomes are outlined in Table 2.1.

<table>
<thead>
<tr>
<th>Biome</th>
<th>Mean Root Biomass</th>
<th>% Mean Root Biomass In Top 30 cm</th>
<th>Root/Shoot Ratio</th>
<th>Mean Maximum Root Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperate Grassland</td>
<td>1.4 kg m⁻²</td>
<td>83 %</td>
<td>3.7</td>
<td>3.7 ± 0.5 m</td>
</tr>
<tr>
<td>Temperate Deciduous Forest</td>
<td>4.2 kg m⁻²</td>
<td>65 %</td>
<td>0.23</td>
<td>2.6 ± 0.2 m</td>
</tr>
<tr>
<td>Desert</td>
<td>0.4 kg m⁻²</td>
<td>53 %</td>
<td>0.7</td>
<td>5.2 ± 0.8 m</td>
</tr>
<tr>
<td>Tropical Grassland Savanna</td>
<td>1.4 kg m⁻²</td>
<td>57 %</td>
<td>0.7</td>
<td>0.5 ± 0.1 m</td>
</tr>
</tbody>
</table>

Temperate grassland species tend to invest heavily in fine shallow root systems, given the high proportion of root biomass in the top 30 cm of soil, high root to shoot ratio and low mean root biomass. By contrast, temperate deciduous forest species have much greater mean root biomass below 30 cm, along with a much lower root/shoot ratio (Jackson et al. 1996). Despite this, their average maximum rooting depth is still less than
that of grassland species. Desert species have the highest mean maximum rooting depth and a significant amount of root biomass below 30 cm, reflecting the need for access to deeper and more consistent water sources in arid climates (Canadell et al. 1996, Jackson et al. 1996).

Rooting strategies differ between tall-grass, short-grass and leguminous species in North American grassland plants. Tall-grass species tend to have a dense network of fine roots that can extend deep into the soil profile, giving them access to deep soil moisture and inorganic nitrogen. Short-grass and rhizomatous species tend to have similar root systems but are limited to shallow portions (0-0.24 m) of the soil profile, leaving deeper soil moisture and inorganic nitrogen inaccessible. Leguminous species employ deep coarse root systems to access water at all depths of the soil profile but do not significantly affect inorganic soil nitrogen (Craine et al. 2003).

Root diameter has been shown to impact soil moisture content and inorganic nitrogen concentration. Fine root biomass – roots with a diameter less than 1 mm – in grasses and forbs is inversely correlated with soil moisture content and inorganic soil nitrogen concentration, while coarse root biomass – roots with a diameter greater than 1 mm – shows no relationship with either of these factors (Craine et al. 2003).

Water uptake patterns differ between plant functional groups. C_4 grasses [Andropogon gerardii Vitman, Schizachyrium scoparium (Michx.) Nash, and Sorghastrum nutans (L.) Nash] have demonstrated greater reliance on water in the top 30 cm of the soil profile, while C_3 forbs [Lespedeza capitata Michx. and Vernonia baldwinii Torr.] and shrubs [Amorpha canescens and Ceanothus spp.] tend to rely on water below 0.30 m in the soil profile. However, some degree of plasticity exists within these patterns.
All functional groups draw water from shallow depths in times of excess, such as immediately following precipitation events; C$_3$ grasses, forbs, and shrubs switch to deeper sources only when water becomes limited. This plasticity allows species and functional groups to coexist by reducing competition under water-limited conditions (Nippert and Knapp 2007a, Nippert and Knapp 2007b).

Similar resource partitioning has been observed in the Sandhills. In the Nebraska National Forest near Halsey, NE, analysis of water uptake patterns found a greater degree of plasticity in C$_3$ woody species [Pinus Ponderosa P. & C. Lawson and Juniperus virginiana L.] compared to C$_4$ grasses [Schizachyrium scoparium (Michx.) Nash and Panicum virgatum L.]. All four species drew most of their water from the upper soil profile (0.5–0.05 m) when water was plentiful in early summer (May–June); this was particularly true of S. scoparium and P. virgatum, which took nearly 100% of their water from this depth. C$_4$ grasses shifted some of their water uptake (~20%) to deeper depths (below 0.5 m) as the growing season (May–August) progressed and surface soil moisture declined. In contrast, P. ponderosa drew most of its water from both the 0.05–0.5 m and 0.5–0.9 m portions of the soil profile during the growing season, with J. virginiana acquiring most of its water in the 0.5–0.9 m range. Tree species shifted water uptake even deeper (below 0.9 m) in September and may have exploited groundwater at ~ 7 m when soil moisture content reached its nadir in the measured profile (0.05–3 m). This plasticity was also evident in winter months. Although additional access to water did not seem to have an effect on transpiration rates, it may have facilitated the recovery of water potentials in tree species once additional water became available (Eggemeyer et al. 2009).
2.3 The Role of Shrubs

2.3.1 General Overview

Shrubs are common fixtures in grasslands worldwide (Sala et al. 1989, Heisler et al. 2003, Frank and Karn 2005, Zarovali et al. 2007, Pihlgren and Lennartsson 2008, Letts et al. 2010). However, their ecological functions can vary greatly based on region, species composition and grassland type. For instance, shrubs have been found to increase biomass, protein concentration, and rates of regrowth in nearby grass stands compared to stands without shrubs (West 1989). There are numerous potential reasons for these observed benefits: shrubs as a functional group may positively impact the water budget via increased soil water infiltration, soil water capacity, and winter snowmelt. They may also enhance nutrient cycling via increased litter, creating nutrient “islands” that aid the growth of nearby plants, or provide protection from erosion and herbivory. Shrubs tend to possess an extensive network of deep thick roots that enhance erosion control, and those same networks may also improve access to deep water or nutrients via hydraulic lift (West 1989).

Field studies have documented many of these beneficial effects. Semi-arid grasslands with encroaching mesquite species (*Prosopis velutina* Woot.) have been shown to have greater soil microbial biomass, carbon-use efficiency and respiration rates in close proximity to large shrub individuals compared to sites that contained only grass species. *P. velutina* is a nitrogen fixing legume that produces an extensive litter layer, which increases soil carbon content and enhances soil microbial activity (Cable et al. 2010).
Retama sphaerocarpa (L.) Boiss. has been shown to create more favorable microclimatic conditions under its canopy, sheltering nearby vegetation from temperature and irradiance extremes (Moro et al. 1997). Hydraulic lift has been observed in sagebrush (Artemisia tridentata ssp. vaseyana) to the benefit of nearby grass species (Agropyron desertorum (Fisch. ex Link) Schult. and Agropyron spicatum Pursh) (Richards and Caldwell 1987).

Shrubs may also aid restoration in badly degraded grassland. Aloe species (Aloe secundiflora Engl.) were successfully used to restore severely overgrazed semi-arid rangeland in Kenya by transplanting individuals alongside newly seeded perennial grasses. The treatments with shrubs fared significantly better than plots that had been given similar physical protection with thorny branches and control plots that had no protection. This indicates that the benefits of A. secundiflora extend beyond physical protection from grazers (King and Stanton 2008).

The presence of glaucous dog rose (Rosa dumalis Bechst.) in several grassland pastures in Sweden was shown to have a positive effect on plant community biodiversity despite hindering the establishment of nearby seedlings of other plant species. R. dumalis’ role as a grazing refuge afforded nearby plants protection and greater chances of successful reproduction, outweighing the negative competitive effects (Pihlgren and Lennartsson 2008).

Certain shrubs have also been observed to have detrimental effects on their surrounding community. For instance, increases in the density of shrub populations were observed to have a negative effect on herbaceous forage quality and quantity in two separate grasslands in Greece and Germany; this negative impact was most likely the
result of reduced light availability to the herbaceous understory. The shrub species observed were kermes oak (*Quercus coccifera* L.), downy oak (*Quercus pubescens* Willd.), almond-leaved pear (*Pyrus amygdaliformis* Vill.), Jerusalem thorn (*Paliurus spin-a-cristii* Mill.) and common hawthorn (*Crataegus monogyna* Jacq.) for the study site in Greece; species studied in Germany were *Crataegus* spp., dog rose (*Rosa canina* L.), European ash (*Fraxinus excelsior* L.) and common dogwood (*Cornus sanguinea* L.) (Zarovali et al. 2007, Kesting et al. 2009).

Similar shrub-forb-grass interactions have been observed in tall-grass prairies in the central United States. Studies conducted at Konza Prairie found that the presence of roughleaf dogwood (*Cornus drummondii* C.A. Mey.) significantly reduced light availability, aboveground net primary production, carbon uptake and nitrogen-use efficiency for the herbaceous understory (Lett and Knapp 2003, McCarron et al. 2003, Lett and Knapp 2005).

### 2.3.2 *Amorpha canescens* in the Nebraska Sandhills

Although *A. canescens* has long been known as a common member of North American grasslands (Weaver and Fitzpatrick 1934b) its exact role is not well-characterized; available literature is limited at best, especially regarding its role in the Nebraska Sandhills.

*Amorpha canescens* has been described as the most ubiquitous species of upland prairie after grasses, occurring at nearly three-quarters of all upland sites. It is also quite common in lowlands and well-drained wetlands, occurring at nearly half of all sites (Weaver and Fitzpatrick 1934b). It is deeply rooted, reaching depths from 2 to 5 m and
branching out 1.2 m or more; this rooting strategy minimizes competition with grass species, allowing _A. canescens_ access to deeper water and nutrients in times of drought. _A. canescens_ is a nitrogen-fixing legume and can improve soil fertility, potentially providing a more favorable environment for understory species (Weaver and Fitzpatrick 1934b, Stubbendieck and Kottas 2007).

### 2.4 Plant Physiology

#### 2.4.1 Differences in Photosynthetic Pathways

Plants belong to one of three major functional groups based on their photosynthetic type: C₃, C₄, or Crassulacean Acid Metabolism (CAM), each of which follows a distinct metabolic pathway. The C₃ pathway is the most common and most primitive pathway, and uses ribulose biphosphate carboxylase (Rubisco) to acquire and fix CO₂ into a 3-carbon compound called 3-phosphoglycerate in the first metabolic step (Larcher 2001a). The C₄ pathway utilizes phosphoenolpyruvate (PEP) carboxylase to acquire and fix CO₂, converting it into an intermediate 4-carbon compound before Rubisco acts on it in a later metabolic step. The use of PEP carboxylase as the initial carboxylating enzyme – coupled with the unique “Kranz” anatomy and bundle sheath cells found in C₄ plants – produces significant physiological and ecological differences between C₃ and C₄ species, which are outlined in Table 2.2.

**Table 2.2 - Comparison of C₃ and C₄ photosynthesis (Larcher 2001a, Fitter and Hay 2002a).**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C₃</th>
<th>C₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The C₄ pathway has several distinct advantages over its C₃ counterpart. C₄ plants are able to maintain lower internal CO₂ concentration (Cᵢ), which confers greater water-use efficiency due to the steeper CO₂ concentration gradient between the atmosphere and plant intercellular space. The difference in Cᵢ stems from two main factors: the Kranz anatomy of C₄ plants – in which mesophyll cells are arranged radially around bundle sheath cells – and the use of PEP carboxylase as the initial carboxylating enzyme instead of Rubisco. These traits work in tandem to concentrate CO₂ at the site of fixation, remove the inhibitory effect of O₂ and improve photosynthetic efficiency at higher temperatures (>30 °C) via the elimination of apparent photorespiration (Larcher 2001a, Fitter and Hay 2002a).

C₄ photosynthesis represents a distinct ecological adaptation. It occurs mainly in tropical plants, particularly those that experience high temperature, high irradiance and limited water availability at some point during the growing season. However, C₃ and C₄ species frequently co-occur, and there is no evidence that C₄ photosynthesis is inherently

<table>
<thead>
<tr>
<th>Primary CO₂ acceptor</th>
<th>RuBP (substrate: CO₂)</th>
<th>PEP (substrate: HCO₃⁻)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating Cᵢ (ppm)</td>
<td>220–260</td>
<td>100–150</td>
</tr>
<tr>
<td>Carbon isotope ratio in photosynthates (δ₁³C)</td>
<td>-20 to -40‰</td>
<td>-10 to -20‰</td>
</tr>
<tr>
<td>Apparent photorespiration</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Temperature response from 20–40 °C at 330 ppm CO₂</td>
<td>Usually slight</td>
<td>Strong</td>
</tr>
<tr>
<td>Water-use Efficiency</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>
superior. Instead, the chief advantages of C₄ photosynthesis are its greater water-use efficiency, ability to withstand higher temperatures, and lack of apparent photorespiration. C₃ plants will often outcompete C₄ plants in cooler environments where water is not limiting, owing to their simpler anatomy and lower energy (i.e., ATP) requirements (Fitter and Hay 2002a).

2.4.2 Plant Water Relations

Water Potential

Water potential represents the ability of a given unit of water to do work, and is defined as “the free energy per unit volume of water” (Fitter and Hay 2002b). It is represented by \( \Psi \) and measured against the zero potential of pure water under ambient temperature and pressure. Water potential increases with temperature and contains two main components: solute potential and matric potential. Solute potential is the degree to which the presence of dissolved ions and other contaminants alter the solution from that of pure water, while matric potential represents the forces of cohesion in water, hydrophilic surfaces and capillary action. Both solute potential and matric potential are negative components (i.e. \( \leq 0 \)) (Fitter and Hay 2002b). The water potential of a given solution is therefore defined as:

\[
\Psi = \psi_s + \psi_m
\]

where \( \psi_s \) is the solute potential and \( \psi_m \) is the matric potential (Fitter and Hay 2002b).

Water potential in plant tissues can also be raised by hydrostatic (turgor) pressure:
\[ \Psi = \psi_s + \psi_m + \psi_p \]

where \( \psi_p \) is the positive hydrostatic potential (Fitter and Hay 2002b).

Water potential within the leaf is determined chiefly by matric forces from the cell walls and intercellular spaces (i.e. the apoplast) and by solute concentration in the cell vacuole. Thus:

\[ \Psi_{apo} = \psi_m \]

and

\[ \Psi_{vac} = \psi_s \]

therefore:

\[ \Psi = \Psi_{vac} + \Psi_{apo} + \psi_p \]

where \( \Psi_{vac} \) is the water potential of the vacuole, \( \Psi_{apo} \) is the water potential of the leaf apoplast and \( \Psi \) represents total plant water potential at the leaf level (Fitter and Hay 2002b).

\( \Psi_{vac} \) is typically lower than \( \Psi_{apo} \), meaning that water will flow from the apoplast into the vacuole. This increases cell turgor, along with an increase in \( \Psi_{vac} (\psi_s) \) as solute concentration decreases; when enough water has flowed into the vacuole such that \( \Psi_{vac} = \Psi_{apo} \), equilibrium is reached and the cell becomes fully turgid (Fitter and Hay 2002b).

Although the vacuole is the primary water storage site in the plant cell, the water relations of the cytoplasm carry much greater significance, given that this is where nearly all plant physiological processes occur. It is assumed that
\[ \Psi_{\text{cell}} = \Psi_{\text{cyt}} = \Psi_{\text{vac}} \]

where \( \Psi_{\text{cell}} \) is the cell water potential and \( \Psi_{\text{cyt}} \) is the cytoplasmic water potential. However, unlike the vacuole, the cytoplasm has a significant matric force potential contribution \( (\psi_m) \) in addition to solute potential \( (\psi_s) \) (Fitter and Hay 2002b).

**Water Uptake**

Water uptake in plants occurs as a result of the water potential gradient between soil water, root xylem, leaf tissue and the atmosphere. This gradient is established and controlled by transpirational water loss that occurs during CO\(_2\) uptake (Fitter and Hay 2002b).

In a non-water-limited environment, water evaporates from the saturated leaf apoplast through the stomata and into the atmosphere. This brings the water potential of the apoplast below that of leaf cells, causing water to move from the leaf cells to the apoplast. This in turn lowers the water potential of leaf cells below that of the xylem, and in turn the xylem water potential falls below that of the soil. This creates the water potential gradient that drives water uptake and movement within plants. The lowest leaf water potential a plant can sustain is effectively the lowest soil water potential it can tolerate before becoming unable to extract any more water from the soil, also known as the permanent wilting point (Fitter and Hay 2002b). The average permanent wilting point for drought-tolerant plants is -3.0 MPa, compared to -1.5 MPa and -0.7 MPa for agriculture crops and aquatic plants, respectively. Plant water uptake patterns vary significantly between species and growth forms (e.g., grasses, forbs, trees) and have shown remarkable plasticity within individual plants in response to seasonal variations in

Water Stress

Plant water stress is measured in terms of the degree to which tissue water content has fallen below that of full turgor. Full turgor is the point at which equilibrium is achieved between the leaf apoplast and leaf cell water potentials, resulting in no net water movement. Leaf water potential therefore represents a valid measure of plant water status. Pre-dawn leaf water potential gives a reliable baseline for plant water status based on the idea that leaf and soil water potentials equilibrate overnight and remain that way prior to the onset of photosynthesis (Fitter and Hay 2002b).

Water stress induces a number of physiological responses. Loss of turgor occurs at all levels of water stress; cell turgor declines rapidly and non-linearly, decreasing greatly at the onset of water stress but stabilizing as the stress worsens. Generally, mild water stress ($\Psi_{cell} \leq -0.5$ MPa) results in some disruption of physiological processes, such as the production of cell wall components, chlorophyll, enzymes, and protein. Moderate stress ($\Psi_{cell}$ between $-0.5$ MPa and $-1.5$ MPa) initiates stomatal closure in order to reduce water lost via transpiration, resulting in a reduction in photosynthetic rate. The onset of severe water stress ($\Psi_{cell} > -1.5$ MPa) severely disrupts plant metabolism and halts photosynthesis. Prolonged stress can result in xylem cavitation, reduced xylem conductance and the formation of embolisms (Fitter and Hay 2002b).
2.4.3 Water-Use Efficiency

Plant water-use efficiency is a ratio of the amount of carbon taken up per unit of water lost:

$$\frac{A}{E}$$

where $A$ is *net photosynthesis* measured in CO$_2$ uptake and $E$ is *transpiration* measured in water loss (Ehleringer et al. 1993). Plants can increase their water-use efficiency by increasing $A$, decreasing $E$ or both. At steady state these terms are defined as:

$$E = vg,$$

and

$$A = \frac{(c_a - c_i) g}{1.6}$$

where $v$ is the vapor pressure deficit between the leaf and the atmosphere divided by total atmospheric pressure, $g$ is the stomatal conductance to water vapor, $c_a$ and $c_i$ are the atmospheric and internal leaf CO$_2$ concentrations, respectively, and 1.6 is the ratio of gaseous diffusivities of CO$_2$ and water vapor in air (Ehleringer et al. 1993). When stomata are open, the amount of water lost via transpiration is determined chiefly by the vapor pressure deficit (VPD) between the saturated sub-stomatal leaf intercellular space and the atmosphere (Fitter and Hay 2002b).

It is also possible to define $A$ and $E$ in terms of stomatal conductance and partial pressures of CO$_2$:
\[ A = g_c (p_a - p_l) = p_a (1 - \frac{p_l}{p_a}), \]

and

\[ E = g_w (e_i - e_a) = 1.6\nu \]

where \( g_c \) is the stomatal conductance to diffusion of CO\(_2\), and \( p_a \) and \( p_l \) are the partial pressures of CO\(_2\) in the atmosphere and inside the leaf, respectively. \( g_w \) is the stomatal conductance of water vapor, \( e_i \) and \( e_a \) are the intercellular and atmospheric vapor pressures, respectively, and \( \nu \) is the vapor pressure difference (Farquhar et al. 1989).

The term \( \nu \) is defined as:

\[ \nu = \frac{e_i - e_a}{P} \]

where \( e_i \) and \( e_a \) are the water-vapor pressures inside the leaf and in the atmosphere outside the boundary layer, respectively, and \( P \) is the total atmospheric pressure.

Taken together, these terms define instantaneous water-use efficiency:

\[ \frac{A}{E} = \frac{c_a (1 - \frac{e_i}{c_a})}{1.6\nu} \]

Note that \( g \) is omitted because both \( A \) and \( E \) utilize leaf stomata for diffusion (Ehleringer et al. 1993). Local atmospheric conditions play a crucial role in determining instantaneous water-use efficiency given the impact of temperature and humidity on \( \nu \). Certain methods have been devised to overcome this, such as the intrinsic water-use efficiency ratio:
which allows for “direct comparison of intrinsic physiological considerations, factoring out the confounding effects of temperature and humidity gradient differences between plants.” (Ehleringer et al. 1993)

In order to measure long-term water-use efficiency and productivity, \( w \) is used:

\[
W = \frac{c_a(1 - \frac{c_v}{c_a})(1 - \phi_c)}{1.6v(1 + \phi_w)}
\]

where \( \phi_c \) is the fraction of carbon lost through respiration and \( \phi_w \) is the fraction of water lost at night if the stomata do not close completely (Ehleringer et al. 1993).

2.5 Stable Isotopes

2.5.1 Isotope Basics

Isotopic variation is common in elements and occurs in both stable and unstable (i.e. radioactive) forms. Many elements useful to biological research (e.g. N, O, H and C) fractionate – or subdivide – into multiple isotopic forms, and differences between these forms can be exploited in scientifically useful ways (Ehleringer and Rundel 1989).

Isotopic variations occur as a result of differences in atomic mass between otherwise identical elements, and typically take the form of additional neutrons in the nucleus. \(^{18}\)O and \(^{16}\)O, for example, represent heavier and lighter isotopes of oxygen, with atomic masses of 18 and 16 amu, respectively. The effect of differences in atomic mass
scales proportionally, meaning that it will be more pronounced between $^1$H and $^2$H, where the atomic mass of the latter is approximately double that of the former, compared to the difference between oxygen isotopes (Ehleringer and Rundel 1989).

Although atomic mass differences are large enough to affect an isotope’s physical and chemical behavior, they are too small to reliably measure absolute isotopic abundances in a given sample, even with high-precision high-accuracy instruments. However, it is possible to detect small differences in relative isotopic abundance through *differential analysis* (McKinney et al. 1950), in which samples of interest are measured against an established standard with known isotopic abundances. The original standard for hydrogen and oxygen isotopes was Standard Mean Ocean Water (SMOW), however this was revised to Vienna Standard Mean Ocean Water (V-SMOW) due to supply limitations. Standards exist for other light elements, such as the PeeDee Belemnite (PDB) standard for carbon, atmospheric air for nitrogen and the Canyon Diablo meteorite (CD) for sulfur (Ehleringer and Rundel 1989).

In order to quantify the differential analysis approach, isotopic compositions of a given sample are calculated and expressed using differential notation:

$$ \delta X (\%) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 $$

where $\delta X_{\text{std}}$ is the isotopic ratio in delta units relative to a given standard and $R_{\text{sample}}$ and $R_{\text{standard}}$ are the absolute isotope ratios of the sample and standard, respectively. Multiplying by 1000 allows the ratio values to be expressed in parts per thousand – also known as *per mil* ($\‰$) – and makes minute differences in absolute ratios more pronounced (Ehleringer and Rundel 1989).
While there are more than a dozen elements with multiple isotopes of interest used in ecological studies, this study focuses chiefly on stable isotopes of carbon, hydrogen and oxygen. Carbon exists in two stable isotopes: $^{12}\text{C}$ and $^{13}\text{C}$. $^{12}\text{C}$ composes 98.89% of naturally occurring carbon, with $^{13}\text{C}$ making up the other 1.11%. Globally, carbon isotopes vary by as much as 100 ‰, although the variation is generally smaller in geological and biological materials (Ehleringer and Rundel 1989).

Oxygen exists in three stable isotopes: $^{16}\text{O}$, $^{17}\text{O}$ and $^{18}\text{O}$. $^{16}\text{O}$ is the most abundant of these and is found in 99.759 % of all oxygen samples. $^{17}\text{O}$ and $^{18}\text{O}$, discovered by Giauque and Johnston (1929), make up 0.037 % and 0.204 % of oxygen samples, respectively. Analytical techniques have focused on $^{18}\text{O}/^{16}\text{O}$ ratios due to the greater abundance of $^{18}\text{O}$ and the larger difference in atomic mass relative to $^{16}\text{O}$. Isotopic ratios in oxygen vary by more than 50 ‰ across the globe and are useful as tracers to track the movement of water within soils (Ehleringer and Rundel 1989).

Hydrogen exists in two stable isotopes: $^1\text{H}$ and $^2\text{H}$, the latter of which is also known as deuterium (D) and was discovered by Urey et al (1932). $^1\text{H}$ makes up 99.985 % of all hydrogen samples while D comprises just 0.015 %. Despite its low abundance, D remains quite useful in scientific studies due to its large atomic mass difference relative to $^1\text{H}$. Hydrogen samples also contain the largest variations in isotopic ratios, with ratios in geological and biological materials varying up to 700 ‰ and precipitation samples by more than 400 ‰. The differences between hydrogen and oxygen isotopes and the behavior of the global hydrologic cycle are such that the isotopic ratios of any given precipitation sample follow a predictable pattern:
This relationship, known as the Global Meteoric Water Line (GMWL) (Craig 1961), varies depending on local and regional climate and hydrology. Local Meteoric Water Lines (LMWL) are often modeled based on local data in order to better characterize the relationship between hydrogen and oxygen isotopes for a given region (Clark and Fritz 1997c).

**Fractionation**

Fractionation refers to the subdivision of elements into different isotopes, and occurs during “phase transitions, chemical or biological reactions and transport processes.” (Ehleringer and Rundel 1989). Processes such as vaporization, condensation and evapotranspiration can cause fractionation and affect the isotopic ratio of a given sample. Precipitation samples, for instance, have a unique isotopic signature in D/\(^{1}H\) and \(^{18}O/^{16}O\) ratios that is the product of fractionation occurring during seawater evaporation and subsequent overland condensation. Plant organic matter, by comparison, will have a unique signature of \(^{13}C/^{12}C\) values based on plant stress, water-use efficiency and photosynthetic pathway. These ratios are further influenced by the latitude, altitude, temperature and overall climate of a given region, given that all of these factors will affect the amount, location and duration of precipitation and the environmental stressors affecting plant metabolism. Therefore it is paramount to have knowledge of local climatic factors when analyzing isotopic data and to have detailed information on the source isotopic values of the samples in question (Ehleringer and Rundel 1989).

Fractionation at its most fundamental level occurs as a result of atomic mass differences between isotopes. For example, deuterium (\(^2H\) or D) has an atomic mass

\[
\delta D = 8 \delta^{18}O + 10 \%
\]
approximately double that of regular hydrogen ($^1$H or protium) due to the presence of a neutron in deuterium’s nucleus that protium lacks. The higher atomic mass means that molecules containing deuterium are more stable and require more energy to break their chemical bonds compared to protium. In a practical sense, this means that lighter isotopes will phase change into higher energy states sooner and participate more readily in chemical reactions compared to their heavier counterparts (Kendall and Caldwell 1998).

Isotopic fractionation occurs under both equilibrium (reversible) and kinetic (nonreversible) conditions. Equilibrium fractionations take place in closed, well-mixed systems after chemical equilibrium is achieved (i.e., the forward and backward rates of reaction become identical), and reflect differences in equilibrium constants between isotopes (O'Leary 1993, Kendall and Caldwell 1998). For example, a bathtub of water inside a sealed room with 100 % relative humidity will exhibit equilibrium fractionation; the rate of condensation matches that of evaporation, and no net change in isotopic ratios occurs in either the liquid water or water vapor. Differential analysis will show the liquid water enriched in heavy isotopes (D, $^{18}$O) relative to the water vapor, given that lighter isotopes (H, $^{16}$O) require less energy to phase-change into higher energy states.

Kinetic fractionations occur in the absence of chemical or isotopic equilibrium (i.e., the forward and backward rates of reaction are not identical). Similar to equilibrium fractionations, kinetic fractionations occur due to atomic mass differences, but are also affected by reaction pathways, reaction rates, and bonding energies of the compounds involved in a given reaction. Kinetic fractionations are typically larger than equilibrium fractionations and tend to partition heavier isotopes into reactants and lighter isotopes
into the products of a reaction, given that lighter isotopes require less energy to break their bonds and dissociate (Kendall and Caldwell 1998).

A special type of fractionation, known as Rayleigh fractionation or Rayleigh distillation, plays an important role in many observed environmental effects on isotopic composition. Rayleigh fractionation refers to an exponential “partitioning of isotopes between two reservoirs as one reservoir decreases in size (Kendall and Caldwell 1998).” This can occur under both equilibrium and kinetic conditions. For example, water vapor condensing out of rain clouds represents a Rayleigh fractionation; the first water to condense out of the cloud mass will be the most heavily enriched in D and ¹⁸O, owing to these isotopes’ greater atomic mass relative to their lighter counterparts. As more water condenses out of the cloud mass, both the remaining vapor and liquid water become progressively lighter over time relative to their original source. (Kendall and Caldwell 1998). Rayleigh distillations form the underlying basis for many observed environmental effects on isotopic composition, such as latitude, altitude, temperature, and continental location.

2.5.2 Carbon Isotopes

Carbon fixation is one of several processes necessary for plant growth and reproduction. The main source of carbon in terrestrial plants is atmospheric carbon dioxide (CO₂), which typically enters the plant through leaf stomata. Different plant species have varying degrees of stomatal conductance; higher stomatal conductance promotes greater CO₂ acquisition at the expense of greater water loss through transpiration, representing an important tradeoff between the potential for growth and the
risk of desiccation. Stomatal conductance is highly plastic, varying between photosynthetic pathways, plant species and even within species across different environments (Farquhar et al. 1989).

Carbon in atmospheric CO\(_2\) comes in three forms: \(^{12}\)C, \(^{13}\)C and \(^{14}\)C; of these, only \(^{12}\)C and \(^{13}\)C are stable, occurring in an 89:1 ratio, respectively. Atmospheric CO\(_2\) typically has a \(\delta^{13}\)C value of -8‰ compared to the standard PDB. Plants discriminate against \(^{13}\)CO\(_2\) during carbon uptake and fixation owing to its larger atomic mass, although this discrimination varies depending on photosynthetic pathway. Discrimination also depends on partial pressures of CO\(_2\) in the leaf and the atmosphere, as well as plant respiration and vapor pressure deficit. Taken together, these biotic and abiotic factors define a given plant’s water-use efficiency (Farquhar et al. 1989).

Photosynthetic pathway greatly affects the \(\delta^{13}\)C values found in the organic matter of plant leaves (Table 2.3), owing to differences in plant water-use efficiency.

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>(\delta^{13})C content of leaf matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_4)</td>
<td>-7 to -15‰</td>
</tr>
<tr>
<td>CAM</td>
<td>-10 to -22‰</td>
</tr>
<tr>
<td>C(_3)</td>
<td>-20 to -35‰</td>
</tr>
</tbody>
</table>

C\(_3\) plants show the lowest \(\delta^{13}\)C values of the three functional groups due to the role of Rubisco as the initial carboxylating enzyme in the early steps of C\(_3\) photosynthesis, which discriminates against \(^{13}\)C in favor of \(^{12}\)C. (Ehleringer 1989). C\(_4\) and CAM plants show less negative \(\delta^{13}\)C values due to their use of phosphoenolpyruvate (PEP) carboxylase as the initial carboxylating enzyme, which does not discriminate against \(^{13}\)C;
however, other processes play a role in determining the final $\delta^{13}C$ values of C$_4$ and CAM organic matter. C$_3$ plants also have the widest range of $^{13}C$ discrimination of the three functional groups (Ehleringer 1989).

Fractionations are common in biological processes and their associated physical and chemical reactions. For instance, lighter isotopes are capable of diffusing across a membrane or open space more rapidly than their heavier counterparts owing to their lighter atomic mass (O'Leary 1993).

Carbon isotope fractionation in plants stems in part from the initial carboxylation reaction necessary for photosynthesis. In C$_3$ plants, Rubisco drives the first step in CO$_2$ fixation:

$$\text{CO}_2 + \text{RuBP} \rightarrow 2 \text{3-PGA}$$

This reaction is the main source of fractionation in C$_3$ plants, resulting in approximately 29‰ depletion of $^{13}C$ in the product relative to the source carbon (i.e. internal leaf CO$_2$ dissolved in water).

In C$_4$ plants, the initial carboxylation enzyme is PEP carboxylase:

$$\text{PEP} + \text{HCO}_3^- \rightarrow \text{OAA} + P_i$$

This reaction results in a product with $\delta^{13}C$ values -2.0‰ compared to the source carbon (HCO$_3^-$). However, the overall reaction begins with CO$_2$, and the final product
has a $\delta^{13}\text{C}$ value of $+5.7\%$ relative to the original source carbon (i.e. internal CO$_2$) (O'Leary 1993).

Carbon isotope fractionation occurs the same basic way in every plant:

$$\text{CO}_2(\text{external}) \leftrightarrow \text{CO}_2(\text{internal}) \rightarrow R-\text{CO}_2$$

The first step involves CO$_2$ diffusion from the external atmosphere into the internal leaf space via the stomata to the site of carboxylation. This is a reversible reaction. The second step involves the irreversible fixation of carbon via carboxylation (O'Leary 1993).

There are two limiting cases. The first entails the complete closure of plant stomata in response to an environmental stressor. This will prevent virtually any CO$_2$ from diffusing into the plant, resulting in the uptake of nearly all internal CO$_2$ via carboxylation regardless of isotope (i.e., $^{12}\text{C}$ or $^{13}\text{C}$). This will negate the fractionation that occurs as a result of Rubisco discrimination in C$_3$ plants; total net fractionation will approach $-4.4\%$, producing organic matter with $\delta^{13}\text{C}$ values at or close to $-12\%$ (atmospheric carbon has a $\delta^{13}\text{C}$ value of $-8\%$) (O'Leary 1993).

The second case entails stomata that are completely open. This will cause the internal and external CO$_2$ concentrations to equalize, thereby negating the diffusional fractionation that occurs as a result of mass differences between $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$. Enzymatic fractionation will be fully realized, however, resulting in net fractionation of $-30\%$ and producing organic matter with $\delta^{13}\text{C}$ values of $38\%$ in C$_3$ plants. Real-world conditions will of course vary between these two extremes, and as environmental
conditions change so will stomatal aperture and by extension the degree of fractionation and internal CO$_2$ concentration (O’Leary 1993).

$\delta^{13}C$ values of plant organic matter vary greatly depending on location and degree of fractionation. Thus, certain precautions must be taken during $^{13}C$ sampling and analysis to ensure reliable results. The best method is to dry and grind the entire plant; when this is not practical (e.g. with trees), plant tissue samples can be used. Care should be taken to use the same tissue from each plant (e.g. the leaves), and age, size and health of the selected tissues must all be comparable if not identical to ensure reliable results (Farquhar et al. 1989).

2.5.3 Hydrogen and Oxygen Isotopes

Terrestrial autotrophic plants derive the majority of their D and $^{18}O$ isotopes from soil water, which itself is derived chiefly from precipitation. Precipitation that initially condenses out of an air mass tends to be highly enriched in D/$^{18}O$ relative to the remaining vapor due to the preferential partitioning of heavier isotopes in lower energy phase states. Thus, as more and more water condenses out of an air mass the remaining water vapor becomes increasingly depleted in D and $^{18}O$. Water from each successive rain event is more depleted in D/$^{18}O$ relative to those that came before, despite containing more heavy isotopes than the residual air mass. This process, known as Rayleigh distillation, occurs non-linearly and forms the basis for many observed effects on isotopic composition of precipitation (Clark and Fritz 1997c). For example, given that the atmosphere’s ability to hold water is temperature-dependent and decreases as temperature decreases, colder air masses will contain less water vapor and tend to be more depleted in
heavy isotopes relative to those found in warmer climates. Several effects have been documented as a result of this relationship, and are described in Table 2.4 (Clark and Fritz 1997b).

<table>
<thead>
<tr>
<th>Effect</th>
<th>δD and δ18O content of precipitation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude</td>
<td>decreases as geographic latitude increases.</td>
</tr>
<tr>
<td>Altitude</td>
<td>decreases as altitude increases.</td>
</tr>
<tr>
<td>Seasonal</td>
<td>increases in summer and decreases in winter.</td>
</tr>
<tr>
<td>Continental</td>
<td>decreases as its associated air mass moves inland.</td>
</tr>
<tr>
<td>Temperature</td>
<td>decreases as temperature decreases.</td>
</tr>
<tr>
<td>Amount</td>
<td>decreases as more precipitation condenses out of its associated air mass.</td>
</tr>
</tbody>
</table>

Latitude has the most far-reaching influence over heavy isotope content, often impacting other effects. For example, the impact of altitude is greater at higher latitudes, producing a 4.8‰ per 100 m difference in δD compared to 1.6‰ per 100 m difference at lower latitudes. The seasonal effects of summer and winter are only significant at latitudes greater than 30°. Finally, the impact of total precipitation amounts is most pronounced in the tropical regions common at lower latitudes (Ziegler 1989).

Fractionations in H isotopes vary depending on the site within the plant and the mechanism involved. Large positive fractionations occur during transpiration at the leaf level, with H2O diffusing out of the plant more readily, leading to an enrichment of HDO in leaf water. Conversely, large negative fractionations occur as a result of biochemical reactions used to synthesize organic compounds within the plant. This results in the depletion of δD values in plant tissue and other organic compounds (White 1989).

Two mechanisms are responsible for H fractionation in leaf water, both of which are tied to evapotranspiration. The first mechanism is the phase change from liquid to
water vapor; at 25°C and thermodynamic equilibrium (i.e. fully saturated air and equal air and water temperature) water vapor will have -80‰ δD compared to the liquid water (White 1989). The second mechanism is the diffusion of water vapor into undersaturated air; HDO diffuses into the air at a slower rate compared to H₂O as a result of its heavier atomic mass. Both mechanisms result in D enrichment in the leaf water relative to plant water that is not subject to evapotranspiration. This enrichment continues until the leaf water reaches an isotopic steady-state, whereupon enrichment ceases as a result of diffusion gradients that equalize the diffusion of HDO and H₂O. Once at steady-state the δD value of transpired water vapor should equal that of the water entering the plant root system (White 1989).

2.6 Stable Isotopes in Plant Research

The origins of stable isotope research in plants are rooted in geochemistry and physical science (Ehleringer and Vogel 1993). While the existence of multiple isotopes in carbon, oxygen and hydrogen has been known for some time (Giauque and Johnston 1929, King and Birge 1930, Urey et al. 1932) it is only in the last 40 years that these isotopes have become viable physiological and ecological research tools. Later findings by Gonfiantini et al (1965) and Wershaw et al (1966) confirmed that hydrogen and oxygen fractionation does not occur during root water uptake, thereby allowing plant water extracted from non-transpiring tissues to be reliably used in isotope and plant water source analyses. DeNiro and Epstein (1979) established that ¹⁸O/¹⁶O ratios in plant organic matter are dependent on plant water alone; ¹⁸O/¹⁶O ratios in atmospheric CO₂ uptake do not play a role.
Transpiration produces significant D and $^{18}$O enrichment in plant water. Water samples taken from non-transpiring tissues above the root crown display isotope values similar to those of soil water, but exchange between the xylem, phloem, and leaf water results in a heavy isotope enrichment gradient as tissue gets progressively closer to the leaf (Ziegler 1989). Certain plants, such as non-woody species, may also transpire from stem tissue; thus, the root crown is the most reliable point to sample plant water for isotopic analysis (Barnard et al. 2006).

Root crown water is useful in determining sources of plant water uptake, particularly if water resource partitioning is occurring. For instance, the large volume of the saturated zone makes its isotopic signature relatively stable, meaning that plants that use groundwater exclusively will not respond to fluctuations in precipitation isotopes. Conversely, plants with no groundwater access should have isotopic signatures that reflect those found in the most recent precipitation events. Plants that have access to both precipitation and groundwater sources are more unpredictable; water uptake patterns will be species-specific, and will affect how much water the plant extracts from either source and the rate at which that water mixes with existing plant water (White 1989).

There are several species-specific effects present with respect to isotopes in plants. Both D/H and $^{18}$O/$^{16}$O ratios vary as a result of evapotranspiration occurring at the leaf level, but only D/H ratios vary as a result of biochemical reactions that synthesize organic compounds within the plant. This difference makes D/H and $^{18}$O/$^{16}$O ratios useful in different ways; D/H ratios give insight into differences in metabolic processes between plant species assuming the water source remains the same, while $^{18}$O/$^{16}$O ratios give insight into plant water-use patterns (Sternberg 1989).
Fractionation of $^{13}$C in plants is dependent on “photosynthetic type, environment, genotype and other factors.” (O’Leary 1993). Nier and Gulbransen (1939) were the first to document $^{13}$C discrimination in plant organic matter relative to the surrounding inorganic substrate, while (Craig 1954) and (Park and Epstein 1961) were the first to study the potential mechanisms behind $^{13}$C discrimination. Their findings suggested that discrimination at the kinetic level during the Rubisco reaction and diffusion through leaf stomata effectively canceled each other out, leading them to conclude that enzymes used during metabolic carbon fixation were responsible for $^{13}$C discrimination (Berry 1989).

$^{13}$C/$^{12}$C ratios in plant organic matter are measured by passing CO$_2$ from the sample in question through a high-accuracy high-precision isotope ratio mass spectrometer to produce the following ratio:

$$ R = \frac{^{13}CO_2}{^{12}CO_2} $$

This means that any materials to be analyzed for $^{13}$C/$^{12}$C concentrations must first be converted to CO$_2$; for plant organic matter this means combustion (O’Leary 1993).

Obtained samples are compared to the PDB standard using the established differential notation:

$$ \delta^{13}C = \left[ \frac{R_{sample}}{R_{PDB}} - 1 \right] \times 1000 $$

Discrimination of $^{13}$C in plants is calculated as follows:

$$ Discrimination = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p/1000} $$
where $\delta^{13}C_a$ is the $\delta^{13}C$ value of air and $\delta^{13}C_p$ is the $\delta^{13}C$ of the plant. The $\delta^{13}C_p/1000$ term in the denominator tends to be quite small and is often dropped altogether (O'Leary 1993).
3 Materials and Methods

3.1 Site description and Species Selection

The study was conducted at the Gudmundsen Sandhills Laboratory (GSL), 11 km northeast of Whitman, NE, USA (1049 m altitude, 42°4’54” N and 101°22’06”). GSL is a research-oriented ranch facility acquired by the University of Nebraska-Lincoln from private land owners in 1978; its 13,000 acreage (5,300 hectares) is composed of upland sandhills and subirrigated meadows used primarily for rangeland pasture.

Climate is semiarid continental, with a mean annual precipitation of 457 mm, 82% of which falls during the growing season (April – September). Mean minimum temperature in January is -31.3 °C and mean maximum temperature in July is 30.1 °C (HPRCC 2011). Soil type is Valentine fine sand, which consists of deep, loose, sandy well-drained soils (USDA Web Soil Survey). Vegetation on site is dominated by C_3 and C_4 grasses with forbs and shrubs also present. Common C_3 grasses include needle-and-thread (*Hesperostipa comata* (Trin. & Rupr.) Barkworth), prairie junegrass (*Koeleria macrantha* (Ledebr.) Schult.), and Scriber’s panicum (*Dichanthelium oligosanthes* (Schult.) var. *scribnerianum* (Nash) Gould). Common C_4 species include prairie sandreed (*Calamovilfa longifolia* (Hook.) Scribn.), sand bluestem (*Andropogon hallii* Hack.), switchgrass (*Panicum virgatum* L.), and little bluestem (*Schizachyrium scoparium* (Michx.) Nash). Forbs including western ragweed (*Ambrosia psilostachya* DC.) and stiff sunflower (*Helianthus pauciflorus* Nutt.) and the leguminous shrub leadplant (*Amorpha canescens* Pursh) were also present.

Two 30 m x 30 m adjacent sites were established on north-facing slopes. One site, referred to as G-L, contained 33% *A. canescens* canopy cover (estimated using the line
intercept method (Canfield 1941)). The other site, referred to as G-NL, contained no *A. canescens*. Sites occupied similar topographic positions and slope gradients (~5-7%) and were grazed only during the dormant period (October – March).

Six species were chosen for this study: C\textsubscript{3} grass species needle-and-thread (*H. comata*) and prairie junegrass (*K. macrantha*), C\textsubscript{4} grass species sand bluestem (*A. halli*) and prairie sandreed (*C. longifolia*), forb species stiff sunflower (*H. pauciflorus*), and shrub species leadplant (*A. canescens*). The G-L site contained all six species, while the G-NL site contained all species except *A. canescens*. All species are common throughout the Sandhills and are major contributors to aboveground net primary production.

### 3.2 Environmental Parameters

Micrometeorological data [i.e., precipitation (mm), maximum and minimum daily temperature (°C), and relative humidity (%)], were obtained from the High Plains Regional Climate Center (HPRCC) weather station, located at GSL headquarters approximately 2 km from the study site. Soil moisture sensors (ML2x ThetaProbe, Time Domain Reflectometry, Dynamax Inc., Houston, TX) calibrated for sandy soils were installed at 0.2, 0.4, and 1.0 m depths at the center of both sites. Volumetric soil moisture (θ\textsubscript{V} or % vol) was measured every 5 minutes and averaged for every 30-minute and 24-hour period and stored on data loggers (CR10X, Campbell Scientific Inc., Logan, UT). Mean daily values are presented.
3.3 Vegetation Biomass and Ecophysiology

Site biomass was measured twice during the study period: once in mid-June and once in mid-August, to capture peak standing crop of C$_3$ and C$_4$ grasses, respectively. Ten 0.25 m$^2$ quadrats were clipped in both sites. All standing vegetation rooted within the quadrats was clipped to ground level; current-year growth was sorted by species and placed in individual paper bags, while standing dead and litter were collected and placed in separate bags. All bags were dried and weighed.

Ecophysiology measurements were taken approximately once every two weeks from June 16$^{th}$ to September 9$^{th}$, 2010 (DOY 167 to 262, respectively). Transpiration (E) and water-use efficiency (WUE) were measured using a Li-Cor LI6400 Portable Photosynthesis System. Five locations were selected in each site one day prior to each sampling date that contained all plant species of interest within a 1 m radius, and were marked with flags to facilitate relocation. Measurements were taken the following day over a 2-day period with consistent weather conditions, with the exception of the September 9$^{th}$ measurements, which were completed in one day. At least half of the individuals per species per site were measured per day. The September 9$^{th}$ measurements did not include K. macrantha or C. longifolia, as these species had entered dormancy.

3.3.1 Water Potential

Seasonal dynamics in plant water potential were measured using a pressure chamber (Model 1000, PMS Instruments, Albany, OR) and nitrogen gas. Measurements were taken once at predawn (1-1.5 h before sunrise) and again at midday the same day (after 13:00 h). A volume reducer was used with grass and forb species to conserve gas.
Single healthy leaves were used to measure water potential in all species except A. *canescens*, in which stems with healthy leaves were used.

### 3.4 Isotope Measurements

#### 3.4.1 Precipitation and groundwater

Precipitation was collected on-site using a rain-bucket with vegetable oil to prevent evaporation between sampling. Precipitation samples were taken approximately two days in advance of plant ecophysiology measurements; no samples were taken prior to the August 1st and September 9th sampling dates (DOY 212 and 252, respectively) due to inadequate moisture. Groundwater samples were taken on the same day as plant ecophysiology measurements from a well within 2.5 km of the study site. Both precipitation and groundwater samples were placed in individual 20-ml polypropylene vials and stored at -10 °C until isotopic analysis.

#### 3.4.2 Plant and soil sampling

Plant root crown tissue was harvested once every two weeks from June 16th to September 9th, 2010 (DOY 167 to 252). Root crowns have been shown to be the least variable with respect to plant water δ¹⁸O signatures (Barnard et al. 2006). Five plants were harvested per species per site, and samples were placed in 20-ml polypropylene vials and stored in plastic bags at -10 °C until isotopic analysis.

Plant leaf organic matter was sampled for δ¹³C values. Several fully developed leaves were taken from 5 plants per species per site once per month over the study period.
Leaves were dried, ground, and analyzed for $\delta^{13}$C at the Ecosystems Analysis Lab at the University of Nebraska-Lincoln.

Soil profiles were sampled using a bucket auger. Profiles were extracted in 0.3 m segments down to 1.10 m, with one profile taken per site per sampling day at the midpoint between plant test species. Samples were double-bagged in Ziploc bags and stored at -10 °C until isotopic analysis. Samples were also analyzed for percent organic matter using loss-on-ignition (LOI) (Heiri et al. 2001) and particle size via high resolution laser diffractometry using a Malvern Mastersizer laser diffraction system (Sperazza et al. 2004), and percent carbon and nitrogen via Klute et al. 1994 using a Costech Analytical ECS 4010 instrument (Klute et al. 1994).

### 3.4.3 Isotope analyses

Precipitation and groundwater samples were analyzed for $\delta^D$ (deuterium) and $\delta^{18}$O at the Water Science Laboratory, University of Nebraska-Lincoln. Samples were analyzed for $\delta^D$ via an online chromium reduction technique (Morrison et al. 2001) on a continuous flow isotope ratio mass spectrometer (EuroPyrOH-3110, Isoprime with Eurovector Pyrolysis Furnace, GV Instruments, Manchester, UK). $\delta^{18}$O analysis was conducted with an automated CO$_2$ equilibration technique (Midwood et al. 1992) connected to an isotope ratio mass spectrometer (GV2003 with an IsoprepB Equilibration System, GV Instruments). Instruments were calibrated daily, with calibration checks interspersed throughout a run. Samples were analyzed in triplicates, averaged and reanalyzed if the standard deviation exceeded the measurement precision (0.2 ‰ for $\delta^{18}$O and 2 ‰ for $\delta^D$).
Plant and soil water samples were extracted and analyzed at the Stable Isotope Mass Spectrometry Laboratory, Kansas State University. Approximately 2 µg of sample was injected into a Picarro water analyzer for determination of δD and δ\(^{18}\)O values. To counteract the memory effect of previous analyses, each sample was run 6 times; the first 3 runs were ignored, while the last 3 were averaged to produce a raw data point. Raw data points were corrected to secondary standards analyzed in each batch of samples, which are calibrated to NIST accepted standards (GISP, SLAP, and VSMOW) (Table 3.1). A working standard of known isotope values was analyzed every 4 samples in order to correct for analyzer drift (Ocheltree 2011).

| Table 3.1 - Standards used in calibration and quality control at Stable Isotope Mass Spectrometry Lab, Kansas State University (Ocheltree 2011). |
|-----------------------------|-----------------|-----------------|
| Standard                  | δD   | δ\(^{18}\)O |
| Arctic Lake Water          | -160 | -20.5          |
| Evian Bottled Water        | -71  | -9.9           |
| KSU Enriched Water         | -2   | 4.1            |
| KSU DI Water               | -34  | -5.4           |

δD and δ\(^{18}\)O values were expressed in parts per thousand (‰) relative to the V-SMOW standard (Gonfiantini 1978)

\[
\delta D \text{ or } \delta^{18}O = \left( \frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000
\]
where \( R = \frac{{}^2\text{H}}{^1\text{H}} \) for deuterium, or \( R = \frac{{}^{18}\text{O}}{^{16}\text{O}} \) for oxygen.

Comprehensive plant water source analysis was performed using the IsoSource software package (Phillips and Gregg 2003, Phillips et al. 2005). Two isotope systems (D, \( {}^{18}\text{O} \)) and three sources (0 – 0.30 m, 0.31 – 0.60 m, and 0.61 – 1.10 m deep soil water) were used with the lowest mass balance tolerances possible. However, similarity of isotopic signatures in the soil profile often confounded IsoSource analysis.

### 3.5 Statistical analyses

Data were analyzed with the SAS statistical package (SAS 2008, SAS Institute, Cary, NC). Repeated-measures analyses and regressions were used to determine site effect and compare species responses, while T-tests were used to determine differences between sites for each measured parameter for each species within dates.
4 Results

4.1 Environmental Parameters

Temperature and VPD (Figure 8.1) fluctuated throughout the growing season, with temperature following a faint bell-curve pattern and VPD peaking towards the end of the sampling period (June 3 to September 11, 2010; DOY 154 to 254). Maximum and minimum daily temperature (36.24 °C and -0.42 °C) were observed on August 7 and September 7 (DOY 219 and DOY 250), respectively, and maximum and minimum VPD (3.12 kPa and 0.01 kPa) were observed on August 22 and June 13 (DOY 234 and DOY 164), respectively.

Total precipitation for 2010 was 498 mm, 8.9 % above the 30-year annual mean of 457 mm (High Plains Regional Climate Center, University of Nebraska, HPRCC). Of the total 2010 precipitation, 86 % (428 mm) fell during the growing season (April - September, DOY 91 to 273) (Figure 8.2). Precipitation during the dormant season (October 2009 to March 2010, DOY 274 to 365, DOY 1 to 90) was 71 mm, 14 % less than the 30-year mean of 83 mm. Precipitation during the 2010 growing season (April – September, DOY 91 to 273) totaled 428 mm, 14 % above the 30-year mean of 375 mm. Monthly precipitation exceeded the 30-year mean in April (58.7 compared to the 49.2 mm long-term mean), June (171.7 compared to 86.3 mm), and July (115.6 compared to 73.9 mm), but fell short of the 30-year mean in May (48.0 compared to the 71.0 mm long-term mean), August (18.8 compared to 53.9 mm), and September (15.0 compared to 40.4 mm) (High Plains Regional Climate Center, University of Nebraska Lincoln). June, July, August and September saw greater departures from the 30-year mean (98.98, 56.48,
-65.12 and -62.94 %, respectively), compared to April and May (19.33 and -32.34 %, respectively) (Figure 8.2).

Volumetric soil water content (VSWC) averaged over the three measured depths (0.2, 0.4, and 1.0 m) was significantly greater (P < 0.05) on the grassland site with A. canescens (G-L) compared to the grassland site without A. canescens (G-NL) (11.58 % compared to 10.74 %), outpacing it by approximately 7.83% from June 3 to September 9 2010 (DOY 154 to 252) (Figure 8.3). Mean VSWC showed noticeable increases following precipitation events on June 7, June 10, June 11, July 3 – July 7, July 21, and August 9 (DOY 158, 161, 162, 184 – 188, 210, and 221, respectively). Mean VSWC in the G-L plot ranged from 15.9 % on July 7 (DOY 188) to 8.3 % on September 9 (DOY 252), while the G-NL site ranged from 15.4 % on June 12 (DOY 163) to 7.5 % on September 9 (DOY 252).

Volumetric soil water content at 0.2 m and 0.4 m depths showed greater variability tracking precipitation compared to the 1.0 m depth (Figure 8.4). The 0.2 m depth ranged from 4.9 – 15.0 % (G-L) and 5.4 – 15.5 % (G-NL), and was typically the driest depth with mean VSWC of 8.2 % (G-L) and 9.0 % (G-NL). The 0.4 m depth ranged from 6.8 – 18.7 % (G-L) and 6.2 – 16.4 % (G-NL), with means of 12.7 % (G-L) and 9.9 % (G-NL). The 1.0 m depth showed the least variability, with a VSWC range of 13.2 – 16.7 % (G-L) and 11 – 15.5 % (G-NL) and means of 15.4 % (G-L) and 13.3 % (G-NL).

Soil particle composition (Figure 8.5) did not differ significantly (P > 0.05) between G-L and G-NL at any of the measured depths. Composition was the most heterogeneous at the surface, with a combined site average of 90 ± 0.66 % sand, 7.5 ±
0.5% silt, and 2.5 ± 0.15% for the 0 – 0.30 m depth. Soil became increasingly sandy with depth at the expense of silt and clay; combined site averages showed 99.4 ± 0.45% sand, 0.4 ± 0.33% silt, and 0.1 ± 0.11% clay for the 0.91 – 1.10 m depth.

Mean organic matter composition (Figure 8.6) decreased with depth, and was significantly greater (P < 0.05) in G-L compared to G-NL at 0 – 0.30 m (0.47% compared to 0.36%) and 0.61 – 0.90 m (0.22% compared to 0.16%) soil depths. G-L and G-NL did not differ significantly (P > 0.05) at 0.31 – 0.60 m (0.23% and 0.24%) and 0.91 – 1.10 m (0.18% and 0.19%) depths. Mean carbon composition (Figure 8.7) did not differ significantly (P > 0.05) between plots at any depth. Carbon composition decreased with depth, with combined site averages showing a mean carbon content of 3.66 ± 0.33 mg g⁻¹, 1.49 ± 0.06 mg g⁻¹, 0.91 ± 0.05 mg g⁻¹, and 0.79 ± 0.08 mg g⁻¹ at 0 – 0.30 m, 0.31 – 0.60 m, 0.61 – 0.90 m, and 0.91 – 1.10 m depths, respectively. Mean nitrogen composition (Figure 8.8) did not differ significantly (P > 0.05) between the two plots at any depth. Similar to carbon content, nitrogen composition decreased with depth; combined site averages for the two plots showed a mean nitrogen content of 0.38 ± 0.03 mg g⁻¹, 0.18 ± 0.01 mg g⁻¹, 0.12 ± <0.01 mg g⁻¹, and 0.10 ± 0.01 mg g⁻¹ at 0 – 0.30 m, 0.31 – 0.60 m, 0.61 – 0.90 m, and 0.91 – 1.10 m depths, respectively.

Above ground dry biomass (Figure 8.9) differed significantly (P < 0.05) between plots. G-L showed significantly more C₃ grass, shrub, and standing dead/plant litter biomass in June compared to G-NL (987.32 compared to 642.48, 242.0 compared to 0, and 2114.76 compared to 1352.48 DM ha⁻¹, respectively). In August, G-L showed significantly more C₃ grass, live, and standing dead/plant litter biomass compared to G-NL (1296.28 compared to 656.2, 2417.88 compared to 1867.96, and 2616.68 compared
to 1492.6 DM ha\(^{-1}\), respectively), while G-NL showed significantly more C\(_4\) grass biomass (882.88 compared to 593.52 DM ha\(^{-1}\)). Differences in forb biomass were not significant (P > 0.05) in either month.

### 4.2 Plant Water Relations

Statistics on measured parameters are outlined in Table 8.1. Repeated measures analysis of variance was conducted on transpiration (E), water-use efficiency (WUE), predawn and midday water potential (\(\Psi_{\text{pre}}\) and \(\Psi_{\text{mid}}\), respectively), and carbon isotope ratio (\(\delta^{13}\)C). Significant differences (P < 0.05) between species were observed for all measured parameters, with E, \(\Psi_{\text{pre}}\), \(\Psi_{\text{mid}}\), and \(\delta^{13}\)C also showing significant differences between dates (P < 0.05). However, only E showed significant differences (P = 0.0082) between G-L and G-NL sites, with rates generally higher in G-NL compared to G-L.

Seasonal dynamics in E and WUE are shown in Figure 8.10, with higher E values typically observed in G-NL compared to G-L. E was significantly higher (P < 0.05) for G-NL A. hallii, C. longifolia, and H. pauciflorus compared to the same G-L species for July 31 (DOY 212; 4.19 compared to 2.54, 1.65 compared to 3.27, 8.93 compared to 6.34 mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\), respectively), with G-NL H. pauciflorus also being significantly higher (P < 0.05) than G-L for September 9 (DOY 252; 3.8 compared to 2.94 mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\)). Water-use efficiency did not differ significantly between sites except for G-L A. hallii on July 31, which was significantly higher (P < 0.05) than its G-NL counterpart (DOY 212; 8.0 compared to 5.42 \(\mu\)mol CO\(_2\) mmol H\(_2\)O\(^{-1}\)). All species reached their greatest WUE on July 13 (DOY 195). C\(_4\) grasses had the greatest mean WUE, followed by H. pauciflorus, A. canescens, and C\(_3\) grasses. Mean seasonal WUE was greater in the
two C₄ grasses compared to the C₃ grasses and forb and shrub species (Table 8.3). *C. longifolia* had the highest mean seasonal WUE of any species (8.62 ± 0.88 G-L and 9.25 ± 1.30 G-NL μmol CO₂ mmol H₂O⁻¹), while *H. comata* had the lowest (4.10 ± 0.35 G-L and 3.90 ± 0.34 G-NL μmol CO₂ mmol H₂O⁻¹).

Predawn (Ψₚᵣₑ) and midday (Ψₘᵢｄ) water potentials differed significantly among sampling dates and species (P < 0.01), but not between G-L and G-NL sites (Table 8.1, Figure 8.11). Exceptions were *H. comata* on August 15 (DOY 226, G-L Ψₚᵣₑ of -0.98 compared to G-NL Ψₚᵣₑ of -0.70 MPa), *K. macrantha* on July 15 (DOY 195, G-L Ψₚᵣₑ of -0.44 compared to G-NL Ψₚᵣₑ of -0.71 MPa), *A. hallii* on August 1 (DOY 212, G-L Ψₘᵢｄ of -1.2 compared to G-NL Ψₘᵢｄ of -1.61 MPa), and *H. pauciflorus* on July 15 (DOY 195, G-L Ψₘᵢｄ of -0.73 compared to G-NL Ψₘᵢｄ of -1.01 MPa).

Mean seasonal water potentials did not differ significantly between sites by species (P > 0.05). Forbs showed the least negative water potential, followed by C₄ grasses, shrubs and finally C₃ grasses. *H. pauciflorus* had the least negative water potential (Ψₚᵣₑ of -0.41 and -0.40 MPa in G-L and G-NL, respectively, and Ψₘᵢｄ of -0.80 and -0.88 MPa in G-L and G-NL, respectively), and showed the smallest shift in water potential from predawn to midday compared to all other species. C₄ grasses had variable water potentials, with *C. longifolia* showing more negative water potential compared to *A. hallii* (*C. longifolia* with Ψₚᵣₑ of -0.52 and -0.49 MPa for G-L and G-NL, respectively, and Ψₘᵢｄ of -1.9 and -1.95 MPa for G-L and G-NL, respectively; *A. hallii* with Ψₚᵣₑ of -0.40 and -0.43 MPa for G-L and G-NL, respectively, and Ψₘᵢｄ of -1.23 and -1.28 MPa for G-L and G-NL, respectively), and a greater shift in water potential between predawn and midday water potential. *A. canescens* showed water potentials in between those of the C₄
grasses, with a $\Psi_{\text{pre}}$ of -0.47 and a $\Psi_{\text{mid}}$ of -1.67 MPa. C$_3$ grasses (*H. comata* and *K. macrantha*) had the most negative predawn and midday water potential (*H. comata* with $\Psi_{\text{pre}}$ of -1.12 and -1.14 MPa in G-L and G-NL, respectively, and $\Psi_{\text{mid}}$ of -3.37 and -3.56 MPa, respectively; *K. macrantha* with $\Psi_{\text{pre}}$ of -0.62 and -0.70 MPa in G-L and G-NL, respectively, and $\Psi_{\text{mid}}$ of -2.9 and -3.04 MPa in G-L and G-NL, respectively).

Analysis of variance (Table 8.1) showed that $\delta^{13}$C ratio differed among species ($P < 0.001$) and reflected differences in photosynthetic pathways between functional groups (C$_3$ vs. C$_4$). Differences in $\delta^{13}$C ratio among sampling dates ($P = 0.0028$) indicated seasonal variability, and was more pronounced on the G-L site than the G-NL site, and more in C$_3$ species than C$_4$ species (Figure 8.12). Seasonally, $\delta^{13}$C increased in both C$_3$ grass species (*H. comata* and *K. macrantha*) towards the end of the growing season, with significantly higher ($P < 0.05$) amounts observed in G-L individuals compared to G-NL on August 17 (DOY 229; -22.86 ‰ compared to -26.03 ‰ in *H. comata*, -24.02 ‰ compared to -27.82 ‰ in *K. macrantha*). C$_4$ grasses (*A. hallii* and *C. longifolia*) showed less change in enrichment over time, although both species showed greater initial enrichment. No significant differences were observed with respect to G-L and G-NL C$_4$ species ($P > 0.05$). $\delta^{13}$C in both G-L and G-NL *H. pauciflorus* remained flat from June 19 to August 17 (DOY 170 to DOY 229) before becoming more enriched on September 10 (DOY 253). $\delta^{13}$C in G-L *H. pauciflorus* was observed to be significantly more enriched ($P < 0.05$) compared to its G-NL counterpart on July 18 (DOY 199; -28.25 ‰ compared to -29.29 ‰). *A. canescens* showed some slight enrichment on August 17 (DOY 229; -26.26‰) before declining to its lowest level of $\delta^{13}$C for the growing season on September 10 (DOY 253; -29.55 ‰).
4.3 Temporal and spatial variation in species water uptake

Temporal and spatial variations in species water uptake were investigated using δD and δ¹⁸O. Plant water δD and δ¹⁸O showed clear seasonal enrichment trends in all species except *A. canescens* (Figure 8.12); the greatest enrichment in these species was observed on August 14 (DOY 226), while *A. canescens* showed the greatest enrichment on July 31 (DOY 212). Plant δD and δ¹⁸O did not differ significantly (P > 0.05) between sites. C₃ and C₄ grasses (*H. comata* and *K. macrantha*, *A. hallii* and *C. longifolia*) exhibited the most δD enrichment (Figure 8.13), followed by the shrub (*A. canescens*) and forb (*H. pauciflorus*) species. δ¹⁸O enrichment differed slightly, being most pronounced in *K. macrantha*, followed by *H. comata*, *A. hallii*, *H. pauciflorus* and finally *A. canescens*.

Regression lines from root crown δD/δ¹⁸O plots show that both C₃ and C₄ grasses had similar degrees of departure from the North Platte Meteoric Water Line (NPMWL) (Figure 8.14); C₃ grasses did not exhibit differences between sites or species, while G-L C₄ grasses showed less of a departure from the NPMWL, with almost identical regression lines between *A. hallii* and *C. longifolia* within the same site. G-NL *H. pauciflorus* showed a noticeably greater departure from the NPMWL relative to its G-L counterpart. *A. canescens* showed the least departure from the NPMWL of any species or functional group.

Soil water δD and δ¹⁸O (Figure 8.15) showed a seasonal enrichment trend in the shallowest depth range (0 – 0.3m) in both plots; water in the G-L plot went from -87/-11.5 ‰ δD/¹⁸O on June 16 (DOY 167) to -54/-3.9 ‰ δD/¹⁸O on September 9 (DOY 252), while water in the G-NL plot went from -83/-10.7 ‰ δD/¹⁸O on June 16 (DOY 167) to -
Isotope variations became less pronounced as soil depth increased; water in the G-L plot in the 0.91 – 1.10 m range went from -82/-10.7 ‰ δD/δ18O on June 30 (DOY 181) to -70/-7.8 ‰ δD/δ18O on September 9 (DOY 252), while water in the G-NL plot went from -78/-10.5 ‰ δD/δ18O on June 16 (DOY 167) to -96/-11.8 ‰ δD/δ18O on September 9 (DOY 252).

Regression lines from soil water δD/δ18O plots showed the 0.91 – 1.10 m depth range in the G-NL plot to be the most similar to the NPMWL (Figure 8.16). Differences existed between sites, with G-NL 0 – 0.30 m, G-L 0.61 – 0.90 m, and G-L 0.91 – 1.10 m all showing stronger departures from the NPMWL compared to the other site at the same depth range. Soil water in the 0.31 – 0.60 m depth range showed similar departures at both sites. Precipitation and groundwater values were plotted for reference, with precipitation showing a regression line similar to that of the NPMWL, while groundwater was flat with little variation.

Precipitation δD and δ18O (Figure 8.17 and Figure 8.18) resembled that of groundwater on June 16 (DOY 167; -83/11.8 ‰ δD/δ18O) and July 14 (DOY 195; -68/-10.6 ‰ δD/δ18O) but became more enriched on July 31 (DOY 212; -50/-8.0 ‰ δD/δ18O) and August 14 (DOY 226; -31/-4.9 ‰ δD/δ18O). C3 and C4 grass species showed root crown isotope values closer to those found in precipitation on June 16, July 14 and July 31, but were closer to shallow soil water values on August 14. Grass species were more enriched than the forb (H. pauciflorus) and shrub (A. canescens) species on all dates. H. pauciflorus consistently had the least enriched root crown values of all species sampled, and often showed less enrichment than soil, precipitation or groundwater values.

Significant differences (P < 0.05) were observed between G-L and G-NL H. pauciflorus
individuals on July 14 (DOY 195; -105/-10.6 ‰ δD/18O compared to -95/-9.9 ‰ δD/18O) and August 14 (DOY 226; -99/-9.6 ‰ δD/18O compared to -91/-8.6 ‰ δD/18O). A. canescens showed equal or greater enrichment than H. pauciflorus on all dates, but less enrichment compared to C3 and C4 grass species. A. canescens showed root crown values consistent with soil water found in the 0 – 0.30 m range on June 16 (DOY 167; soil water value of -87/-11.5 ‰ δD/18O compared to -94/-12.3 ‰ δD/18O) and June 30 (DOY 181; soil water value of -103/-12.8 ‰ δD/18O compared to -106/-13.0 ‰ δD/18O), but shifted to values closer to deeper soil water and groundwater from July 14 to September 9 (DOY 195 to 252).

IsoSource analysis (Figure 8.19) showed that most species derived a majority of their water from the 0 – 0.30 m soil depth range on June 16 (DOY 167), with the exception of G-L H. comata, K. macrantha, and A. hallii, which derived a plurality of their water from this depth. Different functional groups shifted their water uptake to varying degrees as the growing season progressed, and differences were also observed between G-L and G-NL individuals. G-L C3 (H. comata and K. macrantha) grasses alternated between the 0 – 0.30 and 0.31 – 0.90 m depths, using the latter on June 30 and July 14 (DOY 181 and 195) and the former on July 30.

G-L C3 (H. comata and K. macrantha) grasses shifted to the 0.31 – 0.90 depth range on June 30 (DOY 181), split their water uptake between 0 – 0.30 m and 0.31 – 0.90 m on July 14, and C4 grasses (A. hallii and C. longifolia) shifted to the 0.31 – 0.90 depth range on June 30 (DOY 181), shifted back to 0 – 0.30 m from July 14 to August 14 (DOY 195 to 226), and then shifted to 0.91 – 0.10 m on September 9 (DOY 252).
Water uptake in G-NL C₃ grasses was less consistent. G-NL *H. comata* used the 0 – 0.30 m depth range for a majority of its water uptake from June 16 to July 31 (DOY 167 to 212), and then showed an increasing reliance on the 0.61 – 1.10 m range from August 14 to September 9 (DOY 226 to 252). G-NL *K. macrantha* shifted to the 0.31 – 0.60 range for June 30 (DOY 181) before shifting back to the 0 – 0.30 m range from July 14 to July 31 (DOY 195 to 212) and then splitting its water uptake between the 0.31 – 0.61 m and 0.61 – 1.10 m range on August 14 (DOY 226).

G-L *H. pauciflorus* and *A. canescens* took most of their water from 0 – 0.30 m from June 16 to July 14 (DOY 167 to 195). G-L *H. pauciflorus* and *A. canescens* shifted to the 0.31 – 0.60 m depth range on July 31 and August 14 (DOY 212 and 226), respectively, and both species shifted to the 0.61 – 0.10 m range on September 9 (DOY 252). G-NL *H. pauciflorus* shifted to the 0.61 – 1.10 m range from June 30 to September 9 (DOY 195 to 252) and showed less reliance on the 0.30 – 0.61 m range altogether.
5 Discussion

The G-L site featured significantly more C$_3$ grass and standing dead/litter biomass in both June and August and had higher mean soil water content compared to the G-NL site. However, the G-NL site had significantly more C$_4$ grass biomass in August and consistently higher soil water content at the shallowest soil depth (0.2 m) compared to the G-L site. The differences in plant biomass and shallow soil moisture between sites are likely related; greater amounts of water-inefficient C$_3$ grasses will place a greater strain on available soil moisture in the first 0.30 m of soil (Albertson 1937, Weaver 1954, Fitter and Hay 2002b), and more live and standing dead/litter biomass will cause more interception and evaporation of light rains (Naeth et al. 1991). Sites did not differ in soil composition or carbon and nitrogen content; however, the G-L site had a significantly higher amount of organic matter in the 0 – 0.30 m soil depth, which was likely the result of greater plant biomass (Burke et al. 1998).

5.1 Plant water uptake and physiology

In order to use stable isotopes to identify plant water uptake patterns, a clear vertical isotopic gradient must be present in the soil water (Dawson 1993, Brunel et al. 1995, Dawson and Ehleringer 1998). This gradient was not always clearly defined given the abnormally high amount of precipitation in June and July, and only became apparent toward the end of the sampling period in September after an extended dry spell. Over the course of the season, deep soil water (> 1 m depth) resembled the signature of groundwater taken from a nearby well, while water in the shallowest soil depth (0.30 m) most closely resembled that of precipitation and showed a clear seasonal enrichment.
trend. Water taken from below the 1–2 m depth typically resembles that of deep groundwater due to attenuation of seasonal isotope variations (Clark and Fritz 1997a, Eggemeyer et al. 2009). A pulse of water depleted in D and $^{18}$O isotopes was evident in the 0.31 – 0.60 m and 0.61 – 0.90 m G-L soil water on July 31 (DOY 212), possibly reflecting the potential for A. canescens to funnel water from larger rain events deeper into the soil (Lyford and Qashu 1969, Devitt and Smith 2002, Bhark and Small 2003).

Water uptake patterns did not differ between grass species (H. comata and K. macrantha [C₃]; A. hallii and C. longifolia [C₄]). Grasses extracted the majority of their water from recent precipitation events or shallow (0 – 0.30 m depth) soil water during the sampling period (June 3 to September 9, DOY 154 to 252), except for September 9 (DOY 252), when the two grass species that did not go dormant (H. comata and A. hallii) shifted to deeper (0.31 – 0.60 m depth) soil water. This preferential uptake of shallow soil water is similar to the water uptake patterns of grasses found in the shortgrass steppe of Colorado (Dodd et al. 1998), the Corn Belt Region of the Midwest (USA) (Asbjornsen et al. 2008), tallgrass prairie in Kansas (Nippert and Knapp 2007a), and semiarid grassland in Nebraska (Eggemeyer et al. 2009).

Reliance on shallower soil water and a lack of plasticity in water uptake contributed to greater water stress among grass species compared to forbs and shrubs. C₃ grasses (H. comata and K. macrantha) exhibited the most stress, with the most negative $\Psi$ of the species studied, especially between July 31 and August 14 (DOY 195 and 212, respectively) when there was only one heavy rain event and high temperatures. Given that C₃ and C₄ grasses occupied the same terrain and employ similar rooting strategies, both groups had access to the same water resources; therefore, the greater water stress
among C₃ grasses was a product of their lower photosynthetic water use efficiency and lower drought resistance compared to the C₄ grasses on site.

C₄ grasses (A. hallii and C. longifolia) had predictably higher WUE relative to C₃ grasses (H. comata and K. macrantha) due to their Kranz anatomy. This allows them to concentrate CO₂ at the site of fixation by Rubisco via the PEP carboxylation enzyme, resulting in lower internal concentrations of CO₂ within the leaf and the absence of apparent photorespiration. These traits afford C₄ grasses a competitive advantage, allowing them to assimilate more CO₂ per unit of water lost to transpiration (Larcher 2001a, Fitter and Hay 2002a). A. hallii and C. longifolia had lower transpiration rates (E) and were less water stressed (less negative Ψₘ₀) relative to H. comata and K. macrantha over the sampling period, particularly during the drier months of August and September. Similar ecophysiological differences have been observed between these four species in previous studies in the Sandhills (Barnes and Harrison 1982).

IsoSource analysis indicated that H. comata and A. hallii extracted nearly all of their water from the deepest (0.61 – 1.10 m depth) soil at the end of the growing season (September 9, DOY 252); however, although perennial prairie grasses may have roots as deep as 1 m or greater, the vast majority of root biomass is within the first 0.30 m of soil (Albertson 1937, Weaver 1954, Nippert and Knapp 2007a), and the presence of roots at deeper depths does not guarantee water uptake at those depths (Thorburn and Ehleringer 1995, Eggemeyer et al. 2009). In addition, the relatively uniform vertical soil water isotope profile meant that tolerances outside of those recommended by Phillips and Gregg (2003) were necessary to perform the IsoSource analysis on many sampling dates. Therefore, it is unlikely that H. comata and A. hallii were taking up water from this depth.
The C₃ forb (*H. pauciflorus*) and shrub (*A. canescens*) species showed greater plasticity in water uptake patterns compared to the grass species. *H. pauciflorus* was the least water stressed of any species studied, with the highest (least negative) $\Psi_{pre}$ and $\Psi_{mid}$ throughout the growing season, including both the wetter (June, July) and drier (August, September) periods. These water uptake patterns are the product of *H. pauciflorus*’ deep and extensive root system, which allows it to reach depths of up to 2 m while maintaining competitiveness for shallower soil water (Weaver and Fitzpatrick 1934b). This adaptation allowed *H. pauciflorus* to maintain the highest photosynthetic rates during the growing season (Milby 2011).

*A. canescens*’ consistent reliance on deeper soil water throughout the growing season reflects its deeper but less intensive rooting strategy; although its roots typically reach 2 to 5 m deep or more, there is no dense network of shallow fibrous roots. This limits *A. canescens*’ ability to compete for shallow soil water with grasses and other plants (Weaver and Fitzpatrick 1934b) but enables it to tap deeper and more reliable water sources, affording greater survivability and reducing interspecific competition under water-limited conditions.

The partitioning of water resources observed in this study has also been found to occur between tree species and C₄ grasses in semiarid grassland (Eggemeyer et al. 2009), between herbaceous and shrub and tree species (Asbjornsen et al. 2008), and between C₄ grasses and C₃ forbs and shrubs in tallgrass prairie (Nippert and Knapp 2007b).
5.2 The effect of *Amorpha canescens*

*Amorpha canescens* proved beneficial to the surrounding plant community during the study period in several respects; however some of these benefits were offset by negative effects. C₄ grass and C₃ forb species had higher transpiration rates in the G-NL site compared to the G-L site, indicating less favorable conditions for the G-L species. Both C₃ G-L grass species showed significantly higher δ¹³C content in their organic matter relative to their G-NL counterparts toward the end of the growing season when water became limited, indicating lower stomatal conductance as a result of water stress in the G-L species (Fitter and Hay 2002b). This increased water stress was likely the result of lower soil moisture at the 0.2 m depth in the G-L site, particularly after the last major rain event on July 29th (DOY 210).

The G-L site also had higher concentrations of organic matter compared to the G-NL site, indicating more favorable growing conditions for G-L species (Larcher 2001b). Green and senesced leaves of *A. canescens* are high in nitrogen (Norris and Reich 2009), which is incorporated into the soil via leaf composition and root turnover. However, while this can lead to significantly greater soil nitrogen content in the immediate proximity around *A. canescens* (Milby 2011), it does not necessarily lead to higher nitrogen composition throughout the surrounding plant community; up to three-quarters of added soil nitrogen is found within 0.2 m or less of its parent shrub (Schlesinger et al. 1996). Greater organic matter in the G-L site was likely a result of the significantly greater live and standing dead biomass and litter observed in the G-L site (Burke et al. 1998).
A. canescens did not appear to have an effect on plant water uptake patterns of nearby species during the study period. Mean seasonal δD and δ^{18}O values did not differ significantly between sites for any species, and differences in δD and δ^{18}O values between sites for each species by date were inconsistent, when they existed at all. IsoSource modeling showed most G-L species taking more water from deeper (0.31 – 0.60 m) soil depths in the middle of the growing season relative to their G-NL counterparts, although only K. macrantha and A. hallii had robust results (i.e. mass balance tolerance of ± 0.2 ‰ or less).

Regression analysis (δD = m*δ^{18}O + b ‰) for seasonal root crown water values showed almost no difference in C_{3} grasses between sites in terms of departure from the North Platte Meteoric Water Line, indicating water uptake occurred from very similar sources (Clark and Fritz 1997c). G-L C_{4} grasses showed slightly less departure from the NPMWL compared to their G-NL counterparts, potentially indicating water uptake from deeper soil in the G-L site; smaller slopes in δD/ δ^{18}O regression lines indicate lower relative humidity and less mixing prior to water uptake, both of which are more likely at shallower soil depths (Clark and Fritz 1997c). G-L H. pauciflorus produced a peculiar regression line, running nearly parallel to the NPMWL, while G-NL H. pauciflorus produced a regression line that departed the most from the NPMWL of any species. This large range in δD/δ^{18}O values relative to the other species studied could be the result of H. pauciflorus' deep and widespread rooting behavior. Indeed, A. canescens, which also has an extensive root system, produced a regression line very similar to that of NPMWL.

A. canescens may have direct and indirect effects on soil moisture throughout the soil profile. Shrub species found in arid climates have been shown to prevent light rains
(e.g. < 5 mm) from reaching the soil under shrub canopy due to interception and subsequent evaporation (Bhark and Small 2003); however, shrubs have been found to enhance infiltration of more substantial rains on account of stemflow, resulting in wetting fronts up to three times deeper than areas not under shrub canopy (Lyford and Qashu 1969, Devitt and Smith 2002, Bhark and Small 2003). Plant litter and organic matter have also been shown to reduce available shallow soil water through interception of precipitation and subsequent evaporation in mixed prairie (Naeth et al. 1991). The significantly higher amounts of standing dead and plant litter observed in the G-L site – along with the presence of A. canescens – may explain the lower soil moisture observed in G-L at 0.20 m for most of the sampling period relative to the G-NL site; it would also explain the greater soil moisture observed at the 0.40 and 1.0 m depths in the G-L site, as well as the pulse of D and $^{18}$O enrichment seen in the 0.31 – 0.60 m and 0.61 – 0.90 m soil samples taken on DOY 212 given the heavy rain event that occurred just prior.
6 Conclusion

The major goal of this study was to examine the ecological effect of *A. canescens* in the Nebraska Sandhills, particularly its impact on plant water uptake, water stress, and soil water distribution. To this end, we studied 5 species in close proximity to *A. canescens* individuals over the course of the 2010 growing season; these species represented dominant members of the C₃ and C₄ grass and C₃ forb functional groups and are commonly found throughout the Sandhills. We expected *A. canescens* to have beneficial effects on neighboring plants, manifesting in the form of lower water stress, greater water availability, and better performance over the course of the growing season in communities with *A. canescens* compared to those without the shrub. However, the observed effects of *A. canescens* were not so straightforward.

The presence of *A. canescens* influenced the water uptake patterns of its surrounding plant community in indirect and contrasting ways during the study period. The shrub contributed to more favorable growing conditions for C₃ grasses in the form of greater soil organic matter, along with higher nitrogen content in close proximity to *A. canescens* individuals. While this led to greater C₃ grass biomass production when water was plentiful, it contributed to greater water stress among C₃ grasses during drier periods later in the growing season due to increased demands on shallow soil water. These demands were exacerbated by greater amounts of live and standing dead plant biomass and litter around *A. canescens* and the added presence of shrub canopy, all of which likely caused greater interception and evaporation of rainfall – especially the lighter rains that occurred later in the growing season – and deprived shallow-rooted vegetation of a critical water resource. At the same time, *A. canescens* may also have increased the amount of
available soil moisture deeper in the soil profile via stemflow, benefitting more deeply rooted species such as the forb *H. pauciflorus*. There was no evidence that *A. canescens* was directly beneficial to the water relations of the surrounding plant community during the study period.

Water resource partitioning between species was not observed during the wetter periods that characterized the first half of the growing season, with shrubs, forbs, and C₃ and C₄ grasses all predominantly using shallow soil water. However, forbs and shrubs switched to deeper water sources as conditions dried out in the second half of the growing season, while C₃ and C₄ grasses senesced or reduced stomatal conductance to limit water loss. This difference in drought response is mainly due to differences in rooting strategies; *H. pauciflorus* and *A. canescens* utilize extensive root systems that can extend down to several meters, while C₃ and C₄ grass species concentrate the majority of their root biomass in the first 0.3 m of soil.

Overall, it is clear that the presence of *A. canescens* provides some benefit to nearby plants. However, the overall ecological impact of this shrub appears to be quite nuanced and complex, and although *A. canescens* is readily found throughout most of the Nebraska Sandhills, it is clear that much remains to be done in understanding the full measure of its effects on this unique ecosystem.
7 References


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### 8 Tables and Figures

Table 8.1 - Repeated-measures analyses of seasonal courses in measured parameters on grassland with *A. canescens* (G-L) and grassland without *A. canescens* (G-NL) at the Gudmundsen Sandhills Laboratory for C₃ grasses (*H. comata* and *K. macrantha*), C₄ grasses (*A. hallii* and *C. longifolia*), forbs (*H. pauciflorus*), and shrubs (*A. canescens*) from June 3 to September 9, 2010 (DOY 154 to 252). E = transpiration, WUE = water use efficiency, $\Psi_{\text{pre}}$ = predawn water potential, $\Psi_{\text{mid}}$ = midday water potential, $\delta^{13}\text{C}$ = carbon isotope discrimination. Underlined bolded values are statistically significant ($P < 0.05$).

#### G-L vs. G-NL site

All species except *A. canescens* between sites (Prob > F)

<table>
<thead>
<tr>
<th>Source</th>
<th>E</th>
<th>WUE</th>
<th>$\Psi_{\text{pre}}$</th>
<th>$\Psi_{\text{mid}}$</th>
<th>$\delta^{13}\text{C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>0.0002</td>
<td>0.2297</td>
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<td>.003</td>
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Species within G-L site (Prob > F)

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<th>$\Psi_{\text{pre}}$</th>
<th>$\Psi_{\text{mid}}$</th>
<th>$\delta^{13}\text{C}$</th>
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<td>0.2019</td>
<td>0.1152</td>
<td><strong>0.0024</strong></td>
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Table 8.2 - δD (‰) and δ¹⁸O (‰) relationships for the North Platte Meteoric Water Line, soil water at depth ranges of 0 – 0.30, 0.31 – 0.60, 0.61 – 0.90, and 0.91 – 1.10 m, and root crown water for C₃ grasses (H. comata and K. macrantha), C₄ grasses (A. hallii and C. longifolia), forbs (H. pauciflorus) and shrubs (A. canescens) at grassland sites with A. canescens (G-L) and without A. canescens (G-NL) from June 16 to September 9 2010 (DOY 167 to 252).

Table 8.3 - Seasonal means and standard errors for transpiration (E), water-use efficiency (WUE), predawn water potential (Ψᵣₚᵢₑ), midday water potential (Ψₘᵢₐₜ), and δ¹³C content of plant organic matter for C₃ grasses (H. comata and K. macrantha), C₄ grasses (A. hallii and C. longifolia), forbs (H. pauciflorus) and shrubs (A. canescens) at grassland sites with A. canescens (G-L) and without A. canescens (G-NL) from June 16 to September 9 2010 (DOY 167 to 252).
Figure 8.1. Seasonal dynamics in daily minimum and maximum temperature (°C) and vapor pressure deficit (kPa) at the University of Nebraska-Lincoln’s Gudmundsen Sandhills Laboratory from June 3 to September 11 2010 (DOY 154 to 254). Arrows indicate sampling dates.
Figure 8.2. Total monthly precipitation (mm) for 2009 and 2010 compared to the 30-year mean.

Figure 8.3. Seasonal dynamics in daily precipitation (mm) and mean daily volumetric soil water content (VSWC %) down to 1.0 m soil depth on grassland sites with *Amorpha canescens* (G-L) and without *A. canescens* (G-NL) from June 3 to September 11, 2010 (DOY 154 to 254). Arrows indicate sampling dates. Mean is not shown for the G-L site at from June 3 to June 28 (DOY 154 to 179) due to sensor malfunction.
Figure 8.4. Seasonal dynamics in daily precipitation (mm) and daily volumetric soil water content (VSWC %) down to 1m soil depth on grassland sites with *Amorpha canescens* (G-L) and without *A. canescens* (G-NL) from June 3 to September 11, 2010 (DOY 154 to 254). Arrows indicate sampling dates. No data was collected on the G-L site at 1m soil depth from June 3 to June 28 (DOY 154 to 179) due to sensor malfunction.
Figure 8.5. Mean soil particle composition at depth ranges of 0 – 0.30, 0.31 – 0.60, and 0.61 – 1.10 m for grassland sites with *A. canescens* (G-L, black) and without *A. canescens* (G-NL, white) from June 16 to August 14 2010 (DOY 167 to 226).

Figure 8.6. Mean percent organic matter at soil depths of 0 – 0.30, 0.31 – 0.60, and 0.61 – 1.10 m for grassland sites with *A. canescens* (black solid) and without *A. canescens* (white dashed) from June 16 to August 14 2010 (DOY 167 to 226). Note that percent organic matter ranges from 0 to 1%, not 0 to 100%.
Figure 8.7. Mean carbon composition (mg g\(^{-1}\) soil) at soil depths of 0 – 0.30, 0.31 – 0.60, and 0.61 – 1.10 m for grassland sites with A. canescens (black solid) and without A. canescens (white dashed) from June 16 to August 14 2010 (DOY 167 to 226).

Figure 8.8. Mean nitrogen composition (mg g\(^{-1}\) soil) at soil depths of 0 – 0.30, 0.31 – 0.60, and 0.61 – 1.10 m for grassland sites with A. canescens (black solid) and without A. canescens (white dashed) from June 16 to August 14 2010 (DOY 167 to 226). Note that nitrogen ranges from 0 to 0.5 mg g\(^{-1}\), not 0 to 5 mg g\(^{-1}\).
Figure 8.9. Mean above ground dry biomass of year 2010 growth by functional group on grassland sites with *Amorpha canescens* (G-L) and without *A. canescens* (G-NL) in the Nebraska Sandhills in June and August 2010. (C3G = C3 grass, C4G = C4 grass, Forb = forb, Shrub = shrub (predominantly *A. canescens*), AG = annual grass, Live = total current year’s growth, Dead = standing dead material and plant litter). Asterisks indicate a significant difference between sites within functional groups ($P < 0.05$).
Figure 8.10. Seasonal trends in transpiration (E) and water-use efficiency (WUE) for C₃ grasses (H. comata and K. macrantha), C₄ grasses (A. hallii and C. longifolia), forbs (H. pauciflorus) and shrubs (A. canescens) at grassland sites with A. canescens (black) and without A. canescens (white) from June 16 to September 9th 2010 (DOY 167 to 252). An asterisk indicates a significant difference between sites within species (P < 0.05).
Figure 8.11. Seasonal changes in mean predawn and midday water potential (Ψ) and carbon isotope ratios (δ^{13}C) on grassland sites with *A. canescens* (G-L) and without *A. canescens* (G-NL) in the Nebraska Sandhills for C\textsubscript{3} grasses (*H. comata* and *K. macrantha*), C\textsubscript{4} grasses (*A. hallii* and *C. longifolia*), forbs (*H. pauciflorus*), and shrubs (*A. canescens*) from June 3 to September 9, 2010 (DOY 154 to 252). An asterisk corresponding to the lines above (water potential) or below (carbon isotope) indicates a significant difference between sites within species (*P* < 0.05).
Figure 8.12. Seasonal trends in volumetric soil water content (VSWC %) at 0.20 m and mean δD and δ¹⁸O composition of root crown water for C₃ grasses (H. comata and K. macrantha), C₄ grasses (A. hallii and C. longifolia), forbs (H. pauciflorus) and shrubs (A. canescens) at grassland sites with A. canescens (G-L) and without A. canescens (G-NL) from June 3 to September 9, 2010 (DOY 154 to 252).
Figure 8.13. Mean seasonal δD (top) and δ18O (above) composition of root crown water for C3 grasses (H. comata and K. macrantha), C4 grasses (A. hallii and C. longifolia), forbs (H. pauciflorus) and shrubs (A. canescens) at grassland sites with A. canescens (G-L) and without A. canescens (G-NL) from June 3 to September 9, 2010 (DOY 154 to 252).
Figure 8.14. Data points and regression lines of δD and δ¹⁸O composition of root crown water for C₃ grasses (H. comata and K. macrantha), C₄ grasses (A. hallii and C. longifolia), forbs (H. pauciflorus) and shrubs (A. canescens) at grassland sites with A. canescens (G-L) and without A. canescens (G-NL) from June 16 to September 9 2010 (DOY 167 to 252) plotted against the North Platte meteoric water line (North Platte MWL). Regression line equations and R² values are listed in the same order as the regression lines in the legend.
Figure 8.15. Seasonal trends in precipitation, temperature, and soil water δD and δ\(^{18}\)O composition at depth ranges of 0 – 0.30, 0.31 – 0.60, and 0.61 – 1.10 m at grassland sites with *A. canescens* (G-L, black) and without *A. canescens* (G-NL, white) from June 16 to September 9 2010 (DOY 167 to 252) plotted against the North Platte meteoric water line (NP MWL).
Figure 8.16. Data points and regression lines of $\delta^D$ and $\delta^{18}O$ composition of soil water at depth ranges of 0 – 0.30, 0.31 – 0.60, 0.61 – 0.90, and 0.91 – 1.10 m at grassland sites with *A. canescens* (black, dashed) and without *A. canescens* (white, dash-dot) plus precipitation and groundwater from June 16 to September 9 2010 (DOY 167 to 252) plotted against the North Platte meteoric water line (NP MWL). Regression line equations and $R^2$ values are listed in the same order as the regression lines in the legend.
Figure 8.17. Seasonal dynamics in $\delta^{18}$O (‰) content of soil water at G-L (black circles) and G-NL (white circles), groundwater (GW), precipitation (Prec), H. comata (HC), K. macrantha (KM), A. hallii (AH), C. longifolia (CL), H. pauciflorus (HP), and A. canescens (AC).
Figure 8.18. Seasonal dynamics in δD (‰) content of soil water at G-L (black circles) and G-NL (white circles), groundwater (GW), precipitation (Prec), H. comata (HC), K. macrantha (KM), A. hallii (AH), C. longifolia (CL), H. pauciflorus (HP), and A. canescens (AC).
Fraction of uptake

VSWC (%)
Figure 8.19. Seasonal changes in volumetric soil water content (VSWC %) and the fraction of water uptake from 0 – 0.3, 0.31 – 0.90, and 0.91 – 1.10 m soil depths for grassland sites with *A. canescens* (black circles) and without *A. canescens* (white circles) in the Nebraska Sandhills for *C₃* grasses (*H. comata* and *K. macrantha*), *C₄* grasses (*A. hallii* and *C. longifolia*), forbs (*H. pauciflorus*) and shrubs (*A. canescens*) from June 3 to September 9, 2010 (DOY 154 to 252). Solid and dashed lines represent the mean and range of model solutions produced by the IsoSource program, respectively. An asterisk denotes values calculated with a mass balance tolerance of 0.2 ‰ or less. Note that soil water content was recorded at depths of 0.2, 0.4, and 1.0 m. No soil water content data was available for the G-L site from June 3 to June 28 (DOY 154 to 179) due to sensor malfunction.