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Ecosystem Water-Use Efficiency of Annual Corn and Perennial Grasslands: Contributions from Land-Use History and Species Composition

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Abstract

Carbon and water exchanges between vegetated land surfaces and the atmosphere reveal the ecosystem-scale water-use efficiency (WUE) of primary production. We examined the interacting influence of dominant plant functional groups (C_3 and C_4) and land-use history on WUEs of annual corn and perennial (restored prairie, switchgrass and smooth brome grass) grasslands in the US Midwest from 2010 through 2013. To this end, we determined ecosystem-level (*e*WUE) and intrinsic (*i*WUE) WUEs using eddy covariance and plant carbon isotope ratios, respectively. Corn, switchgrass, and restored prairie were each planted on lands previously managed as grasslands under the USDA Conservation Reserve Program (CRP), or as corn/soybean rotation under conventional agriculture (AGR), while a field of smooth brome grass remained in CRP management. The *i*WUEs of individual C_3 plant species varied little across years. Corn had the highest (4.1) and smooth brome grass the lowest (2.3) overall *e*WUEs (g C kg^{-1} H_2O) over the 4 years. Corn and switchgrass did not consistently show a significant difference in seasonal *e*WUE between former CRP and AGR lands, whereas restored prairie had significantly higher seasonal *e*WUE on former AGR than on former CRP land due to a greater shift from C_3 to C_4 species on the former AGR land following a drought in 2012. Thus, differences in grassland *e*WUE were largely determined by the relative dominance of C_3 and C_4 species within the plant communities. In this humid temperate climate with common short-term and occasional long-term droughts, it is likely that mixed grasslands will become increasingly dominated by C_4 grasses over time, with higher yields and *e*WUE than C_3 plants. These results inform

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models of the interaction between carbon and water cycles in grassland ecosystems under current and future climate and management scenarios.

Key words: ecosystem WUE; intrinsic WUE; eddy covariance; carbon isotope ratio; gross primary production; evapotranspiration; switchgrass; restored prairie; C_3; C_4.

INTRODUCTION

The rate of exchange of carbon dioxide and water vapor between plants and the atmosphere during photosynthesis is a process of fundamental importance to understanding plant productivity and terrestrial carbon and water balances (Osmond and others 1982; Ito and Inatomi 2012). At the ecosystem level, rates of stomatal gas exchange are largely influenced by soil water and nutrient availability and prevailing atmospheric conditions (Farquhar and Sharkey 1982; Ponton and others 2006).

Water-use efficiency (WUE), the ratio of net photosynthetic CO_2 assimilation to water loss during transpiration, has been quantified across a broad range of temporal and spatial scales (Steduto 1996). Leaf-level or intrinsic WUE (*i*WUE) is regulated by stomatal conductance and can be estimated from stable carbon isotope ratio ($\delta^{13}C$) of C_3 plant material. Variation in *i*WUE among plants is caused by different environmental conditions as well as genetic (species) variation in leaf gas exchange (Flanagan and Farquhar 2014). Intrinsic WUE is defined as the ratio of net carbon assimilation (A) to stomatal conductance (g_s) (*i*WUE = A/g_s) (Seibt and others 2008). Ecosystem-level WUE (*e*WUE) is defined as the ratio of carbon fixed during CO_2 assimilation (net ecosystem exchange or gross primary production of the overall plant community) to water lost to the atmosphere (evapotranspiration or transpiration) and can be assessed by continuous and simultaneous direct eddy-covariance (EC) measurements of carbon and water fluxes between the canopy and the atmosphere across daily to seasonal time scales.

Land-use conversions change plant community composition and productivity, and thereby can alter *e*WUE. In the US Midwest, much of the agricultural land that exists today was originally tallgrass prairie and oak savanna prior to European settlement (Albert 1995). The increasing desire to conserve soil, water quality, habitat, and biodiversity has led to conservation grassland restoration initiatives by the government, such as the USDA Conservation Reserve Program (CRP), and/or private initiatives on former agricultural lands. However, recent higher commodity prices arising in part from the demand for corn grain as a source of biofuel feedstock has driven farmers to convert many such grasslands to corn (Wright and Wimberly 2013). In the longer term, the US Energy Independence and Security Act of 2007 seeks to produce about half of its 2022 target ethanol biofuel production from cellulosic biofuels. Meeting this target would likely require conversion of croplands and marginal lands not now farmed to grasslands for use as cellulosic biofuel feedstock.

In grasslands, plant species composition may change from one growing season to another in response to environmental variability, disturbance, competition, and succession (Collins and Adams 1983; McIntyre and Lavorel 1994; Suttle and others 2007). For example, in water-limited C_3-dominated grasslands, grazing and competition for soil water regulated species composition (Tsialtas and others 2001). The abundance and distribution of C_3 and C_4 species in mixed grasslands were also shown to be related to mean annual precipitation (Epstein and others 1997). Camill and others (2004) noted that mixed-species restored prairies subjected to regular burning tended to be dominated by C_4 grasses following full establishment. Grasses with C_4 photosynthetic pathways have greater WUE than C_3, and therefore such shifts in community composition can lead to changes in *e*WUE with implications for ecosystem carbon and water balances.

Considering that mixed grasslands are one of the prospective land conversions in the US Midwest, this study examines how changes in the relative abundances of C_3 and C_4 species in mixed grasslands affect *e*WUE, which in turn has implications for ecosystem carbon and water balances. We hypothesized that: (1) land-use history plays a significant role in the magnitude of *e*WUE of the ecosystems regardless of community composition; and (2) changes in *e*WUE in the perennial grasslands will occur with changes in relative abundances of C_3 and C_4 species. Based on 4 years of eddy covariance measurements of carbon and water fluxes together with measurements of carbon isotope ratio of C_3 plant material, we present ecosystem and intrinsic WUEs of annual continuous corn (maize; *Zea mays* L.) and perennial grasslands including switchgrass (*Panicum virgatum* L.), restored native prairie (mixed species, see Appendix S1), and smooth brome grass (*Bromus inermis* L.). The switchgrass and prairie cropping systems reflect candidate cellulosic biofuel crop choices in the region, with corn and brome grass for comparison. Corn, switchgrass, and restored prairie were established on sites with two different land-use histories characterized by different soil organic carbon (C) and nitrogen (N) contents: lands previously under CRP grassland with higher soil C and N contents and lands under conventional corn/soybean rotation (AGR) with lower soil C and N contents. The smooth brome grass, an introduced cool-season C_3 grass widely planted on conservation lands, had been managed as CRP grassland since 1987.

Materials and Methods

Study Area

The study area is part of the Great Lakes Bioenergy Research Center (GLBRC) at Michigan State University, and is located at the Kellogg Biological Station's Long-term Ecological Research site in southwest Michigan (42°24′N, 85°24′W, 288 masl), within the northeastern portion of the US Corn Belt (see http://lter.kbs.msu.edu/maps/images/GLBRC-Scaleup-Fields.pdf for site maps). The climate is humid and continental with a 30-year (1981–2010) mean annual air temperature of 9.9°C and mean annual precipitation of 1027 mm of which, on average, 523 mm falls from May to September (Michigan State Climatologist's Office 2103). The soil is a well-drained sandy loam classified as a Typic Hapludalf according to the USDA soil classification (Thoen 1990). Prior to European settlement and land conversion to agriculture, this region had a mosaic of tallgrass prairies, savannas and oak openings including C_3 and C_4 grasses as well as forbs (Chapman and Brewer 2008). According to presettlement vegetation maps (Albert 1995), the specific lands on which our study fields lie were likely oak-hickory forest, with mixed oak savanna in the vicinity.

Our study sites included three planted grasslands (11–17 ha) that had been managed since 1987 under the USDA CRP and three croplands (11–14 ha) that had been in conventional agriculture (AGR) as corn/soybean rotations for several decades. Fields were converted to no-till soybean in 2009 before being planted in 2010 to either an annual crop (no-till continuous corn) or perennial grasslands (switchgrass or mixed grass/forb restored prairie). Oats were inter-seeded in the switchgrass and restored prairie as first-year nurse crop. The prairie included 19 planted species dominated by C_3 plants and other unplanted grasses that grew on the site (Appendix S1). Corn was planted and harvested each year since 2010, whereas the perennial grasslands were harvested after autumn senescence from 2011 onwards. Since 1987, grass on the former CRP lands had been cut every 3 years and left in place. In addition to these six sites, one grassland site (9 ha) was maintained as CRP grassland vegetation to serve as a reference (Ref), uncut since 2009.

The soils on the former CRP and AGR lands are similar in textural classes, pH, and cation exchange capacity, but differ in bulk density and concentrations of organic carbon and total nitrogen owing to previous land-use (Table 1). In these soils, the organic matter content is positively related to fertility and water-holding capacity (Deal and others 2013).

Eddy Covariance, Meteorological Measurements, and Ecosystem WUE

Eddy covariance (EC) towers containing an LI-7500 open-path infrared gas analyzer (LI-COR Biosciences, Lincoln, NE, USA) and a CSAT3 three-dimensional sonic anemometer (Campbell Scientific Inc. (CSI), Logan, UT, USA) were operated at each site since 2009 to measure CO_2 and H_2O concentrations, wind velocity and sonic temperature, respectively. The LI-7500 s were calibrated every 4 to 6 months. All EC signals were sampled and logged at 10-Hz using a Campbell CR5000 datalogger. The instruments were mounted 1.5–2 m above the average canopy height. Precipitation data were obtained from a nearby weather station (http://lter.kbs.msu.edu/datatables, accessed March 2015).

The raw EC data were processed using the EdiRe software (University of Edinburgh, v 1.5.0. 32, 2012) to produce half-hourly CO_2 (net ecosystem exchange, NEE) and H_2O (latent heat, LE) fluxes. Out-of-range data spike greater than four standard deviations, and time lags between scalars and vertical velocity were removed (McMillen 1988). Planar fit coordinate rotation (Wilczak and others 2001) and corrections to the sonic temperature for pressure and humidity (Schotanus and others 1983), to the CO_2 and H_2O fluxes for frequency response (Moore 1986) and for air density fluctuations (Webb and others 1980), including surface heating of the LI-7500, were applied (Burba and others 2008). All EC outputs were computed using 30-min block averaging without detrending (Moncrieff and others 2004). Instationarity, flux-variance similarity, and friction velocity thresholds were also used to screen out low-quality data with poor turbulent mixing (Foken and Wichura 1996). After applying these quality checks and controls to the 4 years of data, on average about 56% of the CO_2 and H_2O fluxes were retained for WUE analyses. The gaps created due to poor quality data

Table 1. Soil Physical and Chemical Properties for the Top 0.25 m of the Profile at Each Study Site in 2009 Before Land-use Conversion: Sites Were Planted to Corn (C), Restored Prairie (Pr), and Switchgrass (Sw), Converted from Either Conservation Reserve Program (CRP) Grassland or Row-crop Agriculture (AGR); with a Reference (Ref) Site Maintained as CRP Grassland

Site	Area (ha)	Textural class	Soil pH	CEC [meq $(100\ g)^{-1}$]	Bulk density ($g\ cm^{-3}$)	Nitrogen ($g\ kg^{-1}$ soil)	Carbon ($g\ kg^{-1}$ soil)
CRP-C	17	Sandy loam	6.1[a]	6.02[ab]	1.58[b]	2.0[d]	21.2[c]
CRP-Sw	13	Sandy loam	5.9[a]	6.00[ab]	1.66[b]	1.6[c]	18.5[c]
CRP-Pr	11	Sandy loam	6.2[a]	5.46[a]	1.59[b]	1.7[c]	19.5[c]
AGR-C	11	Sandy loam	6.4[a]	8.08[ab]	1.54[a]	1.2[a]	12.2[b]
AGR-Sw	14	Sandy loam	6.4[a]	7.07[ab]	1.79[c]	1.1[a]	10.8[a]
AGR-Pr	13	loam	5.8[a]	8.60[b]	1.69[b]	1.4[b]	13.5[b]
Ref	9	Sandy loam	6.2[a]	6.50[ab]	1.56[b]	1.9[d]	20.9[c]

Means followed by same letter are not significantly different by t test ($P < 0.05$)
Source soil texture, pH, CEC, bulk density, and total carbon and nitrogen—http://lter.kbs.msu.edu/datatables/372
CEC cation exchange capacity

were then replaced by a standardized gap-filling algorithm (Reichstein and others 2005) (http://www.bgc-jena.mpg.de/~MDIwork/eddyproc/ Accessed June 2014). The same algorithm was also used to partition the NEE into gross primary production (GPP) and ecosystem respiration (R_{eco}) based on night-time and temperature measurements. Evapotranspiration (ET) was calculated from LE and latent heat of vaporization.

Ecosystem water-use efficiency (*e*WUE) during the growing season was calculated over daily intervals as a ratio of daily GPP to ET, and over longer periods as the regression slope of the daily GPP vs. ET values (Baldocchi and others 2001):

$$e\mathrm{WUE} = \frac{\mathrm{GPP}}{\mathrm{ET}} \quad (1)$$

where GPP is in g C and ET in kg H_2O. Only high-quality and reliably gap-filled daytime (between sunrise and sunset) data were used. Net carbon gain or loss over time was used to identify the start and end date of the growing season. Data for a day after rain (24 h from the last half-hour rain) were excluded, assuming that soil evaporation and canopy interception (Grelle and others 1997) will result in underestimation of *e*WUE. Periods with LE less than 20 W m^{-2}, which usually occur in early morning and/or evening and cause *e*WUE to be unreasonably high, were discarded. Days with less than 70% of data points remaining after quality filters were not included in the analysis.

Plant Biomass, Composition, Isotopic Analysis, and Intrinsic WUE

Plant biomass was collected at peak standing biomass in August of each year by manual clipping at ground level within 1-m^2 quadrats across 10 stations at each site to determine aboveground net photosynthetic productivity (ANPP), species composition, and carbon isotope ratios. The plant samples were identified and sorted by species, dried, and ground (250 µm sieve). For carbon isotope ratios, we analyzed the top three plant species in terms of contribution to the overall aboveground biomass in each given year. Ground plant tissue was weighed into tin capsules (~4 mg) and analyzed for stable carbon isotope ratios at the University of California, Davis stable isotope facility (http://stableisotopefacility.ucdavis.edu/). The isotopic composition of plants is expressed using standard per mil notation ($\delta^{13}C$; ‰) relative to Vienna-PDB (Maseyk and others 2011).

Intrinsic WUE (*i*WUE, mmol CO_2 mol^{-1} H_2O)—the ratio of net carbon assimilation to stomatal conductance—was estimated from plant $\delta^{13}C$ as follows (Seibt and others 2008):

$$i\mathrm{WUE} = \frac{A}{g_{sw}} = \frac{C_a}{1.6}\left(\frac{b' - \Delta}{b' - a}\right), \quad (2)$$

where A is net assimilation, g_{sw} is stomatal conductance for water vapor, C_a is atmospheric [CO_2] (measured at Mauna Loa) averaged for the months June to August (Keeling and others 2001; http://scrippsco2.ucsd.edu/data/in_situ_co2/monthly_mlo.csv), and a and b' are fractionation constants for diffusion of CO_2 through the stomata and enzymatic reactions by Rubisco and PEP carboxylase, respectively. Plant discrimination against ^{13}C during photosynthesis (Δ) was calculated according to

$$\Delta = \frac{\delta^{13}C_{atm} - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}}, \quad (3)$$

where $\delta^{13}C_{plant}$ was measured from the collected plant material and $\delta^{13}C_{atm}$ is the average for atmospheric [CO_2] observed at Mauna Loa (Keeling and others 2001).

The *i*WUE of the 3 (or fewer) dominant C_3 species at each site was used to compute a biomass-weighed mean *i*WUE according to the contribution of the species to the overall C_3 aboveground biomass. The *i*WUE was also corrected for average daily growing season vapor pressure deficit (VPD) in order to compare *i*WUE with *e*WUE values.

Statistical Analysis

All statistical analyses were conducted using the statistical software R (R Development Core Team 2015, version 3.2.0). The slopes of daily GPP versus ET, representing the overall mean *e*WUE of sites over the growing season, were analyzed using ANCOVA to test for differences in *e*WUE among sites over the study years. The seasonal mean *e*WUE, GPP to ET ratio, was analyzed by the linear mixed model fit using restricted maximum likelihood (REML) to test for differences between sites and over time (Pinheiro and others 2015). Comparisons among the sites and seasons were conducted using Tukey's HSD test with the *P* values adjusted using Bonferroni correction. In all the analysis conducted, values were considered significant at $P < 0.05$. While the seven studied ecosystems were not replicated (that is, $n = 1$), fluxes represent large footprint areas that extend approximately 150–300 m from the towers. In addition, extensive literature examining sources of flux errors (for example, Baldocchi 2003; Richardson and others 2006) support validity of using one EC tower per ecosystem.

Results

The precipitation from May through September, roughly representing the growing season in our study sites, was 568, 510, 227, and 446 mm from 2010 through 2013, respectively. The growing-season precipitation in 2012 was much lower than the 30-year average for May to September (523 mm), whereas the growing-season precipitation of the other years was closer to average.

GPP versus ET Relationship

The daily rates of GPP and ET were positively related during the growing seasons of the study period, and the slopes of these relationships over the 4 years provide an estimate of the overall mean *e*WUE (Figure 1). Mean *e*WUEs for corn and switchgrass did not differ between the sites on

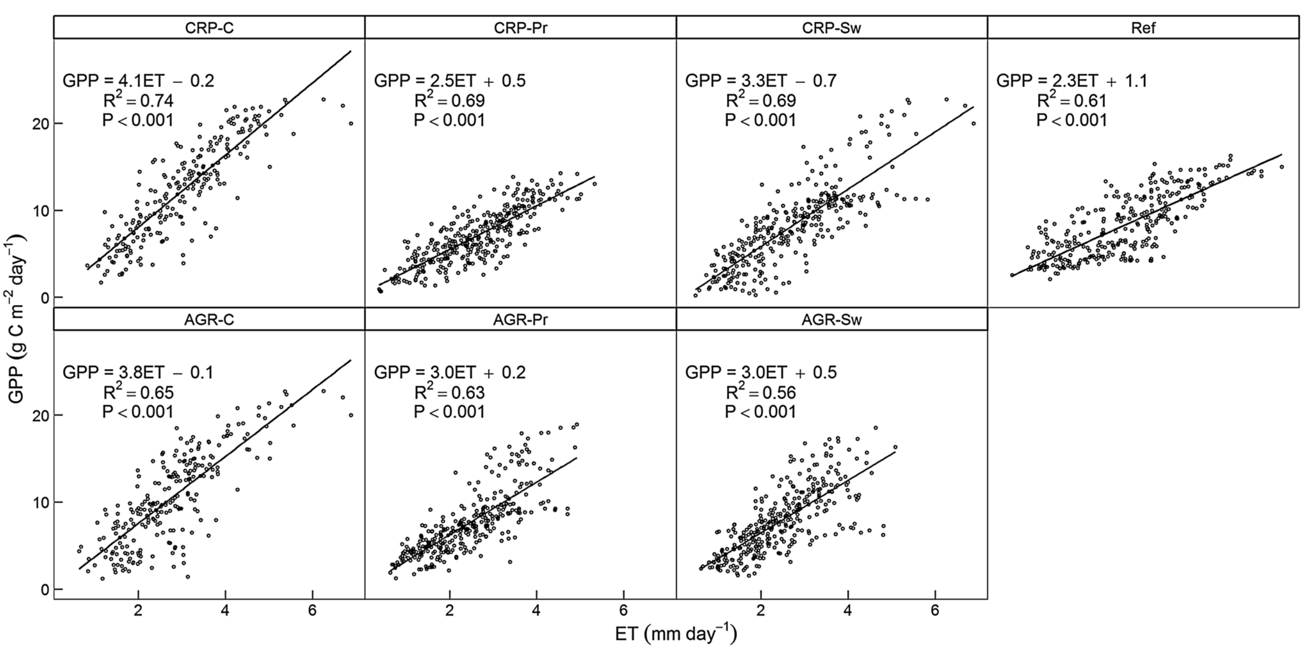

Figure 1. Ecosystem water-use efficiency (*e*WUE) over the 2010–2013 growing seasons as indicated by the regression slopes of daily GPP on ET. Panels show corn (C), restored prairie (Pr) and switchgrass (Sw) sites converted from either Conservation Reserve Program (CRP) grassland or row-crop agriculture (AGR), and the reference (Ref) site maintained as CRP grassland.

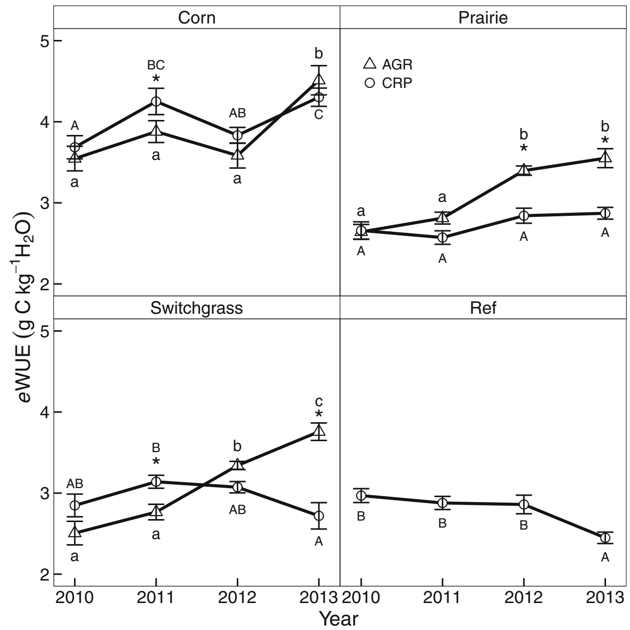

Figure 2. Growing-season ecosystem water-use efficiency (*e*WUE) from 2010 through 2013. *Error bars* represent standard errors of the mean *e*WUEs in each year and the asterisks indicate significant pairwise differences ($P < 0.05$) in a year between the same crops on former CRP and AGR lands and lower and uppercase letters indicate significant difference on former AGR and CRP lands, respectively, across years within a site. Panels show corn, restored prairie, and switchgrass sites converted from either Conservation Reserve Program (CRP, open circles) grassland or row-crop agriculture (AGR, open triangles), and the reference (Ref) site maintained as CRP grassland.

former CRP and AGR lands. Mean *e*WUE for the restored prairie, in contrast, was significantly greater on former AGR than on former CRP land over all years (Appendix S2). The two corn sites had significantly higher ($P < 0.001$), and the CRP-Pr and Ref sites significantly lower, mean *e*WUEs than all other sites, with the Ref site having the lowest *e*WUE. Thus, the rank order of mean *e*WUEs (g C kg^{-1} H_2O) over the 4 years was as follows: CRP-C (4.1) ≈ AGR-C (3.8) > CRP-Sw (3.3) ≈ AGR-Pr (3.0) ≈ AGR-Sw (3.0) > CRP-Pr (2.5) ≈ Ref (2.3).

Seasonal *e*WUE Across Sites and Over Time

The mean growing-season *e*WUEs were not significantly different between sites on the former CRP and AGR lands in 2010 when compared on a crop by crop basis (Figure 2, Appendix S2). In 2011, the mean seasonal *e*WUEs (± se) for the corn and switchgrass on former CRP lands were 4.3 ± 0.2 and 3.1 ± 0.1 g C kg^{-1} H_2O, respectively, significantly greater than those on former AGR lands with *e*WUEs of 3.9 ± 0.2 and 2.8 ± 0.1 g C kg^{-1} H_2O, respectively, but not significantly different between the restored prairie grassland sites on former CRP and AGR lands. During the drought of 2012, the restored prairie on former AGR land had a significantly greater *e*WUE (3.4 ± 0.1 g C kg^{-1} H_2O) than on former CRP land (2.8 ± 0.1 g C kg^{-1} H_2O), but there were no significant differences for corn and switchgrass between the former CRP (3.8 ± 0.1 and 3.1 ± 0.1 g C kg^{-1} H_2O, respectively) and AGR (3.6 ± 0.2 and 3.3 ± 0.1 g C kg^{-1} H_2O, respectively) lands. In 2013, switchgrass and restored prairie on former AGR lands had *e*WUEs of 3.8 ± 0.1 and 3.5 ± 0.1 g C kg^{-1} H_2O, respectively, which were significantly greater than those on former CRP lands with *e*WUEs of 2.7 ± 0.2 and 2.9 ± 0.1 g C kg^{-1} H_2O, respectively.

The corn ecosystems had the highest *e*WUEs in all years. Comparison of the *e*WUEs between and across the perennial grasslands ranged from statistically similar in 2010 to statistically different in 2013 (Appendix S2). During the dry year of 2012, all the grasses had statistically indistinguishable *e*WUEs within their respective land-use history except for corn on former CRP land, which had *e*WUE 3.8 ± 0.1 g C kg^{-1} H_2O, significantly greater than the other grasses.

The *e*WUE of corn on former CRP and former AGR lands fluctuated in the same manner throughout the 4 years (Figure 2). On the other

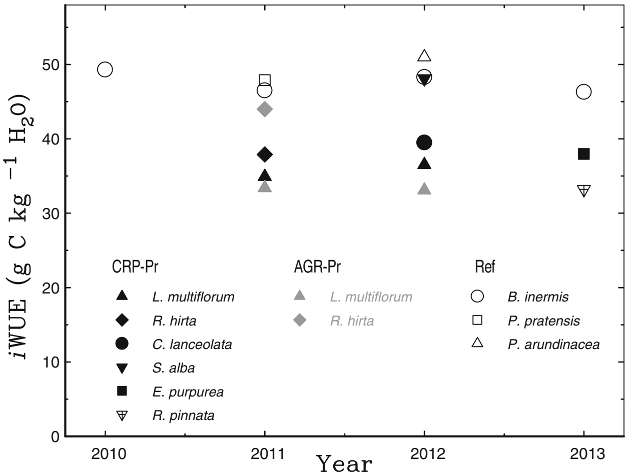

Figure 3. Intrinsic WUE at the CRP-Pr, AGR-Pr, and Ref sites for the dominant C_3 species according to their contribution to aboveground biomass from 2010 through 2013. Each symbol within a year represents one C_3 grass species at a site (more details on species at Appendix S1).

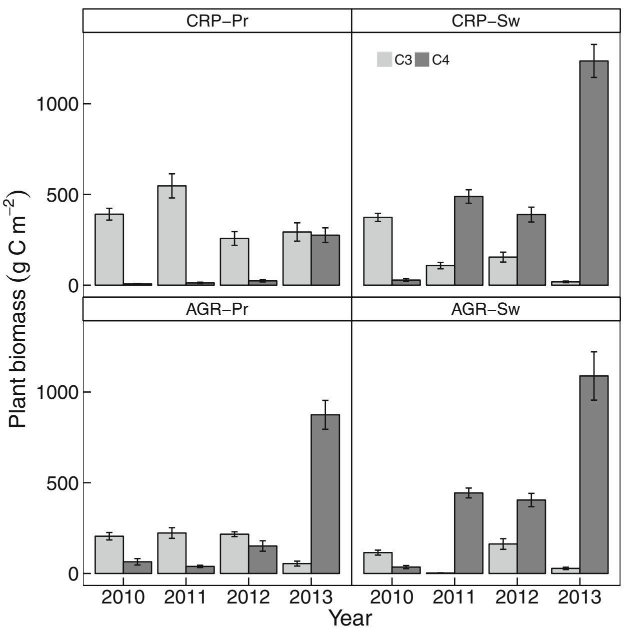

Figure 4. Plant species composition by aboveground biomass (g C m^{-2}) for restored prairie (Pr) and switchgrass (Sw) on former CRP and AGR lands, categorized according to their photosynthetic pathways, from 2010 through 2013. Error bars represent stand error of the mean plant biomass for 10 sampling plots in each ecosystem.

hand, the *e*WUE of switchgrass and restored prairie on former CRP lands did not show statistically detectable trend across the 4 years, but *e*WUE increased on former AGR lands. This is clear from Figure 2 where a divergence in the *e*WUE at the switchgrass sites was observed from 2012 onwards when the *e*WUE of AGR-Sw kept increasing but that of CRP-Sw decreased. Also a divergence in the *e*WUE at the restored prairie sites was observed from as early as 2011 with the *e*WUE of AGR-Pr increasing but that of CRP-Pr remaining more or less constant. The Ref site showed similar *e*WUE through the first 3 years with a significant drop in the last year (Figure 2).

Intrinsic WUE

The biomass-weighted mean *i*WUE of C_3 grasses and forbs calculated from the δ^{13}C carbon isotope ratio within each site was similar across the study years for each species (Figure 3), and ranged between 33 at the AGR-Pr site and 51 g C kg^{-1} H_2O at the Ref site. The difference in *i*WUE among the species was consistent across the years. The C_4 grasses (corn, switchgrass and Indian grass (*Sorghastrum nutans* at the prairie sites)) exhibited δ^{13}C values that were depleted by ~1‰ during the 2012 drought compared to 2011 and 2013 (data not shown).

*e*WUE and the Relative Abundance of C_3 and C_4 Species

The relative contributions of C_3 and C_4 species by biomass (g C m^{-2}) at the restored prairie and switchgrass sites changed over the 4 years, with C_4 grasses becoming increasingly dominant by 2013 in

3 of the 4 sites (Figure 4). Oats served as an annual grass nurse crop in 2010, yielding a higher composition of C_3 than C_4 plants, especially at the switchgrass sites in that year (Figure 4). Originally, both restored prairie sites were planted as polycultures with 19 species dominated by C_3 plants but other unplanted grasses also grew on the sites (Appendix S1). At the CRP-Pr site, the C_3 plants dominated almost completely in the first 3 years, with a small fraction of C_4 grasses (2, 2 and 9% in 2010, 2011 and 2012, respectively). C_3 plants also dominated at the AGR-Pr site, but with considerable fractions of C_4 plants (24, 15, and 41% in 2010, 2011 and 2012, respectively). By 2013, about 94% of the plant biomass was C_4 at the AGR-Pr site but only approximately 48% was C_4 at the CRP-Pr site (Figure 4).

The mean growing-season *e*WUE of all study sites and years combined was positively correlated with the relative contribution of C_4 grasses to total ANPP of the sites ($R^2 = 0.55$, $P < 0.001$, $n = 28$; Figure 5). The relationship with corn excluded from the aforementioned regression was also significantly positive ($R^2 = 0.33$, $P < 0.001$, $n = 20$).

Discussion

The grassland *e*WUE changed from year to year as a function of the relative biomass contributions of C_4 grasses vs. C_3 grasses and forbs (Figures 3 and 4). The *e*WUEs of the perennial grasslands were lower than those for corn, even when the perennials were dominated by C_4 species. The *e*WUE showed little difference during the growing-season drought of 2012 compared to the other years (Figure 2). Southwest Michigan is on the cool and moist end of the range of C_4 grass distribution (Teeri and Stowe 1976), yet the higher *e*WUEs of the C_4-dominated grasslands suggest that C_4 species still have an advantage when soil water availability becomes limiting on the well-drained sandy loam at this location, either ephemerally as commonly occurs late in the growing season or more severely in occasional drought years (Abraha and others 2015; Hamilton and others 2015).

Ecosystem-Level WUE

The overall mean *e*WUE for the four growing seasons, calculated as the slope of daily GPP vs. ET, decreased in the order CRP-C ≈ AGR-C > CRP-Sw ≈ AGR-Pr ≈ AGR-Sw > CRP-Pr ≈ Ref (Figure 1). Thus, corn and the C_4-dominated grasslands generally had higher *e*WUEs than C_3-dominated grasslands including the brome grass of the Ref site and most years for the CRP-Pr site. These results are consistent with other comparisons between C_3 and C_4 crop monocultures (for example, Hendrickson and others 2013). Comparable *e*WUE estimates from GPP versus ET slopes are reported in the literature, for example, 1.7 ± 0.3 g C kg^{-1} H_2O for a grazed grassland site in Central France (Beer and others 2009), 1.74 and 2.04 for temperate grassland and alpine meadow in the North China Plain (Zhu and others 2014) and 5.8 g C kg^{-1} H_2O for a meadow steppe in Northeast China (Dong and others 2011).

The observation that corn had the highest overall *e*WUE among the C_4 grasses is not surprising. First, the production and hence *e*WUE of the perennial grasses are typically lower than that of corn during the first few years of establishment of the perennial system. Second, corn has been improved through many years of selection and genetic breeding for high production. Third, unlike the perennial grasses, corn ecosystems are intensively managed in terms of fertilizer and herbicide application to boost production.

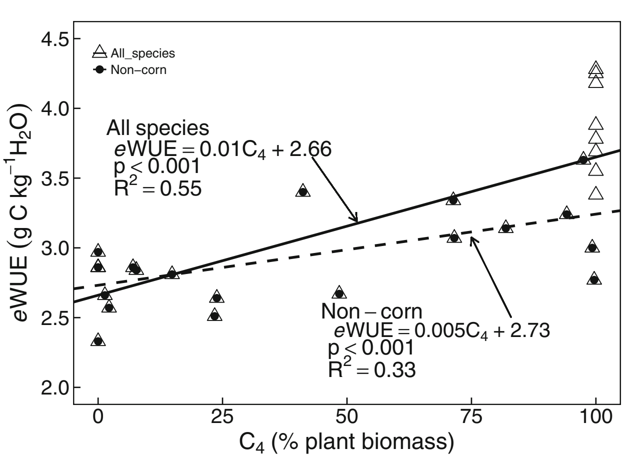

Figure 5. The relation between *e*WUE (from Figure 1) and percent C_4 plant species contribution to total aboveground plant biomass. The solid and dashed lines are with annual corn included and excluded from the regression, respectively.

Cropping systems on the former CRP lands consistently showed a slightly higher correlation between GPP and ET than for the same systems on former AGR lands (Figure 1). This could be due to soil organic matter differences between the former CRP and AGR lands. Sites with CRP land-use history had higher plant biomass and crop residue on the ground than the AGR lands (Zenone and others 2011; Deal and others 2013) and higher soil organic matter in the root zone (Table 1). Higher soil organic matter results in higher water holding capacity (Hudson 1994), likely due to higher infiltration rate and larger number of smaller pores that retain more water. These characteristics can potentially lead to different soil evaporation losses in relation to the total ET. On former CRP lands, evaporation from the soil surface may have been restricted, with ET largely dominated by transpiration, while on former AGR lands soil evaporation may have contributed more to ET. The coefficients of determination (R^2) for regressions of GPP on ET found in the present study are also within the range of earlier reports of 0.74 and 0.63 for a grazed grassland (Beer and others 2009) and a switchgrass field (Wagle and Kakani 2012), respectively.

Interannual Variation in *e*WUE

The *e*WUE of corn showed no trend over the four years, whereas the *e*WUEs of switchgrass and restored prairie were more variable, increasing over time at most sites (Figure 2). The Ref site showed no change in *e*WUE in the first three years, and then decreased in 2013 (Figure 2), likely because of two heavy rainfalls on days of year (DOY) 176 (33 mm) and 200 (39 mm), causing the grass to severely lodge. The grasses did not fully recover after these lodging events and the *e*WUE following the lodging was lower than before the lodging (data not shown). Colder air temperatures following the second rainstorm may also have depressed *e*WUE. The Ref site mean *e*WUE over the 4 years was greatly influenced by the low 2013 value. The switchgrass sites also lodged in response to the second rainstorm, more so at CRP-Sw than at AGR-Sw, which may explain the reduced *e*WUE at the CRP-Sw site in 2013.

The increase in *e*WUEs of the switchgrass and restored prairie communities over the 4 years (with the exception of the CRP-Sw, which decreased towards the last 2 years) could be because the perennial grasses were becoming established following planting. Planted grasslands are known to take a few years to reach their full potential productivity (Parrish and Fike 2005). The perennial grasslands on former AGR lands showed a significant increase in *e*WUE during the drought of 2012 compared to the relatively wetter preceding years, and the 2012 *e*WUE of AGR-Pr site was statistically similar whereas that of AGR-Sw was significantly lower compared to 2013. The 2012 *e*WUEs of the perennial grasslands on former CRP lands were not statistically different compared to the relatively wetter previous and following years despite the drought in that year. These observations suggest that the perennial grasslands use water more efficiently when soil water is limiting.

The greater increase in *e*WUE of the perennial grasslands on former AGR lands than on former CRP lands, especially at the restored prairie sites, is consistent with the observation that the change in species composition towards C_4 dominance was greater in the restored prairie on former AGR than on former CRP lands. C_4 grasses are well known for their higher WUE compared to C_3 grasses, especially under water-limited conditions (Hendrickson and others 2013). Therefore, the higher *e*WUE of the restored prairie observed on former AGR than on former CRP lands can be explained by the greater shift in plant species composition from C_3 to C_4 over the years on the former AGR lands. The change in species composition from C_3 to C_4 plants in the restored prairie was occurring throughout the study period, but it was more pronounced following the drought of 2012 (Appendix S1). Studies on the effects of drought on the relative abundance of C_3 and C_4 species in restored prairies are rare. Tilman (1996) reported similar observations in mixed prairie grasslands where the relative abundance of C_4 grasses increased in response to drought whereas that of C_3 grasses decreased. Allison (2008) also found that two-thirds of C_4 grasses increased in percent cover, whereas most of the C_3 grasses and forbs decreased during a drought year in a humid temperate climate, suggesting that the C_3 plant species were more negatively affected by the drought than the C_4 plant species.

The growing-season *e*WUE values for the annual corn and perennial grassland sites reported here are similar to values reported in the literature. For example, Beer and others (2009) reported *e*WUE of 2.1–4.4, and 1.6–4.0 g C kg^{-1} H_2O for several grasslands and crop-lands, respectively; Xiao and others (2013) reported a growing season *e*WUE of 2.8, 1.1 and 0.9 g C kg^{-1} H_2O for typical steppe, degraded desert steppe and desert steppe, respectively. Ponton and others (2006) also reported an average (±SD) daily *e*WUE of 2.6 ± 0.7 mmol mol^{-1} for a moist mixed-grassland. The Ponton and others (2006) *e*WUE falls in the lower range of our *e*WUEs, but their values were computed during the day when vapor pressure deficit (VPD) is at its maximum.

In sum, land-cover conversion between annual crops and perennial grasslands will have important implications to primary production and *e*WUE in the Midwest region. Food and bioenergy production in the region will likely influence future land conversions, with crop choices driven by commodity prices, conservation programs, aesthetic values, and government biofuel mandates. Land conversions from set aside CRP lands to annual corn increase yield and hence *e*WUE of the land. CRP grasslands were generally in annual crops before conversion, and are unlikely to have been converted to either switchgrass or restored prairie in the past. However, the need may arise if these lands were to be harvested for cellulosic biofuel feedstock, as switchgrass or restored prairie may produce greater yield and have higher *e*WUE than the smooth brome grass commonly planted in CRP grassland. With implementation of the US Energy Independence and Security Act of 2007, many of the recent CRP grassland to annual crop conversions may be reversed. The yield and *e*WUE of these grasses will depend on the grass type that replaces the annual crop, but will probably be lower than that of corn, especially initially during establishment phase. However, the lower production and *e*WUE should be compensated by the better ecosystem service provisions of the perennial grasslands compared to corn (Werling and others 2014). The grassland community composition may also change over time after establishment in response to climatic conditions such as drought, which favors grasses with C_4 photosynthetic pathways that have higher yield and *e*WUE than C_3 grasses.

*i*WUE Compared to *e*WUE

The *i*WUE determined from $\delta^{13}C$ of plant materials could not be directly compared with *e*WUE determined from EC for most of our sites since many were dominated by C_4 plant species whose *i*WUE cannot be accurately estimated from isotopic discrimination. However, the average $\delta^{13}C$ of C_4 grasses measured from 2011 through 2013 at the corn, switchgrass, and restored prairie sites showed a decrease of approximately 1‰ during the drought of 2012 (data not shown), which is a sign of water stress in C_4 plants (Ghannoum and others 2002). Sites with dominant C_3 species, on the other hand, exhibited *i*WUEs 1.2–2.3 times larger than *e*WUE, a difference that is consistent with other estimates of *i*WUE of C_3 plants (Ponton and others 2006; Flanagan and Farquhar 2014; Appendix S3). The *i*WUE estimated from $\delta^{13}C$ is greater than the *e*WUE estimated using EC measurements, likely because of the inclusion of soil and canopy surface evaporation components in *e*WUE that are not accounted for in the isotope-based *i*WUE. It is important to note that the *i*WUE of the same C_3 species remains similar across the years; however, different C_3 species have different *i*WUEs within a particular year (Figure 3; Monson and others 2010).

Although the *i*WUE of the same species did not change much over the years, the relative abundance of a species at a site did change from one year to another. For example, the dominant C_3 species that contribute to the aboveground biomass changed in type and decreased in number from year to year (Figure 3). These changes could create differences in community *e*WUE between years depending on the dominance of specific C_3 species in given year. Thus, both the composition of C_3 and C_4 plant species within a site as well as the overall relative abundance of species with the C_3 and C_4 photosynthetic pathways could change the *e*WUE.

We conclude that ecosystem water-use efficiency (*e*WUE) of planted perennial grasslands is determined to a great extent by the relative abundance of C_3 and C_4 plant species within a given season. Our results also show that land-use history could play an important role in *e*WUE through the strength of the relationship between GPP and ET, presumably related to differences in soil fertility and water-holding capacity as reflected by soil organic carbon and nitrogen content.

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ONLINE SUPPLEMENTAL INFORMATION

Appendix S1. Mean ANPP (g C m^{-2}) by Species and Photosynthetic Pathway Type in the Restored Prairie Sites from 2010 through 2013 Showing Species Composition Change Before, during and Following the 2012 Drought

	Mean ANPP (g C m^{-2})							
Species	AGR-Pr				CRP-Pr			
C_4	2010	2011	2012	2013	2010	2011	2012	2013
Sorghastrum nutans	0.3	31.0	122.1	659.8		10.4	18.6	193.2
Andropogon gerardii		4.8	13.4	171.4		1.0	1.1	51.3
Schizacyrium scoparium	0.3	1.6	15.1	29.7		0.9	1.6	23.2
Panicum virgatum	5.5	0.0	0.8	25.4		0.1		7.7
C_3 and forbs								
Ambrosia artemisiifolia	6.0	3.5		5.0				
Avena sativa	146.2	0.9			286.1	0.3		
Bromus inermis							0.3	4.8
Cassia fasciculata	4.0	7.2	0.3	7.6	1.5	0.6		1.6
Chenopodium album.	0.3				10.0	1.3		
Cirsium arvense								5.2
Conyza canadensis		0.7				1.3	0.2	
Coreopsis lanceolata	0.2	12.8	12.2	0.5	0.6	32.7	53.8	8.2
Dactylis glomerata		1.2				15.6	3.9	15.4
Echinacea purpurea	0.7	0.7	0.3	5.3	0.0	8.0	12.4	52.3
Elymus canadensis				6.4				

Elymus repens	46.3				84.5		4.1	39.1
Erigeron annuus					0.2			2.1
Euthamia graminifolia						2.9	0.8	1.4
Heliopsis helianthoides	0.2	0.3	0.5	2.9	0.1	4.4	4.2	50.9
Lolium multiflorum		112.6	193.9	6.5		67.2	112.5	
Medicago sativa						3.5	8.5	8.1
Monarda fistulosa			0.9	1.6		2.3	14.5	15.0
Phleum pratense						0.7	1.2	
Phytolacca americana	0.1	0.1	0.3					
Poa pratensis				1.4	0.0		1.4	3.5
Ratibida pinnata	0.1	0.5	4.4	3.5		0.8	6.5	69.0
Rudbeckia hirta		75.1	3.5	1.0	3.2	234.4	4.3	3.6
Rudbeckia triloba		5.3				101.2	1.1	0.3
Silene alba	0.0				2.2	66.2	24.2	4.5
Solidago canadensis		0.0				1.0	1.1	6.9
Sonchus arvensis		0.2				1.7		
Taraxacum officinale	0.6	1.4		0.1	0.0	0.4		0.2
Trifolium pratense.					0.0	1.7	0.0	

The mean ANPP is taken from 1-m^2 quadrats of 10 sampling stations. Species occurring only in one season and contributing minimally to ANPP were not included. Blank indicates absence of species and 0.0 indicates presence of species at very low value. Shaded and unshaded rows indicate prairies planted from seed and unplanted grasses that grew on the site, respectively; and *Avena sativa* (oats) was planted in 2010 as a nurse crop.

ONLINE SUPPLEMENTAL INFORMATION

Appendix S2. Analysis of the Mean Seasonal *e*WUE (GPP to ET ratio) Values Among Sites Within a Season Using Linear Mixed Model Fits

All years	AGR-C	AGR-Pr	AGR-Sw	CRP-C	CRP-Pr	CRP-Sw	Ref
2010	3.6^{c}	2.6^{ab}	2.5^{a}	3.7^{c}	2.7^{ab}	2.9^{ab}	3.0^{b}
2011	3.9^{c}	2.8^{a}	2.8^{a}	4.3^{d}	2.6^{a}	3.1^{b}	2.9^{ab}
2012	3.6^{cd}	3.4^{c}	3.3^{bc}	3.8^{d}	2.8^{a}	3.1^{ab}	2.9^{a}
2013	4.5^{d}	3.5^{c}	3.8^{c}	4.4^{d}	2.9^{b}	2.7^{b}	2.4^{a}

Significant values are given at $p<0.05$.

ONLINE SUPPLEMENTAL INFORMATION

Appendix S3. Biomass-weighed Mean *i*WUEs (g C kg^{-1} H_2O hPa^{-1}) and Average Daily Vapor Pressure Deficit (VPD, in hPa) During the Growing Seasons for Restored Prairie at the CRP-Pr and AGR-Pr Sites and for Brome Grass at the Ref Site from 2010 through 2013, Means ± (SD of means)

Cropping system	Year	Growing Season (*DOY range*)	Average VPD (*hPa*)	*i*WUE (g C kg^{-1} H_2O hPa^{-1})
CRP-Pr	2013	111-266	8.8 (0.3)	6.1 (0.4)
CRP-Pr	2012	126-274	14.1 (0.6)	4.4 (0.4)
CRP-Pr	2011	121-266	8.9 (0.4)	6.5 (0.4)
AGR-Pr	2012	126-274	14.1 (0.6)	3.5 (0.0)
AGR-Pr	2011	120-266	8.9 (0.4)	6.5 (0.9)
Ref	2013	111-274	8.8 (0.3)	8.0 (0.0)
Ref	2012	126-274	14.1 (0.6)	5.3 (0.1)
Ref	2011	121-265	8.9 (0.4)	7.9 (0.2)
Ref	2010	102-243	9.5 (0.3)	7.8 (0.0)