

# Altered Belowground Carbon Cycling Following Land-Use Change to Perennial Bioenergy Crops

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## ABSTRACT

Belowground carbon (C) dynamics of terrestrial ecosystems play an important role in the global C cycle and thereby in climate regulation. Globally, land-use change is a major driver of changes in belowground C storage. The emerging bioenergy industry is likely to drive widespread land-use changes, including the replacement of annually tilled croplands with perennial bioenergy crops, and thereby to impact the climate system through alteration of belowground C dynamics. Mechanistic understanding of how land-use changes impact belowground C storage requires elucidation of changes in belowground C flows; however, altered belowground C dynamics following land-use change have yet to be thoroughly quantified through field measurements. Here, we show that belowground C cycling pathways of establishing perennial bioenergy crops (0- to 3.5-year-old miscanthus, switchgrass, and a native prairie mix) were substantially altered relative to row crop agriculture

(corn-soy rotation); specifically, there were substantial increases in belowground C allocation (>400%), belowground biomass (400–750%), root-associated respiration (up to 2,500%), moderate reductions in litter inputs (20–40%), and respiration in root-free soil (up to 50%). This more active root-associated C cycling of perennial vegetation provides a mechanism for observed net C sequestration by these perennial ecosystems, as well as commonly observed increases in soil C under perennial bioenergy crops throughout the world. The more active root-associated belowground C cycle of perennial vegetation implies a climate benefit of grassland maintenance or restoration, even if biomass is harvested annually for bioenergy production.

**Key words:** carbon cycle; root allocation; soil respiration; belowground carbon allocation; bioenergy/biofuels; soil organic carbon; perennial grasses; establishment phase.

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## INTRODUCTION

Terrestrial ecosystems have a pervasive influence on the global carbon (C) cycle, and thereby on climate (Le Quéré and others 2009; Pan and others 2011). A substantial and sometimes dominant

proportion of an ecosystem's greenhouse gas value may be derived from belowground C storage or ongoing sequestration (Anderson-Teixeira and DeLucia 2011), and globally terrestrial ecosystems contain approximately three times more C belowground than aboveground (Schlesinger 2005). Land use profoundly affects belowground C storage; land clearing and tillage for agriculture reduces soil organic C (SOC) by on average 20–40% (Davidson and Ackerman 1993; Murty and others 2002). On a global basis, it is estimated that 40–52 Pg CO<sub>2</sub> has been released historically through plowing of high-C soils such as those of native prairies (Lal 2004). Conversely, agricultural practices that enhance belowground C, including conservation tillage and cultivation of perennial crops, have the potential to restore some of this lost C, with consequent climate benefits (Bernacchi and others 2005, 2006; Lal 2004; DeLuca and Zabinski 2011).

Changes in SOC following land-use change have frequently been quantified (Anderson-Teixeira and others 2009; Davidson and Ackerman 1993; Murty and others 2002; Guo and Gifford 2002), and a number of mechanisms contributing to these changes have been identified. Important mechanisms include altered belowground C allocation to roots (Fornara and Tilman 2008), changes in the depth profile of C inputs to the soil system (Matamala 2008; Frank and others 2004), changes in the quantity or quality of litter inputs (Blanco-Canqui 2010), and altered rates of organic matter breakdown by microbial decomposers—driven, for example, by physical disturbance of soil through tillage (Grandy and Robertson 2007; Zenone and others 2011). Our understanding of the response of belowground C dynamics to land-use change remains limited; however, in that the complete suite of altered belowground C flows following land-use change has yet to be thoroughly quantified through field measurements. Here, we quantify belowground C inputs and outputs over a 3½ year period following land-use change from row crop agriculture to perennial bioenergy crops.

Land-use changes associated with the emerging bioenergy industry are likely to have substantial impacts on the global C cycle (Fargione and others 2010; Melillo and others 2009; Searchinger and others 2008). In the US, for example, the Energy Independence and Security Act of 2007 (US Senate 2007) mandates production of 21 billion gallons of ethanol from cellulosic sources by 2022—a target that would imply widespread land-use change and consequent alteration of biogeochemical dynamics (Davis and others 2011; Robertson and others

2011). Perennial grasses hold promise as second-generation bioenergy crops that could provide ligno-cellulosic material for ethanol while increasing belowground C storage, sequestering C in extensive root structures (McLaughlin and Kszos 2005; Fornara and Tilman 2008; Monti and Zatta 2009; Dohleman and others 2012) and accumulating SOC at rates averaging 40–100 g C m<sup>-2</sup> y<sup>-1</sup> (Anderson-Teixeira and others 2009). Eddy-covariance measurements of ecosystem-atmosphere CO<sub>2</sub> exchange over perennial agroecosystems in Illinois have demonstrated that they act as net C sinks (Zeri and others 2011). However, little is known about the dynamics of belowground C cycling in these ecosystems. Precise measurements of belowground pools and fluxes are critical both to understand the mechanisms behind C sequestration and to predict potential C cycle impacts of altered management practices or climate change.

Here, we quantify multiple components of the belowground C cycle in three potential perennial bioenergy crop agroecosystems—switchgrass (*Panicum virgatum* L.), miscanthus (*Miscanthus x giganteus*), and a native prairie mix—as well as a row crop control [corn–corn–soy (CCS) rotation]. We test the hypothesis that, relative to row crops agriculture, perennial agroecosystems have enhanced root-associated C cycling and greater potential for long-term C sequestration. Specifically, we predict that the perennials will allocate more C to roots and rhizomes, resulting in greater belowground biomass and higher root-associated respiration. However, offsetting this, we expect that reduced litter inputs and cessation of tillage will reduce heterotrophic respiration in the bulk soil. In terms of the net belowground C balance, we predict that—consistent with previous studies (Anderson-Teixeira and others 2009; Zeri and others 2011)—perennial crop establishment will result in net C sequestration.

## METHODS

### Study Site

The study was conducted at the University of Illinois Energy Farm in Urbana, Illinois (40°3'46"N, 88°11'46"W, ~220 m above sea level). Climate is highly seasonal, with monthly average air temperatures averaging below 0°C from December to February and above 20°C from June–August. Mean annual temperature is 11.1°C and mean annual precipitation is 1,042 mm (Illinois State Water Survey historic climate data, averaged from 1979 to 2009). Topography is minimal. The soil, typical of

the region, is a deep and fertile Flanagan (fine, montmorillonitic, mesic aquic Argiudoll) with some low lying blocks of Drummer (typic Haplaquoll). Previously, the land was in continuous cultivation of annual row crops for over 100 years.

## Experimental Design

The experiment was a randomized block design with five replicates of each crop type (miscanthus, switchgrass, prairie, and a CCS rotation). Replicates were arrayed in blocks to accommodate variation in soil fertility and drainage. Four blocks contained 0.7-ha plots for each vegetation type (henceforth, small plots). The fifth block consisted of 3.8 ha plots (henceforth, large plots), which are larger to provide adequate fetch for eddy-covariance measurements of surface atmosphere exchange made there (Zeri and others 2011).

In spring 2008, the plots were planted with maize (May 6, 2008), miscanthus (June 2–16, 2008), switchgrass (May 28, 2008) and a mix of 28 native prairie species (May 29, 2008; see Zeri and others 2011 for species list). Switchgrass and prairie crops were sown with oats (*Avena sativa* L.) as a cover crop. Because of poor establishment, additional miscanthus rhizomes were planted in small pots in 2009 and in the large plot in 2010. Corn (maize) and soy were rotated in a CCS pattern, with corn in 2008, 2009, and 2011, and soy in 2010. Crops were planted and managed according to standard agricultural practices for the region (maize, soy) or best-known management practices (perennials), as described in detail in Zeri and others (2011). Nitrogen fertilizer was applied to corn (168, 202, and 180 kg N ha<sup>-1</sup> in 2008, 2009, and 2011, respectively) and switchgrass (56 kg N ha<sup>-1</sup> in 2010 and 2011), whereas soy, miscanthus, and prairie were not fertilized because of known or suspected nitrogen fixing abilities of these crops (Davis and others 2010; Tilman and others 2006). Corn and soy crops were harvested and chisel-tilled in October or early November and all residue was left on the field. Perennial crops were harvested in the winter following the 2009, 2010, and 2011 growing seasons; harvests occurred in March 2010 (all perennials), November 2010 (switchgrass and prairie)/March 2011 (miscanthus), and November 2011 (prairie)/December 2011 (switchgrass)/January 2012 (miscanthus). Vegetation was cut approximately 10 cm above the ground and then baled using commercial disk-cutting mowers and balers (Case New Holland; Burr Ridge, Illinois, USA).

## Aboveground Biomass and Litter

Aboveground biomass and litter stocks were measured periodically (monthly throughout the growing season, and before and after each harvest) at randomly located positions within each plot ( $n = 2$  for small plots, 4 for large plots). Quadrats were 0.45 × 0.45 m for switchgrass and prairie and 0.75 × 0.75 m for maize and miscanthus (to account for planting row separation). All vegetation (live or dead) was clipped near ground level, and all visible litter was collected. Samples were dried at 60°C for at least 48 h and weighed.

Subsamples were analyzed to determine C content. Dried plant material was ground to pass a 425- $\mu$ m mesh (Wiley mill; Thomas Scientific, Swedesboro, New Jersey, USA) and further ground into a powder with a ball mill (GenoGrinder 2010; BT&C, Lebanon, New Jersey, USA). C concentrations were measured with a flash combustion chromatographic separation elemental analyzer (Costech 4010 CHNSO Analyzer, Costech Analytical Technologies Inc. Valencia, California, USA). The instrument was calibrated with acetanilide obtained from Costech Analytical Technologies, Inc. Average C content (averaged by crop type) was multiplied by dry biomass to convert units from dry biomass to C.

## Belowground Biomass

Root and rhizome biomass was measured during the growing season from 2009 to 2011. Late in the 2008 growing season and five times each throughout the 2009 and 2010 growing seasons, sets of three 5-cm cores were taken using an AMS split core sampler at randomly located positions within each plot ( $n = 2$  for small plots, 4 for large plots; same locations as biomass sampling). Cores were taken to a depth of 30 cm, split into two increments (0–10 and 10–30 cm), and the three replicates from each location combined by depth increment. In 2011, sampling frequency was reduced to once near peak belowground biomass (25 July–5 August; time of peak biomass estimated based on previous years) to allow doubled sampling intensity (4 sampling locations in small plots, 8 in large plots) and paired measurements to greater than 1 m depth. Deep root samples (>100 cm) were taken using a Giddings probe (3.8 cm diameter). At each sampling location ( $n = 4$  for small plots, 8 for large plots), three replicate cores were extracted, divided by depth increment (0–10, 10–30, 30–50, 50–100, and 100+ cm), and replicates combined by depth increment. Samples were

immediately transferred to cold storage (4°C) until processing. Roots and rhizomes were elutriated from the soil with root washing devices as in Roberts and others (1993). Roots and rhizomes were oven dried at 60°C for at least 48 h and weighed. Subsamples were analyzed and C content was calculated as described above.

## Litterfall

In each plot, four 0.6 × 0.6-m quadrats were placed in semi-permanent positions (moved at each harvest), and all new litter was collected periodically. Sampling frequency varied to account for seasonal differences in litterfall and decomposition rates; sampling was most intensive (up to once per week) during summer and fall and less intensive during winter and spring. Litterfall was also collected following each harvest. Samples were dried, weighed, and subsamples were analyzed for C content (described above).

Cumulative litter inputs were calculated for each year, and litterfall during any time gap between the last litterfall collection and harvest was estimated by comparison with subsequent changes in aboveground biomass and litter stocks. Cumulative annual litterfall inputs were calculated for each growing season-harvest cycle (April–March).

## Soil Respiration

Soil respiration ( $R_{\text{soil}}$ ) was quantified using both survey and automated measurements. Survey measurements characterized spatial variation (potential block differences) and separated root and rhizosphere respiration ( $R_{\text{root+}}$ ) from root-free soil respiration ( $R_{\text{rfs}}$ ), whereas automated measurements characterized temporal variation.

In all blocks, survey measurements were used to characterize both  $R_{\text{soil}}$  and  $R_{\text{rfs}}$ . For  $R_{\text{soil}}$ , 20 cm diameter PCV collars (height 11.4 cm) were inserted to a depth of approximately 5 cm at random locations within each plot ( $n = 4$  in small plots, 12 in large plots). At four paired locations in each plot, root enclosure collars (for  $R_{\text{rfs}}$ ) were inserted to a depth of about 50 cm (total height 55 cm). Starting in 2009, depth was reduced to about 30 cm (total height 35 cm) in corn and soy crops as these crops lacked significant root biomass beyond this depth. In the row crop treatment, collars were relocated following tillage. Measurements were made approximately every 3 to 5 weeks between mid-May and late October using a 20-cm diameter gas exchange chamber (LI-8100-103, LI-COR, Inc.). Each survey took 2 to 3 days to complete and was performed between the hours of 0900 and 1700

CST.  $R_{\text{root+}}$  was calculated as the difference between  $R_{\text{soil}}$  and  $R_{\text{rfs}}$ . Statistics were performed in R (R Development Core Team 2008) using a linear mixed-effects model fit by REML, where vegetation type, year, and block were fixed effects, sampling date was a random variable, and variance was scaled as a power of fitted respiration.

In each large plot, continuous  $R_{\text{soil}}$  measurements were made using four 20-cm diameter long-term chambers (LI-8100-101, LiCor, Inc.) connected to an infrared gas analyzer (LI-8011, LiCor, Inc.) through a multiplexer (LI-8150, LiCor, Inc.). Each chamber made one measurement per half-hour period. Soil temperature at 5 cm was measured continuously (Hydra Probe II Soil Sensors; Stevens Water Monitoring Systems, Inc.) and used to fill gaps in the  $R_{\text{soil}}$  record. Measurements began shortly after planting in 2008 and continued through the end of 2011.

Automated  $R_{\text{soil}}$  records were gap-filled and corrected using code written in Matlab® v. 7.8.0. Gap filling was performed by calculating a temperature-independent reference respiration ( $R_{\text{ref}}$ ), filling gaps through linear interpolation of  $R_{\text{ref}}$ , and finally calculating  $R_{\text{soil}}$  based on complete  $R_{\text{ref}}$  and soil temperature ( $T$ ; K) records. Previous analysis determined that this method was the most accurate among several gap-filling methods (Gomez-Casanovas and others 2013). Specifically,  $R_{\text{ref}}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 10°C) was calculated from  $R_{\text{soil}}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and  $T$  (K) based upon the Lloyd and Taylor (1994) model describing the temperature-sensitivity of soil respiration:

$$R_{\text{soil}} = R_{\text{ref}} e^{E_0 \cdot \left( \frac{1}{T_{\text{ref}} - T_0} - \frac{1}{T - T_0} \right)} \quad (1)$$

Here,  $E_0$  is a fitted parameter reminiscent of an activation energy (here, 308.56 K; (Lloyd and Taylor 1994),  $T_{\text{ref}}$  (K) is the temperature at which  $R_{\text{ref}}$  is characterized (here, 283.15 K), and  $T_0$  is the temperature intercept at which  $R_{\text{soil}} = 0$  (227.13 K, following Lloyd and Taylor 1994). Second, gaps in  $R_{\text{ref}}$  were filled using linear interpolation. Third, the gap-filled  $R_{\text{ref}}$  records were compared to simultaneously measured  $R_{\text{ref}}$  values from survey measurements and corrected for any substantial differences. When  $R_{\text{ref}}$  fell outside of one standard deviation of survey measurements (all blocks combined), the difference between the two was used to correct automated  $R_{\text{ref}}$  at that time. Correction factors were linearly interpolated between survey measurements, and the correction applied over the frost-free period of the year (approximately March–November). Finally, the gap-filled,

corrected record of  $R_{\text{ref}}$  was used to calculate  $R_{\text{soil}}$  based on Eq. 1.

## Net Belowground C Balance

Because the crops were harvested annually, there was no net change in aboveground biomass from year to year. Therefore, when calculated from one harvest to the next, the net belowground C balance (counting the litter layer as part of the soil system) is equal to the net ecosystem C balance (NECB)—that is, the net change in ecosystem C storage, whose dominant components in this system are net ecosystem exchange of  $\text{CO}_2$  (NEE) and lateral transfer of C through harvest (Chapin and others 2006). NECB also includes  $\text{CO}$ ,  $\text{CH}_4$ , and volatile organic C (VOC) fluxes and leaching of dissolved organic or inorganic C. In these ecosystems, C leaching is minimal and does not differ significantly among crops (Mark David, personal communication), and fluxes of C compounds other than  $\text{CO}_2$  are negligible (for VOC's at this site, see Miresmailli and others 2012).

NECB was calculated from NEE and harvest data. NEE was estimated using eddy-covariance measurements (large plots only) as described in Zeri and others (2011). NEE was measured continuously beginning shortly after planting in 2008 and continuing through the end of 2011. Records were gap-filled as described in Zeri and others (2011) and summed to produce annual estimates (delineated by calendar year). Uncertainty in NEE estimates associated with random errors or with data gaps was estimated as by Zeri and others (2011). C removed through harvest was subtracted to yield NECB. Miscanthus biomass from the 2011 growing season was harvested in January 2012; however, for the purposes of this analysis, the harvest was counted for the 2011 calendar year.

## RESULTS

### Vegetation Establishment and Total C Stocks

The perennial crops were planted in 2008, and typically require 2–4 years to establish. Whereas the switchgrass and prairie established well and reached “maturity” within about 3 years, the establishment of miscanthus was substantially delayed by poor initial establishment and planting of additional rhizomes in 2009 and 2010. Miscanthus, therefore, did not attain the biomass observed at a nearby site ( $> 1,720 \text{ g C m}^{-2}$ ; Heaton and others 2008) within the first four growing seasons (Figure 1).

Total C stocks (all organic C excluding SOC; that is, C in above- and belowground biomass and litter) were initially low in the perennial agroecosystems, but overtook those of the CCS rotation by 2011 (Figure 1). In the first year (2008), corn C stocks greatly exceeded those of the perennials, peaking at  $1,034 \pm 82 \text{ g C m}^{-2}$  whereas total C stocks remained below  $300 \text{ g C m}^{-2}$  in perennials. However, total C stocks in perennials were closer to those of corn in 2009 (peaking around  $600 \text{ g C m}^{-2}$  compared with  $1,075 \pm 91 \text{ g C m}^{-2}$  in corn) and greatly exceeded those of soy in 2010 (peaking at  $1,623 \pm 349 \text{ g C m}^{-2}$  in miscanthus,  $1,148 \pm 100 \text{ g C m}^{-2}$  in switchgrass, and  $799 \pm 129 \text{ g C m}^{-2}$  in prairie, compared with  $549 \pm 36 \text{ g C m}^{-2}$  in soy). In 2011, C stocks of miscanthus and switchgrass exceeded those of corn throughout the entire year (Figure 1), being more than double that of corn in late July when roots were measured ( $1,118 \pm 178$  in miscanthus and  $1,077 \pm 81$  in switchgrass, compared to  $505 \pm 9$  in corn); however, total C storage in prairie ( $479 \pm 65 \text{ g C m}^{-2}$ ) did not differ significantly from that of corn in late July. The partitioning of C into aboveground biomass, belowground biomass, and litter differed dramatically among crop types (Figure 1).

### Belowground C Allocation

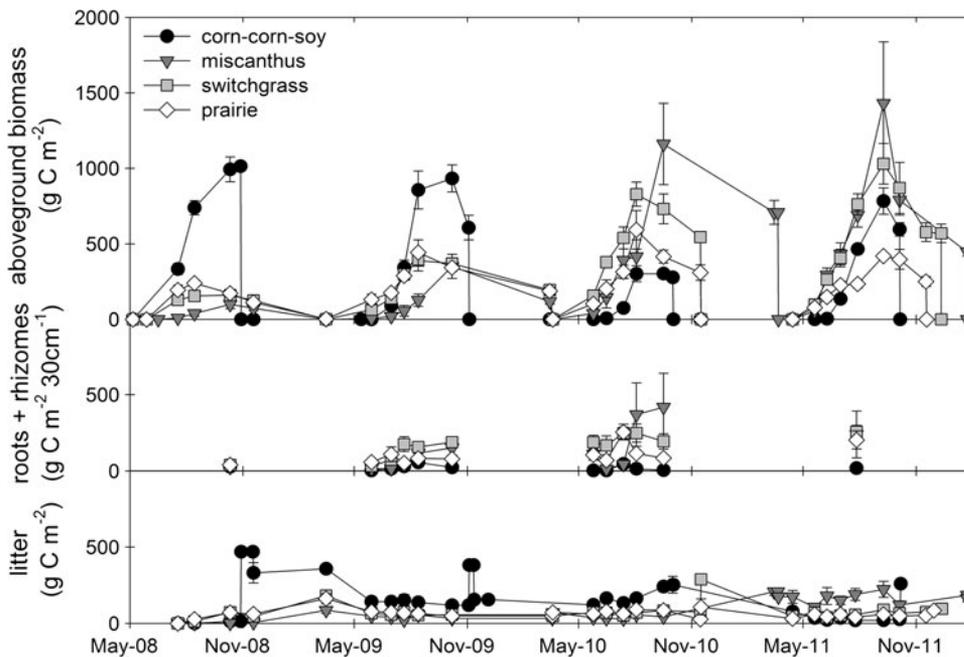
Perennial vegetation allocated far more C belowground to the root system than the row crops (Figure 1). Although there was no significant difference in belowground biomass (to 30 cm depth) at the end of the first growing season ( $p = 0.37$ ), belowground biomass was much greater in all perennial crops than corn in 2009 ( $p < 0.002$ ; date  $\times$  crop n.s.; repeated measures ANOVA in Matlab<sup>®</sup> v. 7.8.0), soy in 2010 ( $p = 0.006$ ; date  $\times$  crop n.s.), and corn in 2011 ( $p < 0.05$ ) (Figure 1).

Differences in belowground biomass were even larger when accounting for roots below 30 cm depth (Figure 2). Deep root cores taken at the approximate time of peak root biomass in 2011 reveal that the perennials had substantive root systems, with root densities greater than  $0.1 \text{ mg dry biomass cm}^{-3}$  extending to a depth of at least 100 cm. In contrast, corn root densities exceeding this threshold were confined to the top 10 cm of soil (Figure 2A). Samples to an average depth of 126 cm (no significant difference in achieved sampling depth among crops;  $p = 0.15$ ) revealed significant between-crop differences in total belowground biomass ( $p < 0.0001$ , block and block  $\times$  crop n.s.; type III SS ANOVA in Matlab<sup>®</sup> v. 7.8.0). Specifically, total belowground biomass was significantly lower in maize

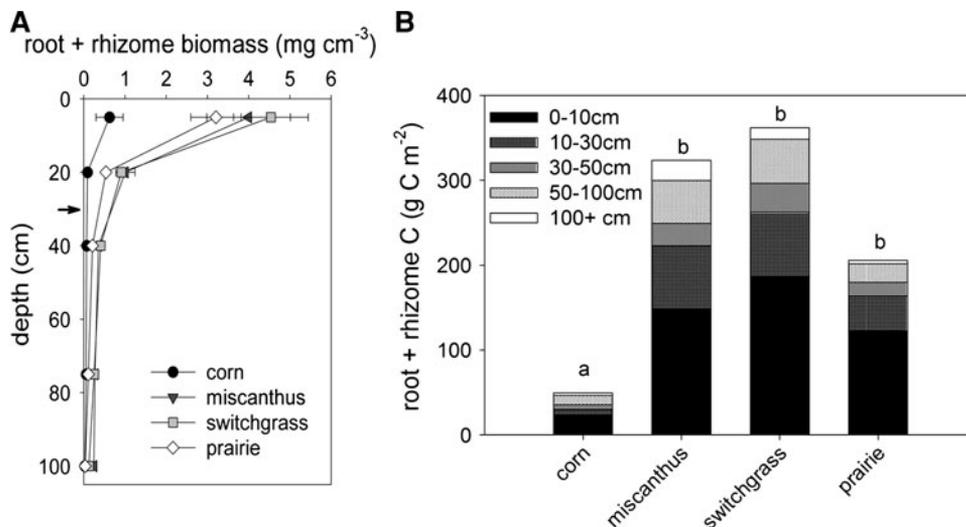
( $49 \pm 15 \text{ g C m}^{-2}$ ) than in all three perennial agroecosystems ( $323 \pm 57 \text{ g C m}^{-2}$  in miscanthus,  $119 \pm 45 \text{ g C m}^{-2}$  of which was rhizome;  $362 \pm 33 \text{ g C m}^{-2}$  in switchgrass; and  $206 \pm 22 \text{ g C m}^{-2}$  in prairie;  $p < 0.05$ ; Figure 2B). This implies that the routine biometric root measurements (Figure 1) missed less than about  $20 \text{ g C m}^{-2}$  for corn but as much as around  $100 \text{ g C m}^{-2}$  for mature perennials.

### Litter Inputs

A second major difference between perennial crops and the CCS agroecosystems was the amount and timing of litterfall (Figure 3A) and the resulting litter stocks (Figure 1). Total litter inputs varied significantly by crop in all years ( $p < 0.001$ ). Specifically, litter inputs from corn in 2008, 2009, and 2011 were significantly greater than those from



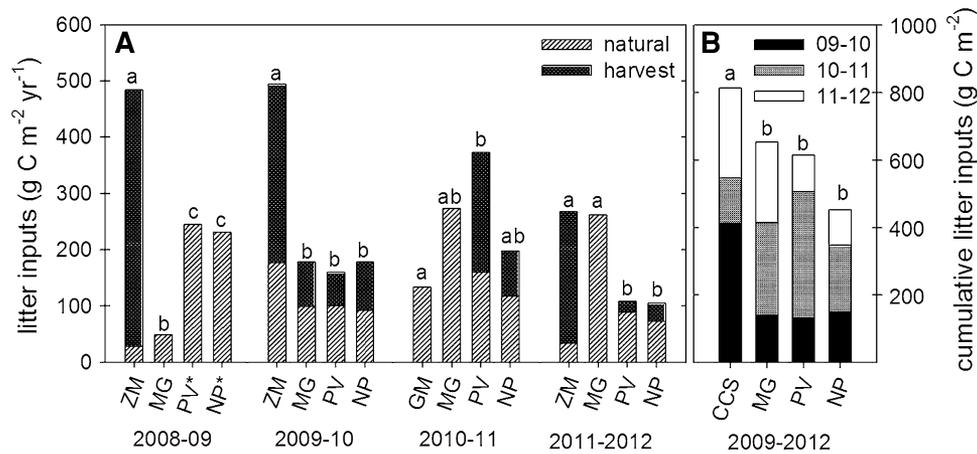
**Figure 1.** C storage in aboveground biomass, roots, and rhizomes in the top 30 cm of soil, and litter in three perennial bioenergy crops (miscanthus, switchgrass, and prairie) and a row crop control (CCS rotation) for the first four growing season-harvest cycles (May 2008–Feb 2011) following perennial establishment in 2008. Error bars 1 SE.



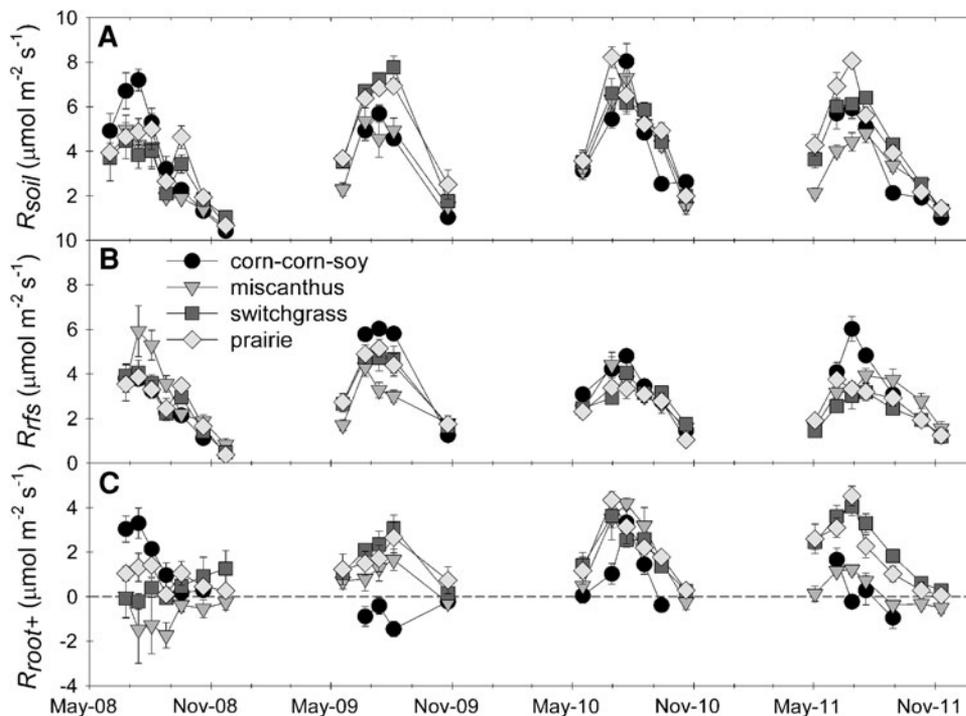
**Figure 2.** Belowground biomass of corn, miscanthus, switchgrass, and prairie in late July–early August 2011. **A** Root and rhizome biomass density as a function of depth (plotted by midpoint depth of each sampling increment). Error bars 1 SE. Arrow The depth of routine root samplings (30 cm). **B** Root and rhizome C storage by depth increment. Letters Significance groupings at  $p < 0.05$ . Average total core depth was 126 cm and did not differ significantly among crops ( $p = 0.15$ ).

perennials ( $p < 0.001$ ), with the exception that miscanthus did not differ significantly from corn in 2011 ( $p > 0.05$ ). Conversely, in 2010, litter inputs from soy tended to be less than those from perennials, but this difference was only significant in the case of switchgrass ( $p < 0.05$ ). As a result, the CCS ecosystem had higher litter stocks than the perennials following corn years (fall 2008–fall 2010; both  $p < 0.001$ ; repeated measures ANOVA in Matlab®

v. 7.8.0) but lower litter stocks in 2011 following a soybean year (Figure 1;  $p < 0.001$ ). The source and timing of litterfall also differed between CCS and perennials (Figure 3A). In corn, over 60% of litterfall occurred at the time of harvest, when all plant biomass except grain was returned to the field as litter. In soy, leaves dropped prior to harvest and the remaining plant material was pulverized through the harvesting process (although some of



**Figure 3.** Annual litter inputs in three perennial bioenergy crops—miscanthus (MG), switchgrass (PV), and prairie (NP)—and a row crop control (corn [ZM]—corn-soy [GM] rotation) for the first four growing season-harvest cycles (May 2008–Feb 2011) following perennial establishment in 2008. To represent each growing season-harvest cycle, litter inputs were summed from April to March of the following year. Letters Significance groupings at  $p < 0.05$ . \*Mowing of PV and NP in summer 2008 resulted in substantial litter inputs.



**Figure 4.** Soil respiration ( $R_{soil}$ ; **A**), heterotrophic respiration in root-free soils ( $R_{rfs}$ ; **B**), and root-associated respiration ( $R_{root+}$ ; **C**) under three perennial bioenergy crops (miscanthus, switchgrass, and prairie) and a row crop control (CCS rotation) for the first four growing seasons following perennial establishment in 2008. Error bars 1 SE.

the pulverized material was returned to the ecosystem, it was below the size threshold of “litter”). Once they reached maturity, perennials generally had higher natural litterfall rates than CCS. Litterfall inputs at time of harvest averaged less than 50% of the annual total for all perennials and were strongly influenced by the efficiency of harvesting equipment and the timing of harvest. In 2010 and 2011, relatively high miscanthus litterfall during fall and winter built up litter stocks, which showed no net increase following harvest.

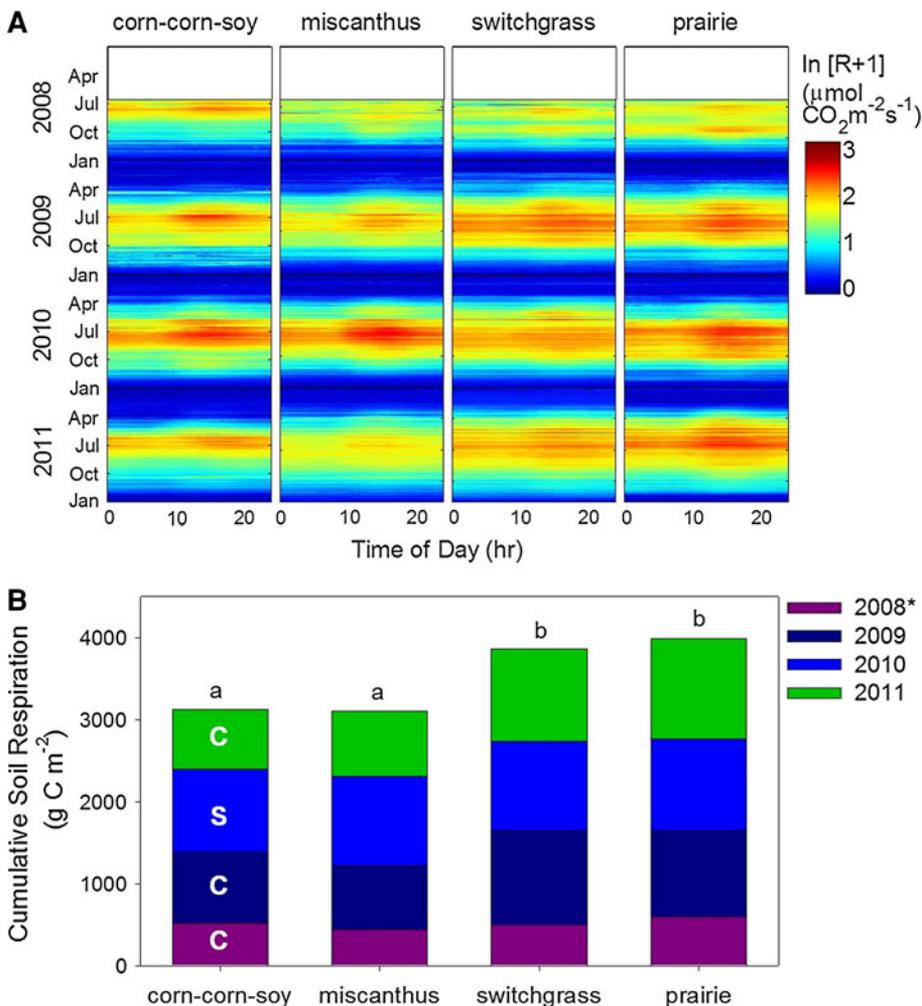
Summed over a full corn-soy-corn rotation cycle from spring 2009–spring 2012, total C inputs from litterfall were higher in the row crop control (935 g C m<sup>-2</sup>) than in the perennial crops (723, 626, and 463 g C m<sup>-2</sup> in miscanthus, switchgrass, and prairie, respectively; *p* < 0.05; Figure 3B).

### CO<sub>2</sub> Efflux Through *R*<sub>soil</sub>, *R*<sub>rfs</sub>, and *R*<sub>root+</sub>

Survey measurements revealed that *R*<sub>soil</sub> (Figure 4A) varied significantly by vegetation type (*p* < 0.001)

and by vegetation type × year (*p* < 0.001) with no significant main effect of year (*p* = 0.87) or block (*p* = 0.40). Initially, during the 2008 growing season, *R*<sub>soil</sub> did not differ by crop except that miscanthus was lower than the others (*p* = 0.005)—a fact that is not surprising given the delayed establishment of miscanthus. Starting in 2009, however, *R*<sub>soil</sub> was consistently higher under perennial vegetation than in CCS (all significant at *p* = 0.05 except switchgrass in 2010, where *p* = 0.06).

Respiration in root enclosure collars (*R*<sub>rfs</sub>; Figure 4B) varied significantly by vegetation type (*p* < 0.001), vegetation type × year (*p* < 0.001), and block (*p* < 0.001) with no significant main effect of year (*p* = 0.10). In 2008, *R*<sub>rfs</sub> was higher under perennials than corn (all *p* < 0.03)—potentially because of more recent soil disturbance from planting, particularly in the case of miscanthus rhizome planting in June 2008. From 2009 on, all perennials had lower *R*<sub>rfs</sub> than CCS (all *p* < 0.05 except prairie 2009, where *p* = 0.11). In the perennial agroecosystems, *R*<sub>rfs</sub> decreased over time (*p* < 0.05).



**Figure 5. A** Continuous record of soil respiration (*R*<sub>soil</sub>) under three perennial bioenergy crops (miscanthus, switchgrass, and prairie) and a row crop control (CCS rotation) for the first 3½ years of development following planting in May–June 2008.

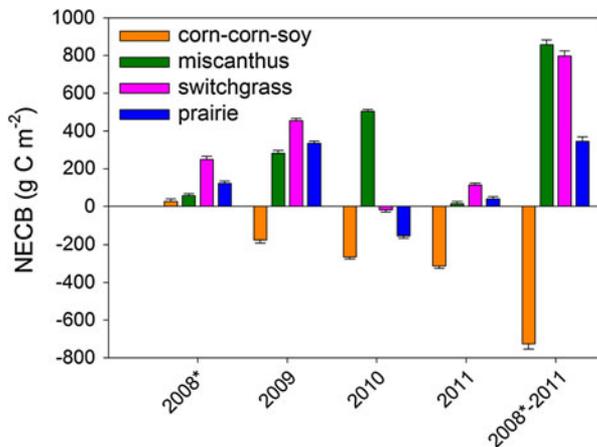
**B** Cumulative annual soil respiration (*R*<sub>soil</sub>) for each crop by year. Corn and soy years are indicated by “C” and “S,” respectively. Letters *a* and *b* refer to significance groupings at *p* < 0.05.

\*In 2008, the sum starts on June 17.

Root-associated respiration ( $R_{\text{root}+}$ , the difference between  $R_{\text{soil}}$  and  $R_{\text{rfs}}$ ; Figure 4C) varied significantly by vegetation type ( $p < 0.001$ ), vegetation type  $\times$  year ( $p < 0.001$ ), and year ( $p = 0.003$ ) with no significant effect of block ( $p = 0.30$ ). In 2008,  $R_{\text{root}+}$  was highest in corn ( $p < 0.05$ )—a fact that is not surprising given that the gross primary production of corn was twice that of the perennials in 2008 (Zeri and others 2011). Starting in 2009,  $R_{\text{root}+}$  of miscanthus, switchgrass, and prairie greatly exceeded that of CCS (all  $p < 0.0001$ ).

## Continuous $R_{\text{soil}}$ Record

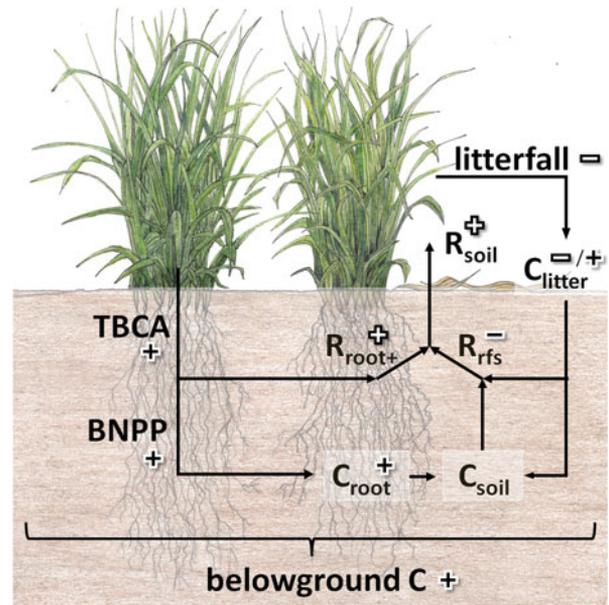
Automated measurements gave a continuous record of  $R_{\text{soil}}$  for the entire 3½-year period (Figure 5A).  $R_{\text{soil}}$  varied seasonally, peaking during the summer and remaining minimal during the winter. Differences between perennials and CCS lay more in the length of the season over which  $R_{\text{soil}}$  was relatively high than in maximum  $R_{\text{soil}}$  (Figures 4, 5). Specifically, the length of the season over which weekly  $R_{\text{soil}}$  exceeded a given threshold tended to be extended in the perennials—particularly switchgrass



**Figure 6.** Cumulative net ecosystem C balance (NECB; sign convention: positive indicates C sink) under three perennial bioenergy crops (miscanthus, switchgrass, and prairie) and a row crop control (CCS rotation) for calendar years 2008\*–2011 and all 3½ years combined. Error bars One standard deviation (see text for details). Note that switchgrass and prairie were harvested twice in 2010 (2009 growth harvested in early spring and 2010 growth in late fall), giving them a negative NECB for the calendar year. Likewise, miscanthus was counted as harvested twice in 2011 (2010 growth harvested in March 2011 and 2011 growth harvested in January 2012 but counted in 2011 budget), \*In 2008, the sum starts in June.

and prairie—relative to CCS. For example, from 2009 to 2011, the average number of weeks per year that  $R_{\text{soil}}$  exceeded  $10 \text{ g C m}^{-2} \text{ week}^{-1}$  was 17.7, 22.7, and 21.3 for miscanthus, switchgrass, and prairie, respectively, compared to only 16.0 weeks for CCS. Similarly, the average number of weeks per year that  $R_{\text{soil}}$  exceeded  $3 \text{ g C m}^{-2} \text{ week}^{-1}$  was 35.7, 39.0, and 38.7 for miscanthus, switchgrass, and prairie, respectively, compared to only 34.3 weeks for CCS.

In all years, annual  $R_{\text{soil}}$  under perennial vegetation was greater than or equal to that of the row crop control (Figure 5B). Annual  $R_{\text{soil}}$  varied significantly by crop type ( $p < 0.0001$ ; type III SS ANOVA in Matlab® v. 7.8.0), being higher in switchgrass and prairie than in CCS and miscanthus ( $p < 0.05$ ; no significant differences within these two pairs). Annual  $R_{\text{soil}}$  also varied by year



**Figure 7.** Schematic diagram illustrating changes in the belowground C cycle under perennial bioenergy crops relative to a row crop control. Figure portrays overall trends for a full row crop rotation cycle from 2009 to 2011. Changes are symbolized as follows: “+”, “–,” and “0” indicate increase, decrease, or no change, respectively. *Solid symbols* A change that tends to cool the climate by removing  $\text{CO}_2$  from the atmosphere; *hollow symbols* a change that tends to warm the climate by adding  $\text{CO}_2$  to the atmosphere. Abbreviations are as follows: *TBCA* total belowground C flux; *BNPP* belowground net primary productivity;  $C_{\text{root}}$  C storage in root or rhizome biomass;  $C_{\text{soil}}$  C storage in soil organic matter;  $R_{\text{root}+}$  root-associated respiration;  $R_{\text{rfs}}$  heterotrophic respiration in root free soil;  $R_{\text{soil}}$  total soil respiration ( $R_{\text{rfs}} + R_{\text{root}+}$ ). Illustration by Beth Yendrek.

( $p < 0.05$ ), being higher in 2010 than in 2009 and 2011 (no significant difference between 2009 and 2011). This result was driven by miscanthus and CCS (year  $\times$  crop:  $p < 0.001$ ), and may be attributable to slow establishment of miscanthus, inherent differences between corn (2009, 2011) and soy (2010), and/or drought in 2011. Summed over the entire measurement period (June 17, 2008–Dec 31, 2011), cumulative soil respiration was significantly higher in switchgrass and prairie ( $3,863 \pm 110$  and  $3,993 \pm 79$  g C m<sup>-2</sup>, respectively) than in miscanthus and CCS ( $3,102 \pm 120$  and  $3,123 \pm 51$  g C m<sup>-2</sup>, respectively;  $p < 0.05$ ).

### Net Belowground C Balance

All three perennial crops were net C sinks over the first 3½ years of development, sequestering an estimated  $826 \pm 26$ ,  $798 \pm 27$ , and  $345 \pm 23$  g C m<sup>-2</sup> for miscanthus, switchgrass, and prairie, respectively (Figure 6; Zeri and others 2011). In contrast, CCS was a net C source, releasing an estimated  $727 \pm 26$  g C m<sup>-2</sup> from June 2008 to December 2011. When delineated by calendar year, perennial grasses were sinks (NECB  $> 0$  at  $p < 0.05$ ) every year with the exception of switchgrass and prairie in 2010, which were harvested twice in that year (2009 growth harvested March 2010 and 2010 growth in November 2010) and miscanthus in 2011, which was counted as harvested twice in that year (2010 growth harvested March 2011 and 2011 growth harvested in January 2012 but counted in 2011 C budget). In contrast, corn was a C source (NECB  $< 0$  at  $p < 0.05$ ) every year except for 2008—likely because the measurement period for this year excluded spring, when the CCS ecosystem is a relatively strong C source ( $\sim 400$  g C m<sup>-2</sup> lost prior to the start of the growing season in 2009 and 2010; Zeri and others 2011).

### DISCUSSION

Belowground C cycling dynamics differed considerably between establishing perennial vegetation and the annually tilled corn–soy agroecosystem (Figure 7). Perennials allocated substantially more C belowground, resulting in much higher belowground biomass (Figures 1, 2; see also Zenone and others 2011), with roots extending beyond a meter in depth (Figure 2; see also Neukirchen and others 1999; Ma and others 2000). Meanwhile, litter inputs to the soil system were generally reduced in the perennial crops during corn years (Figure 3A) and over a full rotation cycle (Figure 3B). In terms of C release from the soil, root-associated respiration

increased because of higher root biomass (Figure 4C) but did not cause equivalent increases in  $R_{\text{soil}}$  (Figure 4A), as respiration from root-free soil was reduced (Figure 4B)—likely due to the cessation of tillage.  $R_{\text{soil}}$  was generally higher, although not always significantly so, under perennials relative to row crops (Figures 4, 5; see also Lobo Alonzo 2004; Al-Kaisi and Grote 2007). Overall, these changes to the belowground C cycle resulted in net belowground C sequestration (Figure 6; Zeri and others 2011).

The observed changes represented fundamental and consequential shifts in belowground C cycling pathways (Figure 7). To begin with, the proportion of C inputs from roots versus shoots shifted dramatically. Under corn and soy, C inputs were dominated by litter inputs, which occurred primarily in the fall, particularly shortly before or at the time of harvest (Figure 3A). In contrast, by inference based on increased belowground production (Figures 1, 2) and root-associated respiration (Figure 4C), total belowground C flux (TBCF; Figure 7) by the perennials represented a dominant portion of the total C input to the soil system. This shift in root- versus shoot-derived C is consequential in that root-derived C is more persistent in soils than is litter-derived C. Root C inputs are more chemically recalcitrant, are more readily stabilized through sorption to clay material, become physically protected in stable aggregates with pore sizes that maintain anoxic conditions and are too small to be accessible to microbial decomposers, and reach depths where microbial biomass is less abundant (Rasse and others 2005; Schmidt and others 2011). As a result, there is a linkage between root biomass and the rate of SOC accumulation (Fornara and Tilman 2008), whereas increased litter inputs do not necessarily increase—and may even decrease—SOC in agroecosystems (Rasse and others 1999, 2005). This differential residence time of root- and litter-derived C implies that the observed shift in the pathways through which C enters the soil system under perennials (Figure 7) may enhance long-term SOC storage even more than would be expected based on the quantity of belowground C inputs alone. An additional implication is that because residue plays a much smaller role in the belowground C cycle of perennials, their C balance may be less sensitive to the fraction of residue harvested for bioenergy production than that of corn (Blanco-Canqui and Lal 2007).

The depth profile of C inputs also shifted dramatically with the conversion of row crop agriculture to perennial vegetation. Under corn and soy, C inputs came predominately as litter at the soil surface (eventually, these were mixed into the

surface soil layer through tillage), and root C inputs were minimal below the top 30 cm of soil (Figure 2). In contrast, under perennial vegetation, the majority of C inputs came through the roots, which were most abundant near the surface but extended to depths of over a meter (Figure 2). Although it remains unclear whether depth per se is important in controlling the rate of soil organic matter decomposition, soil C does tend to be more persistent at deeper layers (Rasse and others 2005). Eventually, the deeper root distribution of perennials results in a generally deeper SOC distribution in grasslands (Jobbágy and Jackson 2000).

The source of CO<sub>2</sub> released from the soil differed substantially between perennials and the corn–soy rotation (Figures 4, 6). Whereas high TBCF by the establishing perennial vegetation fueled significantly increased  $R_{\text{root+}}$  (Figure 4C), higher litterfall inputs under row crops (Figure 3), combined with soil disturbance through tillage, shifted the balance toward  $R_{\text{rf}}$  (Figures 4B, 6). As the majority of respired CO<sub>2</sub> is typically derived from recently fixed C (either root or shoot material), this shift in partitioning of  $R_{\text{soil}}$  between  $R_{\text{root+}}$  and  $R_{\text{rf}}$  is best interpreted as reflecting the shift in root versus litter C inputs (Ryan and Law 2005), as well as the reduction of soil disturbance through tillage (Grandy and Robertson 2007). This study does not allow us to differentiate between old versus recent C sources for two reasons. First,  $R_{\text{rf}}$  includes CO<sub>2</sub> from litter decomposition in addition to breakdown of older soil organic matter. In addition, there is the possibility that high photosynthate input through the extensive root systems of the perennial vegetation has a priming effect on microbial respiration, thereby facilitating decomposition of old SOC (Kuzyakov and others 2000; Fu and Cheng 2002; Fontaine and others 2007; Schmidt and others 2011). Because this priming effect would be associated with the roots (part of  $R_{\text{root+}}$ ), the root exclusion technique used here cannot discern a priming effect. Further research will be required to determine whether there is a significant priming effect in these ecosystems; however, the observation that the perennial systems are net C sinks (Figure 6; Zeri and others 2011) implies that any priming effect is more than offset by C inputs to the soil system.

The increased belowground C allocation of perennial vegetation results in net belowground sequestration by these ecosystems (Figure 7). All three perennial crops acted as C sinks over the first three-and-a-half years of establishment, whereas the corn–soy rotation was a CO<sub>2</sub> source to the atmosphere (Figure 6; Zeri and others 2011).

Because aboveground biomass is harvested annually and litter stocks display no long-term directional change, this net ecosystem C sequestration is attributable to belowground processes. These observations are consistent with observed C cycle changes disproportionately favoring C sequestration as opposed to CO<sub>2</sub> release (Figure 7). Specifically, under establishing perennial vegetation, belowground biomass was 400–750% greater than in CCS (Figure 2), litterfall was reduced by only 20–45% (Figure 3), and annual  $R_{\text{soil}}$  increased by at most 30% (Figure 5). Thus, the shifts in belowground C cycling observed here provide a mechanism for observed net belowground C sequestration by these perennial ecosystems (Figure 6; Zeri and others 2011) and also indicate that the perennial bioenergy crops have ecosystem properties that should favor the build-up and long-term persistence of SOC (Schmidt and others 2011). Although changes in SOC have not yet been quantified at this site, a mass balance approach comparing NECB for 2008–2011 (Figure 6), belowground biomass as of summer 2011 (Figure 2B), and post-2011 harvest litter stocks (Figure 1) suggests that the perennial grasses gained SOC (~370, 340, and 55 g C m<sup>-2</sup> for miscanthus, switchgrass, and prairie, respectively) whereas CCS lost SOC (~1,000 g C m<sup>-2</sup>). Future research will be required to directly quantify changes in SOC in these ecosystems and to close the C budget, as well as to understand how C cycling changes with age of perennial bioenergy crops.

By identifying the mechanisms through which belowground C sequestration occurs, this study reinforces previous findings that, even with annual biomass harvest for bioenergy, perennial bioenergy agroecosystems provide climate benefits through CO<sub>2</sub> sequestration (Anderson-Teixeira and others 2009; Robertson and others 2011; Zeri and others 2011; Gelfand and others 2011). This contrasts with the opposite scenario, where replacing perennial systems with soybean bioenergy crops results in net CO<sub>2</sub> release (Zenone and others 2011; Gelfand and others 2011). Of course, the net climate effect of ethanol production from perennial crops will ultimately depend also on biomass yield and ethanol production efficiency (Fargione and others 2010; Somerville and others 2010), associated changes in N<sub>2</sub>O emissions (Melillo and others 2009; Crutzen and others 2007; Robertson and others 2011), any associated land-use change (Fargione and others 2008; Melillo and others 2009; Searchinger and others 2008), and biophysical climate effects of the land-use conversion (Georgescu and others 2009, 2011; Vanloocke and others 2010; Anderson-Teixeira and others 2012, 2011).

Beyond the implications for the sustainability of bioenergy production, the observed differences in belowground C cycling between establishing perennial vegetation and annual crops clarify the mechanisms through which land-use changes alter belowground C storage. This study documents how land-use change results in simultaneous changes in belowground C allocation and litterfall, which drive changes in belowground biomass, root-associated and bulk soil respiration, and total soil respiration (Figure 7). It demonstrates that land-use change triggers multiple changes in belowground C dynamics, the sum of which determine changes in the belowground C balance. Overall, enhanced root-associated C cycling under perennial vegetation fundamentally alters belowground C dynamics and implies greater long-term capacity of perennial ecosystems to sequester C.

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