

Enhanced evapotranspiration was observed during extreme drought from *Miscanthus*, opposite of other crops

EVA JOO^{1,2,3} , MARCELO ZERI⁴, MIR ZAMAN HUSSAIN⁵, EVAN H. DELUCIA^{2,3} and CARL J. BERNACCHI^{2,3,6}

¹University of California, Croul Hall, Irvine, CA 92697, USA, ²Department of Plant Biology, University of Illinois, 1201 W Gregory Dr., Urbana, IL 61801, USA, ³Carl R. Woese Institute for Genomic Biology, 1206 W Gregory Dr., Urbana, IL 61801, USA, ⁴National Center for Monitoring and Early Warning of Natural Disasters (CEMADEN), Estrada Dr Altino Bondensan, 500, Parque Tecnológico, 12247-016, São José dos Campos, SP, Brazil, ⁵Great Lakes Bioenergy Research Center, W.K. Kellogg Biological Station, Michigan State University, 3700 East Gull Lake Dr., Hickory Corners, MI 49060, USA, ⁶USDA ARS Global Change and Photosynthesis Research Unit, 1201 W. Gregory Dr., Urbana, IL 61801, USA

Abstract

The impact of extreme drought and heat stress that occurred in the Midwestern U.S. in 2012 on evapotranspiration (ET), net ecosystem productivity (NEP), and water-use efficiency (WUE) of three perennial ecosystems (switchgrass, miscanthus, prairie) and a maize/soybean agroecosystem was studied as part of a long-term experiment. Miscanthus had a slower initial response but an eventually drastic ET as drought intensified, which resulted in the largest water deficit among the crops. The substantially higher ET at peak drought was likely supplied by access to deep soil water, but suggests that stomatal conductance of miscanthus during the drought may respond differently than the other ecosystems, consistent with an anisohydric strategy. While there was a discrepancy in the water consumption of maize and switchgrass/prairie in the early time of drought, all these ecosystems followed a water-saving strategy when drought intensified. The gross primary production (GPP) of miscanthus dropped, but was reversible, when temperature reached 40 °C and still provided the largest total GPP among the ecosystems. Increased ET for miscanthus during 2012 resulted a large decline in ecosystem WUE compared to what was observed in other years. The biophysical responses of miscanthus measured during an extreme, historic drought suggest that this species can maintain high productivity longer than other ecosystems during a drought at the expense of water use. While miscanthus maintained productivity during drought, recovery lagged associated with depleted soil moisture. The enhanced ET of miscanthus may intensify droughts through increase supply of deep soil moisture to the atmosphere.

Keywords: eddy covariance, gross primary production, maize, *Miscanthus × giganteus*, net ecosystem productivity, prairie, soybean, stomata control, switchgrass, vapor pressure deficit, water use efficiency

Received 20 September 2016; revised version received 11 March 2017 and accepted 20 March 2017

Introduction

As a consequence of global climate change, extreme weather events (heat waves, drought, etc.) are predicted to become more frequent and intense (Collins *et al.*, 2013). The increase in extreme events is predicted to adversely affect water availability and plant growth and has already become relevant in several regions worldwide (Ciais *et al.*, 2005; Rahmstorf & Coumou, 2011; Coumou & Rahmstorf, 2012). Concerns over global climate change have generated an effort to understand how environmental changes, such as seen in temperature and precipitation, influence net carbon exchange between terrestrial ecosystems and the atmosphere

(Pingintha *et al.*, 2010). In regions where water deficit occurs frequently, plants developed adaptive strategies to cope with drought (Maroco *et al.*, 1997; Borrell *et al.*, 2006; Araus *et al.*, 2008). One adaptation strategy is to conserve water early in the growing season to maintain support for later growth (Sinclair *et al.*, 2005; Zaman-Allah *et al.*, 2011). Stomata closure to prevent water loss can be induced by high atmospheric vapor pressure deficit (VPD) (Sinclair *et al.*, 2005; Fletcher *et al.*, 2007; Kholová *et al.*, 2010) or by root signaling triggered by the dry soil status (Zhang & Davies, 1990).

Large intra- and interspecies variation in the sensitivity of stomata response to VPD has been reported (Maroco *et al.*, 1997; Franks & Farquhar, 1999; Oren *et al.*, 1999), with a break point VPD to induce stomata closure at 1.2 kPa for peanut (Pingintha *et al.*, 2010), 1.7–2.2 kPa

Correspondence: Eva Joo, tel. +1 312 918 9267, fax +1 217 244 6267, e-mail: eva.joo.w@gmail.com

for maize hybrids (Yang *et al.*, 2012), 3 kPa for switchgrass (Wagle & Kakani, 2014), and no indication of a break point up to 3 kPa for C4 turfgrasses (Wherley & Sinclair, 2009). Frequently, a decrease in transpiration at high VPD is observed and referred as the 'feed-forward response'; however, in the case of drought it is unclear whether a stomatal response to cuticular transpiration (Monteith, 1995; Eamus *et al.*, 2008) or a decrease in plant hydraulic conductance due to drying soils (Oren *et al.*, 1999; Macfarlane *et al.*, 2004) dominates the response. Stomata closure of plants has been coupled with reduced transpiration rates (Bernacchi *et al.*, 2007; Wherley & Sinclair, 2009; Yang *et al.*, 2012; Hussain *et al.*, 2013), reduced photosynthesis (Giorio *et al.*, 1999), and lower carbon uptake (net ecosystem exchange, NEE) (Pingingtha *et al.*, 2010; Eichelmann *et al.*, 2016). Ultimately, the relative decline of transpiration, photosynthesis, and NEE largely determines variations in water-use efficiency (WUE) among individual species (Bernacchi & VanLoocke, 2015).

Miscanthus × giganteus (miscanthus) is a potential bioenergy crop that due to its high yield potential, and water-use efficiency may drive land-use change in the Midwestern United States (Heaton *et al.*, 2010). Miscanthus, however, has shown high sensitivity to water deficit in pot experiments, resulting in leaf senescence, lower biomass production (Clifton-Brown & Lewandowski, 2000; Mann *et al.*, 2013b), and reduction in photosynthetic performance (Ings *et al.*, 2013). Ings *et al.* (2013) concluded that miscanthus, in artificial growth environments, continues to maintain high rates of transpiration and physiological activity despite increasing water stress, indicating lack of drought adaptation. In pot experiments, miscanthus may extract all available moisture resulting in loss of photosynthetic function and ultimately susceptibility to drought. Another pot-based experiment demonstrated maintained productivity and a drought-avoidance strategy of another potential bioenergy crop, switchgrass, under a wide range of soil moisture conditions explained by its extensive root development (Mann *et al.*, 2013b). However, the dynamics of species responses to extreme environments are likely to vary between potted experiments and natural growth environments, where rooting depths and competition among neighbors differ, and additional impacts of other environmental drivers, such as heat stress may co-occur. It is unclear whether a similar response would occur under field conditions where deeper roots may have the benefit of accessing deeper water. For example, in a recent study on carbon dynamics, three perennial crops maintained greater annual productivity than the annual crops during severe drought (Joo *et al.*, 2016). A high diversity ecosystem, such as a restored tall-grass prairie, has been proposed as a potential bioenergy feedstock ecosystem (Tilman *et al.*, 2006) and is likely to adopt

a different drought response due to the role of various species responding independently to environmental conditions. Annual crops that presently dominate the Midwestern landscape, such as maize, have more limited root development and therefore are likely to be more sensitive to drought, generally providing lower yields in dry summers.

In this study, we present the combined effect of drought and heat stress on evapotranspiration (ET), net ecosystem productivity (NEP), and water-use efficiency (WUE) of three perennial and one annual agroecosystem. The objectives of this research were to (i) understand the variation behind the responses to extreme drought for one annual and three perennial ecosystems exposed to historic drought conditions and (ii) to assess whether the efficiency of water use at the ecosystem and at the harvest scales vary in response to extreme drought conditions. These objectives are addressed using four ecosystems representing three perennial and one annual ecosystem and build upon a previous study of the same ecosystem (Joo *et al.*, 2016). There we reported that the perennials maintain higher productivity during a drought than the annual row crops, and now we provide mechanistic insights into the dynamics of water use, productivity, and water-use efficiency before, during, and immediately following the drought.

Materials and methods

Climate and site management

Measurements were carried out at the Energy Farm of the University of Illinois at Urbana-Champaign, IL, USA, between 2009 and 2013. The growing season typically starts in April, and summer is generally characterized as warm and relatively wet with temperatures above 20 °C from June to August and mean annual precipitation of 1042 mm (Illinois State Water Survey, average between 1979 and 2009). The soil, typical to the region, is deep and fertile Flanagan (fine, montmorillonitic, mesic aquic Argiudoll) with low lying blocks of Drummer (typic Haplaquoll).

In May 2008, four replicated plots (200 × 200 m each) were established by planting three perennial species, switchgrass (*Panicum virgatum* L.), miscanthus (*Miscanthus × giganteus*), a mixture of tall grass prairie [complete species list in (Zeri *et al.*, 2011)], and a maize (*Zea mays* L.)/soybean (*Glycine max* L.) crop rotation. The maize/soybean rotation consisted of soybean planted every third year, which corresponded to the 2010 and 2013 growing seasons. The maize and switchgrass fields were fertilized by the addition of 168, 202, and 180 kg ha⁻¹ nitrogen in 2008, 2009, and 2011, respectively, for maize, and by 56 kg ha⁻¹ in 2010–2012 for switchgrass, whereas soybean, miscanthus, and prairie were not fertilized on any years based on present management practices (Tilman *et al.*, 2006; Davis *et al.*, 2010; Zeri *et al.*, 2011, 2013). Further information on site management practices can be found in (Zeri *et al.*, 2011, 2013).

Flux and meteorological measurements

Eddy covariance and micrometeorological stations were situated in the center of each plot. The eddy covariance system consists of a three-dimensional sonic anemometer (model 81000VRE; R.M. Young Company, Traverse City, MI, USA) and an open path infrared gas analyzer (IRGA; model LI-7500 upgraded to model LI-7500A in early 2012; LI-COR Biosciences, Lincoln, NE, USA). Each eddy covariance system was accompanied by a meteorological tower, equipped by a set of sensors to monitor temperature and relative humidity (HMP-45C; Campbell Scientific, Logan, UT, USA), up- and down-welling short- and long-wave radiation (CNR1; Kipp & Zonen, Delft, the Netherlands), canopy surface temperature (SI-121 or SI-111 Infrared radiometers; Apogee Instruments, Logan, UT, USA), up- and down-welling photosynthetically active radiation (LI-190; LI-COR, Biosciences, Lincoln, NE, USA), soil heat flux (HFP01; Hukseflux Thermal Sensors B.V., Delft, the Netherlands); and soil moisture and soil temperature (model Hydra Probe II; Stevens Water Monitoring Systems, Inc., Portland, OR, USA). A full description of the eddy covariance system has been published previously (Zeri *et al.*, 2011, 2013).

Ecosystem fluxes were calculated from the 10 Hz data using Alteddy (<http://www.climatexchange.nl/projects/alteddy/>) from 2008 until 2011 and EddyPro (http://www.licor.com/env/products/eddy_covariance/software.html) in 2012–2013. Both software packages employed similar methods for correcting the high-frequency data, including coordinate alignment, correction of the sonic temperature due to the influence of humidity, and compensation of density fluctuations by the WPL term (Webb *et al.*, 1980). The obtained data were filtered for periods of no turbulent mixing during nighttime (Foken *et al.*, 2005), and for cases when more than 30% footprint of the data originated from outside of the plots (Hsieh *et al.*, 2000). Missing data were gap-filled, and the fluxes were partitioned from net ecosystem exchange into ecosystem respiration (R_{eco}) and gross primary production (GPP) as in Reichstein *et al.* (2005) and Zeri *et al.* (2011). Ecosystem water-use efficiency was calculated by dividing daily (e.g., Fig. 3) and yearly (e.g., Table 1) integrated net ecosystem productivity (WUE_{eco}), gross primary productivity (WUE_{GPP}), and harvest (WUE_{H}) by daily and yearly integrated ET, respectively. 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. For all calculations, tables, and figures, harvest values are given to the represented year (so the yield of 2009 for instance is given under the 2009 values, independently that the actual agricultural process and measurement was carried out in the first months of 2010).

Results*The 2012 growing season was among the worst droughts to affect the Midwestern U.S. in decades*

Among the 5 years of this experiment, the mean maximum summer temperatures of ~ 30 °C were typically

Table 1 Productivity and water-use efficiency of the crops in 2009–2013

Year	Species	NEP (g m ⁻²)	GPP (g m ⁻²)	ET (mm)	WUE _{eco} (g m ⁻² mm ⁻¹)
2009	Miscanthus	281	2071	756	0.372
2009	Switchgrass	454	2316	756	0.600
2009	Prairie	335	1826	801	0.418
2009	Maize/soybean	327	2676	835	0.392
2010	Miscanthus	556	1954	758	0.733
2010	Switchgrass	486	2297	757	0.642
2010	Prairie	296	1819	808	0.367
2010	Maize/soybean	-107	1210	704	-0.152
2011	Miscanthus	748	1846	583	1.282
2011	Switchgrass	458	1774	610	0.750
2011	Prairie	218	1326	549	0.397
2011	Maize/soybean	45	1202	522	0.086
2012	Miscanthus	1102	2375	821	1.342
2012	Switchgrass	503	1937	736	0.683
2012	Prairie	341	1937	813	0.419
2012	Maize/soybean	1	1415	617	0.002
2013	Miscanthus	772	1920	614	1.258
2013	Switchgrass	531	1897	755	0.703
2013	Prairie	389	1906	758	0.513
2013	Maize/soybean	12	1285	747	0.016

Annual cumulative values are presented. During the 2012 drought and heat stress, miscanthus had the largest productivity throughout the years, and among the species. Miscanthus achieved the highest water-use efficiency compared to the studied crops.

observed, except for 2011 and 2012, when average daily maximum temperatures reached 35 °C in July (Fig. 1). In particular, the 2012 growing season was considerably drier and warmer relative to long-term mean conditions with prolonged unusually warm temperatures. The daily maximum air temperature often exceeded 35 °C, occasionally reaching 40 °C, between early July and middle September. Moreover, the spring of 2012 was exceptionally warm, with average maximum temperature of 20 °C in March, compared to the 5–12 °C range typically observed in other years.

The monthly photosynthetic photon flux density (PPFD) pattern follows the typical intensity of observed radiation of 1000–1400 mol m⁻² month⁻¹ in the summer, with the highest radiation received over the growing season in 2012 (Fig. 1). Generally the region receives an average PPFD of ~ 1100 mol m⁻² month⁻¹ in May and June, while in 2012 this was 1400 mol m⁻² month⁻¹. The large number of sunny days in 2012 resulted in the highest total PPFD measured between April and September (7489 mol⁻¹ m⁻²) among the studied years. As a combined effect of temperature and light

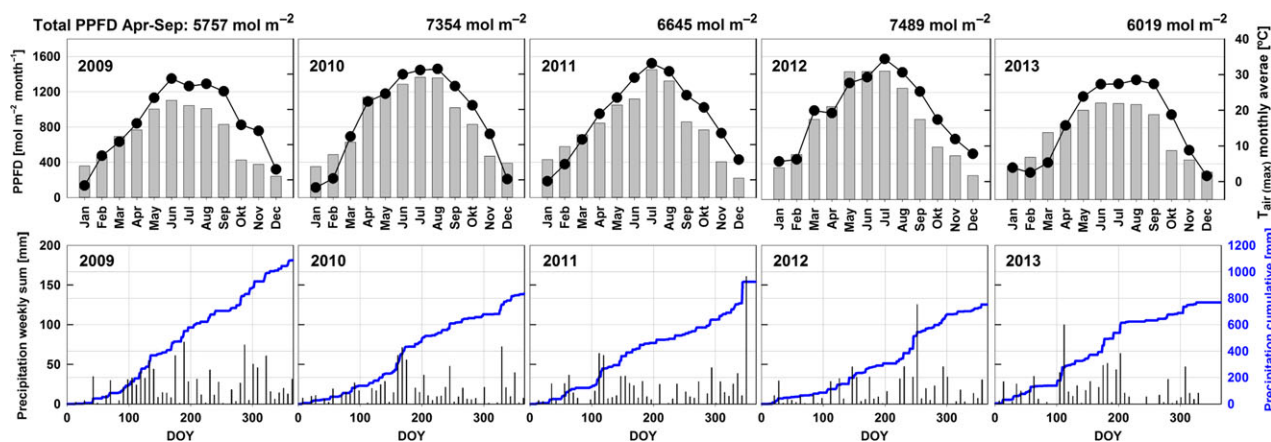


Fig. 1 Meteorological variables during the 5-year-long study including the drought in 2012. Monthly photosynthetic photon flux density (PPFD) is shown as bar plots, while monthly average maximum temperatures are given as line plots. Below, weekly sum (bar) and annual cumulative precipitation (line) is presented. The sunny and hot summer season in 2012 started earlier than what is typical to the region. By the middle of the growing season, plants received approximately only half of precipitation than usual.

intensity, the growing season shifted earlier in 2012 compared to the other years, which is reflected by the development of the plants (Joo *et al.*, 2016).

The annual cumulative precipitation measured on site in 2012 (752 mm) and 2013 (768 mm) was approximately 75% of the long-term mean of 1042 mm (<http://mrcc.isws.illinois.edu/CLIMATE/>), while other years fell within the normal long-term range. The monthly distribution of rainfall events in 2012 was different from the other years, with the lowest amount of precipitation immediately preceding and during the majority of the growing season (306 mm between January and August), which was approximately half typical precipitation (Fig. 1). The 2012 drought appeared together with high temperatures up to 40 °C, which suggest a combined drought and heat stress on the plants, however for simplicity in what follows we refer to it as drought. A significant precipitation event occurred late in the 2012 growing season which brought the annual total precipitation higher, and this was followed by a very wet beginning of 2013, when the highest amount of cumulative precipitation (620 mm) among the studied years was observed between January and August. Opposite of 2012, the lower precipitation in 2013 occurred well into the growing season and mean temperatures were much cooler during the dry-down period.

The drought of 2012 coupled with high temperatures resulted in VPD being much larger in 2012 compared to the other, nondrought years. A large peak of 4–5 kPa was observed in the summer of 2012, lasting approximately 1 month (Fig. 2). In other years, VPD stayed below 3 kPa with the exception of 2011, when a short period (few days) of 4 kPa was reached.

Miscanthus maintained high ET during the drought relative to other ecosystems, which led to the largest water deficit

Despite similar starts of the growing season and rapid accumulation of LAI (Joo *et al.*, 2016), miscanthus shows a lag in the rate of increase in cumulative ET relative to the other perennial ecosystems (Fig. 2). However, the rate of increase in cumulative ET accelerates around day of year 200 after which cumulative ET for miscanthus meets or exceeds the other ecosystems. The exception to this was in 2013, when at the same time miscanthus already began to reduce its ET (Fig. 2). The maize/soybean ecosystem shows similar early-season responses as miscanthus; however, the planting date for the annual row crops is much later than the emergence date for the perennial ecosystems.

The difference between cumulative precipitation and ET (Cum(P-ET)) between 2009 and 2011 was generally positive, meaning that none of the ecosystems experienced water deficit, which is typically resulted in greater rates of ET than precipitation. In 2010 cumulative P-ET values fell (approximately –100 mm for prairie, where negative values indicate water deficit); however, this occurred close to the end of the growing season and late precipitation caused all ecosystems to finish with surplus moisture. On the other hand, in 2012 water deficit reached between –110 mm (maize) and –284 mm (miscanthus). At the same time, cumulative evapotranspiration of miscanthus reached 800 mm by the end of 2012, which was among the highest values for all species and over the duration of this experiment. The 2012 drought showed the largest variation in ET among the four ecosystems. Following the drought, in

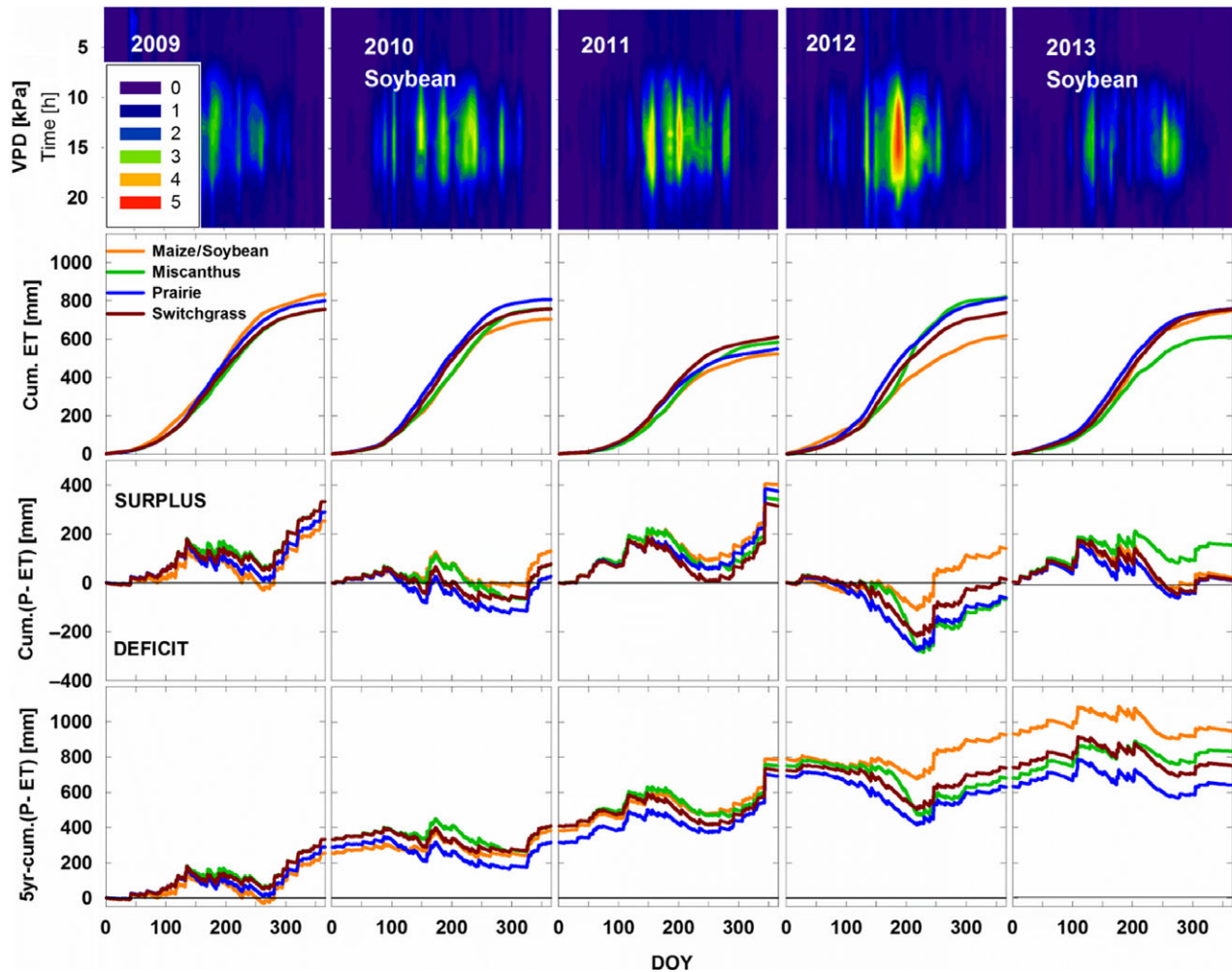


Fig. 2 Vapor pressure deficit over the diurnal time period, the cumulative evapotranspiration for each ecosystem (second row), and cumulative annual (third row) and experiment-long (fourth row) water balance over each year. The four species experienced water deficit in 2012, in accordance with a peak of vapor pressure deficit (VPD). The largest effect was observed for miscanthus with the highest evapotranspiration due to the enhanced transpiration.

2013 cumulative precipitation was generally greater than cumulative ET for all ecosystems. The progression of ET throughout 2013 was similar for all species with the exception of miscanthus, which had the lowest ET. As a result, miscanthus accumulated the largest water surplus among the ecosystems in 2013. The 5-year-cumulative P-ET was nearly the same for all species until the 2012 drought when the miscanthus ecosystem experienced the second largest water deficit, surpassing switchgrass. The 2012 drought had the largest impact on the miscanthus field, miscanthus generally having the best (largest) long-term water content; in 2012, this ecosystem turned to have the second lowest long-term water content, with a slow recharge in 2013. Although available data are limiting and there is large variation in the soil moisture measurements and their sensitivity among the ecosystems (due to methodological

limitation), the deep soil moisture content measured at 100 cm belowground still confirmed the lowest water content level at the miscanthus plot, with a minimum value of approximately 0.05 water fraction by volume (wfv) during the drought, followed by switchgrass (0.19 wfv; Fig. S1). Until DOY 190, an intensive decline of soil water content was observed at the miscanthus field. After the drought, all ecosystems showed soil water content recharge (Fig. S1).

Net ecosystem productivity and water-use efficiency

Daily mean ET, net ecosystem productivity (NEP), and ecosystem water-use efficiency (WUE or WUE_{eco}) for the drought year and the years preceding and following the drought show that miscanthus deviates strongly in all three metrics relative to the other ecosystems (Figs 3

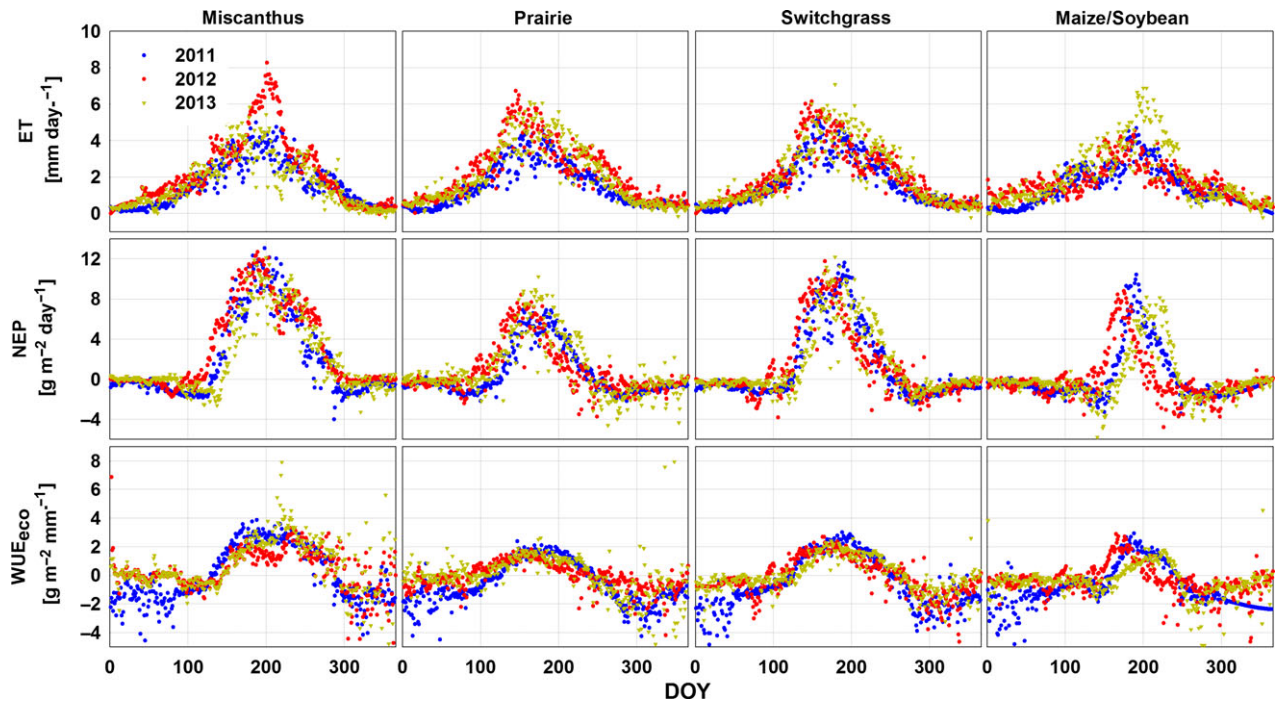


Fig. 3 Evapotranspiration, net ecosystem productivity, and water-use efficiency for each ecosystem during (2012), the year before (2011), and the year after (2013) the drought. A peak in evapotranspiration (ET) was observed for miscanthus in the summer of 2012 when vapor pressure deficit (VPD) reached an average of 4.5 kPa and while net ecosystem productivity (NEP) declined, resulting in a drop of EWUE. On the contrary, switchgrass and prairie reduced ET earlier in the season. In 2013, soybean was planted instead of maize.

and 4). In 2011 and 2013, miscanthus had relatively similar responses of ET and NEP with seasonal maximum values of $\sim 4 \text{ mm day}^{-1}$ and $\sim 12 \text{ g m}^{-2} \text{ day}^{-1}$, respectively. During the drought in 2012 ET of miscanthus peaked at 8 mm day^{-1} , twofold higher than observed in other year and nearly 1.5 times larger than observed for other ecosystems (Fig. 3). At the same time, NEP reached a maximum value at DOY ~ 200 , similar to the previous year. Both ET and NEP of miscanthus declined after DOY ~ 200 , but followed a typical annual curve after DOY ~ 220 (Figs 3 and 4). Despite the drop in NEP, however, miscanthus reached the highest cumulative NEP (1102 g m^{-2} carbon) in 2012 (Table 1), as a prolonged carbon uptake was observed (until DOY ~ 265) compared to the other species (until DOY ~ 200) (Joo *et al.*, 2016). The substantial increase in ET coupled with no net gain in NEP for miscanthus during 2012 resulted a large decline in WUE_{eco} compared to what was observed in 2011 and 2013 (maximum values of $3 \text{ g m}^{-2} \text{ mm}^{-1} \text{ day}^{-1}$).

The other three ecosystems maintained relatively consistent WUE_{eco} throughout the drought relative to the previous years (Fig. 3). Switchgrass and prairie had similar ET and NEP values with a maximum of $\sim 6 \text{ mm}$ and $\sim 9 \text{ g m}^{-2} \text{ day}^{-1}$, respectively, in 2012, which were

nearly the same as in 2013 (Fig. 3). WUE_{eco} of these species followed a relatively consistent annual progression, even during the drought. ET of maize was nearly the same in 2011 and 2012, while NEP was slightly lower in 2012. A decline of WUE_{eco} was observed for this crop as well in 2012; however, it was not as remarkable as for miscanthus. A shift in timing of maximal productivity was observed in 2012 relative to the other years for all species; however, miscanthus maintained a relatively large NEP throughout the entire 2012 growing season. The timing of peak WUE_{eco} for the maize/soybean ecosystem varied among the 3 years, but this variation is largely attributed to management decisions associated with variation in planting date due to differences between maize and soybean as well as meteorological conditions influencing field access.

Throughout the experiment, WUE calculated using gross primary productivity (WUE_{GPP}) was relatively consistent for the perennial ecosystems, particularly for the prairie (Fig. 5b). Switchgrass showed a general decline in WUE_{GPP} throughout the experiment, but in all cases, it was greater than or equal to WUE_{GPP} for the prairie. Miscanthus, however, had greater variation in WUE_{GPP} from year-to-year and did not follow any trend

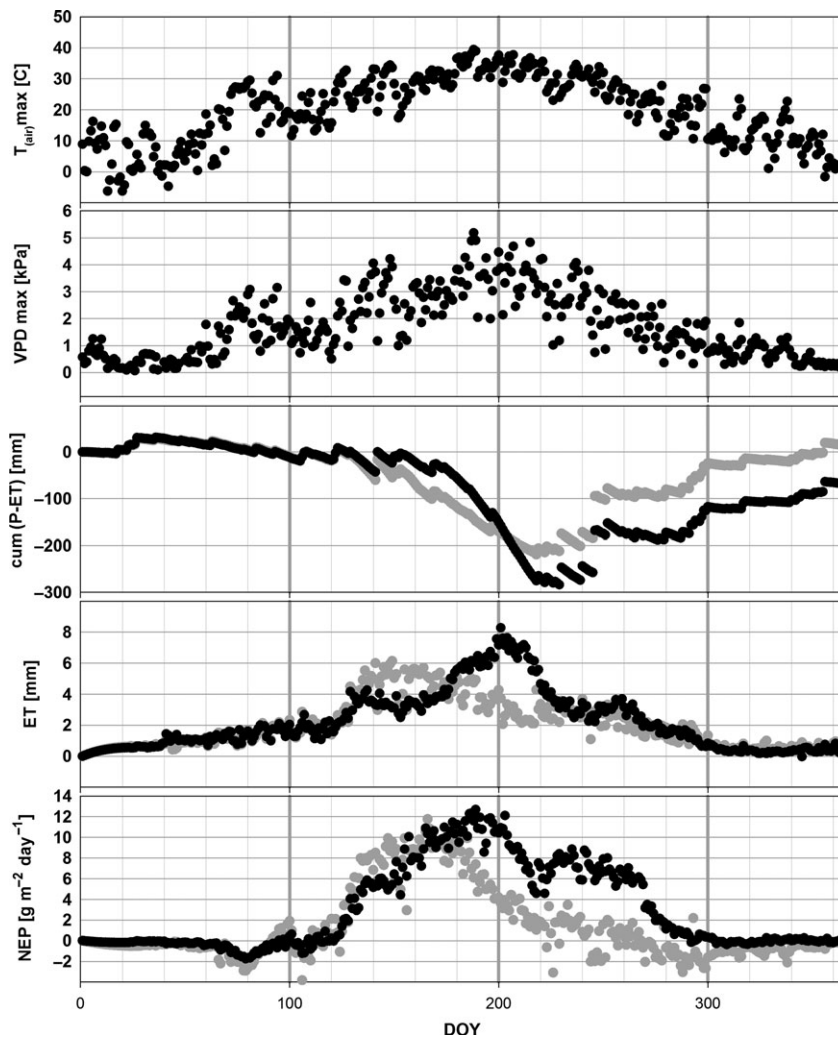


Fig. 4 Dynamics of air temperature (T_{air}), vapor pressure deficit (VPD), cumulative water balance (cum P-ET), evapotranspiration (ET), and net ecosystem productivity (NEP) for miscanthus (black symbols) and switchgrass (gray symbols) during 2012.

as did switchgrass. The maize/soybean ecosystem showed the greatest variability with the maize years (2009, 2011, 2012) having the highest WUE_{GPP} and the soybean years the lowest. The hot and dry conditions 2012, and to a lesser extent in 2011, resulted in lower WUE_{GPP} for maize than the more typical 2009 growing season. With the exception of 2009, the annual row crops had lower WUE_{GPP} values than the perennial ecosystems, although they were similar to prairie in 2011 and in 2012.

Harvest WUE (WUE_{H}), calculated from the carbon in harvested biomass, was substantially more variable across the experiment (Fig. 5a). The miscanthus and switchgrass ecosystems showed a gradual increase in WUE_{H} during the first 1–2 years after which the values stabilized, whereas the prairie ecosystem showed highly variable values of WUE_{H} throughout the experiment. In

2009 and 2011 when maize was planted and in 2010 and 2013 when soybean was planted, WUE_{H} was relatively consistent; however, the 2012 maize WUE_{H} was much lower than observed in the other maize-growing years. All species, other than switchgrass, showed a decrease in WUE_{H} during the 2012 drought. Normalizing ET based on VPD (Fig. 5c) shows that within the perennial ecosystems, miscanthus generally has lower values than the other two ecosystems except during the 2011 and 2012 growing seasons. During the two hot, dry years, all ecosystems had lower ET/VPD than the more typical growing seasons and the variation among the perennial ecosystems was smaller. To calculate this parameter, values of ET and VPD were averaged for the entire dataset of the particular year to make conclusions for the entire ecosystem. Using VPD normalized ET to calculate WUE ($WUE_{\text{GPP}} \times \text{VPD}$), the intrinsic water-use

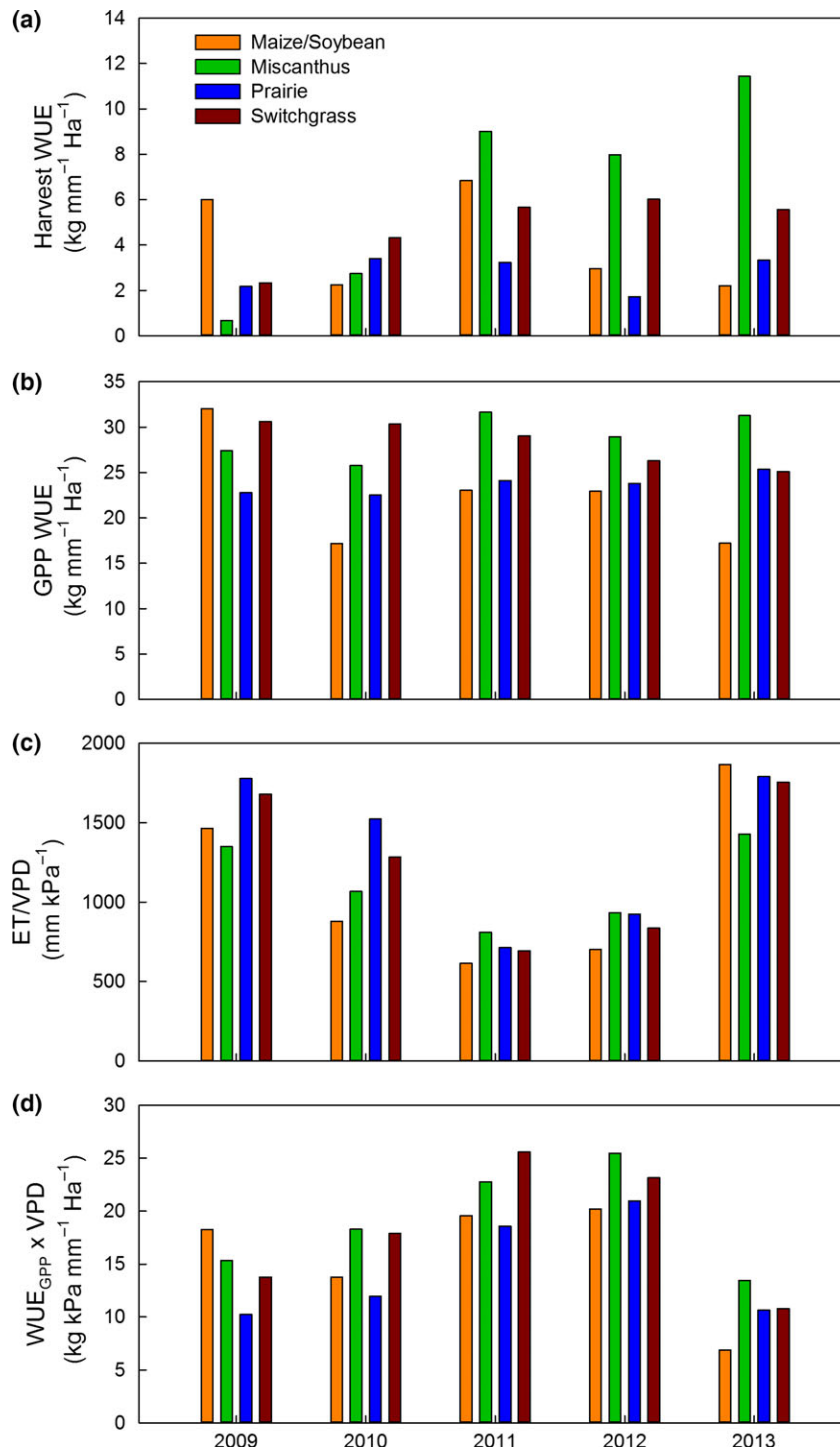


Fig. 5 Water-use efficiency based on harvested biomass (a) and gross primary production (GPP) (b) for each ecosystem over the duration of the experiment. Also shown is the vapor pressure deficit (VPD) normalized evapotranspiration (ET) (c) which is used as a proxy for canopy conductance, and water-use efficiency (WUE) based on GPP using VPD normalized ET (d).

efficiency can be obtained. This resulted in more variation among years than WUE_{GPP} itself (Fig. 5b), and with the exception of 2013, it was lowest in the prairie and

similar between miscanthus and switchgrass. The two hotter and drier years, overall, had the highest $\text{WUE}_{\text{GPP}} \times \text{VPD}$.

Discussion

Water usage dynamics of miscanthus opposite to other ecosystems

Generally crops conserve water use by partial stomata closure at high VPD (Ball *et al.*, 1987; Sinclair *et al.*, 2005; Fletcher *et al.*, 2007; Kholová *et al.*, 2010). Based on the cumulative P-ET curve in 2012 (Fig. 2), miscanthus prevented water loss the longest (until DOY ~170) during the drought compared to the other species that experienced a gradually increasing water deficit starting earlier at DOY ~150. Despite having the second largest water 'surplus' by the end of 2011 and the early-season water conservation, miscanthus had a rapid water use coupled with the lack of precipitation in 2012, which led miscanthus having the largest cumulative water deficit [Fig. 2; Cum(P-ET) and 5 years cum(P-ET)], tied with prairie. In comparison with switchgrass and prairie, miscanthus extracted deep soil water, likely due to its long root system, to supply the large ET. The measured belowground biomass density and depth profile in the summer of 2011 confirmed that the perennials had substantive root systems, extending to a depth of at least 100 cm in contrast to maize that was dominant in the top 10 cm of the soil (Anderson-Teixeira *et al.*, 2013). Among the perennials, switchgrass and miscanthus had extensive root systems below 50 cm, suggesting that these two ecosystems had the ability to reach deep soil moisture in case of a severe drought period. The deep soil moisture content (at 100 cm belowground) of the four ecosystems suggests that indeed miscanthus extracted the most water from this deep layer (Fig. S1). After several years of relatively similar responses among the four ecosystems, the drought in 2012 caused a divergence in the water balance (Fig. 2). While these responses suggest surpluses and deficits in the water balance, it is important to consider that periods when surpluses occur do not directly translate into excess moisture in the area, but instead lead to losses from surface and subterranean flows. Our results also demonstrate that during an extreme drought, miscanthus has the largest flux of water transport of deep soil water content toward the atmosphere (refer to Fig. 3, ET values).

In 2013, the drought recovery year showed strong differences in cumulative ET between miscanthus and the other ecosystems – likely a response to the excessive water use in 2012 resulting in depleted soil moisture, and thus longer recharge of soil moisture. Indeed, switchgrass and prairie recovered to once again have a cumulative surplus of water [Fig. 2, Cum(P-ET)] and had a similar evapotranspiration rate to those observed predrought (Fig. 2, Cum.ET). On the other hand,

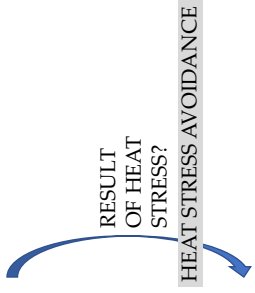
miscanthus showed lower postdrought ET, suggesting possible lag in recovering from the previous year's drought. The difference in the cumulative P-ET between miscanthus and the other perennials in 2013 suggests a greater amount of water needed by miscanthus and for soil recharge. The soil moisture content at 100 cm belowground confirms that by June 2013 the soil at the miscanthus plot reached nearly the same water content (0.3 wfv) as switchgrass (0.35 wfv). It is likely that despite the wet conditions of this year, the very low water availability at the miscanthus field at the start of 2013 limited the ET (and productivity as discussed below), and this suggests that the majority of the precipitation is used to recharge the soil under the miscanthus field in 2013.

In fact, when considering the drought response behavior, the categorization of species as (an)isohydric is based on leaf water potential measurements by definition; however, the observed response of miscanthus relative to the other ecosystems is consistent with anisohydric responses, whereby a species tends to continue evapotranspiring despite experiencing conditions that cause isohydric species to close their stomata. The apparent drought-avoidance water-saving strategy early in the drought allowed for miscanthus to maintain productivity later than for the other ecosystems (e.g., Fig. 4), but at the expense of significant water use later, at the peak of drought, following a drought-avoidance water spender strategy at this time (Table 2). Once the pool of water begins to deplete, the rates of ET will necessarily decline, and likely causing an eventual drop in productivity, as observed later in the drought. The response of the other ecosystems, which tend to follow a response more typical of isohydric species, shows relatively less variation during the drought year and a decline in productivity as soon as the drought conditions intensified. While there was a discrepancy in the water consumption of maize and switchgrass/prairie in the early time of drought, all these ecosystems followed a water-saving strategy when drought intensified, suggesting stomatal regulation in response to increasing VPD. These results highlight that the differences between what are likely isohydric vs. anisohydric species are relatively small during nonstress years but that the differences are amplified during a drought.

The observed stress resistance strategies can be explained by understanding the dynamics of instantaneous water and carbon fluxes of the ecosystems (Fig. 4). At the beginning of drought (*Phase 1*: DOY <170), VPD reached ~3 kPa, which has been reported to trigger stomata closure of several species (Li *et al.*, 2003; Vitale *et al.*, 2007; Aires *et al.*, 2008; Yang *et al.*, 2012). After the water spender behavior (high ET) of switchgrass and prairie at the early stage of drought, the

Table 2 Summary of drought response dynamics of the four ecosystems during the 2012 drought in the Midwestern US.

	Maize	Switchgrass, prairie	Miscanthus
Early drought	Short root system	Relatively deep root system. Access to soil moisture	Deep root system. Access to soil moisture
	Low water consumption	Relatively high water consumption	Low water consumption. Good water use efficiency (WUE)
	Low ET Limited annual yield	High ET High productivity and annual yield	Low ET High productivity and annual yield
Prolonged drought (peak of drought and heat stress)	Water saver	Drought avoidance – water spender	Drought avoidance – but water saver
	Short root system	Relatively deep root system. Available soil moisture at deep soil layers	Deep root system. Exhausting deep soil moisture
	Low water consumption Low ET Limited annual yield	Low water consumption Low ET Reduced productivity. However, average annual yield due to long growing season	High water consumption High ET Reduced productivity. Reduced, but still high annual yield due to long growing season
Stress resistance behavior	Water saver	Water saver	Drought avoidance – water spender
	Isohydic	Isohydic	Anisohydric



[Table 2 and a word in 2nd Paragraph of Materials and Methods was corrected on 7th June 2017 after original online publication.]

decline in ET observed at DOY ~150 (Figs 2 and 4) suggests that these species indeed regulated stomata conductance due to the increasing VPD and decrease in soil moisture, consistent with isohydric responses. On the contrary, miscanthus prevented water loss until the drought intensification, following a water saver strategy in the earlier phase of the drought (VPD ~3 kPa). Our results are consistent with what has been shown for miscanthus under controlled environmental conditions to have no significant change in stomata conductance during mild drought, but a vigorous response of stomata closure during severe drought conditions (Clifton-Brown & Lewandowski, 2000). At DOY 170, the rapid increase in water deficit observed for miscanthus was due to exceptionally large ET until DOY 220. During this time (*Phase 2*; DOY 170–200), ET of miscanthus ranked among the highest observed for all ecosystems for all years with a peak of 8 mm at DOY ~200. This high ET was driven by the peak of VPD, reaching a maximum of 5.1 kPa, combined with extreme high temperatures up to 40 °C between DOY 170 and DOY 220. Although stomata closure to extreme drought is expected and has been measured before (Ings *et al.*, 2013), we believe that our results were additionally influenced by an effect of extreme high temperature (in addition to VPD reaching 5 kPa), which might have triggered the large peak of instantaneous ET for the benefit of a cooling effect, and following a heat stress avoidance strategy at the peak of the drought. In comparison, switchgrass and prairie followed a water saver strategy in response to higher VPD (for prairie refer to the rate of increased water deficit in Fig. 2, or instantaneous ET presented in Fig. 3), or by chemical signaling originating from the roots upon the detection of decreased soil moisture (Schachtman & Goodger, 2008). Crafts-Brandner & Salvucci (2002) reported that leaf-level transpiration rates in maize increased progressively with leaf temperature and peaked above 40 °C, which they suggested indicates that stomata closure was not a factor at higher temperatures. This response is inconsistent with our maize genotypes, but similar to what we observed for miscanthus under field conditions and at the canopy scale. However, our results were more likely complicated by the impact of high VPD, heat stress, and water stress together. Furthermore, previous studies are also generally limited to young plants and/or limited soil depth, whereas the deep roots of miscanthus likely play a large role in its observed drought response. Mann *et al.* (2013a,b) suggested that miscanthus employed a drought tolerance strategy by holding back above- and belowground biomass production, while switchgrass employed a drought-avoidance strategy of growing roots deep into regions of available soil moisture to cope with increasing surface soil

moisture deficit. However at our field, where miscanthus likely already reached a mature stage (4 years old), its deep roots access water that other species could not, and with a water spending strategy typical to anisohydric species, led to the highest water deficit of this species. Aires *et al.* (2008) estimated a potential ET, representing the maximum expected ET from a wet soil-plant surface to be as high as 7 mm for a C3/C4 ecosystem, which would support our results in case of miscanthus lacking the detection of drought by its root system. After DOY 200, during the peak of the drought, the soil moisture that miscanthus was able to reach in deeper soil layers likely became progressively limiting, inducing a lack of water supply for ET (*Phase 3*; DOY 200–220). This likely resulted the leaves to become heat stressed with reduced NEP. The loss in productivity associated with miscanthus at peak drought in response to combined heat and drought stress is supported by Ghannoum (2009), who described a three-phase response to drought of C4 species; Phase 1 mainly controlled by stomata, which may or may not result a decline in CO₂ assimilation rates, followed by a mixed stomatal and nonstomatal Phase 2, and finally a nonstomatal Phase 3, when reduced enzyme activities, early senescence and nitrate assimilation play a dominant role. After this extreme portion of the drought, the cumulative P-ET reached a stable minimum point (approx. –275 mm in the case of miscanthus; *Phase 4*, DOY > 220), followed by recovery associated with precipitation events. Note, that while the largest water deficit during the drought for prairie was similar to miscanthus, the dynamic of ET of prairie was more like that of switchgrass, having an increase in the ET starting early in the season, probably compensating for the lack of precipitation and high temperatures.

Enhanced net ecosystem productivity and reduced water-use efficiency of miscanthus in the drought year

The combination of severe drought and heat stress resulted in a decline of NEP for all ecosystems in the second half of the summer in 2012 (Fig. 3), but the annual cumulative NEP and GPP were still large for the perennials, with the highest annual yield for miscanthus (Table 1). Previous studies have concluded that miscanthus (*Miscanthus × giganteus* in particular) is sensitive to limited water availability (Clifton-Brown & Lewandowski, 2000; Ings *et al.*, 2013; Mann *et al.*, 2013b), which has a strong negative effect on the species' yield production (Heaton *et al.*, 2004). In a glass-house experiment, miscanthus was reported with 56–66% reduction in biomass production due to drought conditions (Mann *et al.*, 2013a). On the contrary, during a severe drought experiment miscanthus was suggested

to employ a drought tolerant strategy; that is, it continued to function despite water stress indicating the lack of drought adaptation (Ings *et al.*, 2013). In our case, the unexpectedly high annual cumulative NEP and GPP were due to the long growing season in 2012 and the deep soil water availability, which also explained the contradiction with the declined NEP during the drought stress (Fig. 4). The high temperature (40 °C) and depleting soil moisture during our field experiment suggest that higher temperatures may be inhibiting photosynthetic carbon uptake by enzyme breakdown, or stimulating autotrophic respiration. Although C4 plants have a higher temperature optimum than C3 plants, photosynthesis is usually inhibited when leaf temperature exceed about 38 °C (Berry & Bjorkman, 1980; Edwards & Walker, 1983), due to the inactivation of Rubisco (Edwards *et al.*, 2001; Ruiz-Vera *et al.*, 2013). As a result of altered ET and NEP during the 2012 drought episode, ecosystem WUE ($WUE_{eco} = NEP/ET$) of miscanthus was slightly reduced (Fig. 3) relative to other years but was higher than other species.

Large intraspecies variation is expected depending on the temperature and drought tolerance of the particular species, supported by our previous observations of these ecosystems (Joo *et al.*, 2016). While miscanthus maintained relatively high productivity until the end of the 2012 growing season, switchgrass and prairie had a clear shift in NEP, with much earlier decline in their productivity in 2012 than in 2011 (Fig. 3). The WUE_{eco} of these two perennial species were much less influenced by the drought, which suggest that the faster response of switchgrass and prairie to drought may provide a more stable performance in the long term. In 2013, the growing season immediately following the drought, all perennials including miscanthus provided similar NEP and WUE_{eco} than in 2011, which was a decline for miscanthus in comparison with the 2012 values (Fig. 3 and Table 1). Postdrought, miscanthus was assumed to be recovering from the drought based on the reduced ET rates relative to other nondrought years, unlike the other perennials during this relatively cool and wet summer. This could explain the drop in the productivity of miscanthus compared to the previous year, and to the long-term trend of increasing annual yields. While miscanthus still provided the largest annual GPP and NEP among the studied ecosystems in 2013, a larger impact is expected in the case of sequenced drought years. Overall, the perennials, especially miscanthus demonstrated better WUE_{eco} than maize and soybean (Table 1).

During the nonstressed years (2009–2010), there was no clear trend in VPD normalized ET, although miscanthus typically had lower values than switchgrass and prairie (Fig. 5). This relationship is a proxy for

integrated canopy conductance (Bernacchi & VanLoocke, 2015), suggesting that miscanthus may have a lower canopy conductance than the other species. During the 2 years where precipitation was limiting (2011 and 2012), including the drought of 2012, the overall ET/VPD was lowest for all species, pointing at a larger withholding of water these years. An alteration among the ecosystems is also noted, miscanthus having the largest VPD normalized ET among the ecosystems, which is consistent with our results suggesting that miscanthus lacks stomata regulation during the onset of drought. All species recovered in the year following the drought, with values higher than any other year, but miscanthus again showed a lower value than the other ecosystems. Despite this, intrinsic WUE_{GPP} based on VPD normalized ET was higher for miscanthus in the postdrought year than for the other species (Fig. 5d). In the case of prairie and switchgrass, this can be explained by the rainy growing season in 2013 (low temperature, high humidity, low VPD), as their annual ET and GPP values were similar to previous years (Figs 2 and 3). On the other hand, miscanthus had additional effects from declined GPP and ET besides the low VPD in 2013. The higher intrinsic WUE_{GPP} of miscanthus also suggests that the canopy conductance of CO₂ to photosynthesis is less limiting for miscanthus than for the other ecosystems.

The 2012 growing season was exceptionally dry and warm in comparison with long-term averages, with maximum daily temperatures reaching 35–40 °C for over a month, which resulted to a peak VPD up to 5 kPa. As a consequence, the drought of 2012 resulted in a large divergence of ET and water deficit among the ecosystems, when all species experienced water deficit, and miscanthus showing divergent responses relative to the other three ecosystems. While switchgrass and prairie showed a gradually enhanced transpiration earlier, miscanthus had a rapid increase leading to a peak of ET later in the drought. At the same time, when temperature reached 40 °C, a drop in NEP was observed, which was reversible after the heat stress was over. Miscanthus, with a more extensive root system likely allowed this species to access deep soil moisture during the drought, which in combination with the large ET resulted in the eventual exhaustion of soil water content, a response consistent with anisohydric species. Overall, miscanthus provided the largest annual NEP, which was due to the early start of the growing season and maintained productivity over the drought relative to the other ecosystems. Although WUE of miscanthus declined slightly during the drought, this ecosystem still provided the best water-use efficiency among the studied ecosystems, independent of the WUE metric. While one can argue that the productivity of miscanthus was

still the highest among the crops after the drought, this ecosystem was observed to be much more sensitive to severe drought (large water loss and exhaustion of soil moisture during the drought, and reduced productivity in the following year), in agreement with controlled environmental studies. Therefore, a continued decline in both the productivity and the enhanced ET is assumed for miscanthus in sequenced drought episodes, while the other crops might follow a direct (short term) response with stable performance in the long term. While our study showed that a wet year following the drought can recharge soil moisture and recover miscanthus, our results of high ET during the drought suggest that a potential ecosystem-climate feedback with large-scale establishment of miscanthus throughout regions exposed to prolonged drought may intensify drought occurrence through maintained supply of moisture to the atmosphere. However, miscanthus could still provide good ecosystem service at wet regions not exposed to extreme high temperatures.

Acknowledgements

The authors are grateful to Timothy A. Mies and his crew for the nonstop management, maintenance, and farm work at the field site. We thank Michael D. Masters, Jesse N. Miller, and undergraduate assistance for their help at the field through the study. This research was funded by British Patrol via the Energy Biosciences Institute.

References

- Aires LM, Pio CA, Pereira JS (2008) The effect of drought on energy and water vapour exchange above a mediterranean C3/C4 grassland in Southern Portugal. *Agricultural and Forest Meteorology*, **148**, 565–579.
- Anderson-Teixeira K, Masters M, Black C, Zeri M, Hussain M, Bernacchi C, DeLucia E (2013) Altered belowground carbon cycling following land-use change to perennial bioenergy crops. *Ecosystems*, **16**, 508–520.
- Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Sciences*, **27**, 377–412.
- Ball JT, Woodrow I, Berry J (1987) A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In: *Progress in Photosynthesis Research* (ed. Biggins J), pp. 221–224. Springer, Dordrecht, the Netherlands.
- Bernacchi CJ, VanLoocke A (2015) Terrestrial ecosystems in a changing environment: a dominant role for water. *Annual Review of Plant Biology*, **66**, 599–622.
- Bernacchi CJ, Kimball BA, Quarles DR, Long SP, Ort DR (2007) Decreases in stomatal conductance of soybean under open-air elevation of [CO₂] are closely coupled with decreases in ecosystem evapotranspiration. *Plant Physiology*, **143**, 134–144.
- Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology*, **31**, 491–543.
- Borrell A, Jordan D, Mullet J, Henzell B, Hammer G, Ribaut J (2006) Drought adaptation in sorghum. In: *Drought Adaptation in Cereals*, (ed. Ribaut J-M), pp. 335–399. The Hawath Press Inc., Philadelphia, PA
- Ciais P, Reichstein M, Viovy N *et al.* (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature*, **437**, 529–533.
- Clifton-Brown JC, Lewandowski I (2000) Water use efficiency and biomass partitioning of three different miscanthus genotypes with limited and unlimited water supply. *Annals of Botany*, **86**, 191–200.
- Collins M, Knutti R, Arblaster JM *et al.* (2013) Long-term climate change: projections, commitments and irreversibility. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Qin D, Plattner G-K *et al.*), pp. 1029–1136. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Coumou D, Rahmstorf S (2012) A decade of weather extremes. *Nature Climate Change*, **2**, 491–496.
- Crafts-Brandner SJ, Salvucci ME (2002) Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. *Plant Physiology*, **129**, 1773–1780.
- Davis S, Parton W, Dohleman F, Smith C, Grosso S, Kent A, DeLucia E (2010) Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in a *Miscanthus × giganteus* agro-ecosystem. *Ecosystems*, **13**, 144–156.
- Eamus D, Taylor DT, Macinnis-Ng CMO, Shanahan S, De Silva L (2008) Comparing model predictions and experimental data for the response of stomatal conductance and guard cell turgor to manipulations of cuticular conductance, leaf-to-air vapour pressure difference and temperature: feedback mechanisms are able to account for all observations. *Plant, Cell and Environment*, **31**, 269–277.
- Edwards G, Walker D (1983) *C3, C4: Mechanisms, and Cellular and Environmental Regulation, of Photosynthesis*. Univ of California Press, Berkeley, CA, USA.
- Edwards GE, Furbank RT, Hatch MD, Osmond CB (2001) What does it take to be C4? Lessons from the evolution of C4 photosynthesis. *Plant Physiology*, **125**, 46–49.
- Eichelmann E, Wagner-Riddle C, Warland J, Deen B, Voroney P (2016) Carbon dioxide exchange dynamics over a mature switchgrass stand. *Global Change Biology – Bioenergy*, **8**, 428–442.
- Fletcher AL, Sinclair TR, Allen LH Jr (2007) Transpiration responses to vapor pressure deficit in well watered ‘slow-wilting’ and commercial soybean. *Environmental and Experimental Botany*, **61**, 145–151.
- Foken T, Göckede M, Mauder M, Mahr L, Amiro B, Munger W (2005) Post-field data quality control. In: *Handbook of Micrometeorology* (eds Lee X, Massman W, Law B), pp. 181–208. Springer, Dordrecht, the Netherlands.
- Franks PJ, Farquhar GD (1999) A relationship between humidity response, growth form and photosynthetic operating point in C3 plants. *Plant, Cell and Environment*, **22**, 1337–1349.
- Ghannoum O (2009) C4 photosynthesis and water stress. *Annals of Botany*, **103**, 635–644.
- Giorio P, Sorrentino G, d’Andria R (1999) Stomatal behaviour, leaf water status and photosynthetic response in field-grown olive trees under water deficit. *Environmental and Experimental Botany*, **42**, 95–104.
- Heaton E, Voigt T, Long SP (2004) A quantitative review comparing the yields of two candidate C4 perennial biomass crops in relation to nitrogen, temperature and water. *Biomass and Bioenergy*, **27**, 21–30.
- Heaton EA, Dohleman FG, Miguez AF *et al.* (2010) Chapter 3 – Miscanthus: a promising biomass crop. In: *Advances in Botanical Research* (eds Jean-Claude K, Michel D), pp. 75–137. Academic Press, Cambridge, MA, USA.
- Hsieh C-I, Katul G, Chi T-W (2000) An approximate analytical model for footprint estimation of scalar fluxes in thermally stratified atmospheric flows. *Advances in Water Resources*, **23**, 765–772.
- Hussain MZ, VanLoocke A, Siebers MH *et al.* (2013) Future carbon dioxide concentration decreases canopy evapotranspiration and soil water depletion by field-grown maize. *Global Change Biology*, **19**, 1572–1584.
- Ings J, Mur LAJ, Robson PRH, Bosch M (2013) Physiological and growth responses to water deficit in the bioenergy crop *Miscanthus × giganteus*. *Frontiers in Plant Science*, **4**, 1–12.
- Joo E, Hussain MZ, Zeri M *et al.* (2016) The influence of drought and heat stress on long term carbon fluxes of bioenergy crops grown in the Midwestern US. *Plant, Cell and Environment*, **39**, 1928–1940.
- Kholová J, Hash CT, Kumar PL, Yadav RS, Kočová M, Vadez V (2010) Terminal drought-tolerant pearl millet [*Pennisetum glaucum* (L.) R. Br.] have high leaf ABA and limit transpiration at high vapour pressure deficit. *Journal of Experimental Botany*, **61**, 1431–1440.
- Li S-G, Lai C-T, Yokoyama T, Oikawa T (2003) Carbon dioxide and water vapor exchange over a Miscanthus-type grassland: effects of development of the canopy. *Ecological Research*, **18**, 661–675.
- Macfarlane C, White DA, Adams MA (2004) The apparent feed-forward response to vapour pressure deficit of stomata in droughted, field-grown *Eucalyptus globulus* Labill. *Plant, Cell and Environment*, **27**, 1268–1280.
- Mann JJ, Barney JN, Kyser GB, Di Tomaso JM (2013a) *Miscanthus × giganteus* and *Arundo donax* shoot and rhizome tolerance of extreme moisture stress. *Global Change Biology Bioenergy*, **5**, 693–700.
- Mann JJ, Barney JN, Kyser GB, DiTomaso JM (2013b) Root system dynamics of *Miscanthus × giganteus* and *Panicum virgatum* in response to rainfed and irrigated conditions in California. *Bioenergy Research*, **6**, 678–687.

- Maroco JP, Pereira JS, Manuela Chaves M (1997) Stomatal responses to leaf-to-air vapour pressure deficit in Sahelian species. *Functional Plant Biology*, **24**, 381–387.
- Monteith J (1995) A reinterpretation of stomatal responses to humidity. *Plant, Cell and Environment*, **18**, 357–364.
- Oren R, Sperry J, Katul G, Pataki D, Ewers B, Phillips N, Schafer K (1999) Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant Cell and Environment*, **22**, 1515–1526.
- Pingintha N, Leclerc MY, Beasley JP Jr, Durden D, Zhang G, Senthong C, Rowland D (2010) Hysteresis response of daytime net ecosystem exchange during drought. *Biogeosciences*, **7**, 1159–1170.
- Rahmstorf S, Coumou D (2011) Increase of extreme events in a warming world. *Proceedings of the National Academy of Sciences*, **108**, 17905–17909.
- Reichstein M, Falge E, Baldocchi D *et al.* (2005) On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm. *Global Change Biology*, **11**, 1424–1439.
- Ruiz-Vera UM, Siebers M, Gray SB *et al.* (2013) Global warming can negate the expected CO₂ stimulation in photosynthesis and productivity for soybean grown in the Midwest United States. *Plant Physiology*, **162**, 410–423.
- Schachtman DP, Goodger JQD (2008) Chemical root to shoot signaling under drought. *Trends in Plant Science*, **13**, 281–287.
- Sinclair TR, Hammer GL, Van Oosterom EJ (2005) Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. *Functional Plant Biology*, **32**, 945–952.
- Tilman D, Hill J, Lehman C (2006) Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science*, **314**, 1598–1600.
- Vitale L, Di Tommasi P, Arena C, Fierro A, Virzo De Santo A, Magliulo V (2007) Effects of water stress on gas exchange of field grown *Zea mays* L. in Southern Italy: an analysis at canopy and leaf level. *Acta Physiologiae Plantarum*, **29**, 317–326.
- Wagle P, Kakani VG (2014) Environmental control of daytime net ecosystem exchange of carbon dioxide in switchgrass. *Agriculture, Ecosystems & Environment*, **186**, 170–177.
- Webb EK, Pearman GI, Leuning R (1980) Correction of flux measurements for density effects due to heat and water vapour transfer. *Quarterly Journal of the Royal Meteorological Society*, **106**, 85–100.
- Wherley BG, Sinclair TR (2009) Differential sensitivity of C3 and C4 turfgrass species to increasing atmospheric vapor pressure deficit. *Environmental and Experimental Botany*, **67**, 372–376.
- Yang Z, Sinclair TR, Zhu M, Messina CD, Cooper M, Hammer GL (2012) Temperature effect on transpiration response of maize plants to vapour pressure deficit. *Environmental and Experimental Botany*, **78**, 157–162.
- Zaman-Allah M, Jenkinson DM, Vadez V (2011) Chickpea genotypes contrasting for seed yield under terminal drought stress in the field differ for traits related to the control of water use. *Functional Plant Biology*, **38**, 270–281.
- Zeri M, Anderson-Teixeira K, Hickman G, Masters M, DeLucia E, Bernacchi CJ (2011) Carbon exchange by establishing biofuel crops in Central Illinois. *Agriculture, Ecosystems & Environment*, **144**, 319–329.
- Zeri M, Hussain MZ, Anderson-Teixeira KJ, DeLucia E, Bernacchi CJ (2013) Water use efficiency of perennial and annual bioenergy crops in central Illinois. *Journal of Geophysical Research: Biogeosciences*, **118**, 581–589.
- Zhang J, Davies WJ (1990) Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. *Plant, Cell and Environment*, **13**, 277–285.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Soil moisture at 100 cm deep during the 2012 drought episode. DOY, day of year; wfv, water fraction by volume.