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# Water Use Efficiency by Switchgrass Compared to a Native Grass or a Native Grass Alfalfa Mixture

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**Abstract** Perennial grass systems are being evaluated as a bioenergy feedstock in the northern Great Plains. Inter-annual and inter-seasonal precipitation variation in this region will require efficient water use to maintain sufficient yield production to support a mature bioenergy industry. Objectives were to evaluate the impact of a May–June (early season) and a July–August (late season) drought on the water use efficiency (WUE), amount of water used, and biomass production in monocultures of switchgrass (*Panicum virgatum* L.), western wheatgrass (*Pascopyrum smithii* (Rydb.) Á. Löve), and a western wheatgrass–alfalfa (*Medicago sativa* L.) mixture using an automated rainout shelter. WUE was strongly driven by biomass accumulation and ranged from 5.6 to 7.4 g biomass mm<sup>-1</sup> water for switchgrass to 1.06 to 2.07 g biomass mm<sup>-1</sup> water used with western wheatgrass. Timing of water stress affected WUE more in western wheatgrass and the western wheatgrass–alfalfa mixture than switchgrass. Water deficit for the western wheatgrass–alfalfa mixture was 23 % lower than western wheatgrass ( $P=0.0045$ ) and 31 % lower than switchgrass ( $P<0.0001$ ) under the May–June stress water treatment, while switchgrass had a 37 and 38 % greater water deficit than did western wheatgrass or western wheatgrass–alfalfa mixture, respectively ( $P<0.001$ ) under the July–August water stress treatment. Water depletion was always greatest in the upper 30 cm. Switchgrass had greater WUE but resulted in greater soil water depletion at the end of the growing season

compared to western wheatgrass and a western wheatgrass–alfalfa mixture which may be a concern under multi-year drought conditions.

**Keywords** Drought · Western wheatgrass · Soil water deficit · Rainout shelter

## Introduction

Globally, agriculture is the largest user of freshwater resources [1, 2], and there are concerns about how biofuel production may impact water consumption and quality [3]. As human population grows resulting in increased demand for fresh water, there will be increased emphasis on water use efficiency (WUE) in biomass production [4]. In the semiarid northern Great Plains, annual productivity of agricultural systems is largely driven by precipitation. Sustainability in the region depends on efficient use of water [5] which is often expressed as WUE or the measure of crop production per unit of water input. Water inputs, in dryland cropping systems, are either stored soil water or precipitation and so WUE can be defined as crop production divided by soil water used plus precipitation [5]. Although WUE primarily has been evaluated in traditional agronomic crops, it can also impact the potential for developing cellulosic biofuels. Therefore, defining WUE of potential cellulosic bioenergy crops is important in developing a sustainable biofuel industry.

Seasonality of precipitation can have important impacts on production. Precipitation in the Great Plains has always been characterized by large inter- and intra-annual variability with high levels of uncertainty in amount and timing of received precipitation [6]. Furthermore, future climate scenarios indicate even more variability in rainfall in the region [7, 8]. Grasslands have the capacity for large increases in biomass in response to high precipitation levels [9] in humid regions, but biomass changes may be more limited at the community level in a semi-arid environment [10]. Seasonality of precipitation can

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also influence relative abundance of functional groups such as C4 grass species [11] which means predicting ecosystem response to climate change needs to include rainfall timing as well as amount [7].

There are several perennial grasses in monocultures or in mixtures that are currently being evaluated as potential bioenergy feedstocks in the northern Great Plains. There has been a serious effort to develop switchgrass as a cellulosic bioenergy crop since the early 1990s [12]. Managed switchgrass monocultures were estimated to produce 540 % more energy than non-renewable energy consumed [13]. Western wheatgrass is a dominant native grass in the northern mixed grass prairie [14] and is being evaluated as a bioenergy feedstock based on its adaptability and stable biomass yields in this region. Alfalfa is often used in grass mixtures to improve biomass yield, quality, and seasonal distribution [15]. Alfalfa also can replace external nitrogen fertilization when grown in binary mixtures with grass [16] and can increase overall productivity with no evidence of a decline in grass yield when interseeded into a rangeland [17].

Warm-season or C<sub>4</sub> grasses such as switchgrass generally have higher WUE than do cool-season C<sub>3</sub> grasses such as western wheatgrass [18, 19]. However, other factors besides photosynthetic pathway affect WUE. Switchgrass biomass production is strongly driven by precipitation, but as precipitation increases, WUE decreases [20]. Precipitation variability can impact switchgrass WUE and yields [21]. In cool-season grasses, WUE was strongly correlated with biomass production [22]. Western wheatgrass was reported to have greater drought tolerance than crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] [23], although western wheatgrass had a lower WUE [24]. This may be related to western wheatgrass's slower but more stable growth pattern [24]. Dryland alfalfa was reported to have greater water use efficiency than crested wheatgrass [25] which indicates alfalfa's WUE should be greater than that of western wheatgrass. Alfalfa WUE also increased with increases in available soil water [26]. Alfalfa can extract soil water to 2.7 m [27] which makes it very drought tolerant. There is limited information available regarding WUE of grass and legume mixtures. Høgh-Jensen and Schjoerring [28] reported that perennial ryegrass (*Lolium perenne* L.) in a white clover (*Trifolium repens* L.)–ryegrass mixture had lower WUE than ryegrass in pure stands, but white clover WUE was greater in a mixture than in a pure stand. These different responses were attributed to N limitation in the ryegrass.

Understanding WUE and how it may be influenced by precipitation timing is critical in developing sustainable cellulosic biofuel systems. We compared the WUE of switchgrass, a native warm-season grass that is a commonly recognized cellulosic biofuel, to western wheatgrass, a native cool-season grass that is indicative of the plant community of the northern Great Plains, and a binary mixture of

western wheatgrass and alfalfa, which is a common producer strategy to improve yields with limited inputs. Because of the regional inter-annual variability and the differing impacts precipitation timing can have on different photosynthetic pathways, we also evaluated the influence of an early (May–June) and a late (July–August) growing season drought on WUE. We hypothesized the early season drought would have a greater impact on western wheatgrass and the binary mixture and the late season drought would have a greater impact on switchgrass.

## Materials and Methods

### Study Site

This study was conducted in 2006 and 2007 at the Northern Great Plains Research Laboratory located near Mandan, North Dakota, USA (100° 54' 56" W, 46° 48' 29" N). The climate is characterized as semiarid continental [29] with cold winters and hot summers. Average annual precipitation and air temperature (1913 to 2012) is 416 mm and 5.3 °C, respectively. A majority of the precipitation (259 mm or 62 %) falls between May and August which are the primary growing months. An evaluation of 94 years of precipitation data from the study site, from the first complete year of data (1914) till the year after the study ended (2008), indicated that approximately 30 % of the years were drier than the long-term average in either May–June or July–August, but these dry periods were either preceded by or followed by normal or above normal precipitation.

An 11.5×30.3-m rainout shelter [30] established on a Parshall fine sandy loam (coarse-loamy, mixed Pachic Haploborolls) was used in this study. Electric motors propel the shelter over the plots when sensors detect rainfall. An overhead sprinkler irrigation system was installed under the shelter to apply water treatments. Soil water measurements were taken using a neutron scattering probe (503DR Hydroprobe, CPN Corp) to a depth of 1.8 m at 0.3-m increments before water treatments were applied and after a killing frost in autumn of each year.

### Treatment Descriptions

This experiment was designed as a completely randomized design. Area under the rainout shelter was divided into six, 7.8×3.9-m plots (main plots). Each plot was randomly assigned to one of three different water treatments (WATER), and each treatment was replicated twice. Since a majority of precipitation in the region is received between May and August, water treatments focused on manipulating precipitation during that period. Water treatments were a CONTROL, with amounts of simulated precipitation water applied to mimic monthly precipitation totals from May through August and two drought treatments, May–June and July–August, where

only 50 % of the May–August precipitation was applied to the plots. The difference between the drought treatments was when the limited irrigation water was applied. In the May–June treatment, 20 % of the limited irrigation water was applied in May and June with the remainder (80 %) applied in July and August. In the July–August treatment, the reverse was true. Twenty percent of the irrigation water was applied in July and August with 80 % being applied in May and June.

Water treatments were applied in 2006, while in 2007 water treatments were more similar to long-term precipitation patterns from May through August. An equipment failure during an electrical storm in June 2007 prevented the rainout shelter from closing. The result was that the drought treatments received approximately 90 % of their water for the season from rainfall during the storm. An analysis of long-term (1913–2000) US Weather Service data from the Northern Great Plains Research Laboratory indicated that only 6 % of the total years of record had two dry years in a row, so a decision was made not to apply water treatments in 2007 but to reestablish the long-term average moisture regime.

Each plot was then divided into three 2.2 m×3.9-m sub-plots seeded to different species or species combinations (SPECIES). SPECIES treatments were: “Sunburst” switchgrass, “Rodan” western wheatgrass, or a western wheatgrass—“Vernal” alfalfa mixture. Grasses were seeded at a rate of 30 seeds per 30.5 cm of row into 3.9 m long rows 0.3 m apart on May 30, 2003. Alfalfa was seeded into mixture plots in 2.2-m rows perpendicular to the grass rows 1 week after sowing the grasses at a rate of 12 per seeds per 30.5 cm of row. Alfalfa rows were also on 0.3 m row spacing. A control plot where plant material was harvested following an autumn killing frost was used to determine end of season biomass. Biomass was estimated by harvesting two 0.05-m<sup>2</sup> rectangular plots to ground level in the autumn. Biomass samples were oven-dried at 55 °C until a constant weight was reached. Biomass yields are reported on a dry matter basis.

#### Water Use

Access tubes were installed in the center of each 2.2×3.9-m sub-plot, for soil moisture measurements via a neutron probe. Soil water measurements were taken to a depth of 1.8 m at 0.3-m increments before simulated precipitation was started, after the killing frost in the autumn and at roughly 2-week intervals during the growing season (May–August).

Water use efficiency (WUE) was measured by modifying the formula for estimating system water use efficiency by Peterson et al. [5]. The modified formula was:

$$\text{WUE} = \text{biomass}/(\text{I} - \text{water} - \text{F} - \text{water} + \text{IRR})$$

where biomass=end-of-season biomass (dry matter yield), I–water=soil water prior to initial irrigation (soil water at planting), F–water=soil water after killing frost (soil water

at harvest), IRR=May-to-August irrigation (growing season precipitation), and names in parentheses correspond to the terminology in the original formula [5]. The formula was developed for annual crops, and therefore, soil water at planting would be more closely related to soil water at greenup for perennial species.

We calculated water deficits as the difference in soil water between the date of the greatest water accumulation in the soil profile to the date of the least water in the soil profile similar to Merrill et al. [31]. In 2006, water deficits were calculated using the entire 1.8-m soil profile to determine dates with the most and least available water for each species and water treatment. Water deficits were then calculated for each 0.3-m soil increments by subtracting minimum available water from maximum available water for each species and water treatment. Water deficit data were analyzed for each species and depth within a water treatment.

#### Statistical Analysis

The study was analyzed as a split plot with water treatment being the main plot factor and species being the sub-plot factor. Data were analyzed using PROC MIXED in SAS [32] with water, species, and year considered as fixed effects, and replication was considered as a random effect. Repeated measures analysis with an unstructured covariance structure was used to evaluate differences between water deficits by soil depth. Biomass yields were log transformed for normality. Means were separated using a Tukey mean separations using  $P \leq 0.10$  to determine significant differences. Actual  $P$  values for significance are given in the text where appropriate.

## Results

#### Irrigation Application

The water treatments were designed to test the impact of drought stress at different critical periods during the growing season. In 2006, irrigation water applied during the May–June treatment was only 23 % of the control for May and June (Table 1). A similar pattern emerged with the July–August treatment where applied irrigation was 20 % of the control for July and August (Table 1). Irrigation water applied to the July–August treatment plots in May and June was 88 % of the control, and simulated precipitation applied to the May–June treatment plots in July and August was 77 % of the control (Table 1). Total simulated precipitation plus naturally occurring moisture applied to the May–June plots in 2006 was 68 % of the control and 71 % of the control for the July–August treatment plots (Table 1). In

**Table 1** Precipitation totals by month in 2006 and 2007 for each water treatment and the long-term (1913–2010) average

|                     | January | February | March | April | May   | June  | July  | August | September | October | November | December | Total  |
|---------------------|---------|----------|-------|-------|-------|-------|-------|--------|-----------|---------|----------|----------|--------|
|                     | mm      |          |       |       |       |       |       |        |           |         |          |          |        |
| <b>2006</b>         |         |          |       |       |       |       |       |        |           |         |          |          |        |
| Control             | 1.78    | 3.56     | 9.14  | 24.13 | 57.91 | 57.91 | 49.53 | 66.04  | 38.10     | 40.13   | 1.27     | 17.27    | 366.78 |
| May–June drought    | 1.78    | 3.56     | 9.14  | 24.13 | 13.21 | 13.21 | 38.1  | 50.80  | 38.10     | 40.13   | 1.27     | 17.27    | 250.70 |
| July–August drought | 1.78    | 3.56     | 9.14  | 24.13 | 50.80 | 50.80 | 9.91  | 13.21  | 38.10     | 40.13   | 1.27     | 17.27    | 260.10 |
| <b>2007</b>         |         |          |       |       |       |       |       |        |           |         |          |          |        |
| Control             | 3.30    | 15.75    | 29.92 | 17.02 | 57.91 | 90.68 | 58.42 | 67.31  | 40.89     | 12.70   | 1.78     | 4.06     | 396.75 |
| May–June drought    | 3.30    | 15.75    | 29.92 | 17.02 | 13.21 | 79.50 | 76.20 | 78.74  | 40.89     | 12.70   | 1.78     | 4.06     | 396.75 |
| July–August drought | 3.30    | 15.75    | 29.92 | 17.02 | 50.80 | 88.90 | 63.50 | 67.31  | 40.89     | 12.70   | 1.78     | 4.06     | 396.75 |
| Long term           | 9.40    | 9.91     | 16.76 | 35.56 | 57.67 | 87.12 | 66.29 | 46.74  | 36.32     | 26.16   | 13.97    | 9.40     | 416.31 |

Monthly precipitation data were the same across treatments in January, February, March, April, September, October, November, and December. Totals for May through August were from applied using irrigation

2007, irrigation water applied was the same for all water treatments. In order to make sure the all water treatments got the same amount during the season, we adjusted July and August irrigation as needed (Table 1).

**Water Use Efficiency**

The WUE and the amount of water used both had a year by water interaction (Table 2). Because of the year by water interaction and the strong *F* values for year, we analyzed WUE and the amount of water used by year. Since WUE is comprised of end of season biomass and the amount of water used, we also analyzed end of season biomass by year.

In 2006, WUE had a water by species interaction. There were no differences in WUE between species for the control treatment (Fig. 1). However, for both of the May–June and July–August treatments, western wheatgrass had a lower WUE than switchgrass. The WUE for the western wheatgrass–alfalfa mixture was similar to switchgrass in the May–

June treatment plots and was similar to western wheatgrass in the July–August treatment (Fig. 1). There were also differences in WUE between water stress treatments within a species (Table 2). The WUE for both the May–June and July–August treatments were significantly greater than the control in western wheatgrass. The WUE for the May–June treatment was greater than the WUE for the control in the western wheatgrass–alfalfa mixture (Table 3). In 2007, there was not a significant interaction, but WUE for western wheatgrass was lower than the WUE for either switchgrass or the western wheatgrass–alfalfa mixture (Fig. 2).

**Water Use**

The amount of water used differed among water treatments and among species in both 2006 and 2007, but there was not a

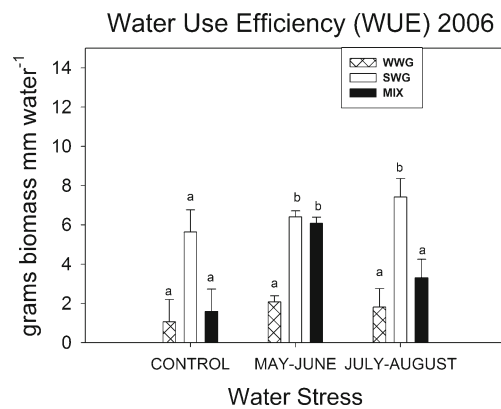
**Table 2** *F* values for water use efficiency (WUE), amount of water used, and biomass yield

| Effect                 | WUE    | Amount of water used | Biomass yield <sup>a</sup> |
|------------------------|--------|----------------------|----------------------------|
| Year                   | 12.9** | 44,511.1***          | 3.3 ****                   |
| Species                | 40.6** | 1.0                  | 45.5*                      |
| Year × species         | 4.7*   | 21.2***              | 2.3                        |
| Water                  | 2.3    | 1,537.4***           | 0.2                        |
| Year × water           | 4.1*   | 2,873.7***           | 0.4                        |
| Species × water        | 1.7    | 1.1                  | 1.3                        |
| Year × species × water | 1.2    | 2.5                  | 0.5                        |

Effects considered in the model include year, vegetation species, and water treatment

\**P*<0.05; \*\**P*<0.01; \*\*\**P*<0.001; \*\*\*\**P*<0.10

<sup>a</sup> Data were log-transformed



**Fig. 1** Water use efficiency (WUE) of western wheatgrass (*WWG*), switchgrass (*SWG*), and a western wheatgrass–alfalfa mixture (*MIX*) under different periods of water stress in 2006. *Control* indicated normal precipitation from May through August, *May–June* indicated 50 % of normal precipitation in May–June but normal precipitation in July and August, and *July–August* indicated normal precipitation for May–June but 50 % of normal for July and August. Letters above bars indicate significant differences between species within a water stress treatment at *P* ≤ 0.10



**Table 3** Water use efficiency (WUE, grams biomass per millimeter of water) of three water treatments within species for 2006

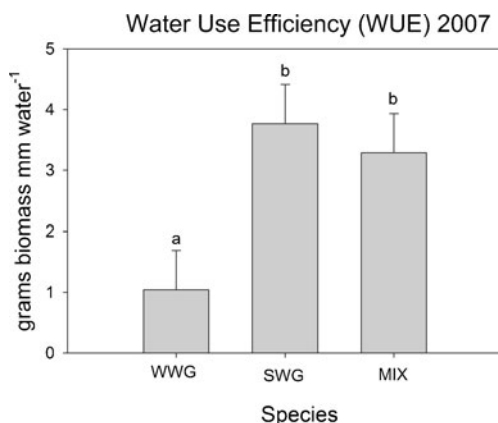
| Water stress treatment | Species            |             |                                    |
|------------------------|--------------------|-------------|------------------------------------|
|                        | Western wheatgrass | Switchgrass | Western wheatgrass–alfalfa mixture |
| WUE                    |                    |             |                                    |
| Control                | 1.07 a             | 5.64 a      | 1.60 a                             |
| May–June               | 2.08 b             | 6.41 a      | 6.08 b                             |
| July–August            | 1.82 b             | 7.42 a      | 3.31 ab                            |
| Standard errors        | 0.21               | 1.34        | 1.0                                |

Letters following numbers within columns indicate significant differences between water stress treatments within a species ( $P < 0.10$ )

water by species interaction. For a majority of species and water treatment combinations, soil water was greatest on April 21 and least on September 21. There were occasional deviations with May 18 being another date with most available water and August 24 and September 6 as dates with least available water. In 2006, the amount of water used in the control treatment was greater than May–June and July–August treatments, and the May–June water stress treatment used more water than the July–August water treatment (Table 4). In 2007, The May–June water treatment used significantly less water than did either the control or the July–August treatment (Table 4). In 2006, switchgrass used significantly more water than either western wheatgrass or the western wheatgrass–alfalfa mixture (Table 4). In 2007, the western wheatgrass–alfalfa mixture used significantly more water than switchgrass (Table 4).

End of Season Biomass

There was a water by species interaction for end of season biomass in 2006. There were no differences among species for



**Fig. 2** Water use efficiency (WUE) for western wheatgrass (WWG), switchgrass (SWG), and a binary mixture of western wheatgrass–alfalfa (MIX) in 2007. Letters above bars indicate significant differences at  $P \leq 0.10$

**Table 4** Amount of water used (millimeters) under three water regimes by different species in 2006 and 2007

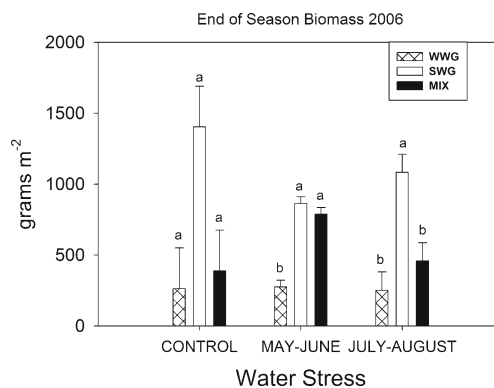
| Water regime            | Species |                                    |          |
|-------------------------|---------|------------------------------------|----------|
| 2006 (mm of water used) |         |                                    |          |
| Control                 | 247.1 a | Western wheatgrass                 | 173.5 b  |
| May–June drought        | 141.5 b | Switchgrass                        | 176.6 a  |
| July–August drought     | 132.5 c | Western wheatgrass–alfalfa mixture | 171.1 b  |
| Standard errors         | 1.2     |                                    | 1.0      |
| 2007 (mm of water used) |         |                                    |          |
| Control                 | 308.2 a | Western wheatgrass                 | 295.7 ab |
| May–June drought        | 273.8 b | Switchgrass                        | 293.5 b  |
| July–August drought     | 304.2 a | Western wheatgrass–alfalfa mixture | 297.0 a  |
| Standard errors         | 1.9     |                                    | 1.1      |

Different letters by column denote differences ( $P < 0.10$ ) between water regimes or species within year

end of season biomass for the control water treatment in 2006 (Fig. 3). There were differences among species in end of season biomass for the May–June treatment ( $P = .0735$ ) and for the July–August treatment ( $P = .0153$ ). End of season biomass for western wheatgrass was lower than for switchgrass or the western wheatgrass alfalfa mixture in the May–June treatment, and end of season biomass for both western wheatgrass and the western wheatgrass–alfalfa mixture was lower than for switchgrass under the July–August water treatment (Fig. 3).

Soil Water Deficit

Soil water deficit was evaluated for each water treatment. There were no significant differences among species for control plots,



**Fig. 3** End of season biomass produced by western wheatgrass (WWG), switchgrass (SWG), and a western wheatgrass–alfalfa mixture (MIX) under different periods of water stress in 2006. Control indicated normal precipitation from May through August, May–June indicated 50 % of normal precipitation in May–June but normal precipitation in July and August, and July–August indicated normal precipitation for May–June but 50 % of normal for July and August. Letters above bars indicate significant differences between species within a water stress treatment at  $P \leq 0.10$

but the water deficit for the western wheatgrass–alfalfa mixture was lower than western wheatgrass ( $P=0.0045$ ) or switchgrass ( $P<0.0001$ ; Fig. 4a) under the May–June water treatment. Switchgrass had a greater water deficit than did either western wheatgrass or the western wheatgrass–alfalfa mixture ( $P<0.0001$ ) under the July–August water treatment (Fig. 4a).

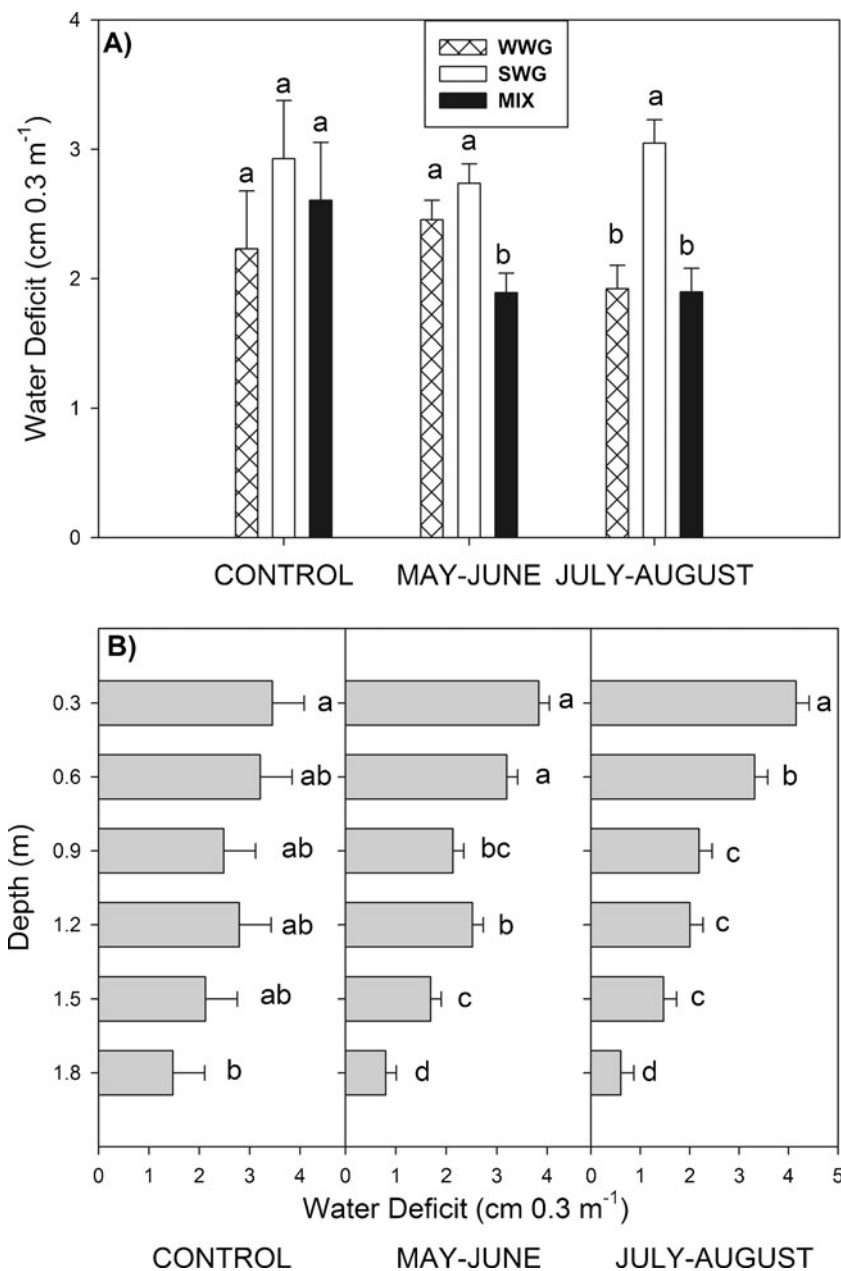
There were differences in water deficit between soil depths for each of the water treatments averaged across species. Under the control water treatment, the 0.3-m depth had a greater water deficit than did the 1.8-m depth ( $P=0.0590$ ; Fig. 4b). Under the May–June water treatment, the 0.3- and 0.6-m depths had significantly greater water deficits than the rest of the depths. The 1.2-m depth had a significantly greater water deficit than the 1.5- or 1.8-m depths, and the 0.9- and

1.5-m depths had significantly greater water deficits than did the 1.8-m depths under the May–June water treatment (Fig. 4b). Under the July–August water treatment, the 0.3-m depth had a greater water deficit than did the other depths, the 0.6-m depth had a greater water deficit than did the all the other depths except 0.3 m, and the 0.9-, 1.2-, and 1.5-m depths had a greater water deficit than did the 1.8-m depth (Fig. 4b).

### Discussion

Developing a sustainable cellulosic bioenergy production system requires information on how efficiently potential bioenergy plants use water especially in semi-arid regions.

**Fig. 4** Soil water deficits for a western wheatgrass (*WWG*), switchgrass (*SWG*), and a western wheatgrass–alfalfa mixtures (*MIX*) grown under different periods of water stress in 2006 and **b** across species within depth increments. *Control* indicated normal precipitation from May through August, *May–June* indicated 50 % of normal precipitation in May–June but normal precipitation in July and August, and *July–August* indicated normal precipitation for May–June but 50 % of normal for July and August. Letters above bars in (a) indicate significant differences between species within a water stress treatment at  $P \leq 0.10$ . Letters beside bars in (b) indicate significant differences between soil depths within water stress treatments at  $P \leq 0.10$



We compared WUE in switchgrass, a major potential cellulosic bioenergy crop, to western wheatgrass, a dominant native perennial grass in the Great Plains, and a western wheatgrass–alfalfa mixture. We hypothesized that season of water stress would affect WUE and water use, and these effects would change with species. We found that (1) switchgrass had three to four times the WUE of western wheatgrass, (2) binary mixtures of western wheatgrass and alfalfa had a WUE similar to that for switchgrass under certain drought conditions, and (3) switchgrass produced greater water deficits in the soil than did western wheatgrass and western wheatgrass–alfalfa. Seasonality of water stress affected the cool-season perennials (western wheatgrass and western wheatgrass–alfalfa) but not switchgrass.

### Water Use Efficiency

One way to enhance WUE is to improve the transpiration use efficiency [1] which would either require producing more biomass with the same amount of water or use less water to produce the same biomass. Biomass differences were the drivers of WUE in our study. Differences in end of season biomass in 2006 mirrored the differences in WUE.

Our study also supported the view that  $C_4$  species have greater WUE than  $C_3$  species [33, 34]. In both 2006 and 2007, the WUE of switchgrass, a  $C_4$  grass, was generally more than three times greater than western wheatgrass, a  $C_3$  grass (Table 1). The WUE for switchgrass ranged from 5.6 to 7.4 g biomass  $mm^{-1}$  water used which was within the range of the 3 to 8 g biomass  $mm^{-1}$  water used reported by Koshi et al. [35] for different harvest and moisture regimes. The western wheatgrass monoculture WUE ranged from 1.1 to 2.1 g biomass  $mm^{-1}$  water used which was similar to the 1.2 g biomass  $mm^{-1}$  water used reported by Frank and Bauer [24]. The limited information on the WUE of binary mixtures and available reports focused on individual components of the mixture [28]. Therefore, our information is some of the first WUE information on binary mixtures as a whole.

Adding alfalfa to western wheatgrass increased WUE (Figs. 1 and 2). It was expected that alfalfa and western wheatgrass would be most detrimentally affected by the May–June water stress period which is the main growing period for cool-season plants in the region. However, WUE for the western wheatgrass–alfalfa mixture under May–June water stress in 2006 and for all of 2007 was very close to that of switchgrass. Incorporating alfalfa into wheatgrass increased biomass yields as reported elsewhere [16]. Legumes that grow over longer periods can improve the seasonal availability of forages into the latter parts of growing seasons [15]. Both factors enhanced WUE of the western wheatgrass–alfalfa mixture and lowered the impact of the May–June stress treatment.

Our data suggested that timing of water stress affected WUE in western wheatgrass and the western wheatgrass–alfalfa mixture but not in switchgrass. Previous reports have suggested that WUE in western wheatgrass did not change with water stress, potentially because western wheatgrass maintains slow levels of growth during drought periods [24]. In contrast, switchgrass has been reported to be impacted by changes in water availability. Earlier reports [20, 35] indicated that WUE declines with increased water availability in switchgrass. Stout et al. [21] indicated soil attributes, such as water holding capacity, make the largest contribution to WUE in switchgrass under variable precipitation scenarios. Soils under the rainout shelter were fine sandy loams [36] with a low water holding capacity, and simulated precipitation was done on a regular basis. Switchgrass plants are deep-rooted [37] and so switchgrass could have partially adjusted to the lower amount of water received under water stress treatments.

### Soil Water Deficit

We calculated soil water deficit by depth as the difference in soil water between the date of the greatest water accumulation in the soil profile to the date of the least water in the soil profile similar to Merrill et al. [31]. Switchgrass had greater soil water deficit than the binary mixture under both water stress treatments. Switchgrass roots are widely distributed in deeper soil horizons than cool-season grasses [38, 39]. However, alfalfa was shown to deplete soil water to a greater extent than other  $C_3$  perennial grasses or wheat (*Triticum aestivum* L.) [25]. Greater switchgrass biomass could have resulted in greater transpiring leaf area which has been linked to increase water use [40], and greater water use can increase soil water deficit. Alternatively, water stress has been reported to lower transpiration in alfalfa and thereby increase WUE [41]. This may explain why the WUE of the binary alfalfa–grass mixture was similar to switchgrass under the May–June water stress treatment.

There were also differences between water stress treatments in soil water deficit by depth (Fig 4). Soil water deficit for each of the six depths was more similar within control treatment than for the other two water treatments (Fig 4). Soil water deficit for all three water treatments was greatest in the upper 30 cm where a majority of the roots are generally located [42]. Drought can result in root mortality, and for both perennial grasses [43] and alfalfa [44], root mortality generally occurs in the surface layer. The potential for increased mortality under water stress in the surface layer and the wide distribution of both switchgrass [38, 39] and alfalfa [45] roots into deeper soil horizons make the significant differences in water depletion at different depths somewhat surprising.



## Conclusions

We found that switchgrass had three to four times the WUE of the common dominant native perennial grass, western wheatgrass, which suggests that switchgrass would be an appropriate cellulosic biofuel crop in semi-arid areas. Binary mixtures with legumes may be one way to improve WUE in C<sub>3</sub> grasses; however, in our study, inclusion of alfalfa with western wheatgrass resulted in more variable WUE during periods of water stress compared to switchgrass. Although switchgrass had greater WUE, it also showed a greater soil water deficit compared to western wheatgrass and the western wheatgrass–alfalfa mixture. Although this is a positive attribute in a single dry year, this may be of concern if switchgrass is periodically rotated into annual crop production or if a multi-year drought occurs.

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