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Photosynthetic traits in C$_3$ and C$_4$ grassland species in mesocosm and field environments

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Abstract

The North American tallgrass prairie is composed of a diverse mix of C$_3$ and C$_4$ plant species that are subject to multiple resource limitations. C$_4$ grasses dominate this ecosystem, purportedly due to greater photosynthetic capacity and resource-use efficiency associated with C$_4$ photosynthesis. We tested the hypothesis that intrinsic physiological differences between C$_3$ and C$_4$ species are consistent with C$_4$ grass dominance by comparing leaf gas exchange and chlorophyll fluorescence variables for seven C$_4$ and C$_3$ herbaceous species (legumes and non-legumes) in two different settings: experimental mesocosms and natural grassland sites. In the mesocosms, C$_4$ grasses had higher photosynthetic rates, water potentials and water-use efficiency than the C$_3$ species. These differences were absent in the field, where photosynthetic rates declined similarly among non-leguminous species. Thus, intrinsic photosynthetic advantages for C$_4$ species measured in resource-rich mesocosms could not explain the dominance of C$_4$ species in the field. Instead, C$_4$ dominance in this ecosystem may depend more on the ability of the grasses to grow rapidly when resources are plentiful and to tolerate multiple limitations when resources are scarce.
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1. Introduction

Central US grasslands are typically dominated by a few highly productive C$_4$ grass species (Epstein et al., 1998). These grasses are accompanied by numerous C$_3$ forb species (Freeman and Hulbert, 1985; Knapp and Seastedt, 1986; Freeman, 1998), which must compete with the dominant grasses for multiple potentially limiting resources, including nitrogen, water, and light (Schulze and Chapin, 1987; Knapp et al., 1998). Thus, in a grassland environment of high-light, warm temperatures, periodic drought, and low nitrogen availability, C$_4$ dominance over the C$_3$ species may result from a combination of mechanisms, including intrinsically higher photosynthetic capacity and resource-use efficiency of C$_4$ photosynthesis (Knapp, 1993; Tezara et al., 1998; Long, 1999) and key adaptations to major biotic and abiotic drivers and stresses in grasslands including fire, grazing by large herbivores, and climatic variability (Seastedt and Knapp, 1993; Ojima et al., 1994; Knapp and Medina, 1999).

The role of intrinsic physiological differences among C$_4$ and C$_3$ species in their relative success in tallgrass prairie assemblages remains unclear. In general, C$_4$ species have lower water and nitrogen requirements than C$_3$ species and reduced stomatal conductance and enzyme requirement per mol of CO$_2$ fixed when measured under controlled laboratory conditions (Pearcy and Ehleringer, 1984; Long, 1999; Sage, 2004). The performance of C$_3$ and C$_4$ species in the field should reflect these inherent C$_4$ advantages (Knapp, 1993). However, the expected physiological advantages of C$_4$ species are often not realized in the field. This discrepancy may result because laboratory studies often poorly reproduce field growth conditions of water, nutrient, and light limitation, or the plant material studied is unrepresentative of field populations (Evans and Seemann, 1989; Wohlfahrt et al., 1999; Gibson et al., 1999). Further, most field studies of photosynthesis have been conducted when some level of interspecific competition exists, rendering comparisons with laboratory studies difficult (Tieszen et al., 1997; Knee and Thomas, 2002; Vitale and Manes, 2005).

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We compared photosynthetic traits in several common tallgrass prairie grass and forb species in environments that differed in resource availability to discern if C_4 advantages common in laboratory studies could be replicated when performed under the prevailing mid-summer growth environment. Species traits were measured in experimental outdoor mesocosms and in upland field sites in an undisturbed annually burned prairie. The mesocosms provided abundant light, nitrogen, and water and minimal variation in these resources similar to laboratory studies, thus enabling us to estimate *potential* photosynthetic traits. By comparison, the field sites represented typical levels of resource limitation common in mid-summer in tallgrass prairie and served as a comparative indicator of *realized* photosynthetic traits. The difference between *potential* and *realized* photosynthesis may allow for a greater differentiation of photosynthetic differences between C_3 and C_4 grassland species. These traits were specifically measured in mid-July, a time that is typically dry, hot, and when the spring pulse in soil N availability has been depleted. This allowed us to test the hypothesis that C_4 grasses in this system have both higher *potential* and *realized* photosynthetic capacity and resource-use efficiency under typical mid-season environmental conditions.

2. Materials and methods

2.1. Study site

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

2.2. Comparative growth environments

2.2.1. Mesocosm facility

Plants were measured in eight 2.6 m³ (1.2 m × 1.2 m × 1.8 m deep) mesocosms containing assemblages of the study species. The mesocosms provided high water, light, and nutrient availability, and favorable soil conditions for root growth. Outdoor mesocosms were chosen for this study to represent the most optimum growing conditions outdoors, for comparison to similar species growing under ambient resource limitations. Individuals were planted in late May 2003 by broadcast seeding the grasses and planting greenhouse-grown seedlings for the forbs. All seeds were from commercial sources. The legumes were not inoculated with *Rhizobium* prior to planting. Planting densities reflect natural stem densities and species relative abundances, and forbs were planted in an identical spatial arrangement in each mesocosm to avoid variation in performance from differing species associations. Mesocosms were frequently weeded to maintain the desired species composition and were watered approximately three times per week to minimize water stress. The soil profile within each mesocosm contained well-mixed A-horizon topsoil in the top 30 cm overlying B-horizon subsoil collected on site. The mesocosms were free draining to allow for natural soil moisture profiles. Average extractable inorganic soil nitrogen measured in September 2003 was 2.80 ± 0.33 (S.E.) $\mu g\,N\,g^{-1}$ soil.

2.2.2. Field sites

KPBS is divided into 60 watersheds of various long-term treatments of fire frequency and grazing by native herbivores. We used the upland regions of the two annually burned, ungrazed watersheds which provide typical field soil moisture, nutrient availability, and competitive interactions. Within each watershed, two separate sites were established where all seven species occurred within a 20 m-diameter circle. Watersheds were burned each April since 1982, including the study year, a typical management practice that maintains C_4 grass dominance and results in saturating sunlight intensities for most individual leaves. Extractable inorganic soil nitrogen from these sites has been reported previously (0.93 ± 0.07 (S.E.) $\mu g\,N\,g^{-1}$ soil; Blair, 1997) from samples collected in September, the same time of year as that measured in the mesocosms.

2.3. Sampling procedure

Sampling was specifically conducted in July when air temperatures were high and mid-season water stress more likely in order to distinguish these responses from previous studies conducted in early summer when water and soil N are not as limiting (Knapp, 1985). Additionally, mid-July is typically the time period for maximum rates of growth in developmentally mature plants prior to late summer senescence (Turner and Knapp, 1996). For gas exchange measurements in the mesocosms, one individual from each species was randomly selected within each mesocosm ($n = 8$ per species). For chlorophyll fluorescence and water potential measurements, 5–7 individuals per species/mesocosm combination were measured and the average value per mesocosm was used in the analysis. Based on the results of the 2003 sampling in the mesocosms, analyses were conducted in the field during the same seasonal period (early July) in 2004. This time period had a nearly identical microclimate to measurements conducted in the mesocosms the previous year (air temperature, relative humidity (RH), solar radiation, and windspeed; Table 1). Cumulative monthly precipitation in 2004 was 29% above average (Table 1), making comparisons with the mesocosms conservative with respect to water stress.

Table 1
Microclimate differences between measurement locations during the periods when data were collected

Measurement location	Average temperature (°C) at 15:00 h	Max.–min. temperature (°C)	Average daily RH (%)	Total daily solar radiation (J cm^{-3})	Average daily windspeed (m s^{-1})
Mesocosms	37.4 (1.0)	40.5–34.0	70.64 (4.75)	2430.3 (182.9)	2.93 (0.24)
Field	34.3 (1.8)	37.5–30.1	66.73 (3.27)	2138.1 (185.7)	2.57 (0.26)

	Cumulative precipitation (mm)				
	April	May	June	July	Total
1982–2003 ($\mu \pm$ (S.E.))	74.1 (10.8)	116.5 (17.3)	118.6 (13.0)	101.3 (19.9)	410.4 (29.9)
1982–2003 (Max.–min.)	233.8–8.5	241.7–15.3	255.6–51.0	385.8–5.7	831.7–166.5
2004	81.5	67.6	213.9	165.1	528.1

Notes: Measurements were performed 07/12–19/03 in the mesocosms and 07/16–23/04 in the field. Standard errors are expressed in parentheses.

For field gas exchange measurements, two individuals from each species were randomly selected from each location, and these measurements were averaged prior to the overall field analysis ($n = 4$ species). Similar to measurements in the mesocosms, 5–7 individuals per species/location were measured for chlorophyll fluorescence and water potential measurements, and the average value was used in the analysis.

2.3.1. Physiological variables

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

Gas exchange measurements were conducted using a LI-COR 6400 IRGA with an artificial red/blue LED light source (6400-02B, LI-COR Inc., Lincoln, NE, USA). Plants were placed inside this leaf chamber and allowed to reach steady-state photosynthesis at ambient C_a (370 μmol mol^{-1}) and at a saturating light intensity (1500 μmol m^{-2} s^{-1}). Leaf temperature was allowed to increase with ambient daily air temperature. To compare changes in the photosynthetic rate (A), as internal CO_2 (C_i) concentration increased as well as potential down-regulation of the photosynthetic process, A/C_i curves were constructed by progressively increasing CO_2 concentrations inside the IRGA cuvette from 40 to 1500 μmol mol^{-1} in twelve steps for the C$_3$ species and from 360 to 0 then 360 to 1500 μmol mol^{-1} in 11 steps for the C$_4$ species. Changes in CO_2 concentration were varied by photosynthetic type to avoid de-activation of Rubisco. Using this measurement protocol, similar photosynthetic rates at ambient C_a (370 μmol mol^{-1}) before and during curve construction suggest steady-state activation of Rubisco was maintained across the range of CO_2 concentrations measured (Long and Bernacchi, 2003). For both photosynthetic types, the majority of steps were measured in the linear (sub-atmospheric CO_2) region

of the curve. Between each CO_2 concentration change, A, g_s, and flow rate were allowed to stabilize. Species order for A/C_i construction was random between consecutive days of measurement, and all measurements were conducted between 9:00 and 15:00 h daily. Fluorescence measurements of $\Delta F/F_m'$ were performed with a modulated fluorometer (OS1-FL, OptiSciences, Tynsboro, MA, USA) concurrent with the gas exchange measurements. For measurements of F_v/F_m, leaves of each species were selected and placed in dark-adaptation cuvettes predawn, allowing at least 30 min prior to measurement. Plant water status was assessed on the measurement dates by measuring predawn and midday leaf water potentials (Ψ) with a pressure chamber (PMS-1000, PMS Instruments, Corvallis, OR, USA).

2.3.2. A/C_i curve modeling

Rates of leaf-level photosynthesis are determined by the minimum of two co-limiting reaction velocities: CO_2 availability limits Rubisco activity, and/or ATP synthesis in the light reaction of photosynthesis limits the rate of regeneration of ribulose-1,5-bisphosphate (RuBP) (Farquhar et al., 1980; von Caemmerer and Farquhar, 1981). To estimate the rate of the limiting reaction, we modeled photosynthesis at varying CO_2 concentrations using photosynthetic pathway-specific models because C$_4$ species require consideration for the spatial separation of initial CO_2 fixation by phosphoenolpyruvate-carboxylase (PEP-C) in the mesophyll cells from the bundle sheath chloroplasts (Berry and Farquhar, 1978). For the C$_3$ species, we used the derivation of the Farquhar et al. (1980) model reported by Medlyn et al. (2002). For the C$_4$ grasses, we used the model presented by Chen et al. (1994) because this model was parameterized using *A. gerardii*. We incorporated the temperature dependence of the Michaelis–Menten coefficients presented by Bernacchi et al. (2001), multiplied by 2.5 to appropriately scale the temperature dependence of the kinetic constants for use in this C$_4$ model (von Caemmerer and Furbank, 1999). Models were fit using the procedure NLMIXED in SAS V9.1 (SAS Institute Inc., Cary, NC, USA).

To determine if changes in photosynthetic rates for mesocosm and field measurements were influenced more by the supply or demand of CO_2, supply functions were calculated (Fig. 4). While the 'demand' of CO_2 is indicated by the photosynthetic rate at a given C_i, the 'supply' is the slope of a line starting

from the intercept at ambient atmospheric CO_2 concentration ($C_a = 370\ \mu mol\ mol^{-1}$) (von Caemmerer and Farquhar, 1984; Lambers et al., 1998). The supply function is affected either by changes in CO_2 assimilation rate, or changes in C_i.

2.4. Statistical analyses

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

3. Results

3.1. Mesocosms

In the relatively resource-rich mesocosms, $A{:}C_i$ curves had higher initial slopes (and greater CO_2 demand per supply) for C_4 compared to C_3 species (Fig. 1). The average maximum difference in the photosynthetic rate between species was less
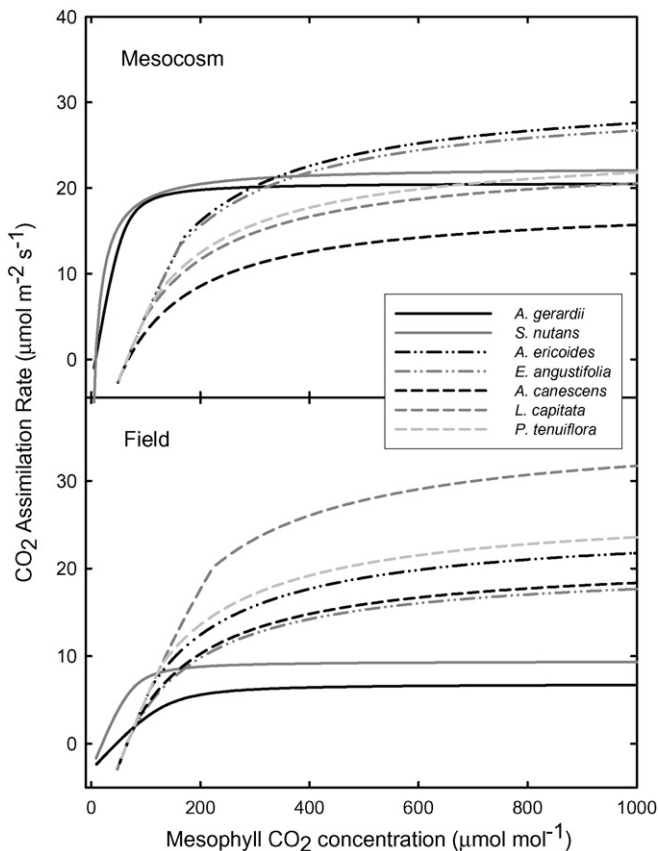

Fig. 1. Representative $A{:}C_i$ curves fit for each species measured in both locations. Each curve was fit to all of the data points for each species ($n = 8$ curves per species). Line types differ according to the three functional groups present (C_4 grass, C_3 forb, C_3 legume).

Table 2
ANOVA results for species effects among the nine variables measured

Response	Mesocosm		Field	
	F-value	P	F-value	P
A_{max}	5.16	0.0004	1.02	0.444
g_s	7.5	<0.0001	7.86	<0.0001
WUE	15.08	<0.0001	1.55	0.2056
F_v/F_m	7.96	<0.0001	11.03	<0.0001
$\Delta F_v/F_m'$	5.15	0.0004	1.6	0.2150
Predawn	6.61	<0.0001	3.3	0.0158
Midday	3.56	0.0054	1.54	0.2093

Notes: Results are presented for both locations. For the mesocosms, d.f. = 6, 48; for the field, d.f. = 6, 18. Instrument malfunction in the field resulted in the loss of $\Delta F_v/F_m'$ values for 1 of the 4 sites measured resulting in d.f. = 6, 12.

than $10\ \mu mol\ m^{-2}\ s^{-1}$ and species within the same functional group responded similarly (Fig. 1). ANOVA results show significant differences were present among species in the mesocosms for each of the variables measured (Table 2). A_{max} was significantly higher for the C_4 grasses than most C_3 species, with the exception of *A. ericoides* (Fig. 2a). Species patterns of stomatal conductance to water vapor (g_s) were similar; except for *A. ericoides* which had significantly higher values (Fig. 2b). C_4 grasses had significantly higher water-use efficiency (Fig. 2c), resulting from comparatively higher A (Fig. 2a) and lower g_s than C_3 species (Fig. 2b). WUE did not vary significantly between C_3 species (Fig. 2c).

Species within a functional group had similar predawn and midday water potentials in the mesocosms (Fig. 3). C_4 grasses had the highest predawn and midday water potentials, while C_3 forbs and legumes did not differ for either water potential measurement (Fig. 3). Measurements of leaf fluorescence (F_v/F_m and $\Delta F/F_m'$) were lower for C_4 species than C_3 (Table 3). While C_3 species had significantly higher values, species differences between C_3 legumes and C_3 forbs were not present for either fluorescence variable.

3.2. Field

In the more resource-limited field sites, A/C_i curves had greater variation among species, with nearly a $20\ \mu mol\ m^{-2}\ s^{-1}$ difference in photosynthetic rates between *L. capitata* and the C_4 grasses at $C_i > 500\ \mu mol\ mol^{-1}$ (Fig. 1). The initial linear slope of the A/C_i curves for C_4 grasses was similar to C_3 species (Fig. 1). For the variables measured, only F_v/F_m, predawn water potential, and g_s varied significantly by species according to the ANOVA results (Table 2). C_4 grasses had the lowest rates for A_{max} compared to C_3 forbs and legumes (Fig. 2a). Among C_3 species, the legumes had a collectively higher A_{max} than forbs, but did not vary significantly. Similar to measurements in the mesocosms, field measurements of leaf fluorescence varied by functional groupings, but not between species within those groupings. Dark-adapted leaf fluorescence (F_v/F_m) was significantly higher for C_3 species than C_4 grasses (Table 3). C_3 species had similarly higher values of light-adapted leaf fluorescence ($\Delta F/F_m'$), but C_3 forbs did not vary significantly from C_4 grasses (Table 3).
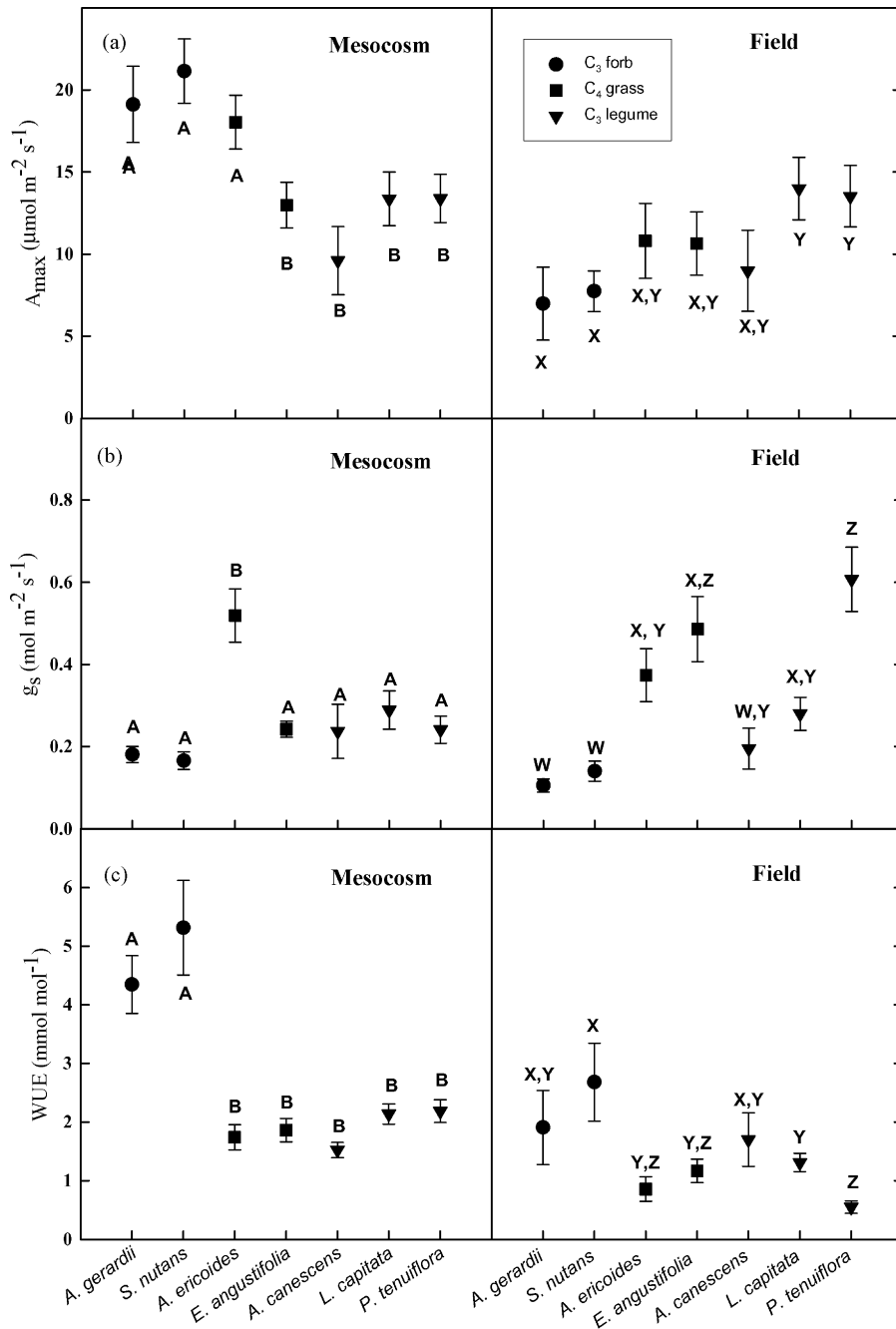
Fig. 2. Mean species response (±1 S.E.) for (a) CO_2 assimilation rate (A_{max}) at the ambient atmospheric CO_2 concentration ($\sim$370 μmol mol^{-1}), (b) stomatal conductance to water (g_s) and (c) water-use efficiency (WUE). Species are arranged by their respective functional group for both the mesocosm and field locations. Points with varying letters indicate a significant difference ($P < 0.05$) using Tukey's HSD.

Water potential measurements in the field differed by functional type. C_4 grasses had the lowest predawn, but highest midday leaf water potential (Fig. 3). Conversely, C_3 legumes exhibited less than 0.2 MPa average change between predawn and midday measurements. C_3 forbs had intermediate values and were not statistically different from either C_4 grasses or C_3 legumes (Fig. 3). Both C_4 grasses had similarly low g_s, while C_3 species varied widely irrespective of functional type (Fig. 2b). Averaged across forbs and legumes, C_3 species had g_s values twice that of the C_4 grasses. Grass WUE was highest for the C_4 grasses, with similar rates among C_3 species (Fig. 2c).

3.3. A/C_i curves between locations

For each of the species examined, C_i either remained the same or increased when supply slopes were compared between mesocosms and the field (Table 4, Fig. 4). For the grass and forb species, decreases in the CO_2 assimilation rate reflect decreases in the demand for CO_2 (Table 4, Fig. 4). For each of the non-legume forb and grass species, A was always higher in the mesocosms for a given C_i when compared to the field (Fig. 4). For the legumes, minor differences were evident in either supply or demand of CO_2 at C_a between locations, but A
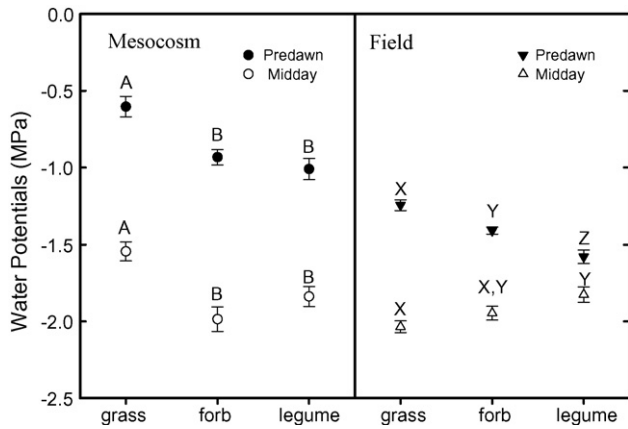
Fig. 3. Mean functional group response (±1 S.E.) for plant water potentials. Predawn and midday leaf water potentials are expressed for both the mesocosm and field locations. Within each measurement location, points with varying letters indicate a significant difference among functional groups ($P < 0.05$) using Tukey's HSD. Predawn values varied significantly ($P < 0.05$) by functional group between measurement locations, but no difference between midday values occurred between locations.

varied between low and high C_i (Table 4, Fig. 4). The legume species grown in mesocosms had a lower CO_2 compensation point ($A = 0$). At high C_i values, mesocosm legumes had lower maximum photosynthetic rates compared to field legumes. At the terminal end of the supply slope (current atmospheric CO_2 concentration = 370 μmol mol^{-1}), no difference between sup-

ply and demand was evident by location for the legumes *A. canescens* and *L. capitata*, and the supply difference for *P. tenuflora* was minor (Fig. 4).

4. Discussion

In the fire-prone tallgrass prairie, the success (productivity and cover) of C_4 grasses has been suggested to stem from alterations in post-fire resource availability (Knapp et al., 1998; Knapp and Medina, 1999). The higher resource capture and use efficiencies (water, N) of these grasses may facilitate success in an environment of high-light, high soil temperature, low available N, and periodic water stress. In this study, we find that when air temperatures are high and resources were relatively more plentiful in the mesocosms, C_4 species had higher rates of photosynthesis and resource-use efficiency than C_3 species. This result supports the premise that C_4 dominance in this grassland is related to the capability of these species to capitalize on resources when they are abundant. However, when species were compared in the more resource-limited field sites, C_4 species did not display a physiological advantage compared to C_3 species. This result is contrary to the view that increased resource-efficiency of C_4 grasses is advantageous when resource availability is low (Knapp and Seastedt, 1986; Seastedt et al., 1991; Ojima et al., 1994; Blair, 1997) and air temperatures are high (under ambient mid-summer field conditions). Indeed, photosynthetic traits for C_3 and C_4 herbaceous species were

Table 3

Average values of dark (F_v/F_m) and light ($\Delta F/F'_m$) adapted fluorescence for both measurement locations

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

Notes: Results are presented as average functional group response because species within each group had similar responses. Varying letters (a and b) following estimates indicate a significant difference between functional groups within locations ($P < 0.05$, Tukey's HSD). Functional groups did not vary significantly between the mesocosm and field locations.

Table 4

Species-specific changes in supply slope for measurement locations

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

Notes: Values for supply function slopes correspond to relationships depicted in Fig. 4. Changes in photosynthetic rate (A) and mesophyll CO_2 concentration (C_i) between locations are reported and correspond to end member values of the supply slopes in Fig. 4. Values are given as increased (+) or decreased (−) changes when comparing the low (field) to high (mesocosms) locations of resource availability.
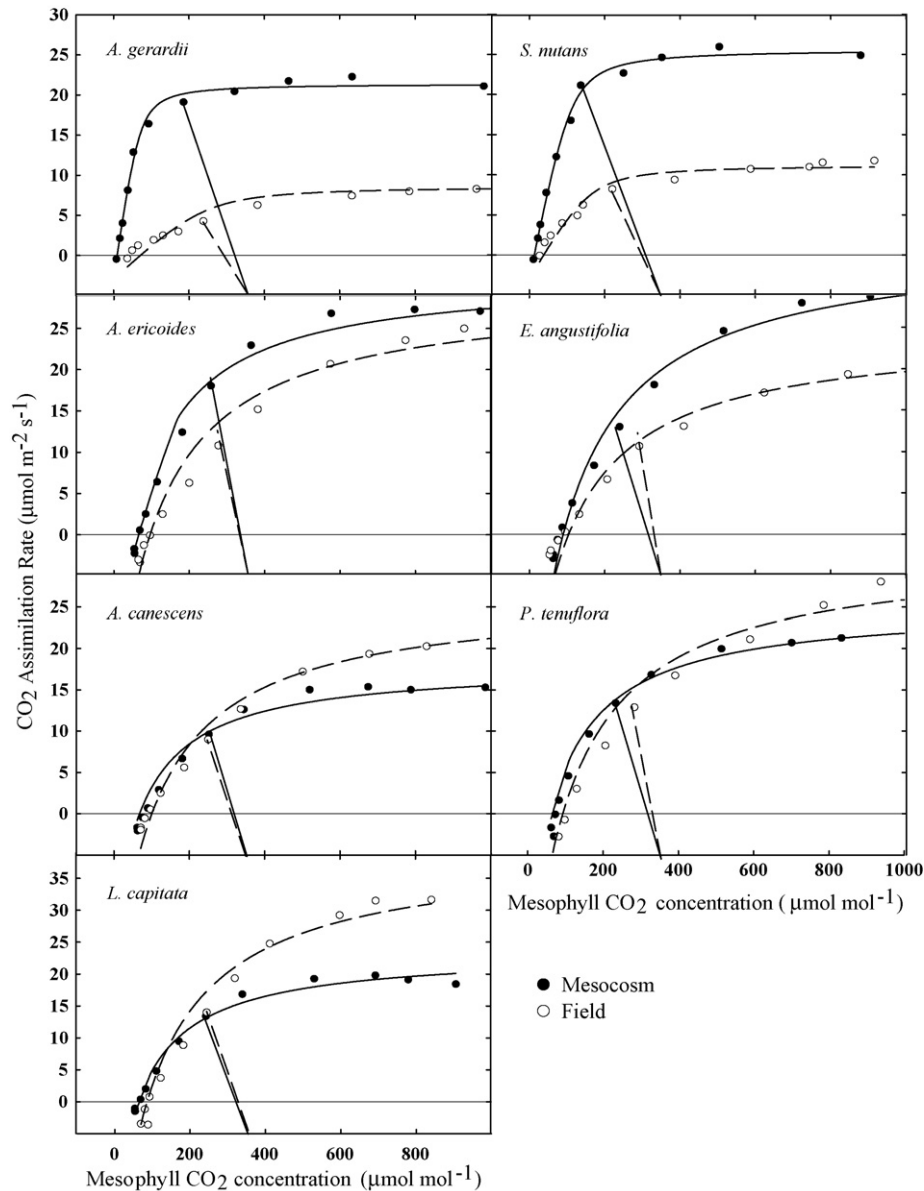
Fig. 4. Representative $A:C_i$ fit for each species separately. A significant ($P = 0.0072$, nDF = 6 dDF = 89) species × location relationship exists for the supply function. Each panel illustrates the CO_2 supply function from C_a (370 μmol mol^{-1}) to the particular C_i associated with that measurement. Species are displayed separately with the solid line expressing the supply function for the mesocosm, and the dashed line for the field.

quite similar when measured in the field. Marked decreases in photosynthesis in the field in the more shallow-rooted C_4 grasses could be due to greater water stress or the availability of soil N. Measurements of stomatal conductance were more variable in the field compared to the mesocosms and predawn water potentials were lower (Figs. 2b and 3). Only leguminous species maintained consistent physiological attributes between the contrasting resource environments.

Leguminous species may be best suited to avoid resource deficiencies in the field. In support of this hypothesis, Reich et al. (2003) reported that legumes allocated a greater proportion of biomass belowground to roots than both forbs and grasses when multiple grassland species were compared. Legumes have also been shown to be nonresponsive to fertilization (Lauenroth and Dodd, 1979; Reich et al., 2003), and therefore may not

exhibit episodic responses to seasonal changes in resource availability similar to other co-occurring species. Towne and Knapp (1996) reported increased abundance of legumes in annually burned watersheds of KPBS relative to unburned prairie. Their results suggest that legumes maybe better adapted than other forb species to tolerate the effects of fire (Towne and Knapp, 1996) and persist through periodic depressions in resource availability. Our results support these previous relationships between legumes and resource availability. Both C_4 grasses and C_3 forbs showed pronounced photosynthetic decreases when measured in the mesocosms versus field, yet legumes remained unchanged (Figs. 1, 2a, and 4).

Measurements of chlorophyll fluorescence followed expected patterns for their respective functional groups. Patterns in chlorophyll fluorescence parameters F_v/F_m and

$\Delta F/F'_m$ were similar within the field and mesocosms with both C_3 functional groups having slightly higher average values than the grasses (Table 3). F_v/F_m and $\Delta F/F'_m$ are generally higher in C_3 species due to lower energy requirements per CO_2 fixed (Pearcy and Ehleringer, 1984; Lambers et al., 1998; Pfündel, 1998). The lack of a noticeable decline in F_v/F_m or $\Delta F/F'_m$ suggests that the interaction of high air temperature with soil N and water availability was insufficient to induce photoinhibition or reduce the rate of electron transport in either measurement location.

The largest reduction in A_{max} between measurement locations was found in C_4 grass species (Fig. 2a). This reduction in photosynthetic rate in the C_4 grasses occurred despite similar rates of stomatal conductance in the mesocosms and field (Fig. 2b). This suggests that the demand of CO_2 for grass species was lower in the field. The measurements of CO_2 supply functions support this observation (Fig. 4, Table 4). For each of the grass and forb species measured, reductions in the photosynthetic rate (Table 4) did not correspond with reductions in C_i or g_s.

Reductions in the rate of A have been attributed to down-regulation and while the exact mechanisms of this process remain unknown, drought, high-light, and high temperature are likely contributing factors (Lambers et al., 1998). Down-regulation following drought among C_4 grass species in the tallgrass prairie has been reported previously (Heckathorn et al., 1997). These authors reported a linear decrease in photosynthetic rate in response to decreases in leaf N content associated with the major photosynthetic enzymes (Rubisco, PEP-C, PPDK) (Heckathorn et al., 1997). Similar to their results, we found significant reductions in A for a given C_i when species between locations were compared (Table 4). This may occur from protective down-regulation of the photosynthetic enzymes among non-leguminous species without concurrent changes in the light harvesting mechanisms within the chloroplast (Table 3) optimizing the use of the two-limiting substrates (CO_2, RuBP) at the ambient C_a (von Caemmerer and Farquhar, 1984). Therefore, for species growing within the upland tallgrass prairies, the combination of frequent drought-stress and fire-induced reductions in available soil N may result in greater decreases in photosynthesis from reduced leaf N, rather than the direct effects of fire or drought alone. The data reported here suggest these resource deficiencies affect C_4 grasses and non-leguminous C_3 forb species similarly (Fig. 4).

Previous studies on KPBS have explored the relationship between leaf-level physiology and C_4 species dominance. Turner et al. (1995) reported similar photosynthetic rates in mid-summer between the dominant C_4 grasses and other C_3 forbs despite the lower tissue N concentration and much higher water-use efficiencies for the grasses. In an experiment relating the photosynthetic rates of 27 species to relative abundance, McAllister et al. (1998), found significantly higher photosynthetic rates for C_4 grasses compared to C_3 plants on sites where C_4 species were most abundant. In general, our results support facets of both studies. In the mesocosms, photosynthetic rates and WUE by the C_4 species were higher (Fig. 2a and c) suggesting a potential mechanism for C_4 dominance: greater competitive ability and growth when resources are available.

However, photosynthetic parameters were similar between all species when resources were more limiting (field). The lack of a difference between the C_3 and C_4 species in the field, despite a lower N use efficiency by C_3 forbs, suggests C_3 species may have greater uptake of N when available, or a greater ability to retranslocate N to storage organs following pulses in availability or the onset of water stress (Jaramillo and Detling, 1992; Heckathorn and DeLucia, 1994, 1996).

The lack of physiological differences between C_3 and C_4 species in the field site may reflect the importance of the seasonal patterns of resource availability in this ecosystem. We specifically chose mid-July for our comparisons as resource availability would be reduced and temperatures would be high, conditions that should highlight the higher use efficiencies of C_4 photosynthesis. Previous work at this site has shown the maximum photosynthetic rate of the C_4 grasses occurs early in the growing season when resource availability is also highest, but photosynthetic rates can be quite low for C_4 grasses during mid-July (Knapp, 1985, 1993). In this grassland, water and available soil N are also highest during April–June and then decline during the remainder of the growing season (July–September). Thus, these results suggest that the greatest advantage of C_4 photosynthesis may not arise from higher use efficiencies when resources are low, but by maximizing growth when resources are high (Briggs and Knapp, 2001). The greater use efficiencies of C_4 species would allow them to remain active over a longer period of the growing season, facilitating increases in activity during pulses of resource availability late in the season when C_3 species may have senesced. We conclude that although higher resource-use efficiencies of species with the C_4 photosynthetic pathway have previously been posited as keys to C_4 grass dominance, especially in the water and N-limited annually burned prairie (Knapp, 1985; Knapp and Seastedt, 1986; Seastedt et al., 1991; Ojima et al., 1994; Blair, 1997), C_4 dominance may result more from greater acquisition of resources when they are plentiful, and less on the higher use efficiencies of the C_4 photosynthetic pathway when resources are limiting. Therefore, in an ecosystem with multiple limiting resources, C_4 grass dominance may depend on the ability to maximize resource capture when available, to grow rapidly, and to persist through periods of resource limitations that may rapidly change within and among years.

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