

Article

## Water Use and Water-Use Efficiency of Three Perennial Bioenergy Grass Crops in Florida

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**Abstract:** Over two-thirds of human water withdrawals are estimated to be used for agricultural production, which is expected to increase as demand for renewable liquid fuels from agricultural crops intensifies. Despite the potential implications of bioenergy crop production on water resources, few data are available on water use of perennial bioenergy grass crops. Therefore, the objective of this study was to compare dry matter yield, water use, and water-use efficiency (WUE) of elephantgrass, energycane, and giant reed, grown under field conditions for two growing seasons in North Central Florida. Using scaled sap flow sensor data, water use ranged from about 850 to 1150 mm during the growing season, and was generally greater for giant reed and less for elephantgrass. Despite similar or greater water use by giant reed, dry biomass yields of 35 to 40 Mg ha<sup>-1</sup> were significantly greater for energycane and elephantgrass, resulting in greater WUE. Overall, water use by the bioenergy crops was greater than the rainfall received during the study, indicating that irrigation will be needed in the region to achieve optimal yields. Species differ in water use and WUE and species selection can play an important role with regard to potential consequences for water resources.

**Keywords:** biomass crops; biofuels; sustainability; water use; transpiration; gas exchange; sap flow; elephantgrass; energycane; giant reed

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## 1. Introduction

Agroecosystems have been managed for years to provide food, fuel and fiber. Demand for these services especially renewable liquid fuels has increased in recent years, as global production of ethanol now exceeds 66 billion L per year [1]. In the USA, this renewable ethanol is currently produced almost exclusively from maize (*Zea mays* L.) grain produced in the Midwestern states. This has led to an interest in producing energy from other more sustainable cropping systems like perennial grasses that do not directly compete with our food systems and are capable of higher fuel yields through lignocellulosic conversion processes [2]. While many perennial tall grass crops are very productive [3,4], bioenergy cropping systems have the potential to greatly diminish less visible ecosystem services [5], especially hydrological regulation at the expense of crop primary production [6,7].

Tall perennial grasses, such as energycane (*Saccharum* spp.), elephantgrass (*Pennisetum purpureum* Schum.), giant reed (*Arundo donax* L.), *Miscanthus* × *giganteus*, reed canarygrass (*Phalaris arundinacea* L.), and switchgrass (*Panicum virgatum* L.) have been evaluated as potential lignocellulosic bioenergy crops [3,4,8–11]. In the southeastern USA, elephantgrass and energycane, both warm-season grasses, have generally been among the most productive perennial grass crops, producing dry matter yields of 20–45 Mg ha<sup>-1</sup> [3,4]. While the C4 grasses like elephantgrass and energycane are generally efficient in terms of water and nutrient use [12,13], many have poor cold tolerance and do not perform optimally at higher latitudes [14]. Therefore, productive C3 grasses like giant reed [8] have also been evaluated for bioenergy use in the region [4]. High dry matter yields of about 30 Mg ha<sup>-1</sup> have been reported in temperate climates for giant reed [8], but dry matter yields of giant reed have generally been less compared to elephantgrass and energycane in the southeastern USA [4].

Thus, while limited data on biomass production have indicated that energycane, elephantgrass, and giant reed are among the most promising perennial grass crops for combustion and lignocellulosic conversion to biofuels in the southeastern USA, the potential impact of these cropping systems on water resources is not well understood. Agroecosystems in general have become a major consumer of water. Agriculture now consumes about 70% of the water used by humans globally, and irrigated croplands (approximately ¼ billion ha globally) in particular consume significantly more water than the ecosystems they replace [15]. Rain-fed cropping systems are more sustainable than irrigated systems, although they might consume more or less water than the plant communities they replace. Despite abundant annual rainfall, the implications of bioenergy cropping systems for the southeastern USA remain a big concern [7], especially since bioenergy cropping systems are likely to displace extensive relatively low input (*i.e.*, no irrigation) pasture lands in the region.

Thus, there is an ever increasing need to achieve greater crop production with less water use and/or more efficient water use [16]. This is important for all crops, but is especially needed for bioenergy crops to allow for production on marginal lands and to minimize competition with food crops. However, an intrinsic property of plant photosynthesis is that water is lost from the plant through stomata to the atmosphere as carbon dioxide is taken up from the atmosphere and assimilated by the plant to be used for biomass synthesis [17]. Nevertheless, there is substantial variation in water use efficiency (WUE; g biomass produced kg<sup>-1</sup> of water transpired) both within and across crops [12,13].

In particular, crops that possess the C4 photosynthetic pathway tend to have a higher water-use efficiency (WUE) compared to C3 crops. In a comprehensive review, Stanhill [12] reported a mean WUE of  $1.6 \text{ g}\cdot\text{kg}^{-1}$  for 51 C3 plants and a mean WUE of  $3.1 \text{ g}\cdot\text{kg}^{-1}$  for 14 C4 plants, however values have been reported as high as  $9 \text{ g}\cdot\text{kg}^{-1}$  for the C4 grass *Miscanthus × giganteus* [13].

Despite the potential implications of bioenergy crop production on transpiration and water resources [6], few data are available on water use of bioenergy crops grown in the field. Therefore, the objective of this study was to compare dry matter yield, water use, and WUE of elephantgrass, energycane, and giant reed, grown under field conditions in North Central Florida. We hypothesized that perennial grass species would differ in total transpiration and temporal patterns of water use, which could help lead to improved selection and use of perennial grass crops for more sustainable bioenergy production in the region.

## 2. Experimental Section

### 2.1. Site Description and Experimental Design

As part of a larger experiment to identify potential tall grass bioenergy crops well adapted to the southeastern USA, a replicated field experiment was conducted in North Central Florida at the University of Florida Plant Science Research and Education Unit ( $29^{\circ}24'38'' \text{ N}$ ,  $82^{\circ}8'30'' \text{ W}$ ), on a very deep, excessively drained fine Candler sand (hyperthermic, uncoated Lamellic Quartzipsamments). The previous crop was bahiagrass followed by winter fallow. The experimental design was a randomized complete block design with four replicates. The main treatment factor was species and included energycane (cv. “L79-1002”), elephantgrass (cv. “Merkeron”), and giant reed (wild Florida population).

### 2.2. Weather and Water Inputs

National Climatic Data Center (Asheville, NC, USA) normal (1971–2000) annual rainfall in nearby Gainesville, FL, is 1228 mm. Average daily air temperature ( $T_{\text{AIR}}$ ) is  $27.2 \text{ }^{\circ}\text{C}$  and  $12.4 \text{ }^{\circ}\text{C}$  in July and January, respectively. For the present study, weather data were collected from the Florida Automated Weather Network (FAWN) weather station located less than 0.5 km N of the field site. Solar radiation ( $Q$ ) and  $T_{\text{AIR}}$  were greater and relative humidity (RH) lower during the 2010 growing season (April to November) compared to the 2009 season (Table 1). These differences were associated with reduced rainfall during the 2010 growing season, especially during the summer months that are typically wetter in the region. Since 2009 was an establishing year, irrigation inputs were greater during 2009 compared to 2010 even though rainfall was also more abundant in 2009. In total, water inputs (rainfall plus irrigation) were 1327 and 990 mm for the 2009 and 2010 growing seasons, respectively.

**Table 1.** Average daily air temperature ( $T_{\text{AIR}}$ ) at 2 m, relative humidity (RH), solar radiation ( $Q$ ), total rainfall and total irrigation for the 2009 and 2010 growing seasons (April–November) at Citra, Florida, USA.

Year	$T_{\text{AIR}}$ (°C)	RH (%)	$Q$ ( $\text{W}\cdot\text{m}^{-2}$ )	Rainfall (mm)	Irrigation (mm)
2009	23.6	79.1	195	897	430
2010	24.4	77.0	213	585	405

### 2.3. Cultural Practices and Aboveground Biomass Yield

In November 2008, plots were established from stem cuttings. Each plot was 6 rows of 6-m length with plant spacing in the row of ~0.5 m for all species. Plots were fertilized with 280 kg N ha<sup>-1</sup> yr<sup>-1</sup> using a 16-4-8 blended granular fertilizer that included minor nutrients in split applications that supplied 90 kg N ha<sup>-1</sup> in mid-April and 190 kg N ha<sup>-1</sup> in June. Known quantities of irrigation (Table 1) were applied to plots during establishment (2009) via overhead irrigation with a linear move system, but thereafter only at sign of early visual drought stress (e.g., leaf rolling). Weeds were removed during establishment mechanically by rotary hoe and subsequently by hand as needed.

To estimate biomass yields in 2009 and 2010, plots were harvested once per year in the fall around late November, prior to anticipated frost. A 4-m section (4 m<sup>2</sup>) from the middle of one of the two inner rows was cut at a 7.5-cm stubble height using a gasoline powered trimmer (Echo, Inc., Lake Zurich, Illinois, USA) and harvested by hand. The 4-m section was immediately weighed green in the field to provide estimates of green yield. The total number of stalks from the 4-m harvested section was counted and used to determine stem population at harvest. Additionally, a four-stalk whole plant subsample was collected, weighed fresh in the field and then dried at 50 °C until a constant dry weight was achieved to determine dry matter concentration at harvest and dry biomass yield. The remaining biomass in each plot was mechanically harvested with a forage harvester.

### 2.4. Water Use and Water-Use Efficiency

Whole plant crop water use (*i.e.*, transpiration) was measured throughout the growing season on each of the three species using sap flow sensors (Dynamax, Houston, TX, USA) installed *in situ* on selected intact plant stems [18,19]. Although time and labor intensive, this heat balance method measures water use under actual field conditions without altering the microclimate (e.g., chamber methods) or the soil profile (e.g., lysimeter methods), and has been used to accurately measure crop water use for sorghum [20], maize [21], and sugarcane [22].

Approximately every 2 to 3 weeks, three to four representative stems from each species were selected from the inner 2 m<sup>2</sup> of the plot for sensor installation (maximum of 16 sensors). Prior to installation, any leaf sheath tissue was removed and stem diameter was measured in two directions (N-S and E-W) at sensor height (equidistant between two internodes) and averaged to estimate stem sap flow area. Before placing sensors on the stem, the area was sprayed with canola oil to maximize sensor contact with the stem. The foam-insulated sensor was then placed on the stem and wrapped with an aluminum-covered bubble wrap to shield the sensor and stem from solar radiation. Finally, a conical shaped plastic piece was wrapped around the stem above the sensor unit to prevent irrigation or rain water from moving downward along the stem toward the sensor.

Once installed, all sensors were left on the stem for 5 to 7 days. A 12-volt deep cycle battery, a CR1000 data logger (Campbell Scientific, Inc., Logan, Utah, USA), and Dynamax software program (Dynagage flow 32-1k ver 1.4.0.1) were used to heat the sensors and to monitor thermocouple temperatures from each of the sensors. The temperature data were recorded at 15-s intervals and averaged every 15 min and stored by the datalogger. Using the input stem area and measured temperature data, the software program directly calculated average tiller water use in  $\text{g}\cdot\text{h}^{-1}$  over the 15-min interval and this was also stored by the datalogger for each tiller. Measurements were repeated approx. every 3 weeks during the growing season until harvest in mid-November.

Measured whole-plant sap flow data were then scaled to estimate daily crop canopy transpiration ( $E_C$ ) per unit ground area for each of the plots. Thus,  $E_C$  was calculated based on the product of the mean measured sap flow ( $\text{g hr}^{-1} \text{cm}^{-2}$  stem area), average stem area ( $\text{cm}^2$ ), and stem density (no. per  $\text{m}^2$ ) per plot. Stem diameter data were collected monthly on 40 randomly (every 4th stem in the inner plot where water use was measured) selected stems at sensor height to obtain the mean stem diameter for each plot. The number of stems in the inner 2 middle rows of the plot ( $8 \text{ m}^2$ ) was also counted monthly to determine stem density. For each day where sap flow was measured, crop canopy transpiration coefficients ( $K_{\text{canopy}}$ ) were calculated as the quotient of  $E_C$  and  $\text{ET}_O$  from the nearby weather station. For days where sensor sap flow was not measured, daily  $E_C$  was estimated as the product of  $\text{ET}_O$  and  $K_{\text{canopy}}$ , where values of  $K_{\text{canopy}}$  were linearly interpolated for each day across the measured values, and from  $K_{\text{canopy}} = 0$  at crop emergence to the  $K_{\text{canopy}}$  value approximately 3 weeks after emergence each year. Total seasonal  $E_C$  was then estimated as the sum of daily  $E_C$  from emergence to harvest. Water use efficiency for each species was calculated from the quotient of harvested dry biomass and total seasonal  $E_C$ .

### 2.5. Soil Volumetric Water Content

In addition to measures of water use by sap flow, soil volumetric water content (VWC) was measured during the 2010 growing season using a time-domain reflectometry (TDR) system (TDR100, Campbell Scientific, Inc.) during the 2010 growing season [23]. Following calibration [24] for the field soil, 15-cm probes (Model CS635, Campbell Scientific, Inc.) were inserted at a 45-degree angle (approx. 10 cm vertical depth) at five depths (0–10, 20–30, 40–50, 65–75, and 90–100 cm) in-row in one of the center two rows of one plot of each of the three species. Soil VWC was measured every 30 min and stored to a CR1000 data logger. Total soil VWC (mm) was then calculated from the product of the measured soil VWC fraction and the depth of the soil between each sensor.

### 2.6. Leaf Gas Exchange and Root Biomass

In the central two rows of each plot, three fully expanded leaves were chosen at random for leaf gas exchange measurements during mid-June in each of the 2009 and 2010 growing seasons. Light-saturated net  $\text{CO}_2$  exchange ( $A_{\text{sat}}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ,  $\mu\text{mol mol}^{-1}$ ), and transpiration efficiency (TE,  $\mu\text{mol CO}_2 \text{mol}^{-1}$  water) were measured on  $6\text{-cm}^2$  leaf area using a LI-6400XT portable open-flow photosynthesis systems (LI-COR Inc., Lincoln, Nebraska, USA). Measurements were made between 1100 and 1300 h under cloud-free conditions at  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density. Reference  $\text{CO}_2$

concentration was set at 400  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  air and flow rate at 500  $\mu\text{mol s}^{-1}$ . Temperature was maintained at 28 °C and relative humidity between 55% and 65%, similar to environmental conditions in the field when measured. Data within species did not differ statistically across years, and were thus pooled and presented as means across both years.

To estimate standing root biomass in each of the species, soil cores were collected from each of the plots in March of the 2010 growing season. A total of 6 (5 cm diam.) cores were collected from four depths (0–10; 10–20; 20–50; 50–100 cm) in each plot. The 6 cores from each depth were pooled to form a single sample for each depth from each plot. The soil for each sample was then sifted through a 1 mm sieve and all the roots were collected and washed and placed in paper bags and then dried at 50 °C until a constant dry weight was achieved to determine standing root biomass.

### 2.7. Data Analyses

Statistical analyses on species effects within year were performed using analysis of variance procedures in the GLIMMIX procedure of SAS (SAS Institute, Cary, NC, USA). Species was treated as a fixed effect and block was treated as a random effect in the model. Residuals from each model fit were analyzed for homogeneity of variance visually and for normality visually and with the Shapiro-Wilk W test. Degrees of freedom were determined using the Kenward-Roger method. Where significant ( $P < 0.05$ ) fixed effects were seen, treatment mean pairwise comparisons were made using the LSMEANS statement with the TUKEY method.

## 3. Results and Discussion

### 3.1. Crop Morphology and Aboveground Biomass Yield

Elephantgrass and energycane were quick to establish and produced relatively high dry matter yields during the 2009 growing season compared to giant reed, which was slower to establish (Table 2). No difference in biomass yield was seen between elephantgrass or energycane during either the 2009 or 2010 growing seasons. However, both energycane and elephantgrass yields were greater than giant reed, but they were only about 37% greater in 2010 compared to 175% in 2009, which was the first growing season following establishment. Dry biomass yields in excess of 35  $\text{Mg ha}^{-1}$  for elephantgrass and energycane were considerably higher than those commonly reported for switchgrass or giant miscanthus [4,25]. Biomass yields in the present study were also considerably greater than those reported for the same species under low input (no irrigation) conditions in the same region [4]. Biomass yields of L79-1002 energycane (49.0  $\text{Mg ha}^{-1}$ ) and elephantgrass (46.5  $\text{Mg ha}^{-1}$ ) under more intensive management were greater than those found in the present study [3]. Dry matter yields of giant reed in Central Italy averaged 29  $\text{Mg ha}^{-1} \text{ yr}^{-1}$  over six years when fertilized, and were also relatively low (19  $\text{Mg ha}^{-1}$ ) during the first harvest following establishment [8]. During 2009, stem densities were greatest in energycane, intermediate in elephantgrass, and least in giant reed (Table 2). During 2010, however, no difference in stem density at harvest was seen among any of the species. Stem diameter was greater in elephantgrass compared to giant reed, and energycane was intermediate between the two (Table 2).

**Table 2.** Treatment means for annual aboveground crop biomass yield, stem density, and stem diameter of perennial grasses during the 2009 and 2010 growing seasons. Species include giant reed (GR), energycane (EC), and elephantgrass (EG).

Species	Dry biomass yield (Mg ha <sup>-1</sup> )		Stem density (per m <sup>2</sup> )		Stem diameter (mm)	
	2009	2010	2009	2010	2009	2010
GR	13.4B †	29.1B	14.5C	24.9A	13.5B	14.8B
EC	38.4A	40.9A	25.3A	26.7A	15.0AB	15.0AB
EG	35.5A	38.9A	19.3B	20.6A	17.1A	16.3A
s.e ‡	2.61	2.75	1.55	2.23	0.73	0.58

† Numbers followed by the same letter within a column do not differ ( $P > 0.05$ ); ‡ Standard error of differences of species means.

### 3.2. Water Use and Water-Use Efficiency

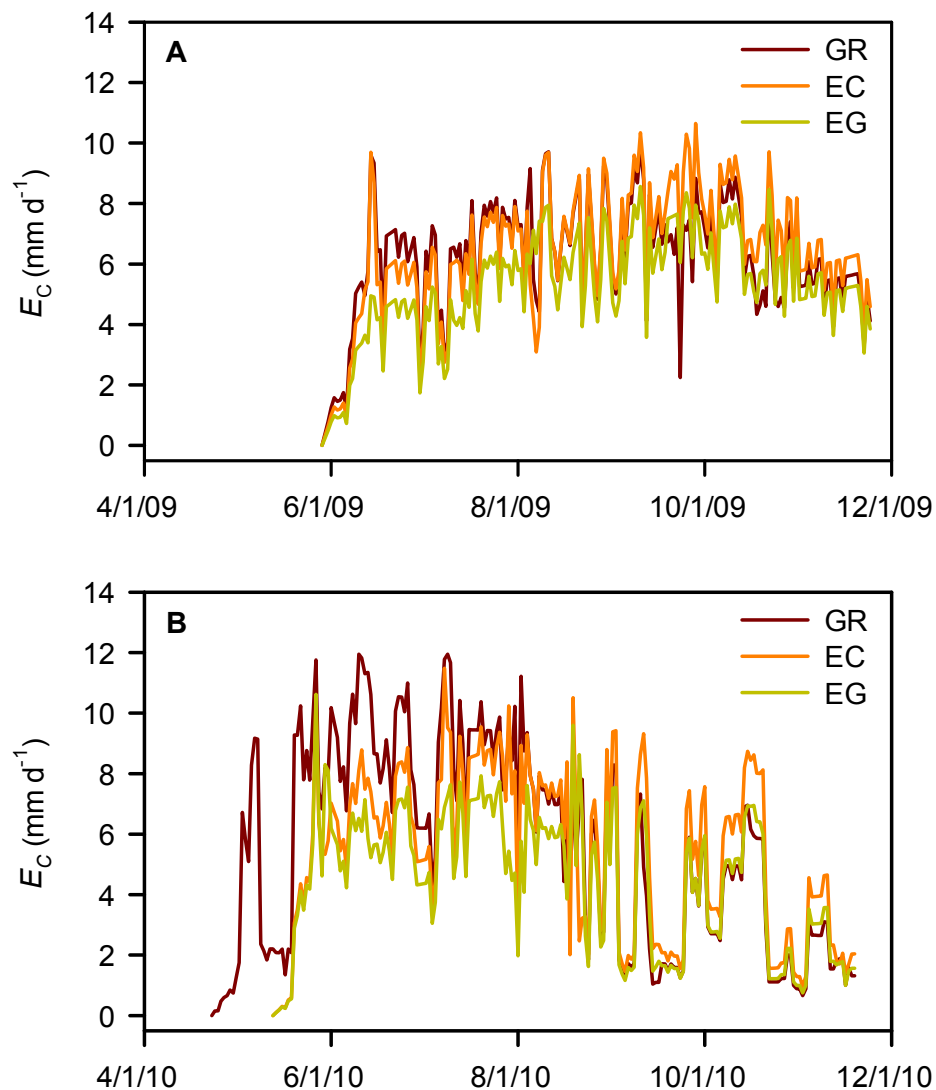
Total seasonal water use,  $E_C$ , during the first year following planting (2009) was greatest for energycane, lowest for elephantgrass, and intermediate for giant reed, which did not differ from either of the other two grasses (Table 3). During 2010,  $E_C$  was greatest for giant reed, intermediate for energycane, and lowest for elephantgrass (Table 3). Seasonal patterns in daily  $E_C$  (Figure 1) indicated earlier emergence during 2010, the second full growing season, especially for giant reed. Thus,  $E_C$  of giant reed tended to be relatively greater early in the growing season, while energycane tended to be relatively greater late in the growing season. Overall, daily  $E_C$  by the grasses was greatest early in the growing season during peak vegetative growth coinciding with high radiation and high evaporative demand. Daily water use rates of maize grown near Ames, IA, were also greatest early in the growing season during growth stages R1 and R2 [26]. During this period, daily  $E_C$  of 5 to 7 mm d<sup>-1</sup> was reported for maize, which was comparable to the daily  $E_C$  for the C4 grasses in the present study. However, the C4 perennial grasses in the current study remained vegetative for much longer periods, resulting in relatively high total seasonal  $E_C$ . High transpiration rates in the present study were comparable to those reported for maize [27], which averaged about 120 mm·mo<sup>-1</sup> and pearl millet (*Pennisetum glaucum* L.) [28], which ranged from 77 to 100 mm·mo<sup>-1</sup> depending on row spacing. Thus, whereas a corn crop can be produced on about 500 mm of water, the C4 perennial grasses in the present study used between 850 to 1100 mm of water. Duration of growing season and management practices are therefore important for  $E_C$  and ET. Seasonal  $E_C$  by the bioenergy grasses in the present study was greater than 787 mm of ET reported for a low input pasture system in the region [29], but less than the 1200 to 1500 mm of year-round annual ET reported for intensively managed bahiagrass (*Paspalum notatum* Flugge) and St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] grown in South Florida [30].

**Table 3.** Treatment means for seasonal crop transpiration ( $E_C$ ) and water use efficiency (WUE; g aboveground dry matter  $\text{kg}^{-1}$  water transpired) of perennial grasses during the 2009 and 2010 growing seasons. Species include giant reed (GR), energycane (EC), and elephantgrass (EG).

Species	$E_C$ (mm)		WUE ( $\text{g kg}^{-1}$ )	
	2009	2010	2009	2010
GR	1113AB†	1177A	1.19B	2.47B
EC	1151A	1035B	3.35A	3.96A
EG	930B	856C	3.84A	4.57A
s.e ‡	77.1	33.3	0.24	0.32

† Numbers followed by the same letter within a column do not differ ( $P > 0.05$ ); ‡ Standard error of differences of species means.

**Figure 1.** Daily crop transpiration ( $E_C$ ) during the 2009 (A) and 2010 (B) growing seasons. Species include giant reed (GR), energycane (EC), and elephantgrass (EG).

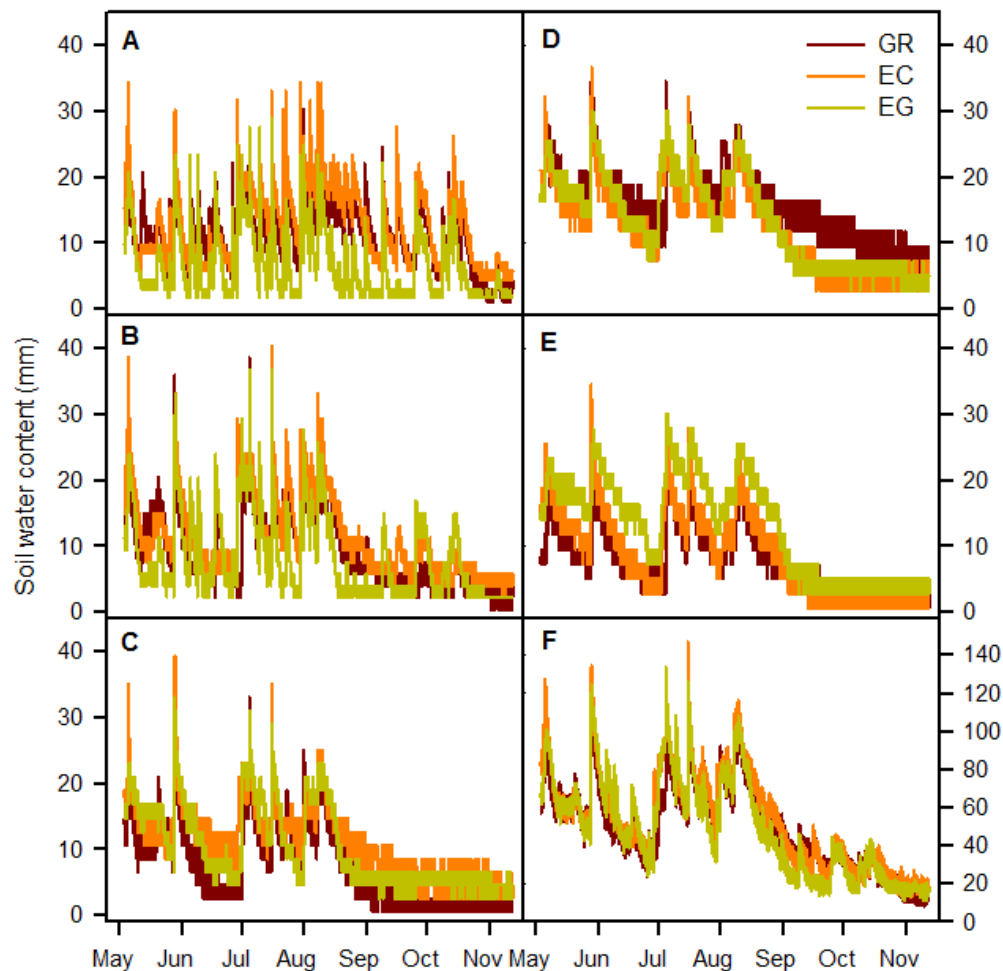




Water-use efficiency was greater for elephantgrass and energycane, which did not differ, compared to giant reed in both the 2009 and 2010 growing seasons (Table 3). In 2010, WUE was generally greater for all species compared to the 2009 growing season. Given comparable or lower seasonal EC and greater biomass yields, WUE was greater for the C4 grasses compared to giant reed the C3 grass. It has long been known that C4 grasses use water more efficiently to produce dry matter [12], which was confirmed for the species in this study under field conditions. Values of WUE ranging from 3.3 to 4.6 g·kg<sup>-1</sup> for the perennial C4 grasses in the present study were comparable to median values of 4.2 reported for grain sorghum (*Sorghum bicolor* L.), 3.9 for pearl millet, and 4.8 g kg<sup>-1</sup> for maize [31].

Soil VWC data were consistent with  $E_C$  data during the peak of the growing season through August, showing generally greater VWC in elephantgrass and lower VWC in giant reed (Figure 2F). Species also differed in stratum where water was preferentially accessed in the soil with elephantgrass preferentially extracting water from the upper soil profile, whereas giant reed and energycane showed greater extraction at deeper depths compared to elephantgrass (Figure 2).

**Figure 2.** Volumetric soil water content (mm) in the soil profile from (A) 0–10 cm; (B) 10–20 cm; (C) 20–30 cm; (D) 30–50 cm; (E) 50–107 cm; (F) 0–107 cm for giant reed energycane and elephantgrass bioenergy crops during the 2010 growing season (May–November).



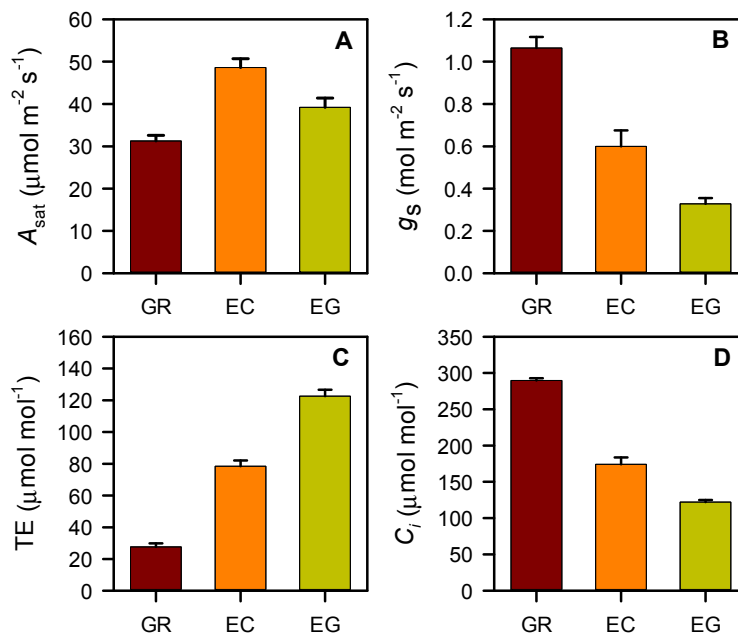
Soil VWC data indicated that the crops used a majority of water inputs during 2010, as soil water drainage below 1 m occurred only on a limited number (3 to 4) of occasions throughout the entire growing season. Across all species daily ET estimated from the soil water budget down to 1 m was approximately 8.5 mm in early July consistent with  $E_C$  during that time. However,  $E_C$  does not include water loss by soil evaporation, but  $E_C$  generally contributes over 90% of ET following canopy closure [32].

Measured values of  $K_{\text{canopy}}$  were commonly in excess of 1.0 and generally closer to 2.0 during mid-growing season (data not shown). High  $K_{\text{canopy}}$  values close to 2.0 have been reported for other crops, including sugarcane [33], but generally do not exceed 1.5. These relatively high values could be due to overestimation scaling sap flow data [33] and/or differences in canopy aerodynamic resistance, which could have been exacerbated by relatively small plot sizes. However, it has been suggested that smaller buffer and fetch areas are typically needed for humid and sub-humid conditions [34].

### 3.3. Leaf Gas Exchange and Root Biomass

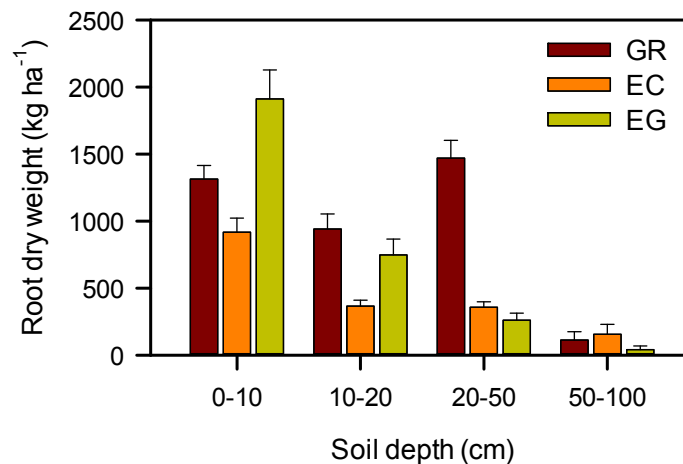
Light-saturated leaf net carbon assimilation,  $A_{\text{sat}}$ , was greatest for energycane, about  $48 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and lowest for giant reed, approximately  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 3A). Leaf stomatal conductance,  $g_s$ , was greatest for giant reed, intermediate for energycane, and lowest for elephantgrass (Figure 3B). This resulted in elephantgrass possessing the greatest leaf TE, followed by energycane, and then giant reed (Figure 3C). Finally, leaf  $C_i$  was lowest in elephantgrass, intermediate in energycane, and highest in giant reed (Figure 3D).

**Figure 3.** (A) Light-saturated net carbon exchange ( $A_{\text{sat}}$ ); (B) stomatal conductance ( $g_s$ ); (C) transpiration efficiency (TE); and (D) intercellular  $\text{CO}_2$  concentration ( $C_i$ ) of fully extended upper canopy leaves averaged across the 2009 and 2010 growing seasons. Species include giant reed (GR), energycane (EC), and elephantgrass (EG). Error bars represent 1 S.E.



Total standing root biomass to 1 m depth was greatest for giant reed ( $3835 \text{ kg}\cdot\text{ha}^{-1}$ ), intermediate for elephantgrass ( $2960 \text{ kg}\cdot\text{ha}^{-1}$ ) and least for energycane ( $1800 \text{ kg}\cdot\text{ha}^{-1}$ ). Root biomass was greater for elephantgrass in the upper 10 cm of the soil compared to energycane and giant reed, but was generally lower for elephantgrass compared to the other two species below 20 cm (Figure 4). This was consistent with the soil VWC data (Figure 2). Root biomass distribution was relatively uniform to a depth of 50 cm in giant reed, whereas root biomass declined more quickly with depth in energycane and elephantgrass.

**Figure 4.** Mean standing root dry weight by soil depth. Species include giant reed (GR), energycane (EC), and elephantgrass (EG). Error bars represent 1 S.E.



In general, the leaf gas exchange and root biomass data supported the observations seen for  $E_C$  and soil VWC. Giant reed transpired more water than elephantgrass and energycane during both growing seasons. Additionally, elephantgrass  $E_C$  was generally lower than that of energycane. Leaf level gas exchange data, also indicated that  $g_s$  was greatest in giant reed, intermediate in energycane, and lowest in elephantgrass. Root biomass was also greatest in giant reed compared to elephantgrass and energycane. However, the difference in  $E_C$  between giant reed and the C4 grasses was not that great overall, and in fact did not differ from energycane during the plant crop growing season. While differences in seasonal  $E_C$  were modest between the C3 and C4 grasses, differences in biomass yield between giant reed and the two C4 grasses were large, as dry biomass yield was substantially greater for the C4 grasses during both growing seasons, especially during 2009 (Table 2). The key implications of these findings are that approximately twice the land area or twice the water would be needed for giant reed to produce a similar amount of biomass as the C4 grasses. Therefore, in low input systems with little or no irrigation, our results indicated that elephantgrass or perhaps energycane would be better suited for bioenergy crop production compared to giant reed and presumably other C3 crop species. While in regions such as frequently flooded marginal lands where available water is not a concern, giant reed might achieve comparable yields and make a suitable bioenergy crop. Although C4 grasses tend to use water efficiently to produce biomass, this efficiency should not necessarily be interpreted as low total seasonal  $E_C$  or the ability to produce high yields with low water inputs [4].

#### 4. Conclusions

Results from the present study not only support the growing concern over water resources with regard to production of biofuel cropping systems, but also help to alleviate this concern. Seasonal  $E_C$  was high, especially for the C3 giant reed, associated with the long growing season for the grasses. However, daily water use by the bioenergy crops was similar to other crops, as was seasonal water use for crops with similar growing seasons. Thus, the implications for water resources will depend in large part on the prior land use of converted bioenergy systems. Still, water use by the bioenergy crops was well above rainfall received during the study, even though precipitation was historically low during both growing seasons, indicating that irrigation will be needed in the region to achieve optimal yields or that reduced yields should be expected without irrigation. Finally, the study demonstrated that differences in species exist and that species selection can play an important role with regard to potential consequences for water resources. The C4 grasses in particular produced greater biomass per unit of water transpired and should be considered for use as bioenergy crops where water limitations to crop production or concerns for ground water recharge exist. Even within the C4 grasses smaller differences existed, indicating that elephantgrass may minimize impacts on water use, while producing similar yields. Further research is needed on more species and genotypes and on management practices to optimize bioenergy crop yields while minimizing the negative impacts on water resources.

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