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Water use efficiency of perennial and annual bioenergy crops in central Illinois

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[1] Sustainable bioenergy production depends upon the efficiency with which crops use available water to produce biomass and store carbon belowground. Therefore, water use efficiency (WUE; productivity vs. annual evapotranspiration, ET) is a key metric of bioenergy crop performance. We evaluate WUE of three potential perennial grass bioenergy crops, *Miscanthus* × *giganteus* (miscanthus), *Panicum virgatum* (switchgrass), and an assemblage of prairie species (28 species), and *Zea mays–Glycine max* rotation, during the establishment phase in Illinois. Ecosystem WUE (EWUE; net ecosystem productivity vs. ET) was highest in miscanthus, reaching a maximum value of 12.8 ± 0.3 kg ha^{-1} mm^{-1} in the third year, followed by switchgrass (7.5 ± 0.3 kg ha^{-1} mm^{-1}) and prairie (3.9 ± 0.3 kg ha^{-1} mm^{-1}); the row crop was the lowest. Besides EWUE, harvest-WUE (HWUE, harvested biomass vs. ET) and net biome productivity-WUE (BWUE, calculated as net ecosystem production − harvest vs. ET) were also estimated for all crops and years. After three years of establishment, HWUE and BWUE were highest in miscanthus (9.0 ± 2 and 3.8 ± 2.9 kg ha^{-1} mm^{-1}, respectively) providing a net benefit to the carbon balance, while the row crops had a negative carbon balance and a negative BWUE. BWUE for maize/soybean indicate that this ecosystem would deplete the soil carbon stocks while using the water resources. Switchgrass had the second highest BWUE, while prairie was almost neutral indicating that long-term carbon sequestration for this agro-ecosystem would be sensitive to harvest timing with an early harvest removing more biomass, and thus carbon, from the field.

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1. Introduction

[2] The production of biofuels from cellulosic plant material is expected to increase worldwide as countries look for alternative sources of energy. In addition, the technology for converting biomass into liquid fuel is rapidly improving with increasing efficiency of the fuel conversion process, i.e., by using less energy during the production of liquid fuel [*Farrell et al*., 2006; *Somerville et al*., 2010]. However, several factors must be considered in selecting economically and environmentally viable feedstocks for biofuel production from cellulosic material, including harvestable yield, net carbon balance, nitrogen pollution, and water use [*Hill et al*., 2006; *Jordan et al*., 2007; *Searchinger et al*., 2008; *Don et al*., 2012]. With respect to the carbon cycle, the best options for biofuel crops are the species that are highly productive in terms of harvestable biomass without depleting soil carbon pools by requiring annual tillage, which drives soil carbon loss, as is the case for the most widely planted crops in the U.S., maize (*Zea mays*) and soybean (*Glycine max*) [*Bernacchi et al*., 2005; *Hollinger et al*., 2005; *Bernacchi et al*., 2006; *Hollinger et al*., 2006].

[3] Perennial species such as miscanthus (*Miscanthus* × *giganteus*) and switchgrass (*Panicum virgatum* L.) have many advantages over annual crops because of reduced fertilizer use and no requirements of irrigation if planted over areas with abundant rainfall [*Lewandowski et al*., 2000; *Clifton-Brown et al*., 2001]. In addition, it has been shown that these species can contribute to increased soil carbon and nitrogen stocks while providing a substantial harvestable biomass [*Heaton et al*., 2004, 2008; *Anderson-Teixeira et al*., 2009; *Davis et al*., 2010]. However, while an extensive root system can improve the soil carbon stocks over time as roots die and stay buried, it can also contribute to a higher water use [*Hickman et al*., 2010; *Vanloocke et al*., 2010]. The efficiency

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of plants in using water while accumulating biomass is an important factor to consider when selecting the best biofuel crop to be planted in a certain location. Water use efficiency (WUE) is the term generally used to refer to the ratio of carbon accumulated over water used during a certain period of time.

[4] A plant can accumulate carbon above ground and below ground and those two components have ecologic and economic benefits. Carbon allocated below ground contributes to soil fertility and carbon sequestration, among other benefits [*Lal*, 2004; *Anderson-Teixeira et al.*, 2009]. Aboveground biomass, which includes the grain and plant material, can be used for food or biofuel production. An ideal plant for biofuel production would be one that maximized both benefits while using less water. Water use efficiency is an important metric when cellulosic biofuels are considered, because it takes into account the benefits—carbon accumulated in soils or harvested—and the environmental impact, i.e., the use of water [*Verma et al.*, 2005; *Suyker and Verma*, 2010, 2012]. There are, however, several definitions of WUE that emphasize agronomic efficiency or ecosystem efficiency [*VanLoocke et al.*, 2012; *Tallec et al.*, 2013]. For example, net ecosystem production (NEP) is the net balance of carbon derived from GPP – R_e, where GPP is the gross primary production and R_e is the ecosystem respiration. The ratio of NEP over total water used during the year (TWU) will be referred as "ecosystem" WUE (EWUE). It should be noted that TWU includes transpiration from plants and evaporation from the soil and leaves. Another metric is the HWUE, after "harvest" WUE, which accounts for harvestable biomass—aboveground only—over total water used. This metric reflects the economic advantage of a given biofuel crop, while accounting for the ecological impact on the water cycle. A third metric is the BWUE = NBP/TWU, or biome WUE, where NBP, the net biome productivity, is assumed to be NEP – harvest. Thus, EWUE, HWUE, and BWUE quantify the ecological and economic benefits from agro-ecosystems normalized by their need for water.

[5] The objective of this work was to use ecosystem fluxes measured using the eddy covariance technique and harvested biomass to calculate the values of EWUE, HWUE, and BWUE to compare WUE between miscanthus, switchgrass, native prairie, and a row crop control over three consecutive years following establishment. The measurements presented in this work are part of ongoing research on the ecosystem impacts of biofuels production in the U.S. Midwest, including the carbon balance of the crops [*Zeri et al.*, 2011; *Anderson-Teixeira et al.*, 2013], nitrogen cycling [*Smith et al.*, 2013], fluxes of volatile organic compounds [*Miresmailli et al.*, 2012], and regional-scale modeling of greenhouse gas fluxes [*Davis et al.*, 2011]. The results show how carbon and water fluxes might evolve in this region during the establishing phase, which can last 2–5 years for miscanthus and switchgrass.

2. Site and Data

[6] The measurements were taken at the University of Illinois Energy Farm, in Urbana, IL, USA (40°3′46.209″N, 88°11′46.0212″W, ~220 m above sea level). Mean annual temperature at the site, averaged between 1979–2009 (Illinois State Water Survey), was 11.1°C, while mean accumulated precipitation was 1041.7 mm per year. Measurements were taken using eddy covariance systems and micrometeorological instrumentation placed at the center of four plots (4 ha, 200 m × 200 m). The plots were established in spring 2008, when standard management practices were applied during planting of four species: maize, miscanthus, switchgrass and a mix of native prairie species. Soybean was planted in 2010 as part of a rotation cycle that includes two years of maize (2008, 2009) followed by one of soybean (2010). The three remaining species were established in 2008 but the plot with miscanthus was replanted in 2010 due to a die-off of rhizomes during the winter of 2008, caused by late planting, low temperatures and soil freezing during the last days of December with little residue cover.

[7] The eddy covariance systems were composed of a three-dimensional sonic anemometer (model 81000 V, R.M. Young Company, Traverse City, MI, USA) and an infrared gas analyzer (model LI-7500, LI-COR Biosciences, Lincoln, NE, USA). This system collected high frequency data (10 Hz) of wind speed, air temperature, and densities of CO_2 and H_2O. Other meteorological variables were collected at the center of each plot, including solar radiation (shortwave and longwave, both incoming and outgoing components), air temperature, relative humidity, air pressure, wind speed and direction, and soil temperature and soil heat flux. More details about the instrumentation and establishment history of this site can be found in *Zeri et al.* [2011] and *Anderson-Teixeira et al.* [2013].

3. Methodology

[8] Fluxes of carbon dioxide and evapotranspiration (ET) between the atmosphere and the vegetation were calculated using the eddy covariance technique [*Goulden et al.*, 1996; *Aubinet et al.*, 2000; *Baldocchi*, 2003]. The fluxes were calculated using the software Alteddy (http://www.climatexchange.nl/projects/alteddy/index.htm) (Jan Elbers, Alterra Group, Wageningen, The Netherlands), which includes all the required corrections for high frequency data. Some of these corrections include the alignment of the coordinate system, the correction of sonic temperature due to the influence of air humidity, the effects of air density on measurements made with an infrared gas analyzer, and surface heating correction of LI-7500 gas analyzer [*Webb et al.*, 1980; *Schotanus et al.*, 1983; *Kaimal and Finnigan*, 1994; *Burba et al.*, 2008]. Data quality was assessed from the stationarity flags generated by Alteddy, following the methods of *Foken et al.* [2004]. Low data quality such as nighttime periods with no turbulent mixing were removed from the data set. A footprint model [*Hsieh et al.*, 2000] was used to identify periods when the fluxes corresponded to areas outside the plot's edges. Records were kept only if more than 70% of the cumulative flux came from the plot's area. Overall, all quality control resulted in a fraction of data rejected of ~30% for daytime and ~50% for nighttime, when using incoming solar radiation to separate day and night periods.

[9] The resulting gaps in the time series of fluxes were then filled using standard gap-filling algorithms [*Reichstein et al.*, 2005; *Zeri and Sá*, 2010], which were also used to estimate uncertainty due to the presence of gaps and to separate net ecosystem exchange (NEE) into ecosystem respiration (R_{eco}) and gross primary production (GPP). Net

Table 1. Carbon Removed Through Harvest for Years 2009–2011, in Tons of C per Hectare

Year	Maize/Soybean Grain Harvest (Date of Harvest) (tC ha^{-1})	Miscanthus Harvest (tC ha^{-1})	Switchgrass Harvest (tC ha^{-1})	Prairie Harvest (tC ha^{-1})
2009	5.02 (maize, 3 November 2009)	0.51 (15 March 2010)	1.77 (15 March 2010)	1.74 (15 March 2010)
2010	1.58 (soybean, 13 October 2010)	2.08 (23 March 2011)	3.27 (17 November 2010)	2.75 (17 November 2010)
2011	3.57 ± 0.1 (maize, 1 November 2011)	5.25 ± 1.1 (26 December 2011)	3.45 ± 0.3 (11 December 2011)	1.78 ± 0.3 (18 November 2011)

ecosystem production, which is required in the calculations of EWUE, is defined as –NEE. EWUE and HWUE were reported in units of [kg ha^{-1} mm^{-1}], where kg refers to the mass of carbon in dry biomass or grain of maize and soybean, and mm refers to the amount of water evaporated and transpired in the field. Total water use was calculated by dividing latent heat flux by the latent heat of vaporization. It should be acknowledged that, according to recent research, the design of a sonic anemometer's transducers (orthogonal or nonorthogonal) can lead to errors in the measurement of the vertical velocity and thus cause inaccuracies in the sensible heat flux on the order of ~10% [*Kochendorfer et al*., 2012; *Frank et al*., 2013]. The errors are expected to propagate to latent heat flux and also CO_2-flux, contributing to the other sources of uncertainty (on fluxes and biomass) that were reported together with the carbon/water-use ratios. Those new corrections were not included in this work due their very recent nature and uncertain impact on fluxes.

[10] For the calculation of HWUE, the amount of biomass harvested must be known, as shown in Table 1. It should be noted that the perennials grown in 2009 were harvested in March 2010, and the harvest of miscanthus grown in 2010 occurred in March 2011. Harvest WUE for each year was calculated using the respective value. However, the values of harvested biomass were grouped in Figure 3a at the end of each year for clarity in the discussion of HWUE and BWUE. Grain yield was converted to carbon using the equation $C = (1 - f_w/100) f_c Y$, where f_w is the grain moisture content, f_c is the fraction of carbon and Y is the yield. The fractions f_w and f_c were, respectively, 15.5% and 0.447, for maize, and 13% and 0.54, for soybean [*Loomis and Conner*, 1992]. Plant biomass for miscanthus, switchgrass and prairie was converted to carbon by multiplying the yield Y by 0.447. Additional details about the field management as well as about the methods of gap-filling, flux partitioning and uncertainty mentioned before can be found in *Zeri et al*. [2011].

[11] The full balance of carbon in an ecosystem should take into account NEP, harvested biomass and other factors such as leaching of organic and inorganic carbon [*Chapin et al*., 2006]. In this study the biome WUE was calculated as the ratio NBP/TWU, where NBP was assumed to be the difference of NEP-harvest, ignoring the leaching of carbon. The average leaching of organic and inorganic carbon can amount to ~0.2 tC ha^{-1} year^{-1}, according to a recent study on dissolved carbon leaching over different ecosystems in Europe [*Kindler et al*., 2011]. This component of the carbon balance represents only 3–4% of NEP of mature miscanthus and switchgrass in 2011, contributing then to a minor source of error on NBP of those agro-ecosystems. This is supported by comparing the average leaching to other sources of error in the carbon balance. For instance, the error of not including the leaching of carbon on NBP lies in the same range of the average error on NEP (composed of gap-filling and random errors, which was estimated for all crops and years to be

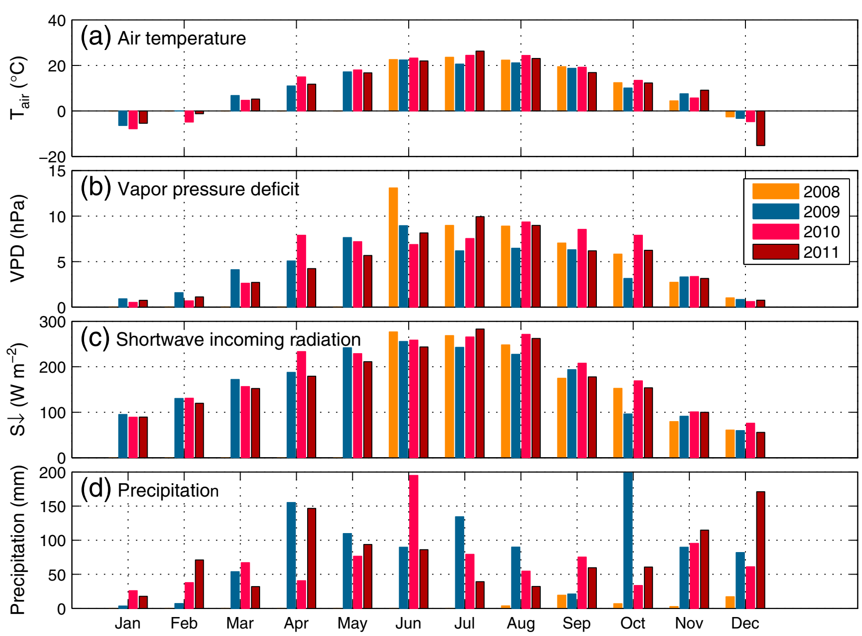

Figure 1. Meteorological variables at the prairie plot during the period.

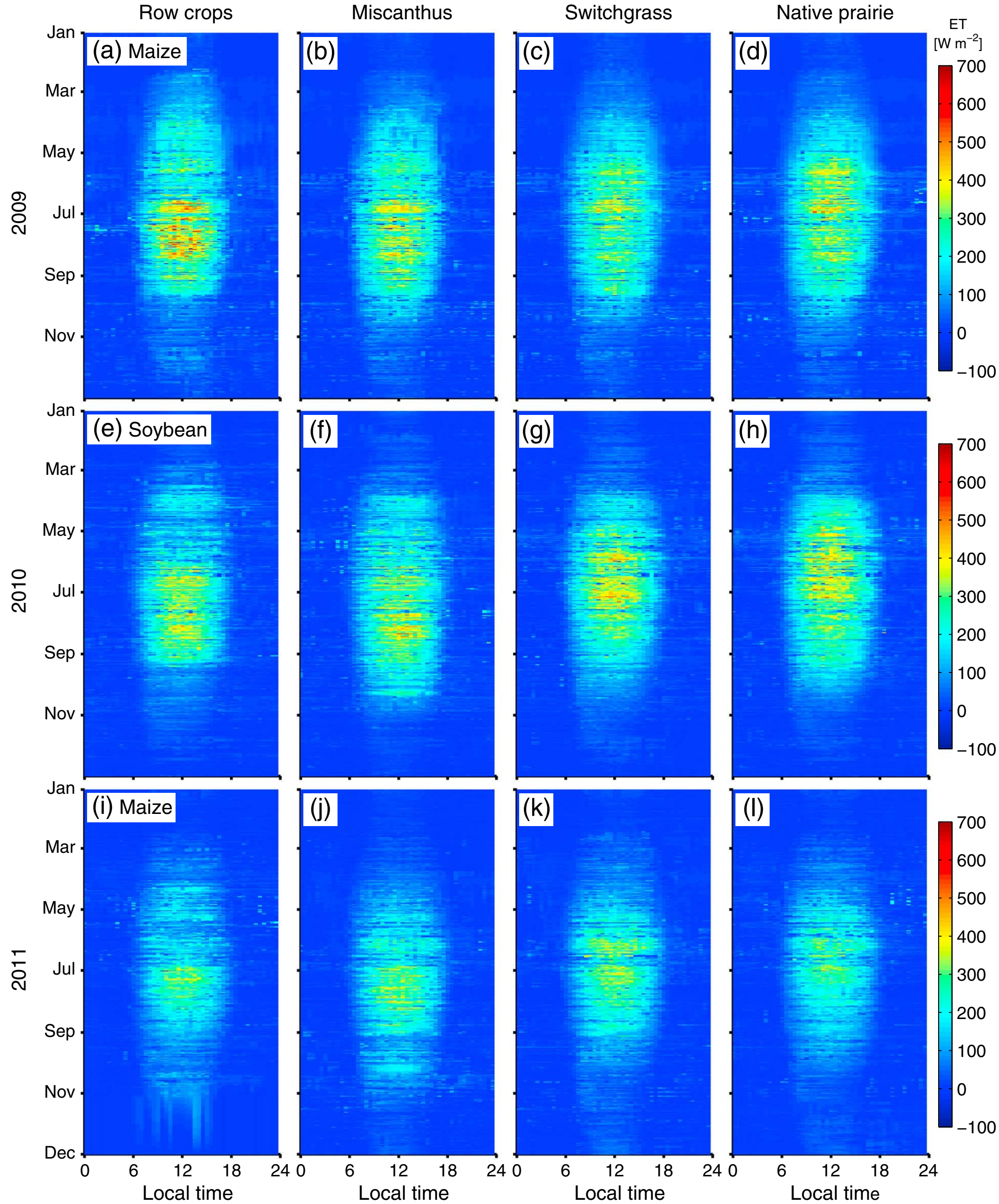

Figure 2. "Fingerprint" plots of evapotranspiration (ET).

~0.22 tC ha^{-1} yr^{-1}) and the error on biomass samplings for 2011 (Table 1), which was ~0.45 tC ha^{-1} yr^{-1}. However, it is likely that this error could affect the carbon balance of prairie, due to its lower NEP, bringing NBP closer to zero (neutral carbon balance).

[12] Analysis of variance (ANOVA, Tukey-Kramer's honestly significant difference criterion, at 5% significance) was performed for the average EWUE based on the multiple versions of NEP and evapotranspiration (ET) generated during the analysis of gap-filling and uncertainty in the fluxes. Since multiple samplings of harvested biomass were only available for 2011, the ANOVA tests for HWUE and BWUE were only performed for that year. All statistical analysis carried out using MATLAB R2011a (The MathWorks, Inc.).

4. Results

[13] Monthly averages of air temperature, vapor pressure deficit, incoming solar radiation, and precipitation are shown in Figure 1. July of 2011 had the highest values of average air temperature and vapor pressure deficit among the four years analyzed in this work. July and August of 2011 had the lowest accumulated precipitation in the period 2009–2011 and, due to the resulting sparse cloud cover, one of the highest values of incoming solar radiation. Total precipitation in 2011 was higher than in 2010, but the distribution of rainfall throughout the year was different. In 2009 precipitation peaked before and during the growing season (April and July), and later in October. The year 2010 was the driest of the three (~800 mm), but

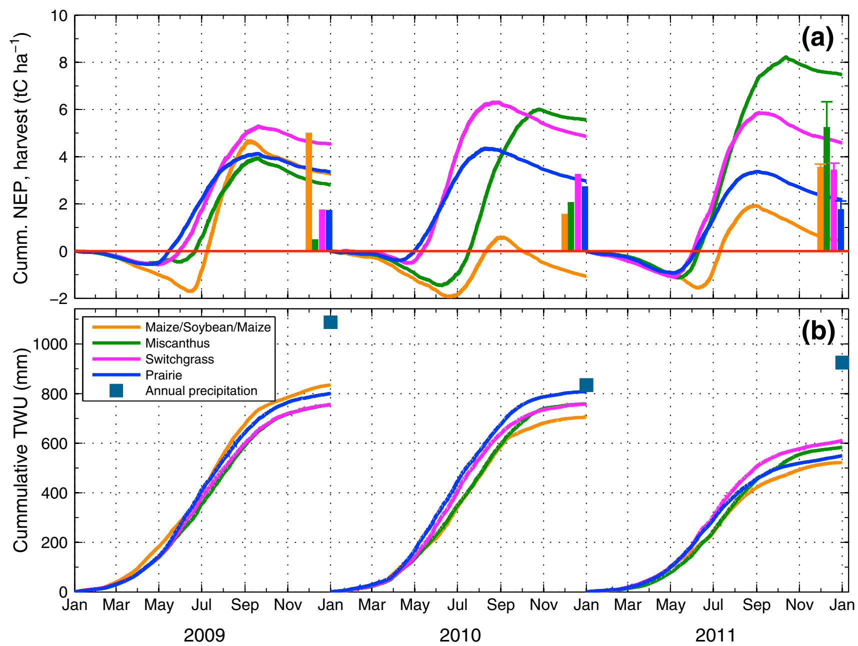

Figure 3. Cumulative fluxes of NEP and harvested biomass (top) and total water used (bottom). Positive values of NEE denote uptake of carbon by the agro-ecosystems. The values of harvested biomass were grouped at the end of the year for clarity. Exact harvest dates can be seen in Table 1. Totals of annual precipitation are shown at the end of 2009, 2010 and 2011.

precipitation peaked in the start of the growing season (June), recharging the soil. Annual rainfall in 2011 was ~900 mm, with a peak in April and decreasing values during the growing season.

[14] Different patterns of precipitation, canopy development and harvest affected the rate of evapotranspiration in different ways throughout the years (Figure 2). Maize had the highest values of ET during the 2009 growing season (from June to September, Figure 2a). Conversely, the drought in 2011 caused the period of highest evaporation to be much shorter (from July to August) and with much lower values (Figure 2i). The period of high ET is associated with the growing season, when the cumulative curves of NEP (Figure 3a) change slope from negative to positive and net carbon uptake commences. The growing season length was similar for all species in 2009 (Figures 2a–2d), since the grasses were in the second year after establishment. Switchgrass and prairie had similar patterns of ET in 2010, but the evapotranspiration decreased sharply after September. Miscanthus was replanted in 2010, causing the period of highest ET to occur later in the growing season (around June) and lengthening the growing season to approximately the end of October (Figure 2f). Miscanthus was not harvested in 2010, which might have contributed to the longer period of evapotranspiration in that year, compared to soybean, switchgrass and prairie. The patterns of ET for 2011 reveal lower values due to the drought, which affected all species.

[15] The perennial ecosystems (miscanthus, switchgrass, and prairie) followed a similar seasonal phenology of CO_2 uptake and release, with maximum accumulated C uptake at the end of the growing season followed by a decrease due to the dominance of soil respiration over photosynthesis after plant senescence, emissions from bare soil following harvest or decomposition of aboveground biomass remaining in the field (Figure 3). To illustrate the carbon balance of these agro-ecosystems, the harvested biomass for each crop and year (Table 1) was displayed at the end of each period in Figure 3a. For miscanthus, the cumulative NEP at the end of the year increased from 2009 to 2011, reaching a maximum of ~7.6 tC ha^{-1} in 2011. The biomass of miscanthus harvested each year also increased from ~0.5 tC ha^{-1} to ~5 tC ha^{-1} but was always lower than the cumulative NEP, resulting in a positive net carbon balance. Switchgrass and prairie also had cumulative NEP higher than harvested biomass and thus net uptake of carbon during the period. The maize/soybean rotation had a strongly positive cumulative NEP at the end of the 2009 maize growing season but the grain harvested at that year caused the agro-ecosystem to be a net source of carbon (release of ~2 tC ha^{-1}). The following period was a soybean year, and was a source of carbon to the atmosphere even before accounting for the harvested grain. In 2011, maize had a low cumulative NEP—due to drought that year—which was not enough to offset the carbon exported through harvest, resulting in a source of carbon to the atmosphere.

[16] During the growing season, evapotranspiration was high, as indicated by the steep slope in the TWU curves (Figure 3b). Following plant senescence between September and November, evapotranspiration decreased sharply and cumulative water use (TWU) decelerated (Figure 3b). This change in slope was in synchronicity with the change in slope in the cumulative NEP curves, denoting the higher evapotranspiration during the growth phase. After plant senescence at the end of the growing season, evaporation from the soil or from water deposited over leaves/postharvest residue continued until the end of the year. In

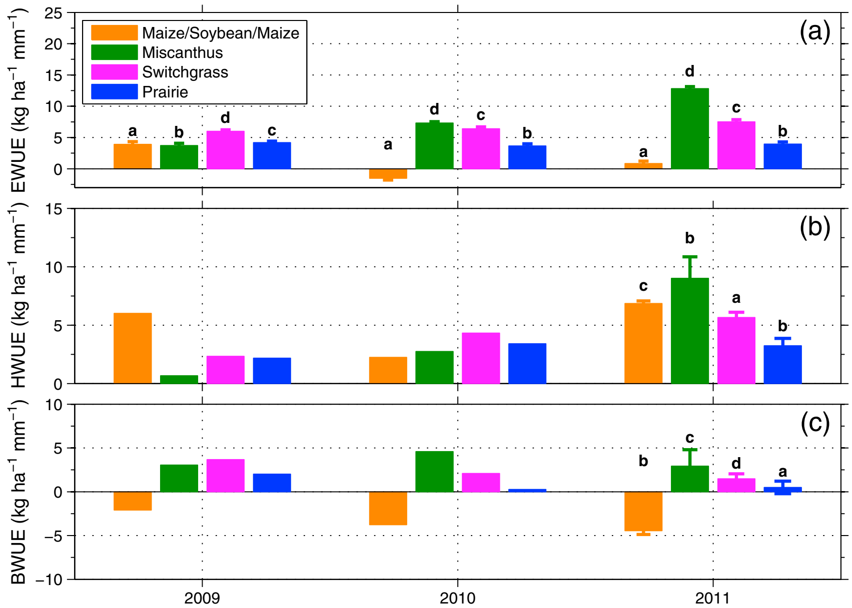

Figure 4. Water use efficiencies—(a) EWUE, (b) HWUE, (c) BWUE (see text for definitions)—of perennial grasses and a conventional row crop rotation (maize in 2009 and 2011, soybean in 2010) from 2009 to 2011. Negative values indicate that the ecosystem was a net C source (i.e., negative NEP for EWUE; harvested C exceeding NEP for BWUE). ANOVA tests for HWUE and BWUE were only performed for 2011, when multiple samplings of harvested biomass were available.

2009 and 2011, the average cumulative TWU for all plots was approximately 70% and 60% of annual precipitation, respectively. In 2010, the lower accumulated precipitation was matched by the evapotranspiration in the prairie plot (~800 mm).

[17] The evolution in time of EWUE, HWUE, and BWUE for each crop is presented in Figure 4. In 2010 and 2011, the mature (or near-mature) perennial bioenergy crops had significantly higher EWUE than the row crop control (Figure 4a). The EWUE of miscanthus increased from 2009 to 2011 because of the increasing value of NEP during the establishment phase (Figure 3). The EWUE of miscanthus in 2011 (12.8 ± 0.3 kg ha^{-1} mm^{-1}) was the highest among all species-years combinations. The EWUE of prairie remained relatively constant (~4 kg ha^{-1} mm^{-1}) between 2009 and 2011, while EWUE for switchgrass increased from 6.01 kg ha^{-1} mm^{-1} to 7.51 kg ha^{-1} mm^{-1} in the same period. The EWUE for maize was 3.92 ± 0.4 kg ha^{-1} mm^{-1} in 2009, a value very close to EWUE of perennials in that year, but decreased to 0.86 ± 0.4 kg ha^{-1} mm^{-1} in 2011, the lowest value among the four crops, due to the drought in Midwest U.S. Soybean had a negative value of EWUE in 2010 because the annual value of NEP was negative (Figure 3). Thus, the EWUE of the perennial grass crops appeared to be not affected by the 2011 drought conditions in comparison with the conventional agriculture.

[18] The values of HWUE in the period evolved in a different way compared to EWUE (middle panel of Figure 4). Maize had an increase of 13% between 2009 (6.0 ± 5.3 kg ha^{-1} mm^{-1}) and 2011 (6.8 ± 0.2 kg ha^{-1} mm^{-1}). The increase of HWUE in 2011, compared to the decrease of EWUE in the same period, was caused by the low annual evapotranspiration in that year, which compensated for the lower harvest of maize in the calculation of HWUE. The HWUE of miscanthus and switchgrass increased from 2009 to 2011, despite the 2011 drought, while the HWUE of prairie was approximately constant between 2010 and 2011 (~3.5 kg ha^{-1} mm^{-1}).

[19] Finally, the values of BWUE for all perennial grasses were positive while maize/soybean had negative BWUE in the period from 2009 to 2011. The grasses had lower harvested biomass (2009) or late harvests (2010) compared to the maize/soybean rotation, resulting in less carbon being exported from the ecosystem. The combination of end-of-year NEP and harvest of maize/soybean resulted in a negative balance in the three years analyzed; this is seen from the harvest (bars in Figure 3a) being subtracted from the end-of-year cumulative NEP (lines in Figure 3a) yielding negative net biome productivity for all three years.

5. Discussion

[20] The calculation of different metrics of WUE reveals that economical or ecological benefits can be obtained from either row crop agriculture or perennial grasses, depending on weather conditions, such as droughts, or the development stage of perennials. However, when both benefits are combined in BWUE, perennial bioenergy crops perform better than row crops such as maize or soybean. In a relatively wet year (2009) when the perennial grasses were still establishing (second growing season), all four crops had fairly similar EWUE. In the following years (2010 and 2011), when precipitation was below average and the perennial grass crops were relatively well established, all three perennial crops had consistently higher EWUE than the row crop control (soy in 2010, maize in 2011). On the other hand, row crops performed better than some perennials when HWUE is considered. Soybean had a HWUE close to

the average of perennials in 2010, and HWUE of maize in 2011 was higher than the value for switchgrass and prairie. However, when BWUE is considered, perennial bioenergy crops present a substantial benefit over row crop agriculture, exporting carbon for ethanol production while adding carbon to the soil.

[21] For two of the three years (2009 and 2011), total water use by all crops was substantially lower than the accumulated precipitation (~30–40% lower). However, 2010 was drier than 2009 and 2011, and TWU was still lower than the annual rainfall, albeit by a much smaller margin (~13%). This implies that the majority of precipitation was returned to the atmosphere through evapotranspiration, leaving little for runoff or groundwater recharge. This illustrates how agro-ecosystems may significantly impact water resources—particularly in drier years—by transferring water from the soil into the atmosphere, depleting belowground water storage and/or reducing the flow in local streams and rivers [*Le et al*., 2011]. On the other hand, reduced precipitation can also impact plant growth, leading to lower evapotranspiration, as occurred in 2011. The different distribution of precipitation throughout the year had a higher impact on plant growth as can be seen in the lower net uptake of carbon by two of the four crops (prairie and maize). The 2011 growing season was dry despite near-normal annual precipitation; this was driven by a peak in precipitation during April followed by very little precipitation in July and August. Thus, precipitation and drought patterns change from year to year, impacting biomass production, carbon sequestration and evapotranspiration [*Dai et al*., 2004]. The production of harvestable biomass and carbon sequestration in soils might be strongly affected in areas subjected to changing precipitation patterns, which should be considered in selecting bioenergy crops to be grown under future climates.

[22] Most of the values of EWUE and HWUE described in this study were similar to modeled values for this region reported in *VanLoocke et al*. [2012]. In that study, maize was found to have EWUE of $\approx 6\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$ in most parts of Illinois. The value of 2009 maize EWUE reported here was lower ($3.9 \pm 0.4\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$), but the difference might be attributed to the higher evapotranspiration measured at the site ($\approx$ 800 mm) compared to the modeled value for the region ($\approx$ 670 mm). The other year of maize cannot be compared because the drought in 2011 reduced NEP and resulted in EWUE of only $0.86 \pm 0.4\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$. The agreement was good for maize HWUE, which was reported to be $\approx 6.8\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$ in the modeling study, a value close to the ones found in this study for 2009 and 2011. *Zwart and Bastiaanssen* [2004] reviewed HWUE of several sites around the world and found a mean value of $8.1 \pm 3.1\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$. *Hickman et al*. [2010] reported HWUE for maize of $8.3 \pm 0.3\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$ for a site near to this experiment in Central Illinois. The higher value of HWUE reported in both studies is justified by the choice of the period used for accounting evapotranspiration. In general, those studies consider only the growing season, excluding from the analysis the periods of the year with high evaporation from the bare soil. In this study, summing TWU for the period from 1 May to 1 October of 2009 (~600 mm) and using this value to calculate HWUE of maize would result in harvest WUE of $8.4\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$, similar to those studies.

[23] EWUE of miscanthus increased from 2009 to 2011, but miscanthus was still in the establishment phase in 2011, resulting in EWUE of $12.8 \pm 0.3\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$, which was lower than the expected value from the modeling study for most of the Midwest US ($15\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$). Similarly, an underestimation of the HWUE for the perennial grasses relative to previous modeling results [*VanLoocke et al*., 2012] was expected given the model assumes a mature crop that was grown beyond the establishment phase, which can last to 2 to 5 years for switchgrass and miscanthus [*Lewandowski et al*., 2000; *Heaton et al*., 2004]. Miscanthus HWUE increased from 2009 to 2011, and the highest value in 2011 ($9.0\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$) was within the range of $8.94–12.52\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$ reported in other experimental studies [*Beale et al*., 1999; *Clifton-Brown and Lewandowski*, 2000; *Hickman et al*., 2010], but lower in comparison with the modeled value ($\approx 13.4\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$). The underestimation in relation with the model can be explained by the establishment phase and model characteristics. While the model simulates a mature canopy with yields exceeding 9 tC $\mathrm{ha^{-1}}$, miscanthus was still in the establishment phase in 2011 due to the natural growth cycle of this crop and also due to management issues after planting, such as the die-off of rhizomes in winter 2008 that resulted in replanting in 2010.

[24] Ecosystem WUE for switchgrass in 2011 ($7.5 \pm 0.3\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$) was similar to the value found for this region in the modeling work. The agreement is the result of the good establishment of switchgrass field since planting in spring 2008 together with the fact that the model used by *VanLoocke et al*. [2012] was evaluated against NEP and evapotranspiration of switchgrass in 2009 and 2010, among other parameters and data from other studies and sites. The model results helped to expand spatially the results found in this study, because the value of EWUE for this site is also characteristic of a larger region extending westward from the US states of Indiana, through Illinois, Iowa and Nebraska. The HWUE of switchgrass in 2010 and 2011 (4.3 and $5.7\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$) compared well with another experimental study [*Hickman et al*., 2010], and with the range in the model (5 to $6.3\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$) if the aboveground biomass assumed by the model for December 31st is taken into account. At that time of year the model assumes less biomass to be harvested due to the conversion of biomass to litter or decomposition, in comparison with the measured harvests. Harvest of switchgrass occurred in mid-November for 2010, and in mid-December, for 2011 (Table 1). The 2011 harvest was close in time to that assumed by the model, which contributed to a better agreement of HWUE between measurements and model.

[25] Finally, prairie had the second best EWUE in 2009 ($4.19\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$), after switchgrass. This value shows that the prairie ecosystem was more efficient than maize ($3.92\,\mathrm{kg\,ha^{-1}\,mm^{-1}}$) when using water to accumulate carbon. In addition, prairie was more resilient to the drought of 2011 considering that its EWUE was reduced by ~5%, compared to a reduction of ~78% for maize. HWUE of prairie for 2009 was the lowest in the period due to the late harvest in March of the next year, according to Table 1. If harvest had occurred in November of the same year (2009), the amount of biomass removed from the field would have been higher, affecting the results of HWUE (increase) and BWUE (decrease). According to aboveground biomass samplings

reported in *Zeri et al.* [2011], extrapolating the last sampling of October to mid-November of 2009 would result in ~3 tC ha^{-1} of biomass to be harvested, what would increase HWUE to ~3.7 kg ha^{-1} mm^{-1}. This value is close to HWUE for 2010 (3.41 kg ha^{-1} mm^{-1}) and 2011 (3.24 kg ha^{-1} mm^{-1}), suggesting a likely range of HWUE for prairie. It is not clear if the reduction in HWUE in 2011 was part of the natural variability or if it was influenced by the drought. In spite of the drought, prairie was the only species that presented an increase in BWUE in 2011 (from 0.26 kg ha^{-1} mm^{-1} in 2010 to 0.73 kg ha^{-1} mm^{-1} in 2011). The resilience of prairie might be attributed to the multispecies characteristic of this ecosystem, which results in an advantage in terms of resources use among different species when subjected to environmental perturbations [*Hooper et al.*, 2005; *Kirwan et al.*, 2009]. A longer time series containing measurements under other extreme climatic events would be necessary to assess with certainty that influence.

[26] The results of HWUE reported in this work relied on the harvested grain for maize and soybean, or harvested aboveground biomass, for the grasses. Maize and soybean were harvested every year after plant senescence. Because the grasses were in the establishment phase, not all of them were harvested every year, and dates of harvest were different, as can be seen in the dates indicated in Table 1. If field harvest is delayed, as happened with 2010 miscanthus, much of the aboveground biomass can be lost during the winter [*Heaton et al.*, 2008; *Dohleman et al.*, 2012], influencing the estimation of HWUE and BWUE. The values of BWUE were positive for the grasses and negative for the maize/soybean rotation. The results for BWUE show higher rates in 2009 and a steady-state between 2010 and 2011, when harvest occurred in the same year of growth (for switchgrass and prairie, in 2010, and all crops in 2011). The values of HWUE and BWUE for 2011 are likely to be closer to what might be expected in following years, because all crops were harvested at the end of the growing season and the grasses, especially miscanthus, are closer to the mature state. Continuous monitoring of the carbon and water cycles is required so that the steady state of those estimations can be assessed with confidence, confirming that the perennial grasses can act as a carbon sink even when significant quantities of biomass are exported from the field for biofuel production.

[27] The ideal biofuel crop should be the one that uses water efficiently to produce biomass and sequester CO_2. In other words, the ideal is maximum BWUE, with both its components (EWUE and HWUE) being positive. The longer the biomass is left in the field, the higher the proportion of plant material lost to decomposition and/or enhancing the soil carbon stocks. In addition, the decision about the best harvest dates for the grasses has to take into account plant physiology characteristics, such as the time required during senescence for the transfer of nutrients from shoots and leaves to rhizomes, as is the case for miscanthus [*Amougou et al.*, 2012; *Dohleman et al.*, 2012]. The evolution of BWUE over the course of crop establishment (Figure 4) suggests that miscanthus will provide the highest climate and economic benefits through C sequestration and biomass accumulation, respectively. The net carbon uptake (NEP-harvest) of miscanthus in 2011 was of approximately 2.25 ± 1.1 tC ha^{-1} yr^{-1} and it is likely to converge to the average net uptake of 0.66 tC ha^{-1} yr^{-1}, as reviewed by *Don et al.* [2012] for European bioenergy crops. In addition, for an agro-ecosystem with a BWUE close to neutrality, such as prairie in 2010 and 2011, the decision about the date of harvest would be crucial to the long-term carbon storage in soils. Early harvest after plant senescence could remove too much biomass and offset the net benefits of carbon stored in the soil. On the other hand, late harvest would decrease the quality of forage, reducing the economic advantage of such crop. According to our measurements, harvesting prairie in mid November led to a positive BWUE even in a dry year. Additional modeling and experimental results would be required to confirm if this would be the case for the whole U.S. Midwest or other regions.

6. Conclusions

[28] Water use efficiency of four biofuel crops was investigated for three years following establishment. The crops were maize/soybean, miscanthus, switchgrass and a mix of native prairie species. The results reported here illustrate the transient phase of the efficiency of use of water in conversion of biomass, because the establishment period for the grasses might range from two (switchgrass and prairie) to up to five (miscanthus) years. Ecosystem water use efficiency, calculated using NEP over TWU, was higher in all three perennial species relative to the row crop control.

[29] When harvest is taken into account and HWUE is calculated, maize had an advantage over perennials in the first year. However, HWUE of maize in 2011 was lower than HWUE of miscanthus, which was much better established. In spite of the superiority of maize HWUE over switchgrass and prairie, the advantage of a row crop for biofuel production wanes when the ecological benefit is integrated, by accounting for the net balance of carbon in the calculation of BWUE.

[30] When NEP and harvest were taken into account and BWUE was calculated, it was revealed that miscanthus had the highest net benefits to the carbon stocks and biofuel production, because removal of biomass from the field still resulted in carbon left in the ecosystem (positive BWUE). The value of BWUE for annual crops was the lowest, indicating that these agro-ecosystems would continue to deplete the soil carbon stocks while using the water resources. Switchgrass was the second best option regarding BWUE, while prairie was almost neutral.

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