Maize and Prairie Root Contributions to Soil CO_2 Emissions in the Field

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ABSTRACT

Increasing soil carbon content via agricultural practices not only enhances the production potential of the land, but also counteracts rising atmospheric CO_2 levels. When predicting production systems' effects on soil carbon, quantifying CO_2 efflux derived from live roots is of particular importance as it is a through-flux and does not signify depletion of soil carbon. This field study aimed to measure and compare soil CO_2 emissions derived from roots in annual and perennial agroecosystems. We used periodic 48-hour shading over two growing seasons to estimate root growth-derived CO_2 in continuously grown maize (CC) with grain and 50% stover harvested each year, unfertilized reconstructed tallgrass prairie (P), and the same prairie grown with spring nitrogen fertilization (PF), both which had biomass harvested post-frost. In CC, P, and PF root-derived CO_2 contributed to 28, 31, and 30% of each crop's respective growing season cumulative CO_2 emissions in 2012, and 19, 24, and 28% in 2013, respectively. Season-cumulative root-derived CO_2 was not proportional to end-of-season belowground biomass (BGB): P had nearly twice the BGB of PF, but their cumulative root-derived fluxes were not significantly different in either year. A significant proportion of soil CO_2 emissions is derived from roots, making it a critical process to consider when comparing or modeling soil emissions of cropped or prairie soils. Using BGB alone may not be a useful proxy for estimating root contributions.

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Abbreviations: CC, continuously grown maize; BGB, end-of-season belowground biomass; P, un-fertilized reconstructed prairie; PAR, photosynthetically active radiation; PF, nitrogen-fertilized reconstructed prairie; SOM, soil organic matter; UAN, urea ammonium nitrate.

Increasing soil carbon (C) content via agricultural practices not only enhances the production potential of the land, but also counteracts rising atmospheric CO_2 levels. In the United States, production of agriculturally–derived biofuels strives to reduce the release of fossil CO_2 per the Renewable Fuels Consumer Protection and Energy Efficiency Act of 2007 (US White House, 2007) and should therefore rely on cropping systems that maintain or improve the C content of the soil. Many cellulosic ethanol plants currently use maize (*Zea mays* L.) stover as biofuel feedstock (DuPont, 2015; POET, 2013), but there is great uncertainty surrounding soil C sustainability of stover removal (Mann et al., 2002; Wilhelm et al., 2007). Dedicated perennial biofuel crops such as switchgrass (*Panicum virgatum* L.) or reconstructed prairies could offer an alternative feedstock option that may improve soil C sustainability (Dietzel et al., 2015; Varvel et al., 2008). Although long-term studies have identified these perennial grass systems as soil C builders (Anderson-Teixeira et al., 2009; Matamala et al., 2008), exact mechanisms are not well understood, and the impact of yearly biomass harvests is unclear (Fornara and Tilman, 2012; Rasse et al., 2005).

Predicting a cropping system's long-term soil C trajectory is challenging. Direct measurements are subject to large errors

due to high spatial variability of soil C and slow changes (Cambardella et al., 1994; Garten and Wullschleger, 1999), offering little insight into mechanisms for C storage. Approaches that quantify C inputs, outputs, and their subsequent balance provide an indication of how systems build or deplete C reserves over time. However, creating a C budget is made difficult by quantifying soil C lost through microbial decomposition of soil organic matter (SOM) (Cahill et al., 2009; Kucharik et al., 2006). Soil C lost via SOM decomposition diffuses as CO_2 from the soil surface. In vegetated soils, belowground portions of plants also produce CO_2 that diffuses from the soil, directly via respiration, and indirectly by exuding C compounds that are immediately oxidized by microbes living in the rhizosphere. The substrate for root-derived CO_2 is C that was recently fixed via photosynthesis; this flux is therefore simply recycling C back to the atmosphere and is not a net loss of soil C. The recycled C from roots mixes with the SOM-derived C, resulting in a CO_2 flux from the soil surface that requires careful interpretation.

One of the simplest paradigms for modeling root respiration assumes two basic components: maintenance and growth respiration (Amthor, 2000). The starvation method, also called the shading method, was developed to estimate respiration due to root growth under laboratory settings (Penning de Vries, 1972). In controlled conditions, shading for 48 h starves the plant of photosynthate and temporarily slows or stops root growth and thus reduces growth-derived CO_2– the difference between root respiration before and after shading estimates root growth-derived CO_2 (McCree, 1974). In a field setting, the microbes living in the rhizosphere that feed on root exudates will also be affected and the reduction in their activity should be equally suppressed by shading. Therefore, this same technique can be applied in a field setting to estimate the contribution of root growth-derived CO_2 to soil CO_2 emissions (Bahn et al., 2009; Kuzyakov, 2006). By comparing soil-surface CO_2 fluxes under full sun and various levels of shading, one can deduce the root growth-derived CO_2 contribution as the portion of soil flux that is responsive to light. Assuming the root-maintenance and SOM components are less tightly linked to photosynthesis, the shading method allows the exploration of light-derived contributions to the soil-surface CO_2 flux. This method has been successfully used in grasslands (Craine et al., 1999; Wan and Luo, 2003) but we are unaware of studies that have used this technique in agroecosystems, nor any that compare root growth-derived CO_2 in perennial and annual systems. Side-by-side comparisons of systems with different growth habits provide much needed insight into field dynamics of root respiration.

Systems with more root biomass typically exhibit higher soil CO_2 emissions (Brye et al., 2002; Daigh et al., 2014) This may be due to higher root maintenance respiration, root growth-derived respiration, or 'priming', a process by which root exudates stimulate microbial decomposition of SOM (Dijkstra et al., 2006; Kuzyakov, 2002). Studies have found a linear relationship between root biomass and soil CO_2 emissions in both managed (Han et al., 2007) and natural (Kucera and Kirkham, 1971) ecosystems; end-of-season root biomass of both annual and perennial systems has been used to estimate root contributions to soil CO_2 emissions (Koerber et al., 2010; Zhang et al., 2009). Maize and prairie systems have exhibited a wide range of end-of-season belowground biomass (BGB) in Iowa (1 Mg ha^{-1} to over 9 Mg ha^{-1}; Jarchow et al., 2014); we hypothesized that a crop's growing season-cumulative root growth-derived CO_2 is proportional to the BGB of the system regardless of growth habit. In addition to the amount of root growth-derived CO_2 released, the percentage of soil surface CO_2 emissions attributed to roots is an often-used value in C budgets. We hypothesized that the large amount of root-derived CO_2 produced by prairies would outweigh both the priming effect of roots and the faster maize root growth rates (Dietzel et al., 2015), resulting in a higher percentage of root-derived CO_2 in the prairies than maize. Therefore, we tested the following hypotheses: (i) the percentage of soil CO_2 emissions derived from root growth is higher in perennial prairie systems compared with an annual maize system, and (ii) the growing-season-cumulative CO_2 flux derived from root growth is linearly related to the end of season belowground biomass of the system.

We tested our hypotheses by using the shading method over two growing seasons in a field experiment to estimate the contribution of root growth-derived respiration to soil-surface CO_2 flux in three biofuel cropping systems: continuously grown maize, unfertilized prairie, and nitrogen fertilized prairie.

MATERIALS AND METHODS

Site, Design, and General Management

The field experiment was conducted during two growing seasons beginning in May 2012 through October 2013 at the Iowa State University South Reynoldson Farm in Boone County, Iowa (41°55′ N 93°45′ W). For a complete site description and history, please see Jarchow et al. (2014). The predominant soil types are Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquoll) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludoll) (USDA–NRCS, 2014). In 2007, soil sampling to 15 cm indicated a mean soil pH of 6.7 and mean organic matter concentration (Schumacher, 2002) of 51 g kg^{-1}. For more details on soil physical and chemical properties, please see Daigh (2013). Subsurface drainage installation was completed in spring of 2008 prior to the establishment of cropping systems; the site has been managed without tillage since establishment. Five potential biofuel systems were arranged as a spatially balanced complete block design (van Es et al., 2007) with four replicates in 27-m by 61-m

plots. Only three of the five cropping systems were investigated in this experiment: continuous maize (CC) of a 104-d maturity hybrid, 5-yr old 31 species-seeded reconstructed tallgrass prairie (P), and a spring nitrogen (N) fertilized 5-yr old prairie of the same seed mix (PF). The prairie seed mix contained, by weight, 12% C_3 grasses, 56% C_4 grasses, 8% legumes, and 24% non-leguminous forbs (Prairie Moon Nursery, Winona, MN). A detailed plant list can be found in Jarchow and Liebman (2013).

At planting, CC received 87 (11 May 2012) and 90 (17 May 2013) kg N ha^{-1} (32% urea ammonium nitrate [UAN] injected at 7.6 cm depth). Based on a late spring soil nitrate test (Blackmer, 1997) conducted when maize plants were 15–30 cm tall, CC plots were side-dressed with 112 (12 June 2012) and 67 or 157 kg (28 June 2013) N ha^{-1} in the form of 32% UAN injected every other row. The variability in fertilizer application reflects variability in the late spring nitrate test. Lime, phosphorus, and potassium were applied based on soil tests to the maize plots as needed. All pests and weeds were adequately controlled using chemicals. Grain was harvested from CC plots after physiological maturity (25 Sept. 2012 and 9 Oct. 2013, respectively), followed by shredding and windrowing of the stover. Approximately 50% of the stover was baled and removed from the plots. Maize grain yields on a dry weight basis were 8.0 and 9.0 Mg ha^{-1} in 2012 and 2013, respectively. County averages were 8.1 and 8.6 Mg ha^{-1} in 2012 and 2013, respectively (USDA–NASS, 2014).

On 28 Mar. 2012 and 26 Apr. 2013, PF received 84 kg N ha^{-1} (28% UAN broadcast). Prairie biomass (both P and PF) was harvested on 10 Oct. 2012 and 28 Oct. 2013, after a killing frost (see Nichols et al., 2014 for more details on biomass sampling methods). In 2012 and 2013, the amount of standing biomass before harvest was 4.7 and 6.1 Mg ha^{-1} in P, respectively, while standing biomass of PF was 7.6 and 9.4 Mg ha^{-1}, respectively. Both prairies were mowed to a height of 3 to 10 cm, and all loose biomass was removed. From standing biomass in P and PF, 2.8 and 5.2 Mg ha^{-1} was harvested in 2012, and 4.3 and 7.2 Mg ha^{-1} was harvested in 2013, respectively. Belowground biomass was measured using an in situ growth core to a depth of 1 m; more details on sampling methods can be found in Dietzel et al. (2015).

A meteorological station located at the research site provided air temperature, solar radiation, wind speed, humidity, and precipitation measurements. Additionally, daily soil volumetric water content and soil temperatures were taken at 5, 10, 15, 30, and 45 cm depths with Decagon 5TE ECH2O sensors and Em50 data loggers (Decagon Devices Inc., Pullman, WA); details about these measurements are provided in Daigh (2013). Stress-degree-days were calculated using the daily high air temperature with a base temperature of 30°C because above this temperature threshold maize development is negatively impacted and the plant is likely to suffer from heat stress (Tollenaar et al., 1979). One stress-degree-day was accumulated for each degree >30°C.

Shading

We used a split-plot design with the three cropping systems (CC, P, and PF) as main plots and three shade levels (0, 50, and 90% reduction in light) as subplots. Three 2 m-tall steel frames covering an area of 4 m^2 were placed in each main plot at the beginning of every growing season, located at least 3 m from the edge of the plot. Frames remained in the same place for the entire growing season except for in maize plots, where frames were partially removed to accommodate N-side dressing. In CC each shaded subplot encompassed one between-plant row receiving N-side dressing, one between-plant row with tractor wheel traffic, and two planted rows; in P and PF structures were placed randomly but aligned with the cardinal directions. Plots were periodically shaded by attaching shade cloth of varying mesh sizes (Greenhouse Megastore) to the top and three sides of the frame, leaving the north face open to facilitate air flow. Shade cloths were attached to the frames at night, remained in place for two to three full daylight cycles (64–76 h) depending on rain events (see *Soil-Surface CO$_2$ Flux* below), and were then removed. Shade treatment was randomly assigned to subplots, restricted by main plot. Shade treatments were rotated for subsequent rounds of measurements using a William's Latin Square design in time, ensuring that every subplot received the same sequence of shade treatments. This was done to avoid a subplot randomly receiving 90% shade treatment in consecutive sampling periods, as chronic shading could permanently affect plant growth. Shade treatments were applied every one to two weeks, again depending on rain forecasts, from May through September of both 2012 and 2013.

Data Collection

Due to equipment availability, from May to August 2012 shading and all flux-associated measurements were staggered between the four blocks. Two randomly chosen blocks received shading on Day 1, while the other two received shading the following day (Day 2). Leaf photosynthesis, soil CO$_2$ flux, soil temperature, and soil moisture were measured on the first group of blocks on Day 4, while the second group was measured on Day 5. These consecutive day measurements were treated as replicates, barring significant weather changes from one measurement day to the next (such as rain). In September 2012 and for the entire 2013 growing season, shading was applied to all four blocks on Day 1 and all measurements were made on Day 4. In the prairies, a total of 9 and 13 samplings occurred in 2012 and 2013; in the maize plots we sampled 6 and 10 times in 2012 and 2013, respectively (exact dates are presented in Supplemental File 1).

Photosynthesis

Leaf-level photosynthesis was measured using a portable infrared gas analyzer (LI-COR 6400, LI-COR, Inc., Lincoln, NE) with an leaf chamber attachment. In 2012, photosynthesis measurements were made between 1000 and 1300 h CST concurrently with soil-surface CO$_2$ flux measurements. In 2013, photosynthesis measurements were made on Day 3, and soil measurements on Day 4. Photosynthetically active radiation (PAR) was measured above and below the ceiling shade cloth at each sampling (AccuPAR LP-80, Decagon Devices Inc., Pullman, WA). In CC, four maize plants were randomly selected from within each shaded plot, and photosynthesis was measured by setting the LED chamber to the shaded PAR value on the last fully expanded green leaf before tasseling, and afterward on the ear leaf. Shaded prairie plots contained a diverse plant community whose composition shifted throughout the year due to

varying growth habits (Jarchow and Liebman, 2013). In 2012, for each sampling we chose one species that appeared in every subplot and measured photosynthesis of three leaves from that species in each subplot. In 2013, we instead chose three plants at each sampling that we felt best represented the community of the individual shade plot on that day and measured on the last fully expanded leaf of those plants. Many prairie grasses have narrow leaves; for plants with leaves that did not have sufficient leaf area to fill the measurement chamber, the width of the leaf was measured and leaf area was calculated using geometry. This calculated leaf area was used to calculate the net μmol of CO_2 uptake m^{-2} s^{-1}. These measurements were only used to confirm the step-wise effect of shading on photosynthesis.

Soil-Surface CO_2 Flux

Four 9-cm diameter PVC collars were installed within the area encompassed by each shade structure. In the prairie treatments, the four collars were randomly placed within each of the three shaded subplots, ensuring the collar was free from live vegetation. Maize collars were carefully placed to account for spatial variability (Daigh et al., 2014; Rochette et al., 1991), with one collar in a wheel-traffic row, one in a side-dressed row, and the remaining two between maize plants in a planted row.

In 2012, collars were installed in the prairie treatments in May and collars were installed in maize plots in mid-June following N side-dressing; all collars were removed in October. In 2013, collars were again installed in May in the prairie treatments, while maize collars were installed 1 wk after emergence, were removed for side-dressing, and were then re-installed for the remainder of the growing season. All collars were inserted to a depth of ~3 cm, with approximately 2 cm remaining above the soil surface. We waited at least 1 wk after collar installation to measure soil-surface CO_2 fluxes.

Soil-surface CO_2 flux measurements were taken using a portable infrared gas analyzer (LI-COR 6400, LI-COR, Inc., Lincoln, NE) with a soil respiration chamber (LI-COR 6400-09), coupled with soil temperature at 7 cm depth within 30 cm of the collar (LI-COR thermocouple attachment). Volumetric water content of the soil was measured within 2 h of flux measurements to a depth of 5 cm (Field Scout TDR 100, Spectrum Technologies, Aurora, IL). Flux measurements were conducted by placing the soil chamber over the PVC collar, reducing the CO_2 concentration below the ambient air concentration, and measuring the CO_2 concentration change over an interval of 10 to 30 s. The instrument measured CO_2 concentration against time, and reported the slope of the line at the ambient air concentration as the soil CO_2 flux (LI-COR Biosciences, 2012). All measurements were conducted in the morning between 0800 and 1200 h CST. When the site received more than 25 mm rain, we waited at least 24 h after the precipitation event before measuring.

To confirm that the soil CO_2 flux responded to shading and stabilized after shading through two full daylight cycles, we performed semi-continuous soil CO_2 flux, temperature and moisture measurements from 7–9 July 2012 independently from the main experiment measurements. On July 7 and 8 we measured soil CO_2 flux, soil temperature, and soil moisture (as described above) at approximately 0600, 1000, and 1800 h, and then at 1000 h on July 9.

Data Analysis

Soil CO_2 flux outliers were assessed as follows: for each crop at each date, a linear model was fit using the *lm* function in R. Carbon dioxide flux was the dependent variable, with block, shade, soil temperature, and soil moisture as fixed effects. Fluxes with residuals greater than 4 were removed from the dataset. Approximately 1% of all data was removed as a result. This outlier-free data set was used for all subsequent calculations.

Effect of Light Availability on Photosynthesis

The percent reduction in ambient light was analyzed using the MEANS procedure in SAS (SAS Institute, 2011). The effect of shading on photosynthesis was analyzed using the MIXED procedure of SAS. Year, date, crop, shade, and their two-, three-, and four-way interactions were fixed variables and block was random. The effect of shade was analyzed for each crop on each date, and effects were considered significant at $p < 0.05$.

Correction for Shading Effects on Soil Temperature and Moisture

Our interest was in isolating the effect of PAR on soil-surface CO_2 emissions, but shading has the potential to alter the micro-environment of the affected area. Soil temperature and moisture have been identified as drivers of soil CO_2 production (Mielnick and Dugas, 2000; Skopp et al., 1990), and shading-induced changes in these variables could be confounded with changes in PAR. We corrected for changes in soil temperature using an exponential function as described in Supplemental File 2. In general, temperature correction was warranted before canopy closure occurred. In 2012, fluxes were adjusted to account for the effect of shading on soil temperature on June 1, June 12, and June 24 sampling dates (both P and PF). In 2013 samples taken on June 3 (P), June 13 (CC, P, and PF), June 18 (PF), June 29 (P), July 27 (P), and August 1 (P) warranted temperature adjustment.

Estimating CO_2 Derived from Root Growth

At each sampling date, we evaluated the temperature-corrected CO_2 fluxes from each crop versus the percentage of ambient light each plot received. We fitted a regression line to the data using the *lme* function from the *nlme* package in R (Pinheiro et al., 2014) with shade as a fixed effect and block as a random effect. The results from the *lme* function were used to estimate the flux under full sun and extrapolate a flux under 0% ambient light (i.e., 100% shade); the difference between the two was assumed to be CO_2 derived from root growth (Fig. 1a). Instances in which more light corresponded to lower CO_2 fluxes were assumed to have no root growth-derived contribution (Fig. 1b) to ensure conservative estimates of root contributions.

Analyzing Semi-Continuous Measurements

Results were analyzed using the MIXED procedure of SAS using the REPEATED statement. We selected the heterogeneous autoregressive as the best structure for the variance-covariance matrix of the residuals based on AIC criteria. Block was assigned as random, and all other effects and their interactions as fixed. Comparisons were made using the PDIFF statement, and unless otherwise specified differences were considered significant at $p < 0.05$.
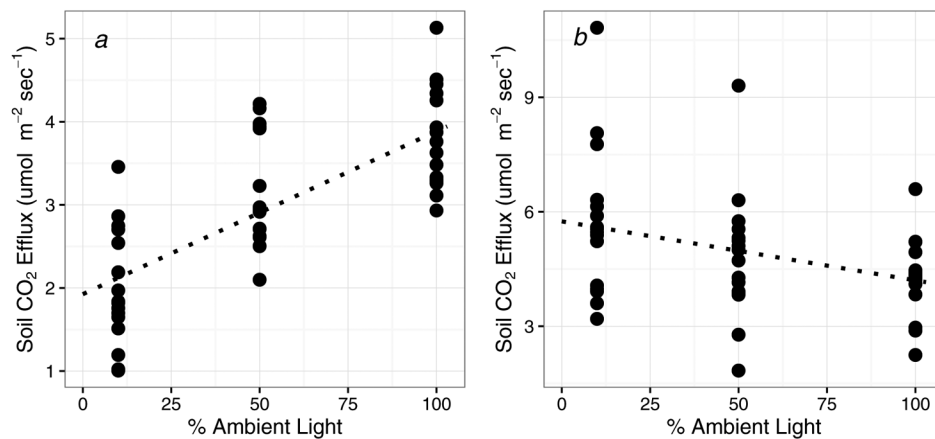
Fig. 1. Root growth-derived CO_2 flux was estimated using linear regression; points represent replicate values (n = 16), dotted line is linear regression; note the different vertical scales. (a) On 29 June 2013 in the unfertilized prairie, soil CO_2 efflux increased with increasing incident light, CO_2 from root growth was calculated as the difference between the temperature-corrected flux at 100% ambient light and the extrapolated value at 0% ambient light. (b) On 6 July 2013 in the unfertilized prairie, soil CO_2 flux decreased with increasing light, no temperature correction was warranted and we assumed no root growth-derived CO_2 contribution on this date.

Calculating Percentages and Total Root-Growth Emissions

To compare growing season values, we summed root growth–derived CO_2 and soil CO_2 values from every sampling period taken throughout the growing season. Cumulative sums are sensitive to missing data; stop-gap measures used in these cases are described in Supplemental File 3. We calculated the percentage of each crop's growing season CO_2 emissions attributed to root growth-derived CO_2 by dividing each crop's cumulative root growth–derived emissions by the cumulative soil emissions in each year. The assumption of normality for the percentage data was assessed using the *nlme*, *lsmeans* (Lenth, 2016), and *lme* packages in R; all assumption criteria were met. Both the percentages and absolute totals were compared using the MIXED procedure of SAS with year, crop, and their interaction as fixed effects and block as random. Comparisons were made using PDIFF and ESTIMATE statements.

RESULTS

Weather

In 2012, the site experienced season-long drought coupled with extremely high temperatures, while in 2013, the site experienced a wet spring followed by drought and warm temperatures relative to historical averages (Fig. 2).

Photosynthesis

Over the 2012–2013 growing seasons, the 50% shade treatment resulted in an average of 54% (±3% SD) reduction in PAR, while the 90% treatment achieved an average of 87% (±2%) reduction. A reduction in PAR consistently and significantly reduced leaf level photosynthesis in all crops on all dates (e.g., Fig. 3) except for the last sampling date in CC (24 Sept. 2013) 2 wk before harvest when no significant difference was found.
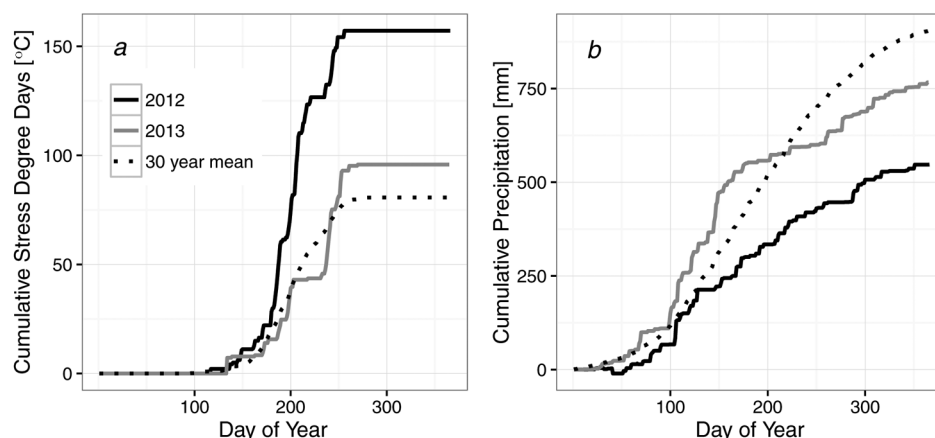

Fig. 2. Summary of study site weather 2012–2013 presented with 30 yr means recorded at a distance of 24 km from the study site. (a) Cumulative stress-degree-days. (b) Cumulative precipitation.

Soil Respiration

The semi-continuous measurements taken in July 2012 showed a marked reduction in soil CO_2 flux during the first daylight cycle, especially in the prairie systems (Fig. 4).

In all three systems, CO_2 fluxes under each shade treatment at 24 h were not significantly different from values at 63 h, indicating the values had stabilized within the two full daylight cycles we allotted for regular measurements.

Reducing the amount of light available did not always reduce soil CO_2 fluxes (e.g., Fig. 1b). More details are presented in Supplemental File 4; instances where reducing light increased soil CO_2 fluxes were assumed to have no CO_2 contribution from root growth.

Effects of Soil Moisture and Temperature on Soil Respiration

In 2012 and 2013, soil temperatures during sampling ranged from 13 to 32°C and 12 to 28°C while volumetric water content readings ranged from 10 to 67% and 8 to 77%, respectively. We observed no consistent relationship between soil moisture at a 5-cm depth and the degree of shading or with measured CO_2 emissions (Supplemental File 2). Under these conditions we assumed that no correction was warranted regarding soil moisture. During early vegetative stages before canopy closure occurred, we found that shading reduced soil temperature at the 7-cm depth. We corrected for these effects using a Q10 model (Davidson et al., 2006; Supplemental File 2).
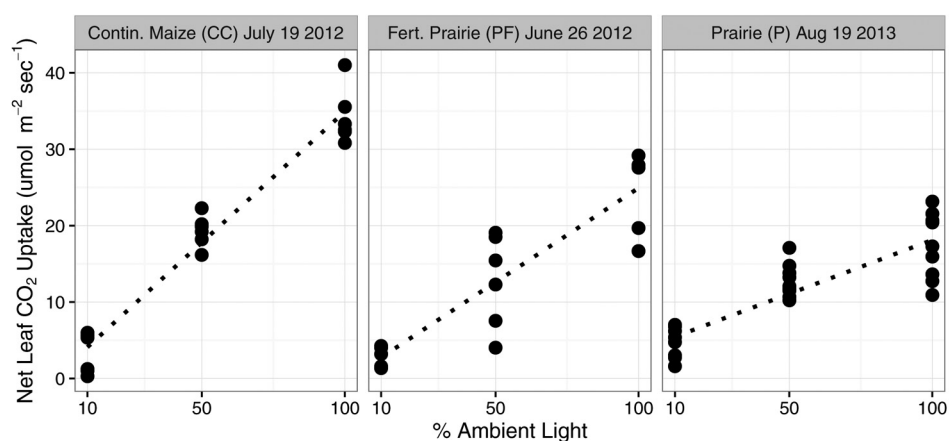

Fig. 3. Plant leaf-level photosynthetic responses to 10, 50, or 100% ambient light at representative days in each cropping system; points represent each replicate ($n = 6$), dotted lines are linear regressions fit to the data for visual interpretation only.
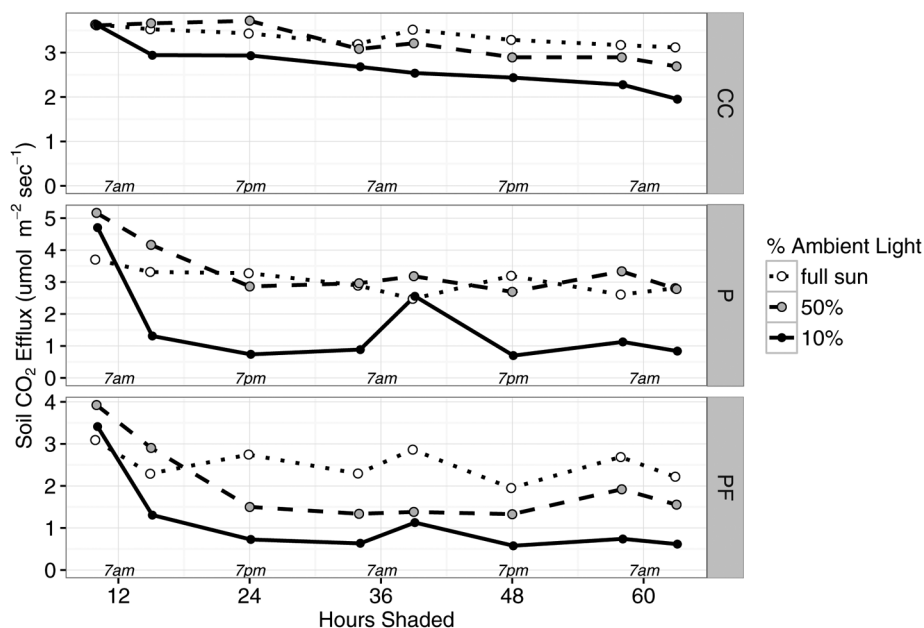

Fig. 4. Semi-continuous soil CO_2 flux measurements taken after applying shade at 1900 h on 7 July 2012. Points represent means of 16 measurements; jointed trend lines are added for visual interpretation only. CC, continuous maize; P, unfertilized prairie; PF, fertilized prairie.

Percentage of Cumulative Growing Season CO_2 Emissions Attributed to Roots

The percentage of soil emissions attributed to roots across growing seasons was higher in all systems in 2012 than in 2013, although only significantly in P ($p = 0.0003$) and CC ($p = 0.02$; Fig. 5). The perennial systems had significantly larger percentages than maize in both years, averaging 7% higher over the 2 yr. The differences between prairies was inconsistent between years; in 2012 the root growth-derived percent in P and PF was not significantly different, while in 2013 that of PF was significantly larger than of P ($p = 0.03$).

Cumulative Root-Derived CO_2

Over the growing season, the mean cumulative root growth-derived CO_2 did not differ between prairies in either year, but overall it was higher in the perennial systems than in the annual maize system ($p < 0.0001$; Table 1).

DISCUSSION

Shading Reduced Photosynthesis, but Not Always Soil-Surface CO_2 Flux

Our semi-continuous measurements confirmed that in early July of 2012, the effects of reducing photosynthesis were manifested in soil CO_2 emissions within 24 h, consistent with other studies that found newly assimilated

photosynthate transport in herbaceous plants occurred within 24 h (Davidson and Holbrook, 2009; Kuzyakov and Gavrichkova, 2010; Warembourg and Estelrich, 2000). Studies have shown photosynthesis is a significant driver of soil CO_2 emissions in forests (Curiel Yuste et al., 2007; Davidson et al., 1998; Hartley et al., 2006; Vargas et al., 2011), un-managed grasslands (Bahn et al., 2006; Gomez-Casanovas et al., 2012; Norman et al., 1992) and winter barley (Moyano et al., 2007); our study demonstrates the linkage exists in both summer annual and perennial cropping systems as well.

Instances in which less available light corresponded to an increase in soil CO_2 emissions were unexpected (Fig. 1b). To our knowledge, no studies have reported this phenomenon. Shade-induced increases were only observed in the prairie systems (P and PF), and the magnitude of increase was more pronounced in P compared with PF. Neither the timing nor magnitudes of these instances were consistent from year to year. These events occurred exclusively when the average soil moisture at a depth of 10 to 30 cm of each system was <20% volumetric water content, although the expected response (i.e., a decrease in soil CO_2 flux with shading) was also observed at similar soil moistures. We observed no relationship between instances of shade-induced increases in CO_2 and average soil temperatures at depth (0–10, 10–30, and 30–60 cm layers). Although the phenomenon only occurred in the prairies, during those sampling periods
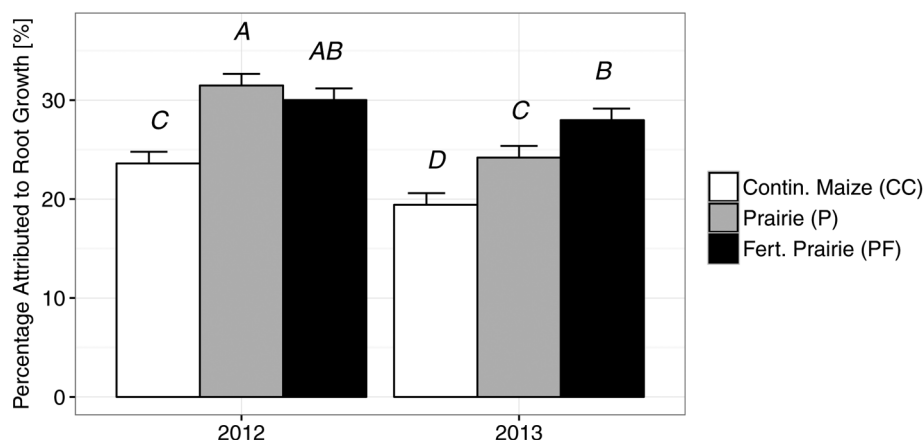

Fig. 5. Mean percentage of cumulative growing season soil-surface CO_2 emissions attributed to root growth in 2012 and 2013; error bars represent standard errors of the means ($n = 4$); means are compared across years with different letters indicating significant differences ($p < 0.05$).

Table 1. Cumulative growing season root-derived CO_2 flux. End-of-year belowground biomass values from a 1-m sampling depth are provided for comparison (Liebman et al., 2013).

	2012		2013	
	Root-derived CO_2	Belowground biomass	Root-derived CO_2	Belowground biomass
	µmol CO_2 m^{-2} s^{-1}	Mg ha^{-1}	µmol CO_2 m^{-2} s^{-1}	Mg ha^{-1}
Continuous maize (CC)	3.61 (0.20) b†	1.5 (0.16) c	5.38 (0.33) b	2.0 (0.16) c
Prairie (P)	8.64 (1.35) a	11.0 (0.20) a	11.38 (0.49) a	10.1 (0.58) a
Fertilized prairie (PF)	8.39 (1.5) a	6.7 (0.76) b	11.76 (1.93) a	5.8 (0.47) b

† Means, standard deviations, and statistical comparisons are presented; means within the same column not followed by the same letter are significantly different ($p < 0.05$).

the soil temperature and moisture at depths of 7 cm and 5 cm under 10% ambient light were not statistically different between cropping treatments. The prairies' different soil structure and rooting depths may play a role in this unexpected result. As the soil dries it shrinks, which may allow faster diffusion of CO_2 from deeper soil layers. This effect may be more pronounced in the prairies as their roots are distributed deeper and soils with perennial vegetation have been shown to have larger and longer macro-pores as compared with maize (Blanco-Canqui, 2010; Daigh, 2013; Jarchow et al., 2014). Additionally, the differences in annual and perennial growth habits may contribute to the fact that this was not observed in the maize treatment. It is possible that during seed set or flowering, combined water and light stress may cause perennial systems to prioritize above-ground growth and translocate carbohydrate resources from root reserves, thus increasing root-respiration in response to light deprivation (Connor et al., 2011). Yet another possible factor is that perennial grass root shedding of fine root hairs after extended periods of drought could increase soil CO_2 effluxes by providing a flush of easily-metabolized carbohydrates for soil microbes (Huang and Fry, 1998). We compared the timing of the shade-induced soil CO_2 increases to a growth study previously done at this site (Dietzel et al., 2015) using thermal time; we found our observations corresponded to times of net decreases in prairie root mass in 2012, but not consistently in 2013.

We would expect plants in the unshaded plots to have a much higher transpiration rate. This higher transpiration rate might indirectly result in lower detectable root respiration because during times of high transpiration rates, trees transport a significant amount of root-respiration CO_2 through the xylem, thus by-passing soil-surface measurements (Grossiord et al., 2012). This transpiration-induced re-routing of root-derived CO_2 could explain our observations, although whether this same phenomenon occurs in herbaceous plants is unknown.

Higher Percentage of CO_2 Emissions were Attributed to Root-Derived CO_2 in Prairies than in Maize

As we expected, a higher percentage of the total growing season soil flux in the perennial systems was derived from roots compared with the annual maize system (Fig. 5), with the prairies averaging 29% and the annual maize 22%. Using natural isotope abundances, Rochette et al. (1999) found root contributions accounted for 27% of growing season soil emissions in a Canadian maize system. In Germany, Moyano et al. (2007) used root exclusion methods in winter barley and found that root-derived CO_2 effluxes accounted for 17% of the growing season soil CO_2 emissions. In un-managed prairie of the US Midwest, Wan and Luo (2003) found light-dependent respiration contributed

23% of the annual soil surface CO_2 emissions. Our values are therefore well within the range of similar assessments of root-derived respiration. However, the prairie values from our study are a slight underestimation as there was already green biomass present when we began our measurements in May, and we assumed there was no root-derived CO_2 when shading induced higher CO_2 fluxes. It should be emphasized that the values from our study are for the growing season and are not valid for whole year extrapolations, which may explain the difference between our conservative prairie estimate (29% for the growing season) and that of Wan and Luo (2003; 23% for the year).

The higher values in 2012 likely reflect the drought and subsequent lack of soil moisture. Microbial decomposition of SOM was potentially suppressed, while root growth in the perennial systems was promoted in response to water shortage (Dietzel, 2014; Liebman et al., 2013). The ranking of the systems was not consistent across years (Fig. 5); the prairies had the same percentage of root-derived CO_2 in the dry and hot year (2012), but the PF had a higher root contribution than P in the wetter year (2013). Additionally, although the maize system was lowest in both years, it was significantly different between years, being lower in the wetter year. These differences indicate that the percentage of CO_2 emissions due to root growth is not fixed for a given system. The root- and soil-derived components respond differently to environmental conditions, possibly on a daily or even hourly timescale. Using fixed proportions, as it is often done, might result in inaccuracies in the calculation of cropping system carbon budgets. It is possible that using a biophysical-based model that incorporates the dynamics we describe here will produce more accurate estimates of both SOM- and root-derived CO_2 on a field scale, and therefore improve our estimates of carbon cycling in annual and perennial systems.

Cumulative Root-Derived CO_2 was Not Proportional to Total Belowground Biomass

Contrary to our hypothesis, total growing season root-derived CO_2 was not proportional to BGB. In both years, P contained nearly twice the BGB of PF, yet the two systems produced the same amount of root-derived CO_2 (Table 1). This may be due in part to the different species and functional group makeup in P and PF (Jarchow and Liebman, 2013) since root respiration varies with plant type (Reich et al., 2003). It may also be due to the complicated nature of perennial root systems: at any given time the BGB is a mix of live, growing, dying, and dead roots. Additionally, the growth component of root respiration is thought to depend on both growth rate and nitrogen content (Thornley and Johnson, 1990), although no field studies to our knowledge have confirmed this. In a study done from 2010 to 2011 at this same site, P had higher peak root growth rates but lower root nitrogen content

compared with PF (Dietzel et al., 2015). Therefore, the higher nitrogen content of PF roots could result in higher root growth respiration per unit of root compared with P, resulting in the two systems exhibiting similar amounts of root growth respiration over the growing season.

CONCLUSIONS

We found that perennial prairie systems' total growing season soil-surface CO_2 emissions have a higher percentage of CO_2 derived from root growth and associated exudates compared with an annual maize system. The cumulative amount of CO_2 produced from root growth processes was not proportional to the end-of-year biomass of the systems. During both years, the fertilized prairie had roughly half the belowground biomass of the unfertilized prairie, but released the same amounts of root growth-derived CO_2 over the growing season. Our results suggest that the growth component of root respiration contributes a substantial amount to the soil-surface CO_2 flux in the field. As such, the CO_2 resulting from root growth processes is an important factor to consider in carbon budgets and agroecosystem carbon cycle modeling.

Supplemental Materials Available

Four supplemental files are available with the online version of this article, with additional material available at http://miguezlab.agron.iastate.edu/publications.html.

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