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# Conversion to bioenergy crops alters the amount and age of microbially-respired soil carbon

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ABSTRACT

Bioenergy cropping systems have the potential to supply plant biomass as lignocellulosic feedstock for biofuels and bioproducts that will reduce reliance on fossil energy. Identifying the effects of alternative bioenergy cropping systems on soil carbon (C) is necessary to assess the sustainability of renewable fuels. We measured the response of soil organic carbon (SOC) pools to four bioenergy cropping systems using soils collected at the establishment of the field trials and after five years in two soils of contrasting texture: a fine-textured silt loam (Mollisol) in south central Wisconsin and a coarse-textured sandy loam (Alfisol) in southwestern Michigan, USA. Crop management followed region-specific practices with no till with the intention of reducing soil C losses from cultivation. Cropping systems included an annual monoculture (continuous corn), two perennial monocultures (switchgrass and hybrid poplar), and a perennial polyculture (restored native prairie). Using a 365-d laboratory soil incubation and a three-pool model, we estimated sizes and turnover times of SOC in surface (0–10 cm) and deeper soils (25–50 cm). After five years, all soils had less bioavailable C as measured by microbial respiration. To determine potential differences in soil C turnover under annual and perennial monocultures, we measured radiocarbon abundance ($^{14}C$) of bulk soils and respired $CO_2$ under corn and switchgrass. Respired-$CO_2$ was more depleted in $^{14}C$ over time, indicating preferential respiration of relatively "old C" after five years. Decreased microbial respiration rates after five years of bioenergy cropping systems offer the potential for the eventual reduction of soil C losses after conversion to no till. However, the $^{14}C$-$CO_2$ data suggest that previously-protected C pools may become depleted over time, especially with continued removal of plant inputs. Results show that conversion of conventional field-crop agriculture to bioenergy cropping systems may not provide belowground C benefits in the short term, as indicated by reductions in the size of the active pool. Reduced total soil respiration over the length of the study, as well as system-specific differences in soil respiration (monoculture annuals < monoculture perennial < polyculture perennials), suggest C loss may decline in perennial polyculture systems as they age. SOM pools responded differently to bioenergy crop management on soils with contrasting texture, highlighting the importance of geography in predicting belowground consequences of intensified agricultural production.

## 1. Introduction

The global production of bioenergy cropping systems increased fivefold from 2000 to 2010 in response to demand for non-fossil energy sources as a means to mitigate rising atmospheric carbon (C) concentrations (Davis et al., 2012; Anderson-Teixeira et al., 2013; Warner et al., 2013). Given this increased pressure on existing and new agricultural lands (Lark et al., 2015), identifying crops that can maintain or enhance soil resources is desirable for sustainable bioenergy crop production (Robertson et al., 2008, 2014, 2017; Anderson-Teixeira et al., 2009; Cotton et al., 2013; Pawlowski et al., 2017).

Crop effects on soil C in the North Central US vary with crop selection and management practices (Tilman et al., 2006; Tiemann et al., 2015). Conversion from annual to perennial crops generally results in soil organic C (SOC) gain attributable to increased root inputs (Lemus and Lal, 2005; Anderson-Teixeira et al., 2013; Sanford, 2014; Tiemann

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and Grandy, 2014; Pawlowski et al., 2017), but the response of SOC to changes in plant inputs and agricultural management is often confounded by the size and heterogeneity of the soil organic matter (SOM) pool (Weil et al., 2003).

SOM is composed of a mixture of compounds at different stages of decay with varying turnover times, which are influenced by multiple biochemical and physical mechanisms that regulate the accessibility of organic matter to decomposers (Collins et al., 2000; Sierra et al., 2011; Torn et al., 2013). A number of approaches have been developed to separate SOM into functionally distinct pools based on chemical properties, size, location in the soil matrix, or bioavailability, including aggregate, density, and biological fractionation. These fractionation methods isolate pools that are more sensitive to changing environmental conditions than the bulk soil (Christensen, 2001; Wagai et al., 2009; Schrumpf et al., 2013; Cates et al., 2016).

In the biological fractionation method, microbial respiration rates over a long-term incubation are used to define an active ($C_a$) and a slow ($C_s$) soil carbon pool, which is further constrained by the size of a resistant carbon pool ($C_r$) that can be isolated by acid hydrolysis (Paul et al., 2001a, 2001b). The $C_a$ represents labile, fresh C inputs from roots and plant litter that are easily accessible to microbial decomposers; this pool typically has turnover times of less than one year (Paul et al., 2001a, 2001b; Six et al., 2002). The $C_s$ cycles on decadal scales and is theorized to represent C that is not rapidly mineralized by microbes, most likely because of occlusion within soil aggregates or sorption to soil particles (Parton et al., 1993; Six et al., 2002). The $C_r$ is a chemically determined pool that represents C that is assumed to be biochemically inaccessible to decomposers and has longer turnover times than can be captured during laboratory incubations. While the mechanisms contributing to the long-term persistence of the chemically isolated $C_r$ pool may vary from soil to soil (Schmidt et al., 2011; Lehmann and Kleber, 2015), the lab incubation approach is well-suited for measuring differences in the size of the C pool available for microbial decomposition on annual to decadal periods and in response to management (Collins et al., 2000; Paul et al., 2001a; Conant et al., 2011).

Radiocarbon ($^{14}C$) measurements can help constrain estimates of C turnover times from the three-pool model (Trumbore, 2009). Atmospheric $^{14}C$ production was relatively constant before 1950, followed by a spike in the 1960s resulting from nuclear weapons testing. The $^{14}C$ signature of SOC can be used to determine if there was an enrichment or depletion of 'bomb' C in the soil, suggesting shorter or longer turnover times, respectively (Trumbore, 2000). Analysis of respired $^{14}C$-$CO_2$ can provide information on the SOM pools accessed by microbes, elucidating changes in C cycling dynamics in response to agricultural management.

We measured the effects of different bioenergy cropping systems on soil C cycling at two agricultural experimental stations on two soils with contrasting texture: a silt loam (Mollisol) in Wisconsin and a sandy loam (Alfisol) in Michigan. We compared two sites representing important agricultural regions because a soil's capacity to store C is influenced by abiotic factors, including climate and soil texture (Kleber et al., 2007; Sierra et al., 2011). Cropping system responses may vary geographically, with implications for the sustainability of bioenergy production. We asked the following questions: (1) Do the size and contribution of SOC pools with different turnover times vary among cropping systems?; and (2) How did five years of management alter the turnover time or substrate source of respired C?

## 2. Materials and methods

### 2.1. Study sites

We conducted this research at the U.S. Department of Energy (DOE)-Great Lakes Bioenergy Research Center's (GLBRC) Biofuel Cropping Systems Experiments (BCSE) located at the Arlington Agricultural Research Station (ARL) in south central Wisconsin, USA (43°17′45″ N, 89°22′48″ W, 315 m elevation.) and the W.K. Kellogg Biological Station (KBS) in southwest Michigan, USA (42°23′47″ N, 85°22′26″ W, 288 m elevation). Mean annual temperature, mean growing season temperature (May–August), and precipitation were 7.6 °C, 19.6 °C, and 869 mm at ARL and 9.0 °C, 20 °C, and 1027 mm at KBS. The soils at ARL are classified as Plano silt loam (Fine-silty, mixed, superactive, mesic Typic Argiudolls), are characterized by a dark A horizon that is rich in organic matter, and have a pH of 6.6 and 6.3 in soils from 0 to 10 cm and 25–50 cm, respectively. These soils formed under tallgrass prairie on post-glacial loess deposits (Sanford et al., 2016). Soils at KBS are classified as Kalamazoo loam (Fine-loamy, mixed, active, mesic Typic Hapludalfs), have a pH of 6.1 in soils from 0 to 10 cm and 25–50 cm, and were formed under hardwood forest (Sanford et al., 2016). The two sites used in this study have a long history of crop production (corn, soybeans, alfalfa) with roughly 4 years of alfalfa immediately preceding BCSE establishment (Sanford et al., 2016).

The BCSEs are randomized complete block designs with five blocks at each site. At both KBS and ARL, we used soils from three blocks, each containing one 27 × 43-$m^2$ plot of the following four cropping systems: 1) continuous no-till corn (*Zea mays* L.), 2) switchgrass (*Panicum virgatum* L.), 3) hybrid poplar (*Populus nigra* × *P. maximowiczii* A. Henry 'NM6') with an understory of herbaceous weeds, and 4) restored native prairie sown with 18 species at ARL and KBS (see Sanford et al., (2016) for agronomic details).

All cropping systems were established between May and June of 2008. Blocks were chisel-plowed and field-cultivated prior to cropping system establishment after which no additional tillage occurred for the 5-year duration in between sampling for our study. Plant hybrids were selected to maximize productivity and represent local farming practices. Corn hybrids differed between the two sites based on varying needs of advanced traits (e.g., herbicide and insect resistance) and differences in the length of the growing season. Poplar plots were hand planted as small whips. Restored native prairie plots were planted with a mixture of six grasses, three leguminous forbs, and nine non-leguminous forbs (see Sanford et al., (2016) for complete species list). Region-specific crop management was adopted at each site, allowing annual agronomic decisions (e.g., hybrid selection, nutrient management, and herbicide application) to be carried out according to local best management practices and climate considerations, and include N, P and K applications (Sanford et al., 2016). The restored prairie system was not fertilized during the study and no P or K applications were made to the perennial systems. Harvest biomass yields across sites were 60, 56, and 44% for corn, switchgrass, and restored prairie respectively and resulted in post-harvest annual system inputs of 6.7, 3.9, and 3.9 Mg $ha^{-1}yr^{-1}$ at ARL for corn, switchgrass, and restored prairie respectively and 6.6, 1.5, and 4.2 Mg $ha^{-1}yr^{-1}$ at KBS for corn, switchgrass, and restored prairie respectively (Supplementary table 1) (Sanford et al., 2016). Annual inputs for poplar systems, from litterfall and understory (weed) biomass, was 4.4 Mg $ha^{-1}yr^{-1}$ at ARL (Supplementary table 1) (von Haden, 2017).

### 2.2. Soil sampling

Soils were collected in 2008, following the first growing season, and in the fall of 2013, following the fifth growing season, using a 1.22-m long × 8.9-cm diameter Giddings soil probe (model #15-TS GSRTS; Giddings Machine Company; Windsor, CO) at ARL and a 7.62-cm diameter Geoprobe (model 540MT; Geoprobe Systems; Salina, KS) corer at KBS. Soils were collected from each plot to 100 cm depth from three permanent sampling stations and composited per plot (Sanford et al., 2016). These cores were refrigerated until processing, then cut at specific intervals (0–10 cm and 25–50 cm). Soils were sieved to ≤ 2 mm and visible roots and plant material were removed by hand. Soils collected in 2008 were oven dried at 50–60 °C and stored in a cool, dry

facility between 2008 and the time of this study. Soils collected in 2013 were air-dried following sieving.

### 2.3. Soil microbial respiration

To understand how different cropping systems affected microbial respiration, we performed a 365-d laboratory incubation to measure $CO_2$ production. We incubated 4 lab replicates of each sample by placing 25 g of soil into a specimen cup and adding deionized water to reach 60% water filled pore space (Linn and Doran, 1984) calculated using an estimated bulk density of 1.0 g $cm^{-3}$ and particle density of 2.65 Mg $m^{-3}$ (Campbell and Norman, 1998). Specimen cups were placed in 473 ml mason jars, which were sealed with a lid fitted with two 0.635-cm diameter holes for venting (Sanford and Kucharik, 2013) and stored in the dark between collection time points to inhibit autotrophic respiration. After samples were brought to 60% water filled pore space, samples were vented for 24-h prior to the first respired $CO_2$ collection. Samples were maintained at 60% water filled pore space throughout the year-long incubation by adding moisture to the soils at the end of each headspace collection so that we were not measuring the response to water additions.

To collect respired $CO_2$, the headspace of the chamber was first vented with in-house pressurized lab air (~400 ppm). Immediately after sealing the chamber, 10 ml of headspace volume was collected at the initial time point ($t_0$) and 60 min later ($t_{60}$) using a syringe. Air samples were stored in over-pressurized glass Exetainer vials (Labco Limited; Buckinghamshire, UK) until analysis. Air samples were analyzed for $CO_2$ concentrations on an infrared gas analyzer (LI-820 $CO_2$ Analyzer; Li-Cor, Lincoln, NE).

We collected air samples every other day for the first week (days 1, 3, and 5); twice weekly for the following three weeks (days 8, 11, 15, 18, 22, and 25); once weekly for the subsequent four weeks (days 32, 39, 43, and 54); then once every three to four weeks thereafter for the duration of one year (days 74, 95, 115, 136, 158, 187, 222, 257, 285, 327, and 365).

### 2.4. Modeling carbon pool sizes

A three-pool regression model with first-order kinetics was used to define the size and decomposition rate of three operationally distinct C pools (Paul et al., 2001b, 2006; Sanford and Kucharik, 2013):

$$C_{t(t)} = C_a e^{-ka(t)} + C_s e^{-ks(t)} + C_r e^{-kr(t)} \quad (1)$$

where $C_{t(t)}$ is the total soil C at time $t$ and $C_a$, $C_s$, $C_r$, $k_a$, $k_s$, and $k_r$ represent the size of the active pool, slow pool, and resistant pool and their corresponding decomposition rates, respectively.

We used an estimated mean residence time (MRT) of 500 y for the $C_r$ to constrain the model. Paul et al. (2001a, 2001b) reported MRT of 485 and 4400 years for acid-resistant fractions based on radiocarbon ($^{14}C$) measurements from soils collected at ARL and KBS, depending on soil depth, and showed that MRTs >350 years had no effect on modeled parameters. We adjusted the estimated field MRT to a laboratory decomposition rate using a $Q_{10}$ temperature coefficient correction (Paul et al., 2001b):

$$Q_{10} = 2^{(Incub\ Temp\ -\ Field\ MAT)/10} \quad (2)$$

where *Field MAT* is the mean annual temperature for each field site and *Incub Temp* is the measured mean lab incubation temperature of 21.72 °C. The lab incubation temperature was representative of conditions in the field during the growing season. The calculated incubation-based MRT values are for comparison across cropping systems and sites rather than representative of *in situ* field rates.

We used published values for the size of the acid-resistant pool measured via acid hydrolysis (Paul et al., 2001a, 2006). These values were 50% (0–10 cm) and 35% (25–50 cm) for ARL and 45% (0–10 cm) and 30% (25–50 cm) for KBS soils, which are similar to those reported in a recent study at our same sites (Sprunger and Robertson 2018). Our approach assumes no change in the size of the resistant pool during the five years of field trials. Data collected from the ARL and KBS field sites in 2001 (Paul et al., 2001a) and in 2013 (Sprunger and Robertson 2018) show no change in the proportion of $C_r$ over 12 years. We then used calculated $C_r$ and $k_r$ and the daily $CO_2$ flux rate to model $C_a$, $C_s$, $k_a$, and $k_s$ for each incubation jar by nonlinear least squares regression using the NLIN procedure (METHOD = MARQUART) of SAS version 9.3.

We report results for the mean absolute size of each C pool as estimated by the three-pool model as well as the contribution of each modeled C pool to the bulk SOC content (size of pool standardized by size of total soil C for each sample, given as a percent), which allows for comparison among samples with different SOC amounts. Total cumulative respiration over 365 days was calculated with model parameter estimates (equation (1)) and normalized by the size of the bulk SOC pool. Modeled C pool mean residence times are reported in field days and field years, for the $C_a$ and $C_s$, respectively, by converting the decomposition rate using equation (2).

Carbon content was measured on finely ground bulk soils on a Flash 2000 C and N combustion elemental analyzer (CE Elantech, Lakewood, NJ). All samples were run in duplicate with <10% replicate error for total C with aspartic acid and a soil reference material as standards. No presence of inorganic C was detected by an acid effervescence test, hence total C represents organic C.

### 2.5. Radiocarbon analyses

To further investigate the effects of annual and perennial bioenergy cropping systems on SOC dynamics, we measured natural abundance radiocarbon ($^{14}C$) composition of bulk soil and respired $CO_2$ for samples collected from the two depths and the two years under continuous corn and switchgrass at ARL. Incubations and graphitization were conducted at the USDA Forest Service Northern Research Station in Houghton, MI.

To measure $^{14}C$-$CO_2$, the incubation protocol was modified to allow for the collection of a greater volume of headspace. For soils collected from 0 to 10 cm, 25 g of soil were incubated, while for soils from 25 to 50 cm, 100 g were incubated. Following initial wetting to 60% water filled pore space, as per Linn and Doran (1984), and 24 h venting, jars were sealed for ~8 (0–10 cm) and ~10 d (25–50 cm) to accumulate enough $CO_2$ for analysis, based on respiration rates from the 365- d laboratory incubation. After the 8- or 10-d incubation period, the headspace (~14,000 ppm) was collected, purified using a vacuum line, and converted to graphite in the presence of $H_2$ (Vogel et al., 1984). These $^{14}C$-$CO_2$ samples represented the age of the C respired at the beginning of the incubation.

Bulk soils were combusted, and along with the respired-$CO_2$ samples, purified on a vacuum line, then subsequently converted to graphite in the presence of $H_2$ (Vogel et al., 1984). Aliquots of the bulk soil and respired $CO_2$ purified gas samples were hermetically sealed in quartz tubes for subsequent $\delta^{13}C$ analysis at the Stable Isotope Lab at the University of Wisconsin-Madison to correct the $^{14}C$ values for isotopic fractionation (Stuiver and Polach, 1977). Radiocarbon values of the graphitized bulk soils and respired-$CO_2$ samples were measured on a Van de Graaff FN accelerator mass spectrometer (AMS) at the Center for AMS at Lawrence Livermore National Laboratory. Radiocarbon data are expressed as $\Delta^{14}C$, and include background subtraction, correction for isotopic fractionation and correction for decay in the Oxalic Acid 1 standard since 1950 following the conventions of Stuiver and Polach (1977).

Radiocarbon-based mean system ages were calculated using a time-dependent steady-state model (Gaudinski et al., 2000; Torn et al., 2002; Sollins et al., 2006). To estimate the $^{14}C$-mean system age in the model, we used atmospheric $\Delta^{14}C$ records dating back 500 y: 1511 to 1950 (Stuiver et al., 1998), 1950 to 2009 (Hua et al., 2013), and 2009 to 2012 (Niwot Ridge LTER). The $^{14}C$-mean system age of bulk soil and

respired-$CO_2$ represent the average age of C in that sample (Trumbore, 2000; Trumbore and Torn, 2003). As discussed in Sierra et al. (2016), mean system age represents the average of a probability distribution of ages for a non-homogeneous system. Modeling of samples that contain a significant proportion of 'bomb' C (i.e., C fixed since 1950) complicate estimating $^{14}$C-mean system ages because the same abundance of $^{14}$C can suggest two different turnover times (Marín-Spiotta et al., 2008), a longer one reflecting the increasing side of the atmospheric $\Delta^{14}$C curve, and a shorter one reflecting the decreasing side of the curve (Trumbore, 2000). In our study, respired-$CO_2$ values from the 2008 samples yielded mean system ages of 5.6 ± 1.5 y and 113.4 ± 12.3 y. The former is more commonly expected for the active pool, but using the longer estimate did not statistically alter our findings.

### 2.6. Statistical analyses

We first used a three-way ANOVA model to test for the effects of cropping system (corn, switchgrass, poplar, and restored native prairie), year (2008 and 2013), site (ARL and KBS), and their interactions (crop × year, year × site, site × crop, crop × year × site) on incubation-based estimates of C pool sizes and MRT. Data from each soil depth (0–10 cm and 25–50 cm) were analyzed separately. The three-way interaction was not significant for any variable because of high variance, so we conducted separate two-way ANOVAs at each site. All ANOVA models were followed by post-hoc Student's t-tests or Tukey's honest significance difference tests. The effects of cropping system (corn and switchgrass), year (2008 and 2013), and their interaction (crop × year) on $^{14}$C- values and mean system ages of bulk soils and respired-$CO_2$ were tested using a two-way ANOVA model, as these data were collected only for one site, followed by post-hoc Student's t tests. Data were log-transformed to fit assumptions of normality where necessary. All statistical tests were conducted using JMP Pro 11.0.0 software. Significance was determined at the $p = 0.05$ level unless otherwise noted.

## 3. Results

### 3.1. Soil respiration

Total cumulative respiration ($CO_2$-C g kg soil$^{-1}$) and as a proportion of bulk SOC ($CO_2$-C g kg SOC$^{-1}$) in surface soils (0–10 cm) differed among cropping systems by site and by year (Fig. 1). Total cumulative respiration was greater overall at ARL than at KBS, despite reductions from year 1 to year 5 at both sites. After five years of field trials establishment, total C respired was greatest under restored native prairie, followed by poplar, switchgrass, and corn. Surface soils lost a greater proportion of bulk SOC via respiration at KBS than at ARL. At ARL, the fraction of bulk SOC respired was greater under restored native prairie and switchgrass than under corn and poplar systems (Table 1). There were no cropping system effects at KBS (Table 2).

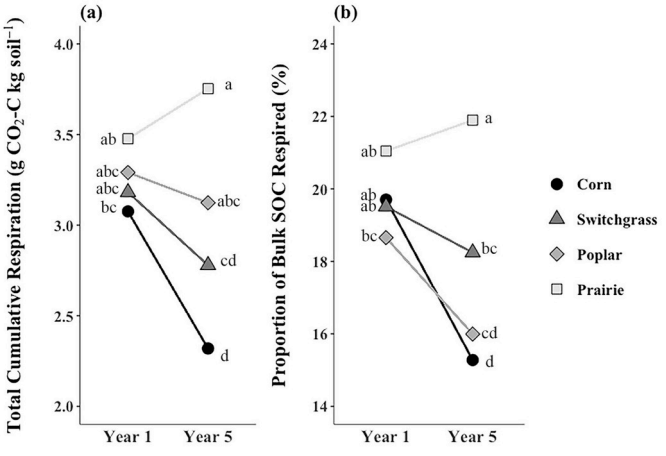


**Fig. 1.** Cropping system = effects from year 1 (2008) to year 5 (2013) on (a) total cumulative respiration ($CO_2$-C g kg soil$^{-1}$) over a 365-d long incubation and (b) proportion of bulk SOC (%) respired from surface soils (0–10 cm) at the two field sites: Agricultural Research Station (ARL), WI and Kellogg Biological Station, (KBS), MI. Lower case letters indicate significant differences ($p < 0.05$).

Soils collected from the 25–50 cm depth showed similar trends, with cropping system, year, and site effects on total cumulative respired C. C respired as a proportion of bulk SOC also differed by cropping system ($p = 0.03$) and site ($p < 0.0001$). Total cumulative respiration was greater at ARL than at KBS, but the amount of $CO_2$ respired as a proportion of bulk SOC was greatest at KBS. At ARL, total cumulative respiration was greater in soils under restored native prairie and poplar systems than corn. At KBS, the proportion of bulk SOC respired was smallest under poplar.

### 3.2. Modeled soil carbon pools

Overall, measured changes in SOC pools after five years of bioenergy cropping were greater in surface soils than in deeper soils, and greater at ARL, with finer-textured soils, than at KBS, with coarser-textured soils. Interpretation of slow pool ($C_s$) dynamics should be guarded as the $C_s$ is calculated from the sizes of the measured active pool ($C_a$), total respiration, and the estimated resistant pool ($C_r$).

Despite differences in total cumulative respiration over the one-year incubation, the average size of the $C_a$ did not differ among the four bioenergy cropping systems, but soils at KBS had a greater proportion of bulk SOC in the $C_a$ pool than at ARL. The response of the $C_a$ pool differed between sites, with reductions in surface soil (0–10 cm) $C_a$ pool sizes and contributions to the bulk SOC pool at ARL after five years of cropping system field trials but no change at KBS.

In contrast to site differences in the size of the $C_a$ pool, average MRTs modeled from the long-term soil incubations did not differ between ARL and KBS. However, cropping system effects on MRT of the $C_a$ pool over time did differ by site. At ARL, surface soil $C_a$ MRT under poplar was shorter than under corn and switchgrass five years after the field trials establishment (Fig. 2a). At 25–50 cm depth, $C_a$ MRT under poplar was shorter than under switchgrass and restored native prairie (Fig. 2b). There were no cropping system effects on $C_a$ MRT from year 1 to year 5 at KBS (Fig. 2).

The size of the $C_s$ pool differed among cropping systems and between sites (Tables 1 and 2), with greater values at ARL than at KBS at both depths, given differences in total cumulative respiration and the size of the resistant pool. In contrast, surface soil (0–10 cm) $C_s$ as a proportion of bulk SOC was greater at KBS than at ARL. At ARL, surface soil $C_s$ was greater under poplar than under switchgrass and corn, with intermediate values in the restored native prairie ($p = 0.05$). $C_s$ as a proportion of bulk SOC increased from year 1 to year 5 ($p < 0.0001$) only in surface soils. At KBS, surface soil $C_s$ pool size was greater under poplar compared to all other cropping systems ($p = 0.05$) and $C_s$ pool as a proportion of bulk SOC did not change over time. On the other hand, deeper soils (25–50 cm) gained $C_s$ over the five years ($p = 0.05$).

### 3.3. Radiocarbon concentrations and mean system ages

Observed losses in modeled soil C pools over the study period suggest that the system was not at steady-state for $^{14}$C-modeling, thus we discuss apparent mean system ages. Bulk soil $\Delta^{14}$C values did not differ between corn and switchgrass after five years of cropping (Fig. 3a). As expected, average bulk soil $\Delta^{14}$C values declined with soil depth from −62.0 ± 12.0‰ (0–10 cm) to −264.6 ± 9.3‰ (25–50 cm), yielding apparent mean ages of 682 ± 103 y and 2898 ± 139 y, respectively.

Respired $CO_2$ was more enriched in 'bomb' C than the bulk soil

**Table 1**
Mean and standard error of three-pool model parameter estimates of pool sizes ($C_a$, $C_s$) and decomposition rates ($k_a$), contribution of pools to total SOC (% of SOC), field-mean residence times ($MRT_f$) of pools based on incubation data, and total cumulative respiration and percentage of total SOC respired over the 365-d incubation (% of SOC) at Arlington Agricultural Research Station, WI in (a) year 1 (2008) and (b) year 5 (2013).

| (a) | Year 1 (2008), Arlington Agricultural Research Station, WI | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Depth (cm) | Active Pool | | | | Slow Pool | | Cumulative Respiration | |
| | | $C_a$ (g $kg^{-1}$) | % of SOC | $k_a$ ($day^{-1}$) | $MRT_f$[a] (day) | $C_s$ (g $kg^{-1}$) | % of SOC | Cumulative Respiration (g $CO_2$-C kg $soil^{-1}$) | % of SOC |
| Corn | 0–10 | 0.27 ± 0.02 | 1.2 | 0.5 | 5.3 ± 0.2 | 11 ± 1.4 | 48.8 | 3.0 ± 0.1 | 14 |
| Switchgrass | 0–10 | 0.34 ± 0.01 | 1.5 | 0.5 | 5.3 ± 0.5 | 11 ± 0.8 | 48.5 | 3.4 ± 0.04 | 16 |
| Poplar | 0–10 | 0.35 ± 0.02 | 1.4 | 0.5 | 5.7 ± 0.5 | 13 ± 1.0 | 48.6 | 3.4 ± 0.1 | 13 |
| Prairie | 0–10 | 0.31 ± 0.02 | 1.4 | 0.5 | 5.3 ± 0.4 | 11 ± 0.3 | 48.6 | 4.0 ± 0.1 | 18 |
| Corn | 25–50 | 0.08 ± 0.02 | 0.9 | 0.3 | 14 ± 5.0 | 6.0 ± 1.0 | 64.1 | 1.1 ± 0.1 | 12 |
| Switchgrass | 25–50 | 0.09 ± 0.03 | 1.2 | 0.4 | 22 ± 17 | 4.5 ± 0.6 | 63.8 | 1.3 ± 0.1 | 18 |
| Poplar | 25–50 | 0.23 ± 0.07 | 1.4 | 0.1 | 52 ± 26 | 10 ± 4.0 | 63.6 | 1.4 ± 0.3 | 8.8 |
| Prairie | 25–50 | 0.46 ± 0.31 | 6.0 | 0.3 | 128 ± 121 | 4.5 ± 0.5 | 59.0 | 1.6 ± 0.1 | 21 |

| (b) | Year 5 (2013), Arlington Agricultural Research Station, WI | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Depth (cm) | Active Pool | | | | Slow Pool | | Cumulative Respiration | |
| | | $C_a$ (g $kg^{-1}$) | % of SOC | $k_a$ ($day^{-1}$) | $MRT_f$[a] (day) | $C_s$ (g $kg^{-1}$) | % of SOC | Cumulative Respiration (g $CO_2$-C kg $soil^{-1}$) | % of SOC |
| Corn | 0–10 | 0.15 ± 0.05 | 0.7 | 0.3 | 9.7 ± 1.3 | 11 ± 0.7 | 49.3 | 2.3 ± 0.2 | 11 |
| Switchgrass | 0–10 | 0.15 ± 0.03 | 0.7 | 0.4 | 7.3 ± 1.1 | 10 ± 0.8 | 49.3 | 3.1 ± 0.2 | 15 |
| Poplar | 0–10 | 0.17 ± 0.03 | 0.6 | 0.6 | 4.5 ± 0.3 | 13 ± 0.7 | 49.4 | 3.0 ± 0.3 | 11 |
| Prairie | 0–10 | 0.21 ± 0.02 | 0.9 | 0.5 | 5.8 ± 0.5 | 12 ± 0.2 | 49.1 | 4.2 ± 0.2 | 18 |
| Corn | 25–50 | 0.05 ± 0.03 | 0.5 | 0.6 | 4.8 ± 1.2 | 6.7 ± 0.5 | 64.5 | 1.1 ± 0.3 | 10 |
| Switchgrass | 25–50 | 0.17 ± 0.04 | 2.1 | 0.04 | 65 ± 11 | 5.0 ± 0.1 | 62.9 | 1.3 ± 0.1 | 17 |
| Poplar | 25–50 | 0.34 ± 0.28 | 3.0 | 2.9 | 1.7 ± 0.8 | 7.1 ± 1.5 | 62.0 | 2.0 ± 0.3 | 18 |
| Prairie | 25–50 | 0.04 ± 0.01 | 0.6 | 0.04 | 70 ± 25 | 4.9 ± 0.1 | 64.4 | 1.7 ± 0.02 | 23 |

[a] Decomposition rates converted to field mean residence times ($MRT_f$) using the reciprocal decomposition rates ($k^{-1}$) scaled to the field environment using $Q_{10} = 2^{[(MIT-MFT)/10]}$, where MIT = mean incubation temperature and MFT = mean annual field temperature.

(Fig. 3). Year 1 surface soil (0–10 cm) respired $\Delta^{14}C$-$CO_2$ was 69.9 ± 20.8‰ and 66.8 ± 2.7‰ for corn and switchgrass, respectively (Table 3). For these $\Delta^{14}C$ values, the time-dependent steady-state models suggested two possible mean $^{14}C$-ages, due to the significant enrichment of 'bomb' C: 6 ± 3.3 y and 115 ± 27 y for corn and 6 ± 0.4 y and 112 ± 4 y for switchgrass (Table 3). We used the more recent $^{14}C$-age to represent the active, fast-cycling C pool (Fig. 3b), but using the older $^{14}C$-age would not alter our findings. The $\Delta^{14}C$ of respired $CO_2$ decreased with soil depth, following trends observed for the bulk soil (Fig. 3b). Across years and cropping systems, $\Delta^{14}C$ of respired $CO_2$ averaged 28.8 ± 14‰ (0–10 cm) and −59.0 ± 12‰ (25–50 cm) (Table 3), corresponding to apparent mean ages of 202 ± 78 y and 663 ± 90 y, respectively. There were no significant differences in respired $\Delta^{14}C$-$CO_2$ between corn and switchgrass, but after five years, respired $CO_2$ became more depleted in $^{14}C$ in surface soils (Fig. 3b). Surface soil respired $\Delta^{14}C$-$CO_2$ changed over the 5 years from 69.9 ± 20.8‰ to −37 ± 31.4‰ for corn and 66.8 ± 2.7‰ to 5.3 ± 8.4‰ for switchgrass. These values represent an increase in apparent mean ages from 6 ± 3.3 y in year 1–529 ± 196 y in year 5 for corn and from 6 ± 0.4 y to 267 ± 35 y for switchgrass.

## 4. Discussion

### 4.1. Reductions in microbially-available carbon pools after five years of bioenergy cropping

All four bioenergy cropping systems in our study experienced reductions in the amount of total cumulative respiration. Similarly, all systems at the Arlington field station (ARL) lost C associated with the active C pool ($C_a$) in surface soils. The $C_a$ was generally less sensitive at Kellogg Biological field station (KBS), where it represented a greater proportion of the bulk SOC pool and which also had greater overall SOC stocks. Estimated from short-term C mineralization rates, the $C_a$ is defined operationally as the fraction of C readily accessible to microbes and is generally the most sensitive to disturbance (Paul et al., 2001a, 2001b). Other studies have reported management-induced alterations to the relative size of bioavailable or labile C pools, as measured using a variety of soil fractionation approaches (John et al., 2005; Jokela et al., 2011; Ramnarine et al., 2015). The $C_a$ pool isolated by our approach (Paul et al., 2001b, 2006; Sanford and Kucharik, 2013) is likely most comparable to soluble organic C produced from litter decomposition or leaching, root and microbial exudates, and to the free, particulate or low-density fraction of SOM (Kaiser et al., 2010). Reductions in $C_a$ pool sizes after five years of cropping are consistent with expectations of the effects of agricultural management on soil C dynamics. Greater soil respiration rates and larger sizes of the $C_a$ pool in samples collected in year 1 can be explained by an initial increase in bioavailable C released by soil aggregate disruption after tillage during the field trial establishment, as has been observed in other studies (Six et al., 2000; Jokela et al., 2011; Six and Paustian, 2014). Subsequent no-till management during the following five years is expected to contribute to increased C storage and reductions in readily available C by incorporation of plant C into soil aggregates, as observed with conversion from conventional-tillage to no-till practices (Kern and Johnson, 1993; Paustian et al., 1997; Lal, 2004). In addition, the larger amounts of bioavailable C early on in the field trials could be explained by the presence of residual nitrogen-rich litter from alfalfa cover or manure applications from the previous management prior to the bioenergy cropping system establishment.

Whereas we did not detect bioenergy cropping system effects on the size of the $C_a$ pool, total cumulative respiration and incubation-based MRTs did differ between monoculture and perennial crops. Generally, soils under restored native prairie and poplar plantations respired more C overall. Poplar systems showed greater reductions in the MRT of the

**Table 2**
Mean and standard error of three-pool model parameter estimates of pool sizes ($C_a$, $C_s$) and decomposition rates ($k_a$), contribution of pools to total SOC (% of SOC), field-mean residence times ($MRT_f$) of pools based on incubation data, and total cumulative respiration and percentage of total SOC respired over the 365-d incubation (% of SOC) at Kellogg Biological Station, MI (a) year 1 (2008) and (b) year 5 (2013).

| (a) | | Year 1 (2008), Kellogg Biological Station, MI | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Depth (cm) | Active Pool | | | | Slow Pool | | Cumulative Respiration | |
| | | $C_a$ (g kg$^{-1}$) | % of SOC | $k_a$ (day$^{-1}$) | $MRT_f$[a] (day) | $C_s$ (g kg$^{-1}$) | % of SOC | Total (g $CO_2$-C kg soil$^{-1}$) | % of SOC |
| Corn | 0–10 | 0.23 ± 0.02 | 1.8 | 0.7 | 3.9 ± 0.5 | 6.5 ± 0.1 | 53.3 | 3.1 ± 0.1 | 26 |
| Switchgrass | 0–10 | 0.23 ± 0.06 | 1.8 | 0.5 | 5.6 ± 0.3 | 6.7 ± 0.3 | 53.4 | 2.9 ± 0.1 | 23 |
| Poplar | 0–10 | 0.19 ± 0.05 | 1.5 | 0.6 | 4.4 ± 0.4 | 7.2 ± 0.4 | 53.7 | 3.2 ± 0.2 | 24 |
| Prairie | 0–10 | 0.19 ± 0.04 | 1.6 | 0.7 | 4.0 ± 0.2 | 6.5 ± 0.04 | 53.5 | 2.9 ± 0.1 | 24 |
| Corn | 25–50 | 0.31 ± 0.10 | 11 | 0.03 | 137 ± 68 | 1.7 ± 0.3 | 58.4 | 0.9 ± 0.1 | 33 |
| Switchgrass | 25–50 | 0.05 ± 0.02 | 1.5 | 0.1 | 21 ± 1.0 | 2.1 ± 0.3 | 67.7 | 1.0 ± 0.1 | 32 |
| Poplar | 25–50 | 0.27 ± 0.12 | 5.0 | 0.1 | 56 ± 39 | 3.4 ± 0.4 | 64.2 | 1.2 ± 0.03 | 23 |
| Prairie | 25–50 | 0.18 ± 0.08 | 7.3 | 0.5 | 84 ± 43 | 1.5 ± 0.1 | 61.9 | 0.9 ± 0.1 | 37 |
| (b) | | Year 5 (2013), Kellogg Biological Station, MI | | | | | | | |
| Treatment | Depth (cm) | Active Pool | | | | Slow Pool | | Cumulative Respiration | |
| | | $C_a$ (g kg$^{-1}$) | % of SOC | $k_a$ (day$^{-1}$) | $MRT_f$[a] (day) | $C_s$ (g kg$^{-1}$) | % of SOC | Total (g $CO_2$-C kg soil$^{-1}$) | % of SOC |
| Corn | 0–10 | 0.30 ± 0.12 | 2.5 | 0.6 | 7.9 ± 3.4 | 6.2 ± 0.2 | 52.7 | 2.3 ± 0.1 | 19 |
| Switchgrass | 0–10 | 0.11 ± 0.02 | 1.0 | 0.4 | 7.1 ± 1.2 | 6.3 ± 0.4 | 54.2 | 2.5 ± 0.2 | 22 |
| Poplar | 0–10 | 0.26 ± 0.07 | 1.7 | 0.6 | 9.2 ± 5.2 | 8.3 ± 1.1 | 53.5 | 3.2 ± 0.4 | 21 |
| Prairie | 0–10 | 0.37 ± 0.22 | 2.9 | 0.5 | 6.3 ± 1.6 | 6.6 ± 0.6 | 52.2 | 3.3 ± 0.3 | 26 |
| Corn | 25–50 | 0.45 ± 0.20 | 10 | 0.4 | 194 ± 100 | 2.6 ± 1.2 | 59.1 | 1.1 ± 0.1 | 26 |
| Switchgrass | 25–50 | 0.27 ± 0.03 | 5.7 | 0.0 | 102 ± 21 | 3.0 ± 1.3 | 63.5 | 1.2 ± 0.4 | 25 |
| Poplar | 25–50 | 0.17 ± 0.06 | 2.5 | 0.5 | 38 ± 29 | 4.4 ± 1.0 | 66.7 | 1.2 ± 0.3 | 19 |
| Prairie | 25–50 | 0.33 | 5.2 | 0.6 | 4.3 | 4.1 | 64.0 | 1.8 | 28 |

Standard error is not reported in year 5 (2013) for restored native prairie systems.
[a] Decomposition rates converted to field mean residence times ($MRT_f$) using the reciprocal ($k^{-1}$) scaled to the field using $Q_{10} = 2^{[(MIT-MFT)/10]}$, where MIT = mean incubation temperature and MFT = mean annual field temperature.

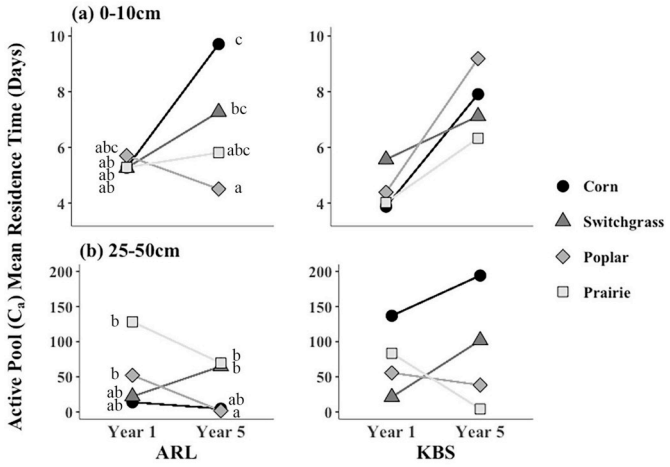


**Fig. 2.** Active pool ($C_a$) mean residence time (MRT) (days) based on incubation data of soils from (a) 0–10 cm and (b) 25–50 cm depths by cropping system at the two field sites: Arlington Agricultural Research Station (ARL), WI and Kellogg Biological Station (KBS), MI. Lower case letters indicate significant differences among years and cropping systems ($p < 0.05$).

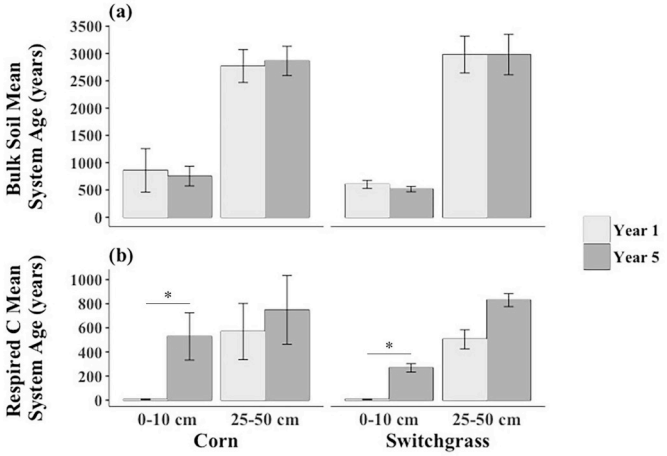


**Fig. 3.** Average radiocarbon-based modeled-mean system ages (years) of (a) respired $CO_2$ and (b) bulk soil collected from 0-10 cm and 25–50 cm under corn and switchgrass at Arlington Agricultural Research Station (ARL), WI in year 1 (2008) and year 5 (2013). Significant differences ($p < 0.05$) are indicated by an asterisk (*).

$C_a$ pool after five years than switchgrass and corn in surface soils, and than switchgrass and restored prairie at depth. These findings could be explained by differences in the quantity and quality of plant litter inputs. Plant residues are an important substrate for microbial respiration (Eisenhauer et al., 2010) and for the stabilization of SOM via increased production of microbial products that can help bind aggregates and sorb to mineral surfaces (Liang et al., 2012). As these were bioenergy field trials, aboveground biomass harvest occurred annually in the corn, switchgrass, and restored prairie. Poplar stands, on the other hand, were harvested at the end of the fifth growing season, prior to the year 5 sampling. Based on annual net primary productivity and harvest yield data from Sanford et al. (2016) measured within the same field trials, corn had the greatest annual aboveground inputs. Switchgrass and restored native prairie were similar to each other and to poplar, estimated from litterfall inputs and understory biomass with data from von Haden (2017). Belowground, von Haden (2017) reported greater inputs in

**Table 3**
Stable and radiocarbon isotope data and modeled-mean system age of bulk soil and respired $CO_2$ from soil samples collected from 0–10 cm and 25–50 cm at Arlington Agricultural Research Station, WI collected in year 1 (2008) and year 5 (2013).

| Treatment | Depth (cm) | Block | Year | Bulk Soil | | | | Respired $^{14}C$-$CO_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | CAMS#'s[a] | $\delta^{13}C$ (‰) | $\Delta^{14}C$ (‰) | Age (y) | CAMS#'s[a] | $\delta^{13}C$ (‰) | $\Delta^{14}C$ (‰) | Age (y) |
| Corn | 0–10 | 1 | 2008 | 167180 | −20.4 | −168.2 | 1647.1 | 167849 | −20.9 | 42.5 | 1.0 or 155.0[b] |
| | | 2 | 2008 | 167182 | −20.0 | −18.0 | 369.3 | 167850 | −22.2 | 56.5 | 3.9 or 127.8[b] |
| | | 3 | 2008 | 167184 | −19.1 | −49.7 | 561.9 | 167840 | −20.1 | 110.7 | 11.8 or 63.2[b] |
| | 25–50 | 1 | 2008 | 167181 | −17.3 | −295.0 | 3339.9 | 167841 | −19.8 | −108.1 | 1035.6 |
| | | 2 | 2008 | 167183 | −16.6 | −224.0 | 2318.8 | 167843 | −17.7 | −10.5 | 332.2 |
| | | 3 | 2008 | 167185 | −16.0 | −248.7 | 2650.4 | 167844 | −13.9 | −12.0 | 339.4 |
| Switchgrass | 0 − 10 | 1 | 2008 | 167186 | −21.0 | −34.6 | 463.2 | 167851 | −21.3 | 61.6 | 4.8 or 119.3[b] |
| | | 2 | 2008 | 167188 | −20.1 | −58.1 | 621.3 | 167852 | −18.8 | 68.5 | 5.9 or 109.0[b] |
| | | 3 | 2008 | 167190 | −19.7 | −71.1 | 719.9 | 167853 | −18.1 | 70.3 | 6.2 or 106.4[b] |
| | 25–50 | 1 | 2008 | 167187 | −17.2 | −267.2 | 2916.0 | 167845 | −14.5 | −54.4 | 594.8 |
| | | 2 | 2008 | 167189 | −16.8 | −232.7 | 2432.6 | 167847 | −14.5 ± 2 | −51.6 | 574.9 |
| | | 3 | 2008 | 167191 | −16.9 | −310.4 | 3591.9 | 167848 | −14.5 ± 2 | −13.5 | 346.7 |
| Corn | 0–10 | 1 | 2013 | 167192 | −19.5 | −112.9 | 1081.9 | 167842 | −13.1 | −61.9 | 468.6 |
| | | 2 | 2013 | 167194 | −18.8 | −34.7 | 464.7 | 167846 | −18.1 | 16.5 | 223.0 |
| | | 3 | 2013 | 167196 | −18.3 | −69.9 | 711.5 | 167860 | −19.2 | −92.1 | 894.2 |
| | 25–50 | 1 | 2013 | 167193 | −17.6 | −297.7 | 3384.9 | 167858 | −14.0 ± 2 | −136.3 | 1308.8 |
| | | 2 | 2013 | 167195 | −16.3 | −236.5 | 2485.2 | 167859 | −14.5 | −49.5 | 561.6 |
| | | 3 | 2013 | 167197 | −16.3 | −253.8 | 2723.4 | 167861 | −13.5 | −19.0 | 375.1 |
| Switchgrass | 0–10 | 1 | 2013 | 167198 | −19.7 | −36.1 | 473.4 | 167854 | −18.9 | 15.1 | 227.7 |
| | | 2 | 2013 | 167200 | −19.3 | −33.9 | 460.1 | 167855 | −16.6 | −11.4 | 336.4 |
| | | 3 | 2013 | 167202 | −18.4 | −56.3 | 609.4 | 167856 | −14.7 | 12.1 | 238.2 |
| | 25–50 | 1 | 2013 | 167199 | −17.7 | −251.0 | 2684.2 | 167862 | −17.0 | −81.6 | 805.5 |
| | | 2 | 2013 | 167201 | −17.2 | −240.6 | 2540.2 | 167863 | −20.2 ± 2 | −75.0 | 752.1 |
| | | 3 | 2013 | 167203 | −16.7 | −317.7 | 3714.3 | 167879 | −18.6 ± 2 | −96.4 | 931.9 |

[a] Sample number for Center for Accelerator Mass Spectrometry (CAMS).
[b] Modeling of samples that contain a significant proportion of 'bomb' C yielded two potential mean system ages.

switchgrass compared to corn at the ARL sites, and Sprunger et al. (2017) measured greater fine root biomass production under switchgrass and restored prairie than under poplar at KBS. Greater respiration of the restored prairie could be explained by greater belowground inputs, although this does not appear to be the case for poplar systems. Instead, differences in tissue quality of poplar and herbaceous leaf litter (average C-to-N ratios of 32) (Ferré et al., 2014) compared to corn stover residues and roots left behind after harvest (average C-to-N ratios of 57) (USDA NRCS, 2011), would lead to the observed faster decomposition rates and shorter SOC MRT under the tree bioenergy cropping system.

After five years, soils gained C in the $C_s$ pool, which is not surprising given that its size is influenced by the size of the $C_a$ pool. The $C_s$ is theorized to be less accessible to decomposers than the $C_a$ pool via protection by occlusion within soil aggregates and by sorption to mineral surfaces (Parton et al., 1993; Six et al., 2002). The accumulation of C in the $C_s$ pool in the deeper soils (25–50 cm) may reflect an increase in the vertical transport of C from upper depths associated with bioturbation or in response to initial tillage (Dimassi et al., 2013) or in root biomass inputs. Given that the $C_s$ represented 51–63% of the bulk SOC, comparable to similar agricultural soils (Paul et al., 1999), its response to land management has implications for overall soil C storage. Gains in slow cycling C pools could lead to more long-term SOC sequestration, although better constraints on its size and turnover are needed before long-term predictions can be made.

### 4.2. Microbes respired C with longer mean system ages after losses of active carbon pools

In contrast to observed differences in soil respiration over the one-year incubation between annual and perennial bioenergy crops, radiocarbon values of bulk soil and respired $CO_2$ at the start of the incubation did not differ between corn and switchgrass. There was also no effect of year on radiocarbon values of bulk soil or of respired C from the 25–50 cm soils. However, surface soil $\Delta^{14}C$-$CO_2$ did change from year 1 to year 5, with values becoming consistently smaller or depleted in $^{14}C$ over time.

We interpret differences in radiocarbon concentrations of respired $CO_2$ to indicate that microbes were accessing different SOM pools after five years of bioenergy crop field trials. Reductions in the size of the biologically-active C pool over time were accompanied by increases in the mean apparent age of respired $CO_2$. We propose two alternate hypotheses to explain these observations (Fig. 4). The first assumes an initial flush of $^{14}C$-enriched recent C immediately following soil disturbance during field trial establishment, followed by a return to background respiration values by year 5. Alternatively, the continued reduction in plant litter inputs via harvest may be responsible for enhanced microbial decomposition of SOM composed of older plant residues, which would lead to respiration of $^{14}C$-depleted C above background (year 1) levels after five years. The key differences between these two hypotheses is whether the amount of 'old' C that was accessed and respired increased after five years of management or remained constant, and what the $\Delta^{14}C$ values and C source are of background soil respiration.

In the first hypothesis, the observed enrichment in $^{14}C$- $CO_2$ from samples collected shortly after the establishment of the field trials could be explained by a temporary flux of recent plant-derived C enhanced by soil disruption after tillage in year 1. Subsequent no-till practices would promote the protection of additional C inputs into soil aggregates, leading to observed reductions in the total amount of respired $CO_2$ and in its apparent age in year 5.

Under the second hypothesis, a shift towards respiration of C with older apparent mean system ages could be explained, not by the initial flush of more bioavailable C at the start of the field trials, but by the overall reduction of total plant inputs with annual harvesting. In this case, $CO_2$ respired from the year 5 soils would be dominated by background fluxes from the slowed decomposition of residual SOC. If this scenario is representative of what is occurring in the field, our findings suggest that 'old' C pools are sensitive to changes in management, and their loss has negative implications for overall effects of bioenergy crop

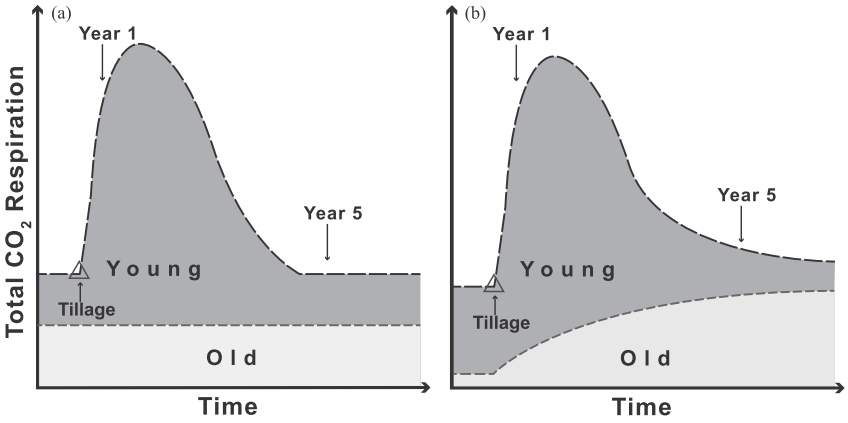


**Fig. 4.** Depiction of two possible scenarios with more $^{14}$C-depleted C respired after five years: (a) initial (year 1, 2008) increased respiration of $^{14}$C-enriched (young) and labile C and a return to background respiration (year 5, 2013) with unchanged respiration of $^{14}$C-depleted (old) C and (b) increased respiration of $^{14}$C-depleted (old) (C) with reduction of respiration of $^{14}$C-enriched (young) C following an initial increase after tillage at the field trial establishment.

management. In an earlier study on a nearby site with the same soil series, Sanford et al. (2012) documented significant losses of SOC at depth even under no-till bioenergy cropping systems. SOC losses at depth may stem from a background flux of older C pools that accrued under tallgrass prairie vegetation, consistent with our findings of an older C signal in heterotrophic respiration following conversion to biofuel cropping systems. Further work is needed to determine if these losses are indeed due to depletion of old C, and whether or not present day cropping systems may be exacerbating these losses as a result of enhanced microbial decomposition or priming (Zhang et al., 2017).

### 4.3. Cropping system effects on microbial activity

Soil microbial respiration, a measure of microbial activity and of C bioavailability, in year-long laboratory incubations differed between perennial and annual crops after five years of experimental bioenergy crop production. The greatest amount of total C respired from surface soils was associated with perennial polycultures, followed by perennials grown in monoculture, and annuals grown in monoculture (i.e., restored native prairie > poplar > switchgrass > corn). This trend suggests potential future gains in bulk SOC associated with more diverse systems, as microbial biomass C pools can be early indicators of changes in SOC content (McDaniel et al., 2014).

Differences in microbial respiration between polyculture perennials and monoculture annual crops have been attributed to differences in belowground litter inputs, which are a dominant source of SOC and a substrate for microbial activity (Rasse et al., 2005). Sprunger et al. (2017) measured greater fine root biomass production with increasing plant species diversity at KBS. Restored native prairie allocated more C to root biomass compared to corn and switchgrass systems, with poplar having the lowest fine root production. Annual and perennial crops also differ in their rooting structure, with high-density roots and enhanced root persistence associated with perennials (Roumet et al., 2006). Increased root biomass also leads to soil aggregate formation and stabilization, which may be one of the most important mechanisms of SOM protection from decomposition in temperate grassland soils (Tisdall and Oades, 1982; Jastrow et al., 1998).

Our findings of increased microbial respiration under diverse perennial crops compared to a single-crop system are consistent with those of other studies showing positive relationships between crop diversity, perenniality, and soil microbial activity (McDaniel et al., 2014; Tiemann and Grandy, 2014; Tiemann et al., 2015). Microbial activity and soil C storage increased with plant diversity in temperate grasslands (Lange et al., 2015). At both sites, the poplar monoculture behaved more like the high diversity perennial plots where total cumulative respiration values of surface soils were similar to those for restored native prairie. These results could be explained by the presence of a thick understory of mixed forbs and grasses (i.e., herbaceous weeds; personal observation).

Despite differences in total soil respiration between the multi-species perennials and the monoculture annuals, we found no difference in the size of the modeled soil C pools or in the $^{14}$C-based turnover time of respired $CO_2$ between switchgrass and corn, specifically. Differences between the two systems may become apparent with time and under alternate management practices such as tillage in corn.

### 4.4. Bioenergy cropping system effects differed by site

Changes in the size and MRT of the $C_a$ after five years of bioenergy cropping were greater at ARL than at KBS, despite similar management practices at both sites. Coarse-textured soils may accumulate SOC in the particulate fraction more rapidly than fine-textured soils, and we did find that the $C_a$ composed a greater proportion of the bulk SOC at KBS. Other studies have reported that biologically available C may be more sensitive to agricultural management in soils with greater clay content (Hassink, 1997; Six et al., 2004; Stockmann et al., 2013). The $C_s$ was similarly more sensitive to cropping systems at the silt loam site (ARL) than at the sandy loam site (KBS). Two key mechanisms for C stabilization in temperate grassland soils (Paul, 1984; Six et al., 2002), microaggregate formation (Oades, 1984) and increased organo-mineral interactions (Lagomarsino et al., 2012), are promoted in fine-textured soils and are especially vulnerable to cultivation. In a separate study, Tiemann and Grandy (2014) reported greater aggregate stability at ARL than at KBS, especially under perennial cropping systems.

ARL is cooler and drier than KBS and we cannot rule out climatic differences explaining the response of the $C_a$ pool, although we would expect greater response in the warmer and wetter site. Regardless of the mechanism, site-specific differences indicate the importance of environmental conditions in predicting the sustainability of bioenergy cropping systems and of the need to test for management effects in different locations.

## 5. Conclusions

Conversion from historical field-crop agricultural systems to bioenergy crops altered the size, mean residence time, and age of SOC pools after only five years. Archived soil samples collected prior to field trial establishment paired with $^{14}$C-analysis provided insights into changes in soil C dynamics. The soil incubation approach to separating SOC into functional pools revealed losses of bioavailable soil C, as measured in the size of the active C pool and in total cumulative $CO_2$ production in a long-term incubation, irrespective of bioenergy crop type. The amount or age of C in the active pool did not differ between perennial and annual cropping systems. Soils lost C associated with the $C_a$ pool. SOC pools were more responsive to changes in agricultural management in the finer-textured soils at ARL than in the coarser-textured soils at KBS and generally under perennial systems compared to annual systems. Carbon respired by microbes in year 5 was $^{14}$C-depleted and had older

mean ages than samples collected at the start of the field trials for both corn and switchgrass. We propose that initial soil disturbance by tillage and continued removal of plant biomass by harvesting, even in these low-intensity cropping systems, have depleted rapidly-cycling carbon, leading microbes to decompose older carbon pools. This finding may be due to an initial flush of $^{14}$C-enriched labile C during soil disturbance at the time of site establishment or a shift to increased respiration of previously protected $^{14}$C-depleted C with reduction of inputs due to annual harvest of aboveground biomass. If the latter mechanism is at play, the loss of soil C with older mean ages has overall negative implications for the long-term trajectory of SOC sequestration under both annual and perennial bioenergy cropping systems. Our results suggest that tailoring management to prevent the release of old, stored C and for the accrual of C in the active and slow pools can contribute to reducing C costs of bioenergy production.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.08.025.

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