

## Original Article

## The influence of drought and heat stress on long-term carbon fluxes of bioenergy crops grown in the Midwestern USA

Eva Joo<sup>1,2</sup>, Mir Zaman Hussain<sup>3</sup>, Marcelo Zeri<sup>4</sup>, Michael D. Masters<sup>2</sup>, Jesse N. Miller<sup>1,2</sup>, Nuria Gomez-Casanovas<sup>1,2</sup>, Evan H. DeLucia<sup>1,2</sup> & Carl J. Bernacchi<sup>1,2,5</sup>

<sup>1</sup>Department of Plant Biology, University of Illinois, Urbana, IL 61801, USA, <sup>2</sup>Energy Bioscience Institute – Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana, IL 61801, USA, <sup>3</sup>Great Lakes Bioenergy Research Center, W.K. Kellogg Biological Station, Michigan State University, Hickory Corner, MI 49060, USA, <sup>4</sup>National Center for Monitoring and Early Warning of Natural Disasters (CEMADEN), 12.247-016, São José dos Campos, SP, Brazil and <sup>5</sup>Global Change and Photosynthesis Research Unit, USDA ARS, Urbana, IL 61801, USA

## ABSTRACT

Perennial grasses are promising feedstocks for bioenergy production in the Midwestern USA. Few experiments have addressed how drought influences their carbon fluxes and storage. This study provides a direct comparison of ecosystem-scale measurements of carbon fluxes associated with miscanthus (*Miscanthus × giganteus*), switchgrass (*Panicum virgatum*), restored native prairie and maize (*Zea mays*)/soybean (*Glycine max*) ecosystems. The main objective of this study was to assess the influence of a naturally occurring drought during 2012 on key components of the carbon cycle and plant development relative to non-extreme years. The perennials reached full maturity 3–5 years after establishment. Miscanthus had the highest gross primary production (GPP) and lowest net ecosystem exchange (NEE) in 2012 followed by similar values for switchgrass and prairie, and the row crops had the lowest GPP and highest NEE. A post-drought effect was observed for miscanthus. Over the duration of the experiment, perennial ecosystems were carbon sinks, as indicated by negative net ecosystem carbon balance (NECB), while maize/soybean was a net carbon source. Our observations suggest that perennial ecosystems, and in particular miscanthus, can provide a high yield and a large potential for CO<sub>2</sub> fixation even during drought, although drought may negatively influence carbon uptake in the following year, questioning the long-term consequence of its maintained productivity.

**Key-words:** maize; miscanthus; switchgrass; carbon balance; ecosystem development; eddy covariance; gross primary production; net ecosystem exchange; prairie.

## INTRODUCTION

Direct combustion and/or conversion to liquid fuel of lignocellulosic plant material holds promise as a more efficient and environmentally beneficial alternative to both fossil fuel use and current maize-based ethanol production. In addition to

biofuel production, certain lignocellulosic feedstocks may provide ecosystem services, such as long-term ecosystem carbon storage. However, variation exists whether an ecosystem is a carbon sink or a source, because of a range of factors including the type of crop (Moureaux *et al.* 2006; Aubinet *et al.* 2009; Béziat *et al.* 2009; Prescher *et al.* 2010; West *et al.* 2010), management practices (Bernacchi *et al.* 2005; Hollinger *et al.* 2005; Verma *et al.* 2005; Ceschia *et al.* 2010), geographical location (Béziat *et al.* 2009; VanLoocke *et al.* 2012) and climatic conditions (Glenn *et al.* 2010; Eichelmann *et al.* 2016). Therefore, direct comparisons of different species are important to estimate the environmental impact of land cover change. Furthermore, extreme environmental conditions are predicted to increase with future climate change and species responses are likely to be highly variable (Collins *et al.* 2013). It is therefore critical to assess which bioenergy feedstocks are best suited to maintain a high productivity and a high sequestration potential during extreme conditions.

Maize, typically grown in rotation with soybean in the Midwestern USA, is presently the dominant bioenergy feedstock globally. Maize has very high nitrogen requirements, and only the grain is generally harvested for ethanol production. With conventional, frequently tilled management, this agro-ecosystem is a net carbon source to the atmosphere (Lal 2004; Sisti *et al.* 2004; Anderson-Teixeira *et al.* 2009; Zeri *et al.* 2011). Two perennials, miscanthus (particularly *Miscanthus × giganteus*) and switchgrass, have been identified as promising advanced bioenergy feedstocks because of their high yield (e.g. 29.6 versus 17.6 t ha<sup>-1</sup> harvestable biomass for miscanthus versus maize) and high energy potential, high water use efficiency and lower fertilization requirements relative to most annual row crops (McLaughlin & Adams Kszos 2005; Heaton *et al.* 2008, 2010; Liebig *et al.* 2008). Low-input high-diversity grasslands have also been considered as potential ecosystems for bioenergy production, as they can provide higher energy output per unit invested energy. Therefore, as alternative bioenergy crops, these species were selected for our study to determine the environmental impact of traditional (maize) and perennial feedstocks (miscanthus, switchgrass, prairie) on the major carbon cycle components of

Correspondence: C. J. Bernacchi. E-mail: bernacch@illinois.edu

the Midwestern USA. Perennial agro-ecosystems act as carbon sinks under typical agronomic conditions (Ma *et al.* 2000; Hansen *et al.* 2004; Clifton-Brown *et al.* 2007; Lee *et al.* 2007; Zeri *et al.* 2011; VanLoocke *et al.* 2012; Zimmermann *et al.* 2012; Wagle & Kakani 2014a); however, there is uncertainty regarding whether these ecosystems will act as long-term carbon sinks, particularly when presented under climate extremes, such as heat stress and drought (Mann *et al.* 2013a).

There is considerable variation in drought tolerance among potential bioenergy feedstocks. For example drought induced in a controlled pot experiment resulted in early senescence in *M. × giganteus* and *Miscanthus sacchariflorus* but not for *Miscanthus sinensis* (Clifton-Brown & Lewandowski 2000). Productivity is maintained through a drought-avoidance strategy for switchgrass grown in pots under a wide range of soil moisture conditions, explained by extensive root development, but not for miscanthus (Mann *et al.* 2013b). However, the dynamics of species responses to extreme environments is likely to vary between potted experiments and natural growth environments, where a range of rooting depths, competition from neighbours and environmental drivers may co-occur.

Observing the long-term dynamics of carbon fluxes into and out of an ecosystem is necessary to assess whether an ecosystem is a net carbon source or sink (Anderson-Teixeira *et al.* 2013). Many measurement strategies cannot accurately assess the long-term impact of extreme climatic events, such as drought, on individual ecosystems. The eddy covariance technique allows continuous measurements of ecosystem fluxes over annual cycles and can be used to evaluate the carbon budgets for different cropping ecosystem by directly measuring the net ecosystem exchange (NEE) of CO<sub>2</sub> (Aubinet *et al.* 1999; Baker & Griffis 2005; Reichstein *et al.* 2005; Moureaux *et al.* 2006). Measuring the carbon removed during harvest coupled with NEE determines the net ecosystem carbon balance (NECB), providing information on the total rate of organic carbon accumulation (or loss) from ecosystems (Chapin *et al.* 2006). Together, these components of ecosystem carbon cycles reflect two major ecosystem services, harvestable yield and carbon storage capabilities.

The objective of this study was to investigate the long-term carbon fluxes, canopy development and yield on four ecosystems. This experiment included the most severe drought affecting the Midwestern USA in the last 100 years (Mallya *et al.* 2013), characterized by a substantial decline in precipitation coupled with extreme temperatures. This study utilized long-term side-by-side measurements of CO<sub>2</sub> fluxes by using the eddy covariance technique coupled with *in situ* meteorological measurements from 2009 to 2013. This research built upon previous research from this experiment (Zeri *et al.* 2011) by adding additional years to the analysis, including the 2012 drought.

## MATERIALS AND METHODS

### Site and climate data

The experiment was conducted at the Energy Farm of University of Illinois Urbana-Champaign (UIUC), IL, USA (40°3′46.209″N, 88°11′46.0212″W, ~220 m above sea level). The climate of the region is seasonal with monthly average air

temperatures averaging below 0 °C from December to February and above 20 °C from June until August. The mean annual temperature is 11.1 °C, and the mean annual precipitation is 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). Prior to plantation of the investigated species in 2008, the land was in continuous long-term cultivation of annual crops.

### Experimental design and management practices

Four plots (200 × 200 m each) were established in the spring of 2008 with maize (*Zea mays* L.; 6 May 2008), miscanthus (*M. × giganteus*; 2–16 June 2008), switchgrass (*Panicum virgatum* L.; 28 May 2008) and a mixture of 28 native prairie species (29 May 2008). The maize plot was established as part of a maize/soybean (*Glycine max* L.) rotation that consisted of a cycle of 2 years of maize (2008–2009, 2011–2012) followed by 1 year of soybean (2010, 2013). Because of the high mortality of newly established miscanthus in 2008, it was replanted in 2010. Nitrogen fertilizer was applied to maize as 168, 202, 180 and 168 kg ha<sup>-1</sup> nitrogen in 2008, 2009, 2011 and 2012, respectively; and switchgrass received 56 kg ha<sup>-1</sup> nitrogen yearly starting in 2010. Soybean, miscanthus and prairie were not fertilized during any years based on present management practices (Tilman *et al.* 2006; Davis *et al.* 2010; Zeri *et al.* 2011, 2013). Maize and soybean were harvested and the fields were chisel-tilled between late September and early November. Perennial species were harvested in the winter following each growing season (Table 1).

### Leaf area and biomass measurements

Leaf area index (LAI) of all species were measured optically (LAI-2200; Li-Cor Biosciences, Lincoln, NE, USA) at weekly intervals during the period of active canopy development. At each plot six to eight subsamples of measurements were made depending on the light availability. Above-ground biomass was determined for each ecosystem at the end of the growing season when the harvested and baled biomass was dried and weighed. Carbon content was measured from six samples from each ecosystem that were dried at 60 °C, homogenized, ground through a 425 μm pore size mesh in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) and ground into a powder with a ball mill (Geno/Grinder 2010; BT&C, Lebanon, NJ, USA). Plant material was weighed in tin capsules for carbon and nitrogen analysis with an elemental analyser (Costech 4010 CHNSO analyser; Costech Analytical Technologies Inc., Valencia, CA, USA) by using acetanilide and apple leaves (National Institute of Science and Technology, Gaithersburg, MD, USA) as standards.

Carbon export was calculated from the harvested biomass (g m<sup>-2</sup> year<sup>-1</sup>) multiplied by the measured fractional carbon content. The plots were harvested by using modified hay balers, and all bales were weighed for each plot to provide one biomass harvest number per plot. The Energy Farm consists of replicated plots, but only one plot of each ecosystem is

**Table 1.** Characteristics associated with productivity of the four ecosystems from 2009 to 2013

Year	Species	Emergence/ harvest	Start of productivity	End of productivity	Absorbed PPF (growing season) (mol m <sup>-2</sup> )	NEE (g m <sup>-2</sup> )	R <sub>eco</sub> (g m <sup>-2</sup> )	GPP (g m <sup>-2</sup> )	LUE (growing season) (g mol <sup>-1</sup> )
2009	Miscanthus	6 May/19 Mar.*	24 May	30 Aug.	3678	-281 ± 30	1741 ± 72	2071 ± 78	0.56
	Switchgrass	26 Mar./19 Mar.*	29 Apr.	16 Sep.	5821	-454 ± 17	1847 ± 58	2316 ± 60	0.40
	Prairie	8 Mar./19 Mar.	22 Apr.	27 Aug.	5854	-335 ± 17	1480 ± 51	1826 ± 54	0.31
	Maize	24 May/3 Nov.	8 Jun.	6 Sep.	3453	-327 ± 29	2389 ± 196	2676 ± 198	0.77
2010	Miscanthus	2 May/23 Mar.*	8 Jun.	26 Oct.	6449	-556 ± 16	1394 ± 51	1954 ± 54	0.30
	Switchgrass	12 Apr./19 Nov.	27 Apr.	10 Aug.	4826	-486 ± 25	1771 ± 74	2297 ± 78	0.48
	Prairie	25 Mar./17 Nov.	19 Apr.	4 Aug.	4760	-296 ± 20	1492 ± 61	1819 ± 64	0.38
	Soybean	31 May/12 Oct.	28 Jun.	27 Aug.	3659	107 ± 19	1316 ± 61	1210 ± 64	0.33
2011	Miscanthus	30 Apr./23 Jan.*	13 May	11 Oct.	5862	-748 ± 20	1090 ± 47	1846 ± 51	0.31
	Switchgrass	12 Apr./11 Dec.	4 May	31 Aug.	5149	-458 ± 22	1283 ± 85	1774 ± 87	0.34
	Prairie	29 Mar./18 Nov.	10 May	23 Aug.	4444	-218 ± 15	1082 ± 37	1326 ± 40	0.30
	Maize	23 May/6 Oct.	12 Jun.	23 Aug.	3627	-45 ± 22	1148 ± 65	1202 ± 68	0.33
2012	Miscanthus	21 Apr./22 Jan.*	29 Apr.	30 Sep.	6277	-1102 ± 40	1280 ± 71	2375 ± 81	0.38
	Switchgrass	23 Mar./28 Nov.	3 May	28 Aug.	6060	-503 ± 18	1435 ± 52	1937 ± 55	0.32
	Prairie	17 Mar./20 Nov.	7 Apr.	28 Jul.	3601	-341 ± 13	1595 ± 57	1937 ± 59	0.54
	Maize	5 May/20 Sep.	29 May	18 Jul.	3374	-1 ± 11	1414 ± 73	1415 ± 74	0.42
2013	Miscanthus	8 May/23 Mar.*	28 May	21 Oct.	4981	-772 ± 37	1149 ± 52	1920 ± 63	0.39
	Switchgrass	1 May/12 Dec.	19 May	15 Sep.	3880	-531 ± 24	1365 ± 44	1897 ± 50	0.49
	Prairie	7 Apr./26 Nov.	24 Apr.	24 Aug.	3427	-389 ± 21	1579 ± 54	1906 ± 58	0.56
	Soybean	23 May/28 Oct.	23 Jun.	28 Aug.	2346	-12 ± 13	1273 ± 39	1285 ± 41	0.55

The error given for NEE, R<sub>eco</sub> and GPP is the interquartile range, taking into account the gap-filling error and the random error. Asterisks denote a harvest date that occurred in the following calendar year.

PPFD, photosynthetic photon flux density; NEE, net ecosystem exchange; GPP, gross primary production; NEE, net ecosystem exchange.

large enough to accommodate eddy covariance measurements. Therefore, the biomass measurement for the eddy covariance plots are reported only for the plots where the sensors were situated, but error estimates for biomass are reported as the standard error for all five replicate plots.

Net ecosystem carbon balance was computed by subtracting the amount of carbon removed from the ecosystem via harvest from the annual net ecosystem productivity (NEP) (Chapin *et al.* 2006). Other fluxes of carbon from the ecosystems, including leaching of dissolved organic and inorganic carbon or methane losses, were not included as leaching of organic and inorganic carbon is calculated as 20 g m<sup>-2</sup> year<sup>-1</sup> or about 1–2% of observed annual NEE (M. David, unpublished). For maize, NECB was calculated by subtracting carbon removed from the ecosystem from cumulative NEE, which was harvested grain for maize and soybean and total biomass harvest from the perennial ecosystems. Emergence and senescence dates for each ecosystem (Table 1) was determined based on visual inspection of archived images (Axis 211M network camera, Axis Communications Lund, Sweden) collected hourly throughout the experiment. The start and end dates of productivity period was based on the first day that net daily NEE became negative in the spring (start of the productivity period) and then became positive in the fall (end of the productivity period).

### Flux and meteorological measurements

Each eddy covariance system consisted of a three-dimensional (3D) sonic anemometer (model 81000VRE, R.M. Young

Company, Traverse City, MI, USA) and an open-path infrared gas analyser (IRGA; model LI-7500; Li-Cor Biosciences) situated in the centre of each ecosystem plot. Each eddy covariance system included meteorological sensors to monitor temperature and relative humidity (HMP-45C; Campbell Scientific, Logan, UT, USA), up- and down-welling short- and long-wave radiation (CNR1, Delft, Kipp & Zonen, the Netherlands), canopy surface temperature (SI-121 or SI-111 infrared radiometers; Apogee Instruments, Logan, Utah, USA), up- and down-welling photosynthetic photon flux density (PPFD) (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). Soil heat flux plates (two per plot) were buried at a 10 cm soil depth, while all the other sensors were mounted at 4.5 m above ground. Up- and down-welling PPF results were used to calculate absorbed PPF by subtracting the incoming and the reflected radiation. The sonic anemometer and the LI-7500 sensors were elevated after plant growth to ensure that the sensors remain above the roughness canopy layer. A full description of the eddy covariance system was published previously (Zeri *et al.* 2011, 2013).

### Data processing and quality control

Ecosystem fluxes were calculated from the 10 Hz data by using ALTEDDY (<http://www.climatexchange.nl/projects/alteddy/>) from 2008 until 2011 and EDDYPRO ([http://www.licor.com/env/products/eddy\\_covariance/software.html](http://www.licor.com/env/products/eddy_covariance/software.html)) in 2012–2013.

Comparisons among the software packages show negligible differences in analysis of identical data inputs (Mauder *et al.* 2008; Fratini & Mauder 2014). The high-frequency data were processed for coordinate alignment, correction of the temperature measurements by the sonic anemometer due to the influence of humidity (Schotanus *et al.* 1983) and compensation of density fluctuations occurring in the IRGA measurements (Webb *et al.* 1980). A double-rotation scheme was used to align the coordinate system to the main wind direction and make the average vertical velocity zero (Kaimal & Finnigan 1994). Data were also corrected for high-frequency data losses due to sensor separation (Moore 1986). At low turbulence conditions, the CO<sub>2</sub> storage flux was calculated as described previously (Aubinet *et al.* 2001). By using the method of Foken *et al.* (2005), the night-time data with no turbulent mixing were removed from the data set, and the data were filtered by the  $u$  threshold. The software packages used the same footprint model (Hsieh *et al.* 2000) to identify periods when the fluxes were outside the edges of the plots. If less than 70% of cumulative flux came from within the plot area, the records were removed. Quality control of the data applied by filtering out unreasonable fluxes, outside of the accepted range of  $-70 < \text{CO}_2 \text{ flux} < 30 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $-20 < LE < 600 \text{ W m}^{-2}$ ,  $-100 < H < 300 \text{ W m}^{-2}$ ,  $-40 < T_{\text{air}} < 50 \text{ }^\circ\text{C}$ ,  $0 < S_{\text{in}} < 1500 \text{ W m}^{-2}$  (Zeri *et al.* 2011). Missing data were gap-filled and the fluxes were partitioned from NEE into ecosystem respiration ( $R_{\text{eco}}$ ) and gross primary production (GPP) (Reichstein *et al.* 2005; Zeri *et al.* 2011).

Energy balance closure for the years 2011–2013 was calculated similar to the years of 2009–2010, as reported previously (Zeri *et al.* 2011) based on previously published methodology (Nobel 1974; Zeri *et al.* 2011). Referring to the same time interval as reported earlier (Zeri *et al.* 2011), energy balance closure is provided in Supporting Information Fig. S1, where  $R_n$  is the net radiation,  $H$  is sensible heat flux,  $LE$  is latent heat flux,  $G$  is soil heat flux,  $S_g$  is the heat storage in the upper soil layer and  $S_{\text{ph}}$  is the energy stored by photosynthesis. Our results (Supporting Information Figs. S1 & S2) show an imbalance in the energy budget closure between 13 and 27%, similar to previously reported values of approximately 20% (Twine *et al.* 2000; Wilson *et al.* 2002; Meyers & Hollinger 2004; Foken *et al.* 2006).

## Error estimates

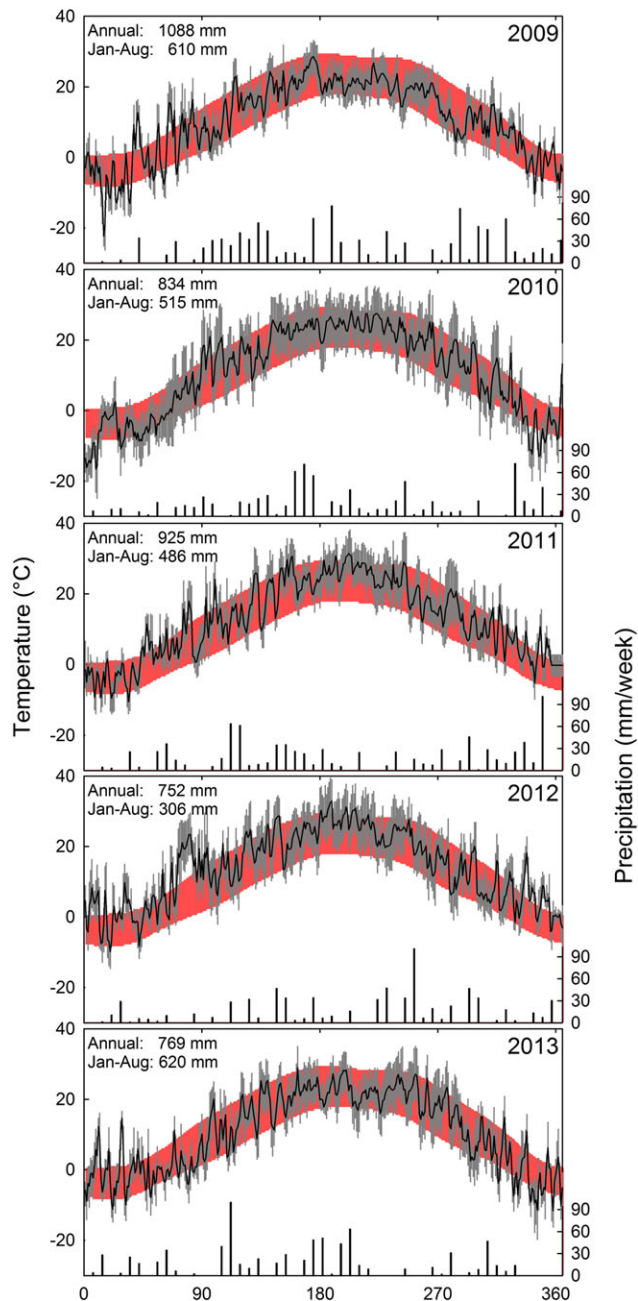
The resulting cumulative fluxes for these four agro-ecosystems are subjected to many error terms. For instance providing significance tests for NECB requires error estimates on the yield measurements; therefore, conclusions are limited to observed trends. Several error terms are associated with the eddy covariance technique when comparing multiple ecosystems, including different methods for making spectral corrections, the influence of 3D effects such as drainage and advection, underestimation of eddy covariance fluxes due to inability to measure low-frequency contributions, coordinate systems and night-time flux measurements (Massman & Lee 2002). Previous reports from the same experiment demonstrated that energy balance closure is within 15% for all four ecosystem

measured, which included data from 2008 to 2010 (Zeri *et al.* 2011). This level of error associated with energy budget closure was similar to error estimates in carbon fluxes (Twine *et al.* 2000) and energy budget closure estimates from the additional years of this study (Supporting Information Fig. S1). Estimates of error associated with gap filling for these ecosystems were about  $20 \text{ g m}^{-2} \text{ year}^{-1}$  carbon for 2008–2011 (Zeri *et al.* 2011) but have been reported as high as  $60 \text{ g m}^{-2} \text{ year}^{-1}$  carbon for other ecosystems (Black *et al.* 2007; Eichelmann *et al.* 2016). These errors suggest that the interpretation of whether all ecosystems act as carbon sources or sinks needs to be critically assessed. Here we provide error estimates for NEE as a combination of gap-filling error and random error (Table 1), which were calculated as the interquartile ranges of a sample of annual sums derived from the gap-filling and uncertainty routines. For the error of  $R_{\text{eco}}$ , confidence intervals were calculated for the parameters in the soil respiration model. Next, those intervals were used to generate 100 different versions of  $R_{\text{eco}}$  and the error reported is the interquartile range of this sample. Finally, the error of GPP was calculated as the square root of the sum of errors of NEE and  $R_{\text{eco}}$  squared ( $\text{GPP}_e = \sqrt{\text{NEE}_e^2 + R_{\text{eco}}^2}$ ). It should be noted that error associated with the design of the sonic anemometer's transducers can result in errors in the vertical wind velocity and can cause inaccuracies in the sensible heat flux on the order of ~10% (Kochendorfer *et al.* 2012; Frank *et al.* 2013), which was not included in our estimates. Furthermore, the nature of eddy covariance experiments makes spatial representation in statistical analysis prohibitively difficult. Making standardized measurements under identical forcing conditions minimizes the sensor and environmental influences that can drive differences among these four ecosystems (Massman & Lee 2002); however, inherent variation in the edaphic conditions across the UIUC Energy Farm is confounded with the observed ecosystem differences. Despite the lack of discrete spatial replication of eddy covariance, the inherent variation in spatial representation associated with a constantly changing footprint coupled with the replication over time suggests that large differences, particularly greater than the sum of known inherent errors and biases, in ecosystem fluxes over time and between ecosystem types represent meaningful influences of biological responses to environmental forcing. For example the difference in carbon being released from the maize ecosystem and being taken up by miscanthus suggests that despite the potential error associated with the measurement, our conclusion of the different source/sink potential of these species remain valid.

## RESULTS

### Meteorological conditions and vegetation development

A dominant meteorological feature for this experiment was the considerably warmer and drier conditions during part of 2011 and much of 2012 relative to long-term means (Fig. 1). During the 2009, 2010 and 2013 growing seasons, the daily mean temperatures, particularly during the growing season,



**Figure 1.** Daily mean (solid black line; left axis) and daily temperature minimum and maximum (grey error bars) for each year of the experiment along with 30 year mean monthly maximum and minimum temperatures (red bands). The weekly total precipitation is also plotted (right axis). Inset for each year is annual precipitation and the total precipitation that fell between 1 January and 1 August for each of the five years.

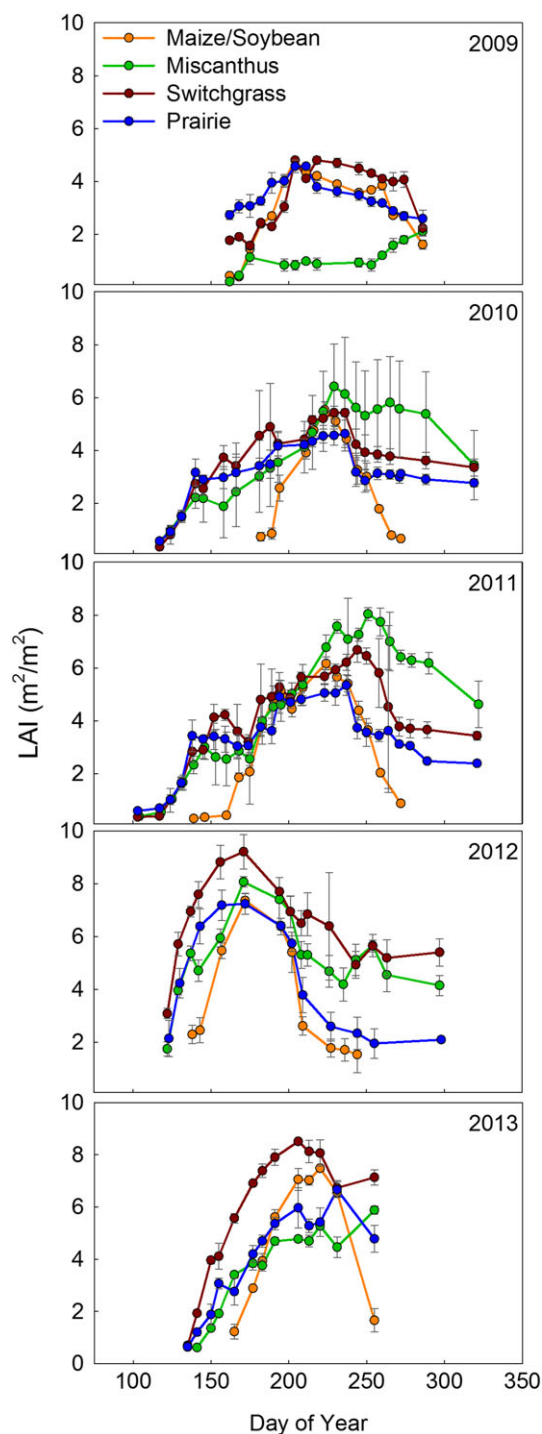
generally fell within the typical range of historic mean temperatures (within the red bands in Fig. 1). However, the mean daily temperatures during 2011 and 2012 were frequently higher than the long-term mean daily maximum temperatures, often exceeding 35°C between early July and middle September. Moreover, late winter and early spring of 2012 was exceptionally warm, with an average daily temperature

of 20°C in March. All years with the exception of 2009 (Fig. 1) fell short of the long-term (1981–2010) mean annual precipitation rate for the area of  $\sim 1050 \pm 170$  mm (<http://mrcc.isws.illinois.edu/climate/>). The distribution of rainfall events over time was generally consistent for all years with the exception of 2012, when the total amount of precipitation from January through August was approximately half relative to the other years (Fig. 1). The 2012 drought was followed by a relatively typical growing season in 2013, when the highest amount of cumulative precipitation (620 mm) was observed between January and August among the studied years. Prolonged extreme high temperatures of up to 40°C were reached during the 2012 drought, resulting in a combined drought and heat stress effect.

Detectable ecosystem productivity (net CO<sub>2</sub> uptake) was observed typically  $\sim 3$  weeks after emergence but varied among species and year (Table 1). In 2012 emergence of all ecosystems was observed 1–3 weeks, and the start of productivity 1–5 weeks earlier relative to other years. The end of productivity occurred up to 5 weeks earlier in 2012 than what is typical to each of the particular ecosystems; however, miscanthus maintained its productivity 4–7 weeks longer than the other crops. Growing season absorbed PPF was the highest for miscanthus, followed by the other perennials, except for 2009, because of poor establishment of miscanthus. Miscanthus generally showed the largest light use efficiency (LUE), particularly in the drought year of 2012. The C<sub>3</sub> crop, soybean, had an LUE of 0.19 and 0.26 g mol<sup>-1</sup> in 2010 and 2013, respectively, providing much lower values than the C<sub>4</sub> crops.

All perennial species showed a monotonic increase in the annual maximum LAI from establishment until the end of 2011 for miscanthus and until the end of 2012 for switchgrass and prairie (Fig. 2). In 2012, switchgrass reached the highest LAI among the species in that year, and it was the highest LAI switchgrass achieved in all years. In 2012 the annual maximum LAI of miscanthus was nearly the same as in 2011; however, the maximum LAI appeared around day of year (DOY) 171 (LAI = 8.1 m<sup>2</sup> m<sup>-2</sup>) in 2012, while the maximum occurred much later, DOY 229 (LAI = 6.4 m<sup>2</sup> m<sup>-2</sup>) and DOY 251 (LAI = 8.0 m<sup>2</sup> m<sup>-2</sup>), in 2010 and 2011, respectively. Despite higher precipitation in 2013, all perennials had lower maximum LAI values relative to those for 2012. During 2013, switchgrass reached the highest LAI, followed by soybean, while the LAI of miscanthus was less than that for the year before.

Prior to 2012 the progression of LAI within the season for the perennial species increased relatively consistently as the growing season progressed. All perennials, independent of year, had a longer growing season than maize or soybean. A warmer spring in 2012 led to rapid and early increases in LAI for the perennial grasses ( $\sim$ DOY 130) but also declined much earlier (in June) relative to all other years. Maize in 2012 also had an earlier start of the growing season resulting from an earlier planting date compared with other years and showed a similar early decline in LAI. During the 2013 growing season, the LAI for all ecosystems remained near the seasonal maximum for a longer duration compared with that for 2012.



**Figure 2.** Leaf area index (LAI) measured over each growing season for the four ecosystems. Symbols represent the mean  $\pm$  one standard error of the mean ( $n$  ranges from 1 to 4). In 2010 and 2013 soybean was planted instead of maize.

### Annual carbon fluxes

Daily, seasonal and annual patterns of NEE differed among the four ecosystems (Fig. 3). Net carbon sink potential (negative NEE representing carbon sink) was higher in maize relative to the perennials early in the establishment

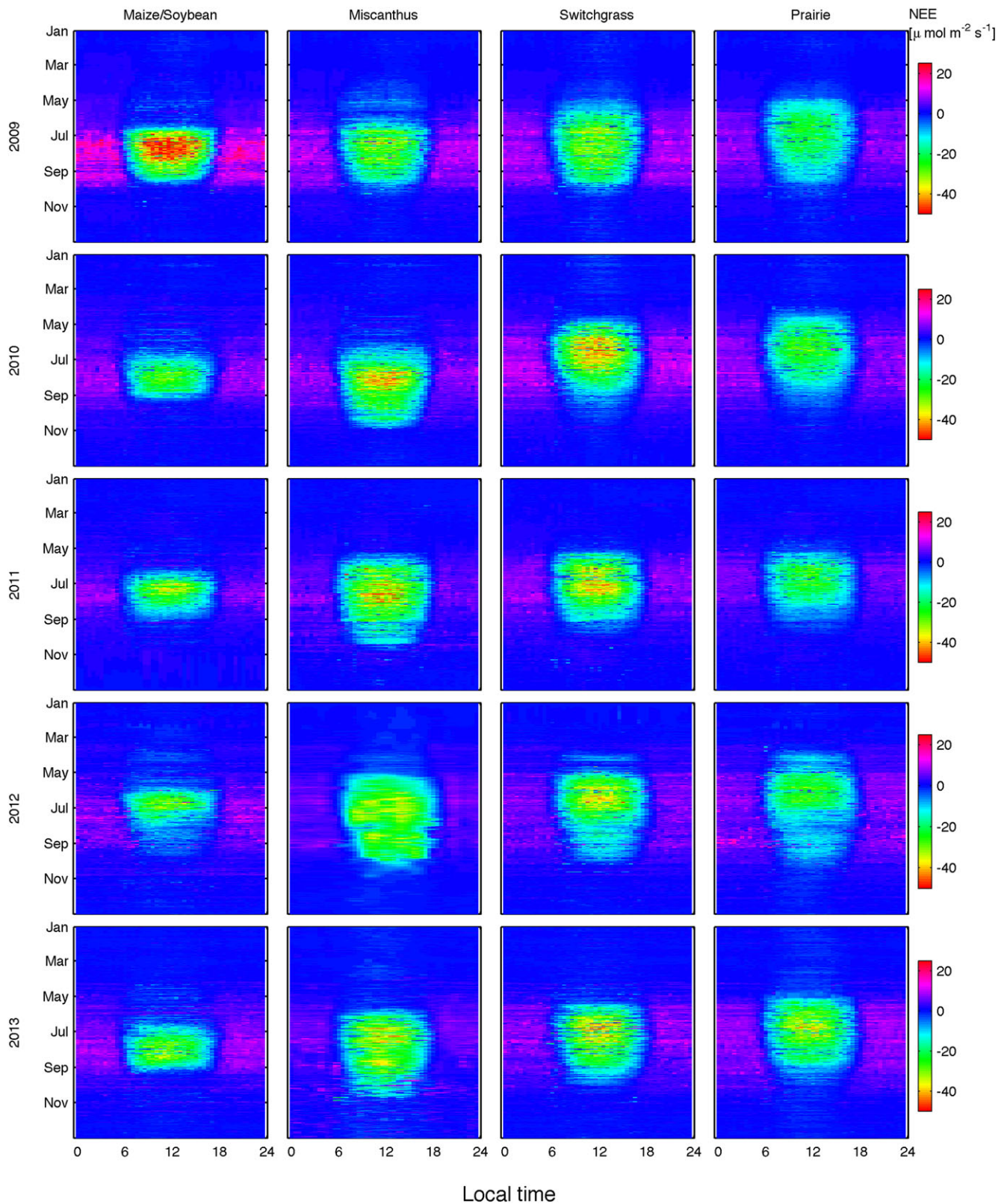
phase (2009); however, the growing season length for the perennial ecosystems became much longer in the following years. Growing season carbon sink potential for maize in 2011–2012 was similar to observations for soybean in 2010 and 2013 except that maize became a net carbon sink earlier in the growing season. In 2012 net carbon uptake began earlier for all ecosystems than observed in previous years; however, it began to decline much earlier in the growing season, with the exception of miscanthus. Maize showed a relatively abrupt decrease in net carbon uptake at the beginning of July, much earlier than for maize or soybean in other years (Fig. 3). Around the same time, the intensity of NEE in the switchgrass and prairie ecosystems was similar to that for maize, although the relative change in NEE was not as exaggerated as observed for maize (Fig. 3). Miscanthus maintained a relatively high carbon sink throughout much of the growing season, particularly relative to the other species.

When carbon fluxes were accumulated over time for each of the five growing seasons, switchgrass and prairie ecosystems were relatively consistent with their cumulative NEE patterns over the duration of the experiment (Fig. 4); however, in 2011 the GPP values of these species were lower than in previous years. This resulted in the prairie having nearly equal cumulative GPP ( $1326 \pm 40 \text{ g m}^{-2} \text{ year}^{-1}$  carbon) and therefore similarly low  $\text{CO}_2$  sink potential to that of maize in 2011. Miscanthus became a stronger carbon sink each year until 2013, when it was lower than 2012. By the end of 2012 miscanthus showed the highest net  $\text{CO}_2$  sink ( $-1102 \pm 40 \text{ g m}^{-2} \text{ year}^{-1}$  carbon) among all species over the duration of this experiment. In 2012 all species with the exception of miscanthus had reduced GPP by the middle of July (at DOY of  $\sim 200$ ; Fig. 4). That year's miscanthus  $R_{\text{eco}}$  ( $1280 \pm 71 \text{ g m}^{-2} \text{ year}^{-1}$  carbon) was nearly the same as in the previous years, but GPP ( $2375 \pm 81 \text{ g m}^{-2} \text{ year}^{-1}$  carbon) was higher. Miscanthus GPP was lower in 2013 than in 2012, which corresponded to a lower carbon sink in 2013.

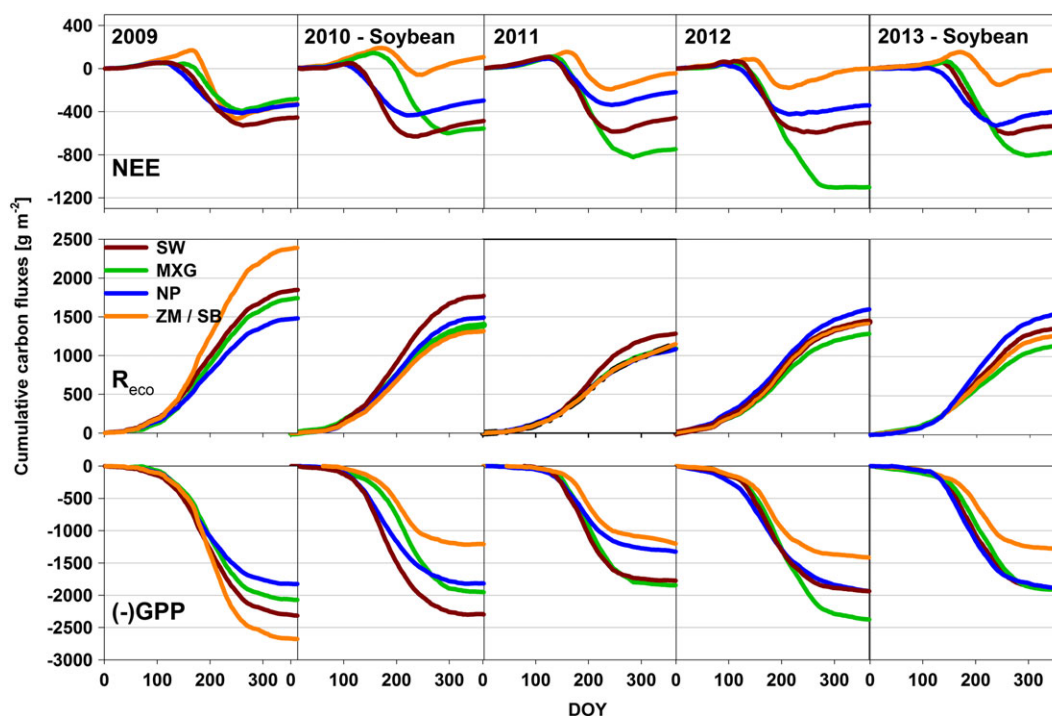
### Yield and net ecosystem carbon balance

Both switchgrass and prairie had the highest carbon export, approximately  $300 \text{ g m}^{-2}$  in yield in 2010 relative to any other year (Fig. 5b). Post 2010, switchgrass yields maintained remarkably similar yields, while prairie yields declined, particularly in 2012. Beginning in 2011 miscanthus yields consistently exceeded that of all other species, despite needing to be replanted in 2010 (Fig. 5b). With the exception of prairie, yields of the perennials during the 2012 drought were similar relative to non-drought years. Maize, however, had the lowest harvested carbon ( $182 \text{ g m}^{-2} \text{ year}^{-1}$ ) in 2012 relative to previous non-drought years (2009 and 2011), with yields nearly as low as observed for soybean (2010 and 2013).

Net ecosystem carbon balance was calculated based on measurements of NEE (Fig. 5a) accounting for the harvested carbon removed from the fields (Fig. 5b). Five-year mean NEE suggests that the row crops are carbon neutral (Fig. 5a)



**Figure 3.** Fingerprint plots of the gap-filled half-hourly net ecosystem exchange (NEE) data calculated by using the eddy covariance technique for maize, soybean, miscanthus, switchgrass and restored prairie. In 2010 and 2013 soybean was planted instead of maize. The data are presented over the diurnal time course (X-axis) over the entire calendar year (Y-axis). Positive values represent net carbon from the ecosystem to the atmosphere (carbon source), while negative values are net carbon flux from the atmosphere to the ecosystem (carbon sink).



**Figure 4.** Cumulative net ecosystem exchange (NEE), ecosystem respiration ( $R_{eco}$ ) and gross primary production (GPP) for switchgrass (SW), miscanthus (MXG), prairie (NP) and maize (ZM)/soybean (SB) crops for each year of the experiment. Fluxes are cumulative based on the day of year (DOY) for each year. Positive fluxes of NEE correspond to net carbon source towards the atmosphere, and GPP is defined as carbon uptake from the atmosphere to the ecosystem (negative GPP values are plotted to show sink of carbon from the atmosphere).

but become a large carbon source to the atmosphere when accounting for harvested carbon (Fig. 5c). Within the 5 years, only 2009 showed maize or soybean to be carbon neutral, whereas all other years were large carbon sources (Fig. 5c). Five-year mean NECB showed the perennial ecosystems as being carbon sinks, with miscanthus showing the largest carbon sink potential (Fig. 5c). However, within years there were many instances where the NECB values of switchgrass and prairie ecosystems were shown as carbon neutral, consistent with the 95% confidence limits overlapping zero (Fig. 5c).

Accumulating NEE over the duration of the experiment (Fig. 6a) demonstrated that all four ecosystems were net sinks of  $\text{CO}_2$  from the atmosphere, with miscanthus being the largest net carbon sink, followed by switchgrass, prairie and finally the maize/soybean ecosystem that was nearly zero. All three perennial ecosystems maintained their role as carbon sinks after the harvested biomass was taken into account to determine NECB; however, the carbon in the harvested biomass represented almost half the net  $\text{CO}_2$  sink strength of the perennials (Fig. 6b). Despite relatively large differences in cumulative NEE between switchgrass and prairie ( $\sim 6 \text{ t ha}^{-1}$ ; Fig. 6a), these two ecosystems ended with relatively similar NECB values at the end of the experiment. As with cumulative NEE, miscanthus had the most carbon storage potential associated with NECB (Fig. 6b). Note that, although the harvest of the biomass accumulated in 2013 occurred after the start of 2014, this carbon export was included near the end of the 2013 growing season (Fig. 6b).

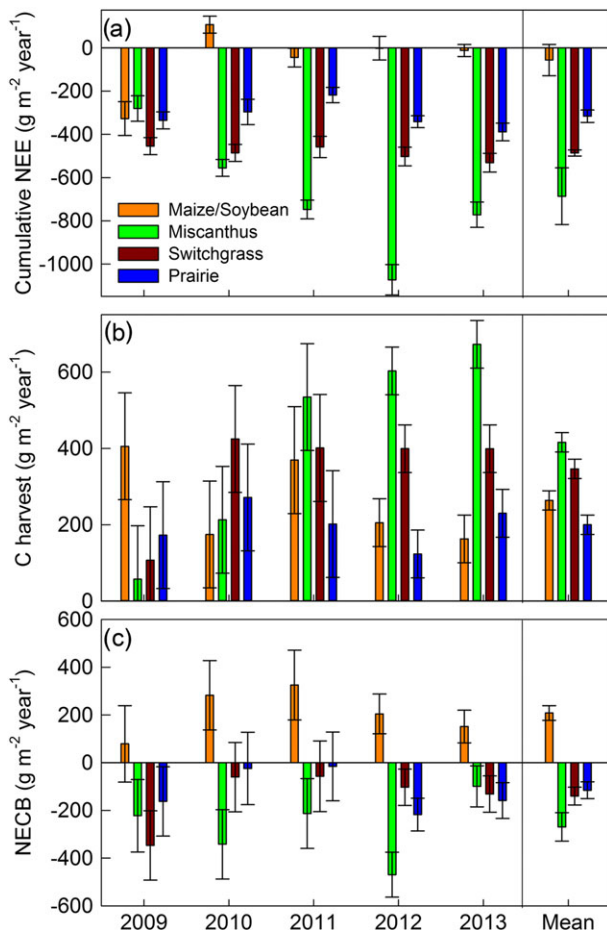
## DISCUSSION

This study characterized the carbon fluxes over five consecutive years (Fig. 1), including a severe drought (Karl *et al.* 2012), for four different ecosystems that are current (maize/soybean agro-ecosystem) or potential (miscanthus, switchgrass and prairie) feedstocks for bioenergy production. The establishment phase (2009–2010) of the crops was reported previously (Zeri *et al.* 2011), and it was concluded that the perennial ecosystems acted as carbon sinks during the establishment phase and stored more carbon than the maize/soybean ecosystem which was a large carbon source. Here we present the fluxes of carbon over three additional years, representing a drought and post-drought recovery. The results suggest that perennials reached maturity within 3–5 years. The results also indicate that while the drought conditions had a large negative impact on the yield of the annual row crop maize in 2012, the perennial grasses, with the exception of the prairie, maintained high productivity (yield, NEE, GPP) throughout the drought period with little impact on the accumulated carbon in following year, except for miscanthus which showed a slight decline in NEE.

### Long-term development of energy crops and their yields

The time required for a perennial grass ecosystem to reach maturity is highly variable, yet it is an important consideration when assessing the suitability of a perennial grass as a

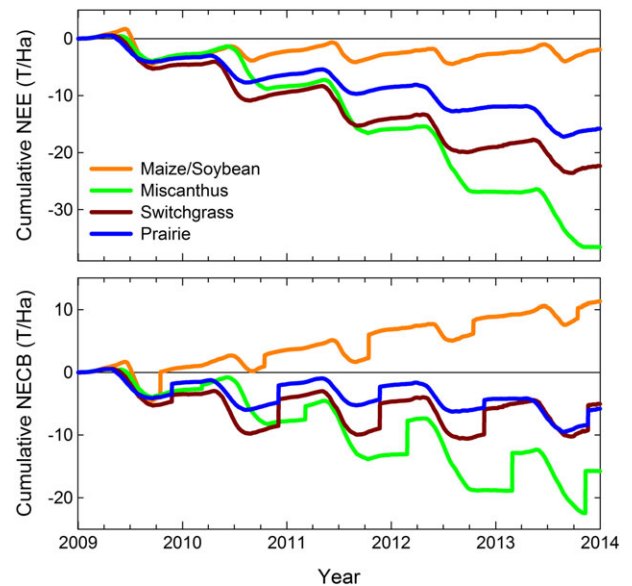




**Figure 5.** Annual cumulative net ecosystem exchange (NEE) (a), carbon export (b) and net ecosystem carbon balance (NECB) (c) for the four ecosystems. Error bars within a year for NEE, C harvest and NECB correspond to the 95% confidence interval, while error bars for the 5 year means are the standard error of the mean of all years within an ecosystem type. In 2010 and 2013 soybean was planted instead of maize.

bioenergy feedstock (Heaton *et al.* 2004, 2010; McLaughlin & Adams Kszos 2005; Arundale *et al.* 2014). From 2009 through 2012 the peak LAI of the perennial ecosystems increased progressively (Fig. 2), consistent with the establishment phase, leading to the maturity phase of perennial grassland ecosystems (Mitchell *et al.* 1998; Zeri *et al.* 2011; Lesur *et al.* 2013). On the other hand, the GPP of the three perennials (switchgrass, miscanthus and prairie) showed no increase between 2009 and 2011 growing seasons (Fig. 4), indicating relatively constant photosynthetic rates. The consistent GPP coupled with gradually increasing biomass is likely explained by less carbon being allocated to roots relative to shoots as the ecosystems mature. Growing season peak LAI values of the perennials in 2012 were among the highest throughout the study (Fig. 2), likely driven by the perennial ecosystems reaching maturity earlier from an earlier start to the growing season associated with warmer than average spring temperatures.

The development of these or similar perennial grasses has been characterized as having an initial development phase from 1 to 3 years, followed by mature ecosystems from 5 to



**Figure 6.** Cumulative net ecosystem exchange (NEE) (top) and cumulative net ecosystem carbon balance (NECB) (bottom) from Fig. 4 plotted as a continuous series over the duration of the experiment for each of the four ecosystems.

11 years followed by the potential for declining growth in years 12 and beyond (Clifton-Brown *et al.* 2007). Our results are consistent with the initial phase of this characterization, suggesting that full maturity was reached within 3–5 years after establishment of the perennials. However, the circumstances associated with the drought and heat stress can influence whether maturity had occurred by 2012. Our results confirm that the high variability of production among years and locations is generally largely affected by climatic conditions (Frank *et al.* 2004; Taylor *et al.* 2013; Arundale *et al.* 2014; Wagle & Kakani 2014b).

## CO<sub>2</sub> exchange and carbon budget

Previous studies reported the large variation in carbon sink/source potential as being largely attributed to species distribution and/or climatic conditions (Janssens *et al.* 2003; Soussana *et al.* 2007; Ceschia *et al.* 2010; Prescher *et al.* 2010). Our mixture of 28 native species at the prairie field site was a net atmospheric carbon sink with average NEE of  $-316 \pm 64 \text{ g m}^{-2} \text{ year}^{-1}$  (2009–2013; Fig. 5a), which supports previous observations that low-input high-diversity grasslands can sequester carbon ( $2.7 \pm 0.29 \text{ Mgha}^{-1} \text{ year}^{-1}$ ) (Tilman *et al.* 2006). Switchgrass and prairie were similar regarding carbon uptake, but switchgrass had a larger carbon sink potential ( $\text{NEE} = -486 \pm 32 \text{ g m}^{-2} \text{ year}^{-1}$  between 2009 and 2013; Fig. 4) coupled with larger harvestable carbon export (Fig. 5b). However, switchgrass has previously been found to be close to carbon neutral in the USA, because the harvested biomass is large and compensates for NEP gains (Wagle & Kakani 2014b). When accounting for harvested biomass, NECB for both prairie and switchgrass fields were small carbon sinks, with 5 year mean NECB values of  $-116 \pm 34$  and  $-140 \pm 37 \text{ g m}^{-2} \text{ year}^{-1}$ , respectively (2009–2013; Fig. 5c). Switchgrass at our field site, however, started with a largely negative carbon balance but

quickly became carbon neutral. Maize and soybean were neutral over the annually integrated NEE, which corresponded with large positive NECB values for these crops. Studies in the USA have often reported net carbon losses of typically less than  $100 \text{ g m}^{-2} \text{ year}^{-1}$  carbon from maize and soybean rotations (Baker & Griffis 2005; Verma *et al.* 2005). However, our measurements highlighted a relatively high NECB (large carbon source) of the maize/soybean ecosystem of between 80 and  $320 \text{ g m}^{-2} \text{ year}^{-1}$  carbon (Fig. 5c). Our results directly contradict results from a nearby maize/soybean ecosystem, where a slight carbon sink was observed (Bernacchi *et al.* 2005; Hollinger *et al.* 2005; West *et al.* 2010). However, this previous experiment was conducted over a no-till maize/soybean agro-ecosystem grown in an annual rotation relative to the conventionally tilled maize/soybean agro-ecosystem of this study with two consecutive years of maize for every 1 year of soybean. There is limited information in the literature on the NECB of miscanthus. Our results indicate that miscanthus had the largest carbon sink and the largest above-ground biomass production compared with the other crops. Miscanthus also maintained the largest negative NECB throughout this study, with the only exception in 2009 during the establishment year and in 2013, which was the year immediately following the drought.

### Effect of drought

The warmer than average temperatures in March 2012 led to an earlier start of the growing season as indicated by recorded emergence dates (Table 1), providing an opportunity for a longer than average growing season. However, the lack of precipitation during the growing season induced decreased carbon uptake and LAI in June for the crops relative to other years. Consistent with expectations, maize grain production in 2012 was much lower than normal (Fig. 5b); however, GPP in 2012 was similar to that in the previous year (Fig. 4), suggesting that maize exhausted available soil water prior to the initiation of reproductive development. In 2013, soybean did not appear to be influenced by the 2012 drought, reaching the second largest LAI after switchgrass and similar harvest as in 2010, suggesting adequate soil water replenishment prior to the start of the growing season.

No considerable effect of drought and heat stress on the long-term development (LAI, biomass production and GPP) of switchgrass and prairie was observed. Switchgrass has been reported to be drought tolerant (Rehm 1990; Reynolds *et al.* 2000; Sanderson & Reed 2000; Heaton *et al.* 2004), benefitting from intensive root development during drought stress (up to  $-3.71 \text{ MPa}$  soil moisture tension) (Mann *et al.* 2013b). An investigation on the impact of the 2012 drought on a 6-year-old switchgrass plantation in Canada reported significantly lower GPP ( $-1354 \pm 23$  compared with  $-1430 \pm 50 \text{ g m}^{-2}$  carbon) and lower carbon sink potential (NEE  $-380 \pm 25$  compared with  $-430 \pm 30 \text{ g m}^{-2}$  carbon) in 2012 than in 2013, respectively (Eichelmann *et al.* 2016). Despite this, higher yields were observed during the drought relative to the recovery year in 2013 (Eichelmann *et al.* 2016). The reported NECB values of  $106 \pm 45$  and  $-59 \pm 45 \text{ g m}^{-2}$  carbon in 2012 and 2013

(Eichelmann *et al.* 2016), respectively, indicate that their ecosystem acted as a carbon source. The results presented here (Table 1; Fig. 5) show that switchgrass is a carbon sink both during and immediately following the drought (Fig. 5), although in 2010 and 2011 the ecosystem is carbon neutral. Relative to the all other ecosystems, miscanthus had the highest productivity and biomass production and the drought year is no exception (Table 1).

Carbon harvested from the switchgrass ecosystem was remarkably consistent over the duration of this experiment, including during the drought year. Miscanthus harvest increased steadily throughout the experiment, although the rate of increase was much lower starting in 2011. The drought did not appear to have any detrimental impact on the harvest for either switchgrass or miscanthus (Fig. 5). Maintenance of consistent productivity for miscanthus and switchgrass during the drought is likely driven by access to soil moisture at deeper layers associated with deeper roots (Neukirchen *et al.* 1999) and a perched water table at the field location. The established root system and soil types at field conditions explain the discrepancy with the previously observed reduced productivity of miscanthus, which had been conducted by using artificial growth conditions (Clifton-Brown & Lewandowski 2000; Ings *et al.* 2013; Mann *et al.* 2013a,b).

Miscanthus had lower cumulative NEE and GPP in 2013 relative to 2012, suggesting a legacy effect of the 2012 drought, likely driven by a lag in the rates of replenishment of soil moisture. Miscanthus is shown to evapotranspire more than the other ecosystems in this study (Hickman *et al.* 2010; Zeri *et al.* 2013). The high productivity in 2012 will inevitably correspond with high water use, suggesting that the soil moisture pool is depleted. Despite lower productivity as measured using eddy covariance, there was no observable latent drought effect on 2013 harvest (Fig. 5). The disparity between the fluxes and the biomass measurements could potentially be explained by altered biomass allocation patterns, with the drought year leading to increased allocation of carbon below ground. The 2009 establishment year showed higher values of LUE for all ecosystems relative to other years (Table 1). While there were clear species differences in LUE, there did not appear to be a shift in LUE during the drought year (Table 1).

### Comparison of ecosystems from the environmental perspective

Many factors need to be considered when determining the suitability of a given species for land-use change. Research trials in the USA have reported that miscanthus productivity is among the highest recorded within temperate climates (Heaton *et al.* 2010) and that it could require 87% less land to produce the same amount of biomass as a low-input high-diversity mixture of prairie species (Heaton *et al.* 2008). *M. × giganteus* has been demonstrated to have a stronger response to water deficiency than switchgrass (Heaton *et al.* 2004; Mann *et al.* 2013b). Looking at the long-term NEE, NECB and yield (Figs. 4–6), we found

miscanthus to have the highest CO<sub>2</sub> uptake from the atmosphere and the largest productivity among the ecosystems included in our experiment. However, drought and heat stress appears to have had little effect on this species during the drought, but they did influence net carbon uptake in the following year. Switchgrass showed similar drought resilience but without any apparent legacy effect. Both the prairie and row crop ecosystems were strongly impacted by the drought; however, relative to non-stress growing conditions (e.g. 2010, 2013) the prairie ecosystem appears to be more resilient to drought than the row crops. Drought continually threatens harvest of all crop species and can potentially influence other ecosystem services such as carbon storage. Therefore, identifying species that are more resistant to drought, in terms of both harvested biomass and ecosystem carbon fluxes, can help to maintain productivity in a changing environment. The research here suggests that the perennial crops are more resistant to drought and heat stress than annual row crops.

## CONCLUSIONS

The impact of the 2012 drought and heat stress on long-term development and on the atmospheric effect of carbon exchange for four ecosystems was investigated. Three perennial bioenergy crops, miscanthus, switchgrass and a high-diversity prairie and maize/soybean rotation were compared simultaneously over 5 years after establishment of the fields. The LAI and biomass measurements suggest that the perennials likely reached full maturity during the first half of the measurement campaign; however, a drought occurred in what was likely the first year of maturity for the perennial ecosystems. While yields were reduced in maize and prairie during the 2012 drought, they were consistent for switchgrass and miscanthus. Miscanthus showed the highest GPP in 2012; however, it dropped in the year following the drought. The highest CO<sub>2</sub> sink potential was attributed to miscanthus over the duration of this experiment, which reached its maximum in 2012. Our observations suggest that miscanthus can provide high yield and large potential for CO<sub>2</sub> fixation from the atmosphere; however, short-term drought tolerance, likely from increased access to soil moisture, potentially led to long-term drought-induced consequences on carbon assimilation in the following year, which questions whether the short-term stress tolerance comes at a consequence of hysteresis effects following a drought.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**Figure S1.** Representative energy balance closure for each of the four ecosystems. Data presented are from 1 July to 31 August 2013.

**Figure S2.** Data from Supporting Information Figure S1 plotted over the diurnal time course. The storage of energy by photosynthesis  $S_{ph}$  was multiplied by 10.