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Early accumulation of active fraction soil carbon in newly established cellulosic biofuel systems

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ABSTRACT

We examined relative changes in soil C pools shortly after the establishment of six perennial and two annual bioenergy cropping systems that differed in diversity (monoculture vs. polyculture). Perennial systems included two monocultures (switchgrass, *Panicum virgatum*; and miscanthus, *Miscanthus* × *giganteus*) and four polycultures including hybrid poplar (*Populus* sp.) + herbaceous understory; mixed native grasses, successional vegetation, and restored prairie. Two annual systems included no-till continuous corn (*Zea mays*) and rotational corn (corn-soybean (*Glycine max*)-canola (*Brassica napus*)). Each crop was planted in a full factorial design at both a moderate fertility Alfisol and a high fertility Mollisol site. Relative differences in active, slow, and passive C pools in surface soils, where C changes are most likely to be detected early, were evaluated with 322-day laboratory incubations followed by acid hydrolysis to infer different pools from exponential decay curves. Five years post-establishment, active C pools under perennial polycultures at the Alfisol site were up to twice those under annual and perennial monocultures, and followed the order hybrid poplars (696 ± 216 μg C g^{-1} soil, n = 5 replicate blocks) ≈ native grasses (656 ± 155) ≈ restored prairie (638 ± 44) > early successional (500 ± 54) ≫ continuous corn (237 ± 68) ≈ rotational corn (180 ± n.a.). Active C pools in perennial monocultures were similar to those in continuous corn: switchgrass (274 ± 29) ≈ miscanthus (299 ± 9). In contrast, differences in active C pools among crops at the more fertile Mollisol site were not detectable except for greater pools in the restored prairie and rotational corn systems. At both sites, slow and passive C pools differed little among systems except that slow pools were greater in the poplar system. That diversity rather than perenniality itself led to greater active C pools suggests that polycultures might be used to accelerate soil C accumulation in bioenergy and other perennial cropping systems.

1. Introduction

Soil carbon (C) is crucial for improving soil structure, increasing biological activity, and increasing nutrient and water availability, all of which lead to healthier and more productive soils (Lal, 2011; Seremesic et al., 2011). Globally, soil C can serve as a source and sink of CO_2: when net primary production exceeds decomposition, soil C accumulates, and when soil heterotrophs respire CO_2 at a faster rate than C accumulates, soil C is lost. Since soils globally hold twice as much C as the atmosphere (Swift, 2001), the stabilization and growth of soil C pools is crucial for stabilizing atmospheric CO_2 (Paustian et al., 2016). Sequestering C in agricultural soils is especially attractive as stored C will also enhance soil health and crop yields (Lal, 2011). Well-studied approaches to enhancing soil C in croplands include slowing decomposition by converting to no-till management and increasing C inputs with cover crops or additional crop residue (Hutchinson et al., 2007; Jarecki and Lal, 2003; Johnston et al., 2009).

Planting perennial vegetation in place of annual crops is another strategy that can increase soil C (Post and Kwon, 2000; McLauchlan et al., 2006; Kell, 2011). Perennial species typically exhibit extensive root systems that can contribute large amounts of C belowground (Dupont et al., 2014). Further, perennial field crops are no-till by nature and thus decomposition is often slowed as compared to annually tilled systems (Syswerda et al., 2011). For example, Ferchaud et al. (2016) found that conversion from annual row crops to semi-perennial cropping systems sequestered 0.93 t C ha^{-1} yr^{-1}. Plant diversity can also enhance soil C accumulation: grassland communities with increased diversity have been found to sequester up to five-fold more C compared to monocultures of the same species (Steinbeiss et al., 2008; Fornara and Tilman, 2008; Lange et al., 2014).

Perennial cellulosic bioenergy crops may thus provide a significant potential for increased soil C sequestration (Robertson et al., 2011), especially if planted in diverse mixtures. However, while conversion to perennial vegetation has proven to be effective for increasing total soil

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C, the proportion of C accruing in active versus more recalcitrant slow and passive pools is poorly understood (McLauchlan et al., 2006). The active pool best reflects soil fertility and consists of freshly deposited material such as plant residues, root exudates, and microbial necromass and typically has a mean residence time (MRT) of less than a year. In contrast, the slow pool is comprised of material that has been stabilized through physical and biochemical processes and has an MRT that ranges from a few years to a decade or more, and the passive pool, with an MRT of thousands of years, consists of non-hydrolyzable C often closely associated with soil mineral surfaces (Paul et al., 2001a; Wander, 2004).

The value of examining individual C pools in soil is to provide insight into mechanisms favoring soil C turnover and persistence, and also to identify soil C trajectories before changes in total soil C can be detected gravimetrically (Leifeld and Kögel-Knabner, 2005). In Midwest USA soils, from 10 to 70 years are typically required to detect total soil C change (Necpálová et al., 2014).

Several techniques have been used to separate and quantify pools of soil C including biological, chemical, and physical methods. Active and slow pools are readily measured with physiochemical approaches that measure the amount of particulate organic matter size and density fractions (e.g., Cambardella and Elliott, 1992; Wander, 2004; Culman et al., 2012) and with biological approaches that measure the amount of CO_2 mineralized in long-term laboratory incubations (e.g., Robertson and Paul, 2000; Paul et al., 2001b; Sanford and Kucharik, 2013; Riggs and Hobbie, 2016). Passive pools can be estimated by chemical fractionation with acid hydrolysis (Paul et al., 1997, 1999; Sanford and Kucharik, 2013). Paul et al. (1999), for example, used long-term incubations to show that poplars accumulated more C in the active pool compared to a conventionally tilled annual system, and as well that both early successional and poplar systems accumulated more slow C. Collins et al. (2010) used long-term incubations to show that the slow C pool under five year-old switchgrass stands was 13% greater than in nearby uncultivated native soils.

Here we used long-term soil incubations in the laboratory together with acid hydrolysis to investigate changes in C pools five years after planting eight candidate biofuel cropping systems at two sites with similar climates but different soils, one a moderately fertile Alfisol soil in Michigan USA developed under forest, and the other a highly fertile Mollisol soil developed under prairie vegetation in Wisconsin USA. Cropping systems ranged in perenniality and diversity from continuous no-till corn to restored prairie. We used a three-pool model fit to incubation results to identify active, slow, and passive pools in the surface horizons of all systems.

Our overall objective is to evaluate the influence of different bioenergy cropping systems and soil types on active, slow, and passive C pools in order to better understand changes in soil C following land use conversion, and in particular to identify trends even before total C differences can be detected. Thus, we examined 1) whether diverse perennial biofuel cropping systems have greater and more persistent active, slow, and passive soil C pools in surface soils compared to annual cropping systems, 2) whether plant diversity (monoculture versus polyculture) influences active, slow, and passive C pools differently, and 3) whether soil type influences relative changes in soil C pools in different systems.

2. Materials and methods

2.1. Site description

We examined soil C pools in the Great Lakes Bioenergy Center's Biofuel Cropping System Experiment located at the Kellogg Biological Station (KBS) Long-Term Ecological Research Site in Michigan, USA (Alfisol) and at Arlington Agricultural Research Station (ARL) in Wisconsin, USA (Mollisol). At KBS mean annual precipitation and temperature are 1005 mm yr^{-1} and 10.1 °C, respectively. KBS Alfisols developed under eastern deciduous forest vegetation and are moderately fertile well-drained loamy mesic Typic Hapludalfs of comingled Kalamazoo and Oshtemo series with five distinct horizons: Ap (0–30 cm), E (30–41 cm), Bt1 (41–69 cm), 2 Bt2 (69–88 cm), 2E/Bt (88–152) (Robertson and Hamilton, 2015). Prior to establishment in 2008 the pH of surface soils (0–10 cm) was 6.1, total soil carbon was 14.3 g C kg^{-1}, and texture was 63% sand, 31% silt, and 6% clay (http://data.sustainability.glbrc.org).

At ARL mean annual precipitation and temperature are 833 mm yr^{-1} and 7.4 °C, respectively. ARL Mollisols developed under native prairie vegetation and are highly fertile silty loam mesic Typic Argiudolls in the Plano Series (Sanford et al., 2012), with five horizons: Ap (0–23 cm), A (23–36 cm), Bt1 (36–48 cm), Bt2 (48-79 cm), and Bt3 (79–109 cm). Prior to establishment in 2008 the pH of surface soils (0–10 cm) was 6.6, total soil C was 22.4 g C kg^{-1} (Sanford et al., 2016), and soil texture was 9% sand, 66% silt, and 25% clay (http://data.sustainability.glbrc.org). Prior to this experiment both sites were under annual row crops that were conventionally managed.

2.2. Experimental design and systems

The Biofuel Cropping System Experiment is a randomized complete block design with five replicate blocks at each site consisting of eight biofuel cropping systems, including continuous no-till corn (*Zea mays* L.), rotational no-till corn as a corn-soybean (*Glycine* max L.)-canola (*Brassica napus* L.) rotation with each phase of the rotational corn system (corn, soybean, and canola) present each year, and monoculture and diverse perennial crops. The perennial monoculture systems are switchgrass (*Panicum virgatum* L.) and miscanthus (*Miscanthus* × *giganteus*) crops. Hybrid poplar (*Populus nigra* × *P. maximowiczii* 'NM6') was planted as a biculture with a nurse crop of oats, replaced in succeeding years with an herbaceous understory (24% cover) dominated by *Crepis capillaris* and *Conzya canadensis*. The other diverse perennial systems included a five species native grass mix (*Andropogon gerardii*, *Elymus canadensis*, *Panicum virgatum*, *Schizachrium scoparium*, and *Sorghastrum nutans*), an early successional community abandoned from agriculture at the time of experiment establishment, and an 18-species restored prairie system consisting of C4 and C3 species including legumes (described below). The monoculture switchgrass system consisted of the Cave-in-Rock variety, while the switchgrass in the native grass mix and restored prairie were of the Southlow variety. Each of the 100 plots within the Biofuel Cropping System Experiment is 27 m × 43 m (0.12 ha) and plots are separated by a > 15 m wide mowed alley.

Prior to planting in early spring 2008, all plots were tilled to a depth of 20.3 cm with a chisel plow and secondary soil finisher. The annual row crops were subsequently planted in late spring, and thereafter managed without tillage. Planting rates for corn and soybeans were 70,000 and 78,000 seeds ha^{-1}, respectively. Canola was planted at 4.5 kg ha^{-1}. The switchgrass, native grasses, and restored prairie systems were then planted in summer 2008 with a brillion-type native plant seeder. Seeding rates for switchgrass were 7.5 kg ha^{-1}, for the native grasses 1.6 to 2.4 kg ha^{-1}, and for restored prairie 0.4 to 1.2 kg ha^{-1}. Both the miscanthus and the poplar systems were planted by hand in May 2008 at densities of 17,200 rhizomes ha^{-1} and 2778 cuttings ha^{-1}, respectively. Miscanthus failed at the Mollisol site due to winterkill in winter 2008–2009 and was replanted in spring 2010 (Sanford et al., 2016).

The composition of the early successional system reflects the 2008 soil seed bank and natural colonization by annual and perennial herbaceous species. At the Mollisol site, the early successional system was dominated by *Elymus canadensis and Ambrosia trifida*. At the Alfisol site, annuals made up 79% of the early successional system; dominant species were *Setaria faberi* and *Conzya canadensis*. The Shannon-Weiner diversity index for the early successional community was 1.94 and 2.10 for the Mollisol and Alfisol sites, respectively. More details on species

composition for the native grasses, early successional and restored prairie systems are available at https://data.sustainability.glbrc.org/datatables/421.

Nitrogen fertilizer application varied by cropping system. Corn received on average 167 kg N ha^{-1} y^{-1} as urea-ammonium nitrate. Canola received 176 kg N ha^{-1} y^{-1} as urea-ammonium nitrate. The switchgrass, miscanthus, native grasses, and early successional systems each received 56 kg N ha^{-1} y^{-1} of urea-ammonium nitrate. The poplars received a single pulse of urea-ammonium nitrate fertilizer in 2010 at a rate of 155 kg N ha^{-1} at the Alfisol site and 210 kg N ha^{-1} at the Mollisol site. The restored prairie and soybeans were not fertilized.

2.3. Soil sampling

Intact soil cores were collected in November 2013 at both sites from blocks 1–3 with hydraulic direct-push soil samplers (Geoprobe; Salina, KS at the Alfisol site and Giddings; Windsor, CO at the Mollisol site). Three 1 m deep cores (7.6 cm diameter) were taken at three designated sampling stations within each plot and divided into four different depths: 0–10 cm, 10–25 cm, 25–50 cm, and 50–100 cm. Cores within each plot were composited by depth interval and sieved to 4 mm. However, results for only the 0–10 cm layer are reported in this analysis. All living roots were extracted prior to sieving.

2.4. Long-term incubations

Long-term laboratory incubations were used to estimate size and turnover rates of the different soil organic C pools (Paul, 2001a; Grandy and Robertson, 2007; Sanford and Kucharik, 2013). The laboratory experiment was a two-site by ten-treatment full factorial design. Treatments included all eight cropping systems plus soybean and canola phases of rotational corn. Incubations included surface soils (0–10 cm) for all systems. Two analytical replicates, incubated separately, were treated as subsamples for 2 sites × 10 cropping systems × 3 replicate blocks × 2 analytical replicates = 120 incubation jars. Twenty-five grams of fresh soil were placed in 237 mL glass Mason jars after adjusting soil to 55% water-filled pore-space according to Franszluebbers et al., 2000 following a pilot study to determine optimal water content for C mineralization.

Throughout the experiment soils were kept in the dark at 25 °C. Soil moisture was adjusted once per week to maintain a 45–55% moisture content throughout the course of the incubations. CO_2 measurements were taken 11 times over the course of 322 days, with more intensive sampling at the beginning (once per week) and less sampling towards the end (once every 6 weeks). CO_2 production for each sample was determined by injecting 1 mL of headspace into an N_2 carrier gas that streamed through a LI-COR LI-820 infrared gas absorption analyzer (LI-COR Biosciences, Lincoln, NE). An initial CO_2 reading was taken immediately after jars were capped, followed by three subsequent readings 40 min apart. CO_2 fluxes were calculated by regressing CO_2 versus time (Robertson et al., 1999). In between readings, jars were covered with plastic film.

2.5. Acid hydrolysis

To determine the passive or non-hydrolyzable C pool, we performed acid hydrolysis on soils after the last incubation (Paul et al., 1999; Collins et al., 2000; Sanford and Kucharik, 2013). Prior to hydrolysis, we used a dissecting microscope (20 ×) to identify plant material that was then removed with forceps and by flotation in a 5% NaCl solution. Two grams of soils were then refluxed in 6 N HCl (20 mL) at 116 °C for 16 h to oxidize available C and solubilize amino compounds, pectins, and cellulose (Sollins et al., 1999). The remaining material was then washed by centrifugation, dried, and ground for total C and N analysis performed with a CHNS Elemental Analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia, CA).

2.6. The three-pool decomposition model

In order to determine active, slow, and passive pool sizes and decomposition rates we used non-linear regression results from C mineralization rates, acid hydrolysis, and total C in a three-pool model with first-order kinetics (Paul et al., 1999; Sanford and Kucharik, 2013):

$$C_{t(t)} = C_a e^{-ka(days)} + C_S e^{-ks(days)} + C_p e^{-kp(days)} \quad (1)$$

where C_t = total soil organic C at time t; C_a, C_s, and C_p = C in active, slow, and passive pools, and k_a, k_s, and k_r = decomposition rates for each fraction. In order to estimate the active C pool (C_a) and decomposition rates, we determined the first order derivative of Eq. (1) via non-linear regression:

$$\text{Cumulative C mineralization} = C_a * k_a e^{(-ka*days)} + C_s * k_s e^{(-ks*days)} + C_r * k_r e^{(-kp*days)} \quad (2)$$

where C_a, C_s, and C_r = active, slow, and passive C pools respectively; and k_a, k_s, and k_r = decay constants for the active, slow, and passive pools, respectively (Paul et al., 1999).

The size of the passive C pool (C_r) was determined by acid hydrolysis. Once the active and passive C pools were determined, the slow C pool was calculated by subtracting the passive C and active C pools from total C:

$$C_s = C_t - C_a - C_r \quad (3)$$

where C_s, C_t, C_a, and C_r are defined as above.

Mean residence times (MRTs) were then calculated by taking the inverse of the decay constants for the active, slow and passive pools. In our model, k_r was set at 8.3×10^{-6} d^{-1}, a previously determined value for both sites by ^{14}C decay (Paul et al., 2001a), which is equivalent to a mean residence time of ~1000 yr. Laboratory estimated MRTs were scaled to in situ MRTs by using a Q_{10} correction ($2^{(T_{lab} - T_{field})/10}$) where T_{lab} is the laboratory incubation temperature (25 °C) and T_{field} is the mean annual field temperature (9.9 °C for the Alfisol site, 6.8 °C for the Mollisol site) (http://data.sustainability.glbrc.org/protocols/122). Automated weather stations at both sites were used to measure field temperatures.

2.7. Statistics

The non-linear regression function in SAS (Proc NLIN, version 9.4; SAS Institute, Cary, NC, USA) was used to estimate the active pool (C_a) and the decay rates for the active, slow, and passive pools (Table S1). Curves were generated for each of three replicate blocks per cropping system per site to estimate active, slow, and passive pools, MRTs, and cumulative C mineralization for every replicate block. Cumulative C mineralization was estimated by using Eq. (2) to calculate a daily value for each of 322 incubation days and then summing over the total period. Cumulative C mineralization, MRT, and active, slow, and passive C pools were analyzed using Proc Mixed of SAS (version 9.4; SAS Institute, Cary, NC, USA). Site and cropping system were treated as fixed effects and block as a random effect. Significant differences were determined at $\alpha = 0.05$ and means were compared with an adjusted Tukey's pairwise means comparison.

3. Results

3.1. Cumulative C mineralization

Cumulative C mineralization differed by cropping system (Fig. 1, $p < 0.05$), but not by site (Fig. 1, $p > 0.1$). At the Alfisol site, cumulative C mineralization ranged from 0.73 ± (0.1, standard error of the mean) to 1.33 ± 0.2 mg C g^{-1} soil (Fig. 1). The native grasses, early successional, and poplar systems mineralized the most C as compared to the rotational corn systems and switchgrass. At the

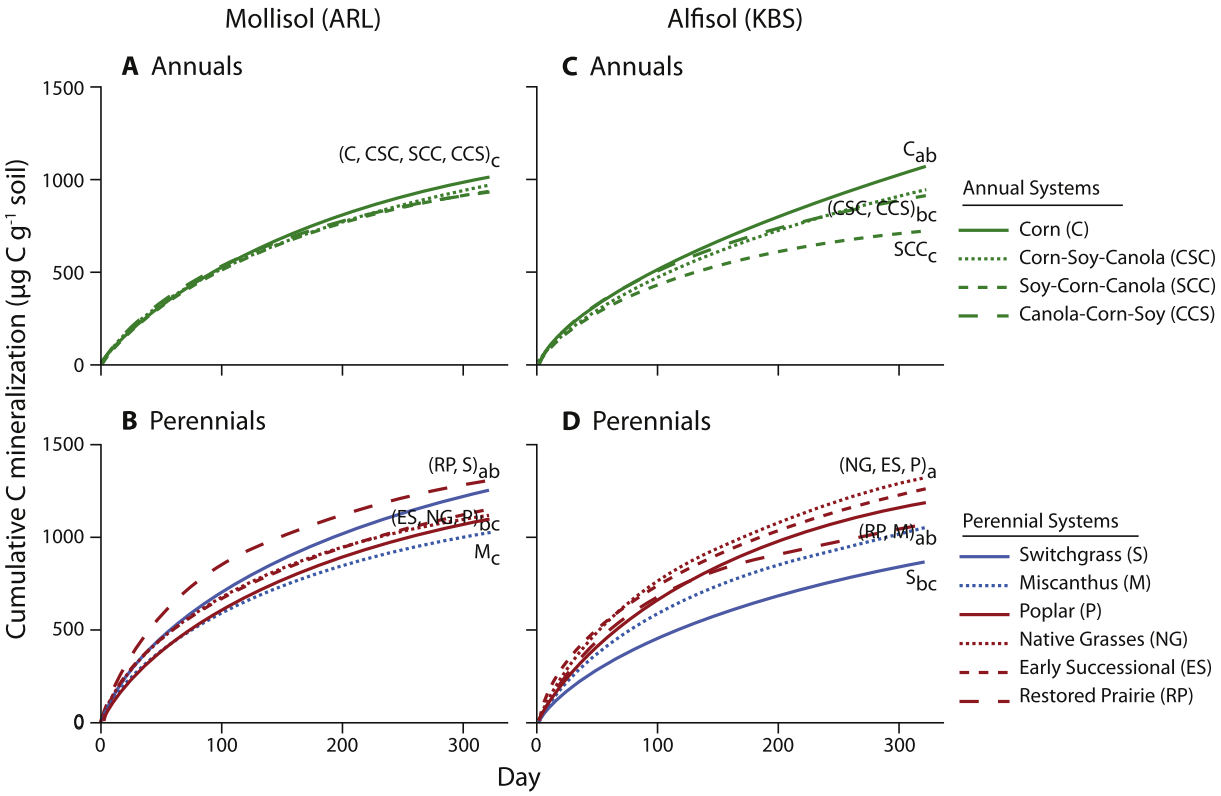

Fig. 1. Cumulative C mineralization from surface soils (0–10 cm depth) over the course of 322 day incubations for the moderate fertility Alfisol (KBS) and high fertility Mollisol (ARL) sites. Systems with different lowercase letters are statistically different from one another ($p < 0.05$, $n = 3$).

Mollisol site, cumulative C mineralization ranged from 0.94 ± 0.01 to 1.3 ± 0.2 mg C g^{-1} soil (Fig. 1). There were three apparent groupings within the eight Mollisol systems: The restored prairie and switchgrass systems mineralized significantly more C compared to the annual cropping systems and miscanthus, while early successional, native grasses, and poplar systems were not statistically different from either group.

3.2. Cumulative C mineralized per g soil C

Cumulative C mineralization expressed on a per g total soil C basis (rather than per g soil), which is a means to normalize against soil type (total C) differences between sites, was significantly greater at the Alfisol site than at the Mollisol site (Fig. 2, $p < 0.0001$). Cumulative C mineralized per g soil C ranged from 62.5 ± 13.0 to 98.6 ± 6.0 mg C g^{-1} soil C at the Alfisol site compared to 43.9 ± 16.1 to 57.1 ± 4.5 mg C g^{-1} soil C at the Mollisol site. There was also an overall significant cropping system effect (Fig. 2, $p < 0.05$). At the Alfisol site, the diverse perennial, corn, and miscanthus systems mineralized significantly more soil C per g total C than did the poplar, switchgrass, and rotational corn systems. At the Mollisol site, the restored prairie, switchgrass, native grasses, miscanthus, and annual systems mineralized more soil C per g total C than did the poplar systems (Fig. 2).

3.3. Trends of C mineralization over time

Most systems appeared to stabilize at a low C mineralization rate by incubation day 322 but a few systems had rates that were still decreasing (Figs. S1–S6). C mineralization rate variability was greatest towards the end of the incubation for most systems. For example, the continuous corn system at the Alfisol site was slowest to stabilize (Fig. S1E). For most soils the decline in C mineralization near day 100 (break in the curve) numerically differentiated the active from the slow C pool (Figs. S4–S6). The stabilization of C mineralization, which for most soils is represented by an asymptotic line close to but not equal to zero, represents the slow C pool (Paul et al., 1999).

3.4. The active C pool

The active C pool in surface soils significantly differed by system (Fig. 3, $p = 0.001$), and by site ($p < 0.05$). At the Alfisol site, there was a clear difference between the diverse perennials systems compared to the monoculture perennials and the annual systems (Fig. 3c): the diverse perennial systems had more than twice the amount of active C as compared to the other systems. At the Mollisol site, there were no apparent differences between the annual and perennial cropping systems except the restored prairie system had the largest active C pool (631 ± 134 µg C g^{-1} soil) compared to all other cropping systems (Fig. 3a). In addition, the poplar and native grasses had a larger active C pool compared to corn (Fig. 3a).

Both site and cropping system had significant effects on the proportion of active C found in the total C pool (Table 1, $p < 0.0001$ and $p < 0.0001$, respectively). At the Alfisol site, the active C pool comprised between 1.3% (soybean-corn-canola) and 6.7% (corn-canola-soybean) of the total C pool. At the Mollisol site, the active C pool comprised between 0.9% (continuous corn) and 2.7% (restored prairie) of the total C pool (Table 1), with the continuous corn system containing the lowest percentage of C in the active pool.

3.5. The slow C pool

Although the size of the slow C pool was statistically indistinguishable between the two sites and across cropping systems (Fig. 3b and d, $p > 0.05$), the proportion of C in the slow pool substantially differed by cropping system and site (Table 1, $p < 0.0001$ and $p < 0.0001$, respectively). At the Alfisol site, the slow C pool comprised between 39% (restored prairie) and 55% (poplars) of total C,

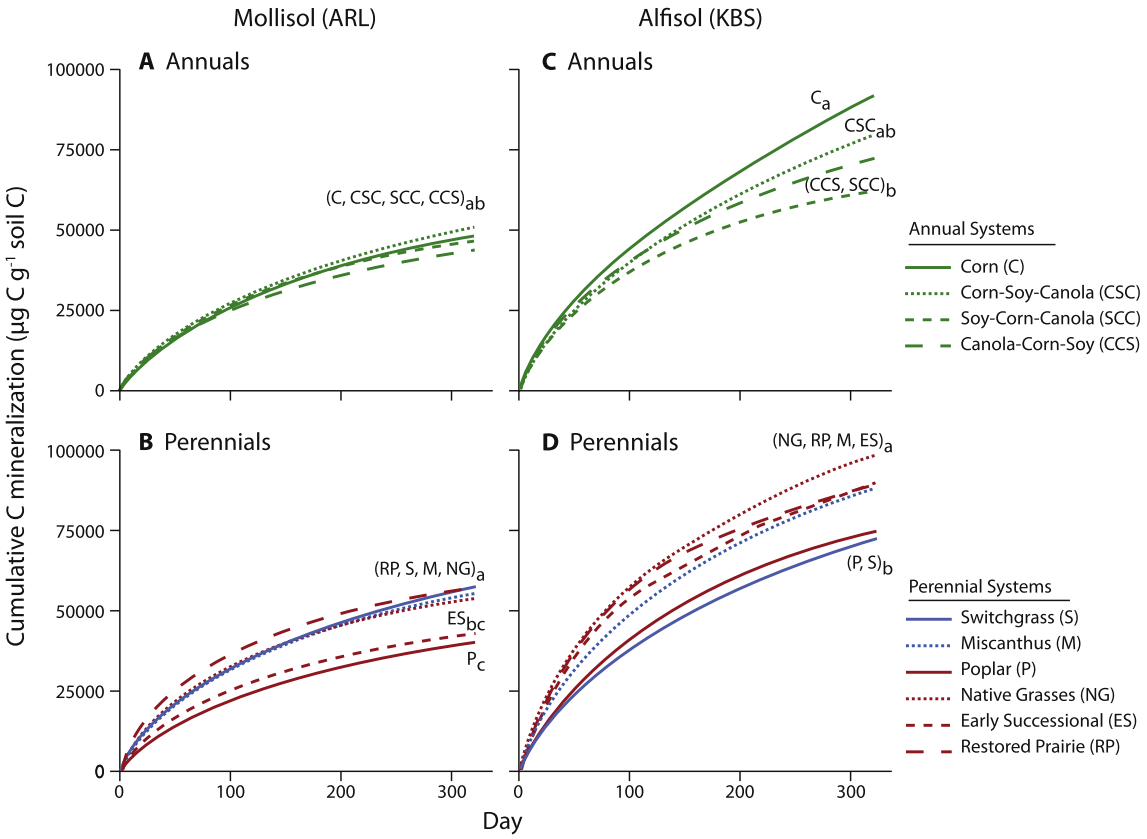

Fig. 2. Normalized cumulative C mineralization per gram of soil C from surface soils (0–10 cm depths) over the course of 322 day incubations for a moderate fertility Alfisol (KBS) and a high fertility Mollisol (ARL) site. For each site, systems with different lowercase letters are statistically different from one another ($p < 0.05$, $n = 3$).

whereas the slow C pool at the Mollisol site comprised between 20% (rotational corn) and 45% (poplar) of total C (Table 1). The size of the slow C pools significantly differed by cropping system ($p < 0.0001$), although distinct trends were not as visible as in the active pool. In addition, there was a marginally significant site by cropping system interaction ($p = 0.06$). At the Alfisol site, the poplars had significantly more C in the slow pool compared to all other systems except native grasses and early successional. At the Mollisol site, the poplar and early successional systems had significantly greater slow C compared to the other systems (Fig. 3b). The next group consisted of the restored prairie, switchgrass, and corn, which had significantly more slow C compared to the native grasses, miscanthus and other annual systems (Fig. 3b).

3.6. The passive (non-hydrolyzable) C pool

We did not detect any differences among cropping systems at either site for the passive C pool (Tables S2 and S3). However the passive C pool was overall significantly greater at the Mollisol site compared to the Alfisol site, with 2.2 times more passive C in the surface horizon. Site had a significant effect on passive C proportions of total C (Table 1, $p < 0.0001$). On average, the passive pool at the Mollisol site comprised 69% of total C compared to 52% at the Alfisol site.

3.7. Mean residence times of soil C

The MRTs of the active C pool differed by cropping system (Tables 2 and 3, $p < 0.001$) but not by site ($p > 0.1$). At the Alfisol site, the poplar system had an MRT of 78 ± 18.8 days, which was significantly longer than all of other cropping systems except for the native grasses, which had an MRT of 69 ± 13.4 days. Continuous corn had the shortest MRT of 33.4 ± 6.7 days. At the Mollisol site, the rotational corn soybean phase and poplar systems had the longest active C MRT at 63 ± 10.8 and 58 ± 6.0 days, respectively. The other systems had MRTs that ranged from 27 to 46 days, whereas miscanthus had the shortest MRT of 27 days.

MRTs of the slow C pool did not differ by site ($p > 0.1$) or cropping system ($p > 0.1$), although there were some noteworthy trends. For example, at the Alfisol site, the native grasses had a longer MRT of 7.9 ± 4.5 years compared to all other systems. At the Mollisol site the longest MRTs for the slow C pool were 4.5 and 4.2 years for the poplar and early successional systems (Table 3).

4. Discussion

Overall, diverse perennial systems by year five had accumulated significantly more active soil C compared to both annual systems and perennial monoculture systems, particularly at the Alfisol site: perennial polycultures including native grasses, early successional, restored prairie, and poplar systems had 50–117% more active C than did monocultures of annual (corn) and perennial (switchgrass, miscanthus) crops. Within the monocultures, there were no active C pool differences between annual and perennial crops: switchgrass and miscanthus had about as much active soil C as corn.

Differences in slow C pools between annual and perennial systems were less apparent. At both sites the poplar system had relatively more slow C than did the annual crops, as did the early successional system at the Mollisol site. There were no detectable differences in passive soil C among cropping systems at either site, though there was substantially more passive C at the Mollisol than at the Alfisol site.

4.1. Active C pool trends

Five years after establishment at the lower fertility Alfisol site, the diverse perennial systems had up to 2.5 times more active C than did

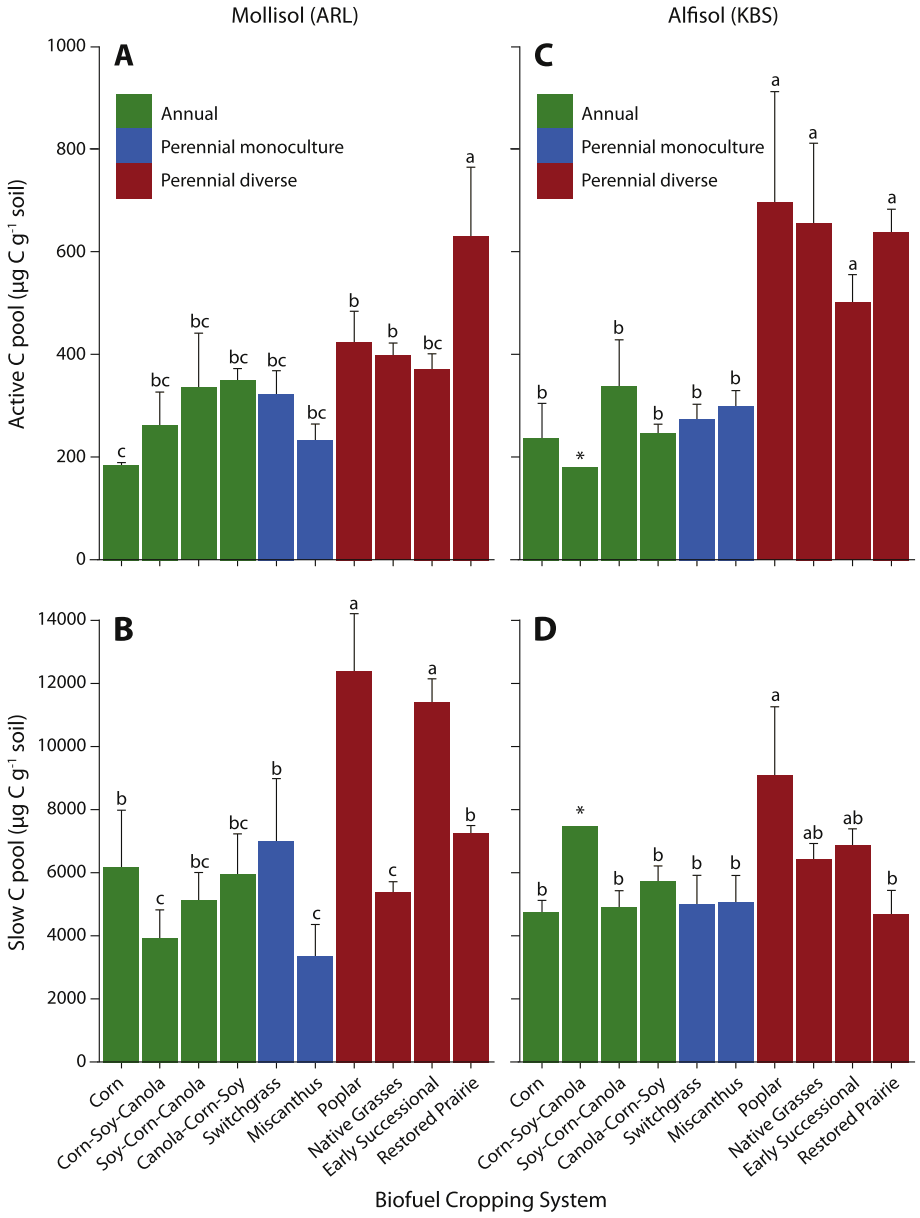

Fig. 3. The active and slow C pools at the high fertility Mollisol (ARL) and moderate fertility Alfisol (KBS) sites. Within each site, systems with different lowercase letters are statistically different from one another ($p < 0.05$). Bars with no letters were not significantly different from one another. Bars are means ± SE, n = 3; asterisk represents n = 1.

the annual systems and monoculture perennials, which were statistically similar to one another. At this site active C pools in perennial polycultures followed the order hybrid poplars (696 ± 216 µg C g^{-1} soil) ≈ native grasses (656 ± 155) ≈ restored prairie (638 ± 44) > early succession (500 ± 54) ≫ continuous corn (236 ± 68) ≈ rotational corn (180 ± n.a.). In perennial

Table 1
Active, slow, and passive C percentages of total C for eight biofuel cropping systems at the Mollisol (ARL) site and the Alfisol (KBS) site.[a]

System	Mollisol			Alfisol		
	Active C (%)	Slow C (%)	Passive C (%)	Active C (%)	Slow C (%)	Passive C (%)
Continuous corn	0.9n.s	28.8b	70.3a	2.0 cd	40.8bc	57.2a
Rotational corn						
Corn-soybean-canola	1.4n.s	20.3c	78.3a	6.7a	44.6bc	48.7ab
Soybean-corn-canola	1.6n.s	24.7bc	73.7a	1.3d	52.1ab	46.6b
Canola-corn-soybean	1.6n.s	28.0bc	70.4a	1.9d	45.4bc	52.7ab
Switchgrass	1.4n.s	31.4b	67.2a	2.3 cd	41.8bc	55.9ab
Miscanthus	1.3n.s	18.1c	80.6a	2.5 cd	42.0bc	55.5ab
Poplar	1.5n.s	44.6a	53.9c	4.2bc	55.3a	40.5b
Native grasses	1.9n.s	25.9bc	72.2a	3.6b	47.4abc	49.0ab
Early successional	1.4n.s	42.5a	56.1b	3.6b	49.0ab	47.4ab
Restored prairie	2.7n.s	31.7b	65.6ab	5.2ab	39.0c	55.8ab

[a] n.s. = not significant across treatments.

Table 2
Mean Residence Times (MRTs) for surface soils (0–10 cm) of eight biofuel cropping systems at the Mollisol (ARL) site for the active and slow C pool. Different letters represent a significant difference ($p < 0.05$) across cropping systems. Laboratory MRT was calculated as 1/k. Field MRT was determined using a Q_{10} correction for the difference in the lab temperature and the field mean temperature at ARL.

System	Active C		Slow C	
	Lab MRT (days)	Field MRT (days)	Lab MRT (years)	Field MRT (years)
Continuous corn	41.7 (3.8)^{b}	147.4 (13.4)	2.1 (0.5)^{a}	7.4 (1.6)
Rotational corn				
Corn-soybean-canola	38.7 (8.3)^{b}	136.6 (29.3)	2.1 (0.3)^{a}	7.4 (0.9)
Soybean-corn-canola	62.6 (10.8)^{a}	221.2 (38.0)	1.6 (0.1)^{a}	5.7 (0.36)
Canola-corn-soybean	46.3 (7.9)^{ab}	163.6 (28)	3.2 (0.3)^{a}	11.3 (1.2)
Switchgrass	31.4 (1.4)^{b}	111.0 (5.0)	2.3 (0.5)^{a}	7.9 (1.8)
Miscanthus	27.0 (4.0)^{b}	95.5 (14.2)	1.4 (0.3)^{a}	4.9 (0.9)
Poplar	57.5 (6.0)^{a}	202.9 (21.1)	4.5 (0.3)^{a}	15.7 (0.9)
Native grasses	39.3 (7.6)^{b}	138.6 (26.9)	2.1 (0.13)^{a}	7.4 (0.5)
Early successional	35.0 (5.1)^{b}	123.5 (17.9)	4.2 (1.2)^{a}	14.6 (4.4)
Restored prairie	39.3 (4.6)^{b}	138.6 (16.2)	2.8 (0.3)^{a}	9.9 (0.9)

Table 3
Mean Residence Times (MRTs) for surface soils (0–10 cm) of eight biofuel cropping systems at the Alfisol (KBS) site for the active and slow C pool. Asterisks represents ($n = 1$). Different letters represent a significant difference ($p < 0.05$) across cropping systems. Laboratory MRT was calculated as 1/k. Field MRT was determined using a Q_{10} correction for the difference in the lab temperature and the field mean temperature at KBS.

System	Active C		Slow C	
	Lab MRT (days)	Field MRT (days)	Lab MRT (years)	Field MRT (years)
Continuous corn	33.4 (6.7)^{c}	94.9 (19.1)^{c}	3.1 (0.5)^{b}	8.8 (1.5)^{b}
Rotational corn				
Corn-soybean-canola	27.3*	77.7*	3.1*	9.0*
Soybean-corn-canola	51.5 (9.4)^{bc}	146.2 (26.6)^{bc}	2.5 (0.1)^{b}	7.2 (0.4)^{b}
Canola-corn-soybean	33.9 (1.7)^{bc}	96.5 (4.7)^{bc}	2.7 (0.2)^{b}	7.7 (0.6)^{b}
Switchgrass	54.0 (13)^{bc}	153.0 (36.2)^{bc}	3.1 (0.3)^{b}	8.9 (0.9)^{b}
Miscanthus	36.8 (4.1)^{bc}	104.4 (11.9)^{bc}	2.2 (0.8)^{b}	6.4 (0.8)^{b}
Poplar	78.2 (18.8)^{a}	222.2 (53.3)^{a}	3.3*	9.4*
Native grasses	69.2(13.4)^{ab}	196.4 (38.1)^{ab}	7.9 (4.5)^{a}	22.6 (12.8)^{a}
Early successional	40.1 (7.9)^{bc}	113.7 (22.6)^{bc}	2.9 (0.8)^{b}	8.1 (2.4)^{b}
Restored prairie	55.8 (1.9)^{b}	158.4 (5.3)^{b}	3.6 (0.5)^{b}	10.1 (1.4)^{b}

monocultures active C pools were similar to those in corn: switchgrass (274 ± 29) ≈ miscanthus (299 ± 9).

The similarity in active C among annual systems and perennial monocultures differs from other studies that have demonstrated greater C accumulation under switchgrass and miscanthus compared to conventionally tilled corn (Liebig et al., 2005). Our results suggest that differences in these other studies may be due simply to tillage rather than crop life history differences: in our study no-till corn accumulated active C at rates similar to the perennial herbaceous monocultures which were, by nature, also no-till. Bonin and Lal (2012) also found similar rates of surface soil C accumulation in no-till corn and switchgrass.

That diversity rather than simply perenniality appears to explain the differences in active C among cropping systems is a novel finding that heretofore has only been noted for natural grassland and forest systems (Fornara and Tilman, 2008; Steinbeiss et al., 2008; He et al., 2013; Lange et al., 2015).

Why might diverse perennial systems accumulate more active C than monoculture perennials? One explanation is root productivity. Perennials tend to have extensive root systems with 3 to 8 times greater biomass (Dupont et al., 2014; Culman et al., 2010; Anderson-Teixeira et al., 2013). Estimates of fine root production at these sites reveal that the diverse cropping systems allocate more biomass to roots compared to monoculture perennials (Sprunger et al., 2017). Since aboveground net primary productivity (ANPP) at this site is equivalent among cropping systems except for higher productivity miscanthus (Sanford et al., 2016), we surmise that greater belowground C inputs and greater aggregate stability may be primary drivers for enhanced C accumulation under the diverse perennial systems.

Greater fine root production with higher productivity (Sprunger et al., 2017) is poorly understood but may be related to greater competition for nutrients or the 'functional composition effect.' This might be particularly evident in the restored prairie system, where N fixation by legumes can facilitate growth of C_4 grasses (Steinbeiss et al., 2008 and Fornara and Tilman, 2008). Greater C accumulation in the polycultures where legumes are absent (poplar, native grasses, and early successional systems) could be the result of greater root foraging, said to be intensified in mixed species systems where competitive root networks are established due to greater nutrient demand (de Kroon et al., 2012).

Increased aggregate stability could also contribute to greater active C under diverse perennial cropping systems relative to corn. Previous work from this same experiment demonstrated that the native grass system at the Alfisol site increased aggregate stability in the > 4 mm soil fraction compared to corn (Tiemann and Grandy, 2015). Aggregate stability allows for the physical protection of nutrients and has been directly linked to soil C sequestration (Six et al., 2000). For example, Grandy and Robertson (2007) found enhanced SOC accrual in macroaggregates under diverse perennial systems at this site.

At the Mollisol site, differences between the annual and perennial systems were much less apparent, with only the restored prairie accumulating more active C than others. There are at least four potential reasons for less differentiation at this site. First, the Mollisol site has substantially greater soil organic matter stores, which would make small changes more difficult to quantify. Total surface concentrations here were 22.4 g C kg^{-1} compared to 14.3 g C kg^{-1} at the Alfisol site. Second, almost 70% of the Mollisol site's soil C is in the passive pool, which may indicate approaching C saturation (Stewart et al., 2008) at which point soils have little capacity to stabilize additional C (Stewart et al., 2007). At the Alfisol site, in contrast, soil C levels are well below saturation (Gelfand et al., 2011), which suggests a greater capacity to build soil C quickly (Anderson-Teixeira et al., 2009; Johnston, 2011). Third, the Mollisol site has more clay (25% vs. 6% at the Alfisol site), which causes C to accumulate at a much slower rate as C approaches equilibrium (West and Six, 2007). Fourth and finally, Tiemann and Grandy (2015) showed that soil C accrual at the Mollisol site is associated with smaller aggregates as compared to the Alfisol site, which means that the Mollisol site is also further along on an aggregation and stabilization trajectory and thus less likely to accrue soil C in more labile pools.

Increases in the active C pool should eventually result in greater accumulation of C in more recalcitrant pools so long as management remains the same. For example, as the active C pool increases, C will transfer into the more recalcitrant pools of C through physical breakdown of organic material and microbially mediated processes (Grandy and Neff, 2008). This filtering effect of molecular C compounds is

driven by selective microbial degradation, whereby more recalcitrant pools accumulate in slow and passive C pools.

4.2. Slow C pool trends

Differences between the annual and perennial systems in the slow C pool were much less pronounced at the Alfisol site; only the poplars at both sites and the early successional system at the Mollisol site accumulated more slow C than did other systems. Although not significant, the native grasses and early successional systems at the Alfisol site had slightly greater slow C pools compared to the annuals and monoculture perennials, following trends visible in the active C pool.

Poplars are the only woody species in this study and previous experiments at a nearby KBS site have also shown that poplars on Alfisols are effective at sequestering slow C (Paul et al., 1999). In the first ten years of establishment, this nearby poplar system added 32–44 g C m^{-2} y^{-1} to the total surface soil C pool (Robertson et al., 2000). Twelve years post establishment, Grandy and Robertson (2007) found that poplars accumulated 37% more total C relative to conventional row crops in the top 5 cm. Likewise, our findings demonstrate that in the first five years of establishment poplars are accumulating twice as much C in both the active and slow pool in the top 10 cm of soil relative to no-till corn.

The poplars' accumulation of C in the slow pool is likely due to coarse and fine root production and turnover since most aboveground biomass (wood) is removed at harvest. However, fine root production results from this site (Sprunger et al., 2017) show that poplars produced fewer fine roots compared to the other polyculture systems. Thus, belowground C accumulation here could be a function of quality rather than quantity. Results from a decomposition experiment in Quebec showed that hybrid poplar roots have a high lignin to N ratio, which could lead to reduced microbial activity and may slow the overall rate of decomposition (Camiré et al., 1991).

The slow C pool can be altered by management but is generally associated with more stabilized pools of C, which greatly influence long-term C sequestration (Wander et al., 2004). The slow C pool is also influenced by physical protection (Grandy and Robertson, 2007), which will give systems with more extensive roots an advantage for building C over time, since roots affect aggregate formation and thus the protection of soil organic matter (Rasse et al., 2005) and contribute disproportionately to soil C pools compared to aboveground biomass (Kong and Six, 2010; Austin et al., 2017). For example, Austin et al. (2017) report that C derived from roots may be preferentially stabilized in soil due to rhizodeposits and their direct mineral associations. Thus, it is reasonable to expect detectable increases in the slow C pool over longer periods of time under systems that have extensive roots systems and that are already exhibiting increases in the active C pool relative to annuals and monoculture perennials.

4.3. Passive C pool trends

That we did not detect differences in passive soil C among cropping systems within our two sites is not surprising given that C in passive pools is generally not influenced by short-term management or biological activity (Wander, 2004). Mean residence times for passive pools at our sites were over 1000 years, consistent with ^{14}C data from these sites: Paul et al. (2001a) found that MRTs for soil C in soils high in clay like those found at the Mollisol site were about 2840 years and other Alfisols at KBS had MRTs of 1435 years.

However the amount of passive soil C differed substantially between the two sites. At the Alfisol site, the passive pool on average accounted for 52% of total C. Nearly identical percentages have been reported from work at other experiments at the Alifsol (KBS) site. For example, Paul et al. (1999) found that the passive pool was 56% and 53% of the total C pool for other KBS corn and never-tilled systems, respectively. In contrast, at our Mollisol site the passive pool on average accounted for 69% of total soil C. Thus, while C accumulation in the active C pool is occurring more quickly under diverse perennials at our Alfisol site, our Mollisol soils are more effective at stabilizing C overall, corroborated by the lower amount of C that is respired per gram of total C (Fig. 2) as well as by Tiemann and Grandy's (2015) findings that the soil C accruing at the Alfisol site is found in more easily degradable pools of C compared to soil C at our Mollisol site.

4.4. Management implications

Our results demonstrate that diverse perennial cropping systems could be used to quickly increase the active fraction of surface soil C in moderate fertility soils, a finding that has several management implications. First, because less fertile soils are further from C saturation, the accumulation of active C and eventually other C pools will provide marginal soils an additional climate benefit when hosting diverse perennial biomass crops as compared to more fertile high C soils because C change plays a major role in the net C balance of biofuel cropping systems (Fargione et al., 2008; Gelfand et al., 2011). This distinction is important and provides further reason to favor marginal over arable lands for growing cellulosic biofuels (Robertson et al., 2017).

Second, our results show greater active C gain with perennial polyculture crops than with perennial monoculture crops, suggesting a higher soil fertility and C sequestration capacity for polycultures. While we do not know from these results how much diversity is necessary to provide an active C advantage, it is clear that some degree of polyculture is as beneficial for cropping systems as it is for natural systems (e.g., Fornara and Tilman, 2008). At our Alfisol site switchgrass and miscanthus monocultures accumulated active C at rates that were only about half of those in the mixed-species stands of restored prairie, early successional, native grasses, or hybrid poplar stands. At our Mollisol site restored prairie resulted in substantial active C gain. At both sites slow C pools accumulated more quickly in the diverse poplar and early successional sites relative to other systems. This polyculture advantage has implications for the design of biofuel systems, conservation set-aside programs, and soil restoration efforts.

Finally, our results emphasize the need to examine biofuel cropping system effects on soil C in contrasting soils (Tiemann and Grandy, 2015). Inherent differences such as soil type, texture and fertility will influence the rate at which soil C accumulates and whether accumulation occurs in labile or more stable C pools. Thus, if we are to accurately determine soil C sequestration potential under biofuel cropping systems, comparative experiments on different soil types should be the norm rather than the exception.

5. Conclusions

We examined surface horizon active, slow, and passive C pools under eight different biofuel cropping systems in contrasting soils five years post-establishment. Overall, we found that soil C accumulation trends were site specific, where accumulation in the active C pool under perennials relative to annuals occurred more quickly at the moderate fertility Alfisol site compared to the high fertility Mollisol site. Slower accumulation in the active C pool at the high fertility site is likely a result of high C and clay contents and smaller aggregates. In addition, a greater proportion of soil C at the Mollisol site was found in the slow and passive pools, which is a sign that soils may be approaching C saturation. In the moderate fertility site perennial polycultures accumulated up to 2.5 times more active C than did annual and monoculture perennial crops, highlighting the importance of plant diversity for enhancing soil C accrual. Perennial monocultures accumulated active C at rates similar to no-till continuous corn. Ultimately, results demonstrate that diverse perennial cropping systems have the ability to rapidly and significantly increase active C at lower fertility sites. That we saw variable soil C accrual trends at contrasting sites also highlights the importance of comparative cropping system experiments on different

soil types to fully understand soil C sequestration potential under a variety of biofuel cropping systems.

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Appendix A. Supplementary data

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Supplemental Information

Diverse perennial bioenergy crops on an Alfisol build active soil carbon faster than monoculture crops

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Figure S1. Observed C mineralization (µg C g^{-1} soil d^{-1}) over the 322-day incubation period for the four annual biofuel cropping systems at the high fertility Mollisol (ARL, A–D) and moderate fertility Alfisol (KBS, E–H) sites. Curves represent goodness of fit and were calculated using output from the three pool model: $C_t = k_a (C_1 * e^{-ka*day}) + k_s (C_2 * e^{-ks*day}) + k_r (C_3 * e^{-kr*day})$. Points are color coded by replicate blocks.

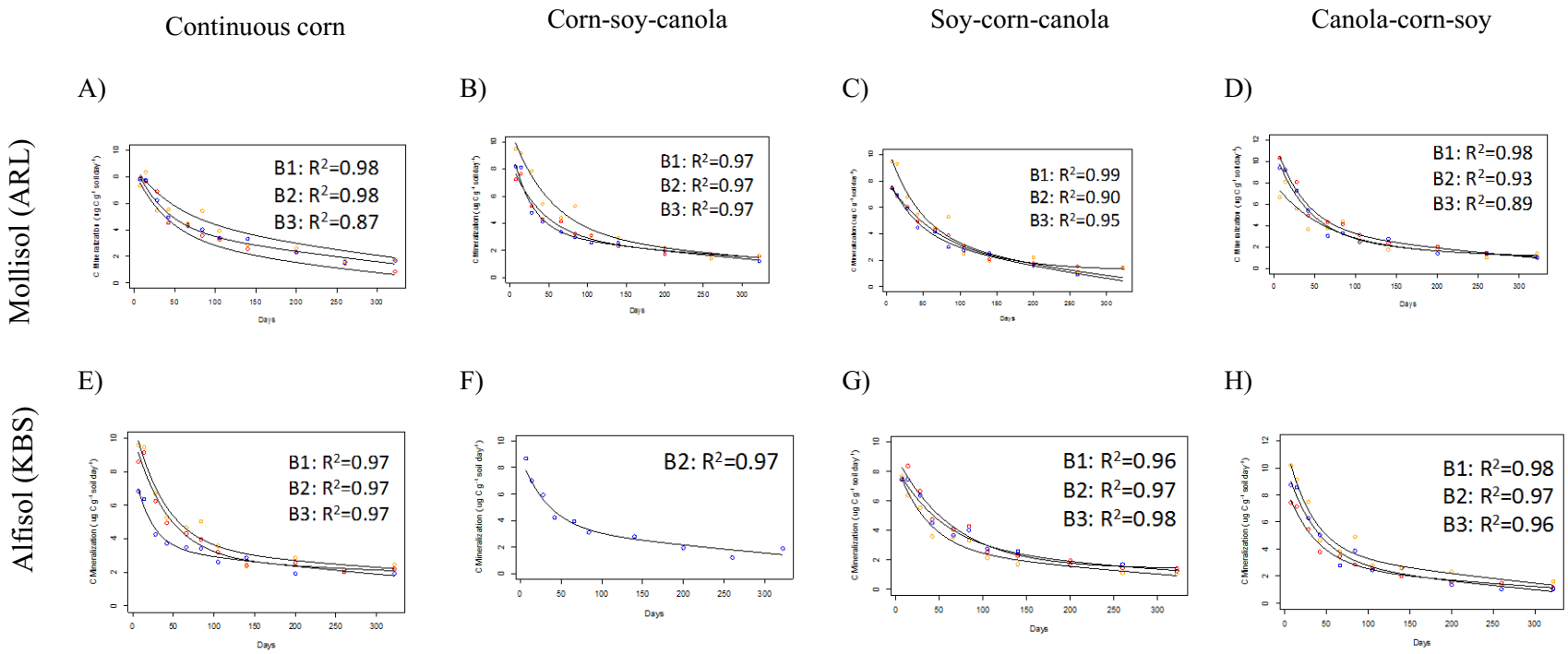

Figure S2. Observed C mineralization (μg C g^{-1} soil d^{-1}) over the 322-day incubation period for the two perennial monoculture cropping systems at the high fertility Molliosl (ARL, A–B) and moderate fertility Alfisol (KBS, C–D) sites. Curves represent goodness of fit and were calculated using output from the three pool model: $C_t = k_a (C_1 * e^{-ka*day}) + k_s (C_2 * e^{-ks*day}) + k_r (C_3 * e^{-kr*day})$. Points are color coded by replicate blocks.

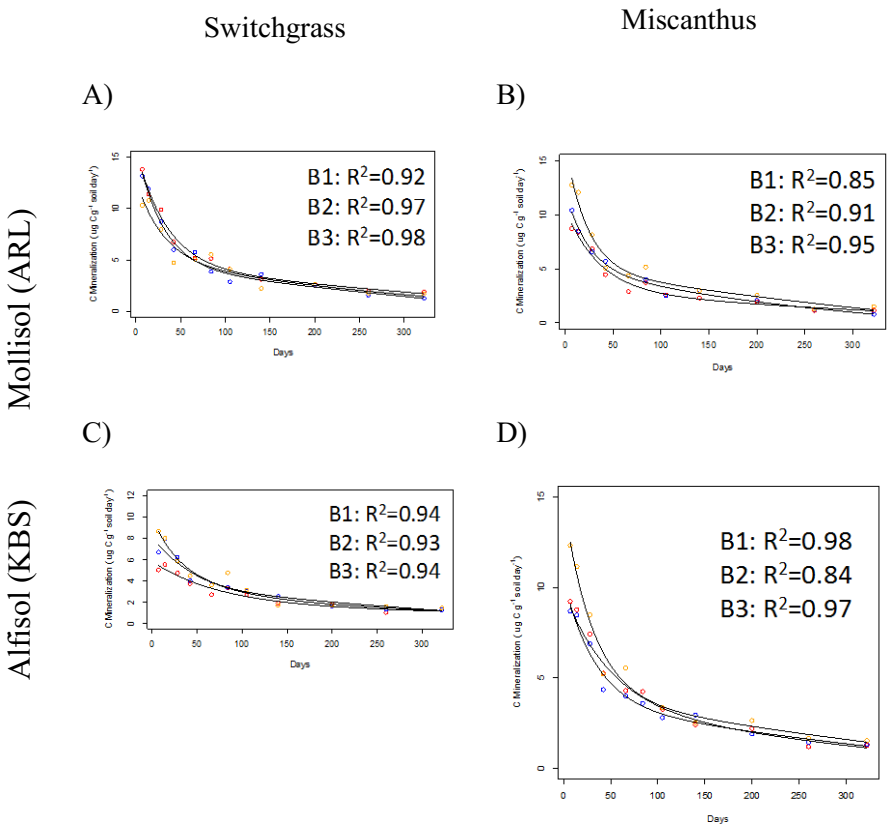

Figure S3. Observed C mineralization (µg C g^{-1} soil d^{-1}) over the 322-day incubation period for the four perennial diverse biofuel cropping systems at the high fertility Mollisol (ARL, A–D) and moderate fertility Alfisol (KBS, E–H) sites. Curves represent goodness of fit and were calculated using output from the three pool model: $C_t = k_a (C_1 * e^{-ka*day}) + k_s (C_2 * e^{-ks*day}) + k_r (C_3 * e^{-kr*day})$. Points are color coded by replicate blocks.

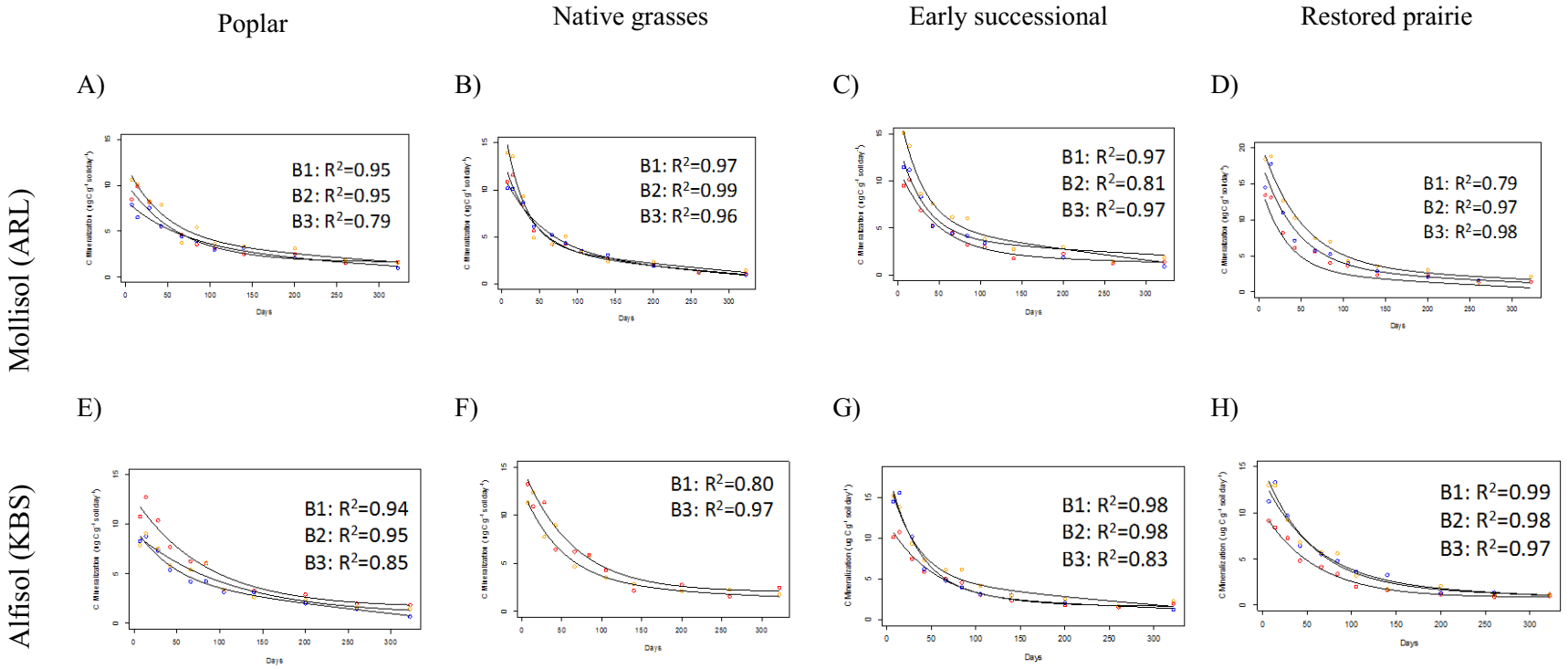

Figure S4. Predicted Mean C mineralization (µg C g^{-1} soil d^{-1}) over the 322 day incubation period (regression of means represented by blue line) for the four annual biofuel cropping systems at the high fertility Mollisol (ARL, A–D) and moderate fertility Alfisol (KBS, E–H) sites. Shaded bands represent standard error from the mean. Each point represents the average value from the corresponding figures in Fig. S1.

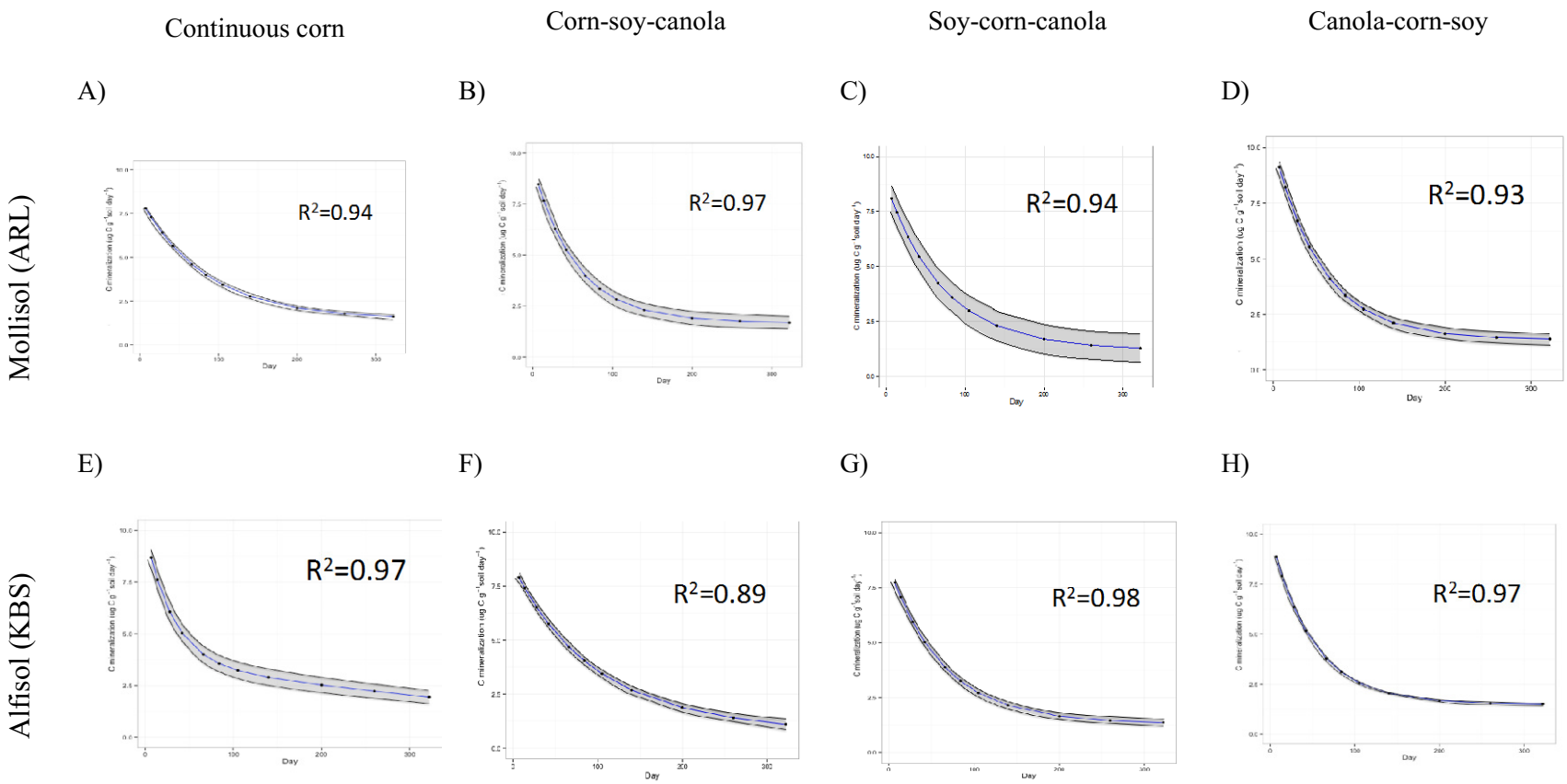

Figure S5. Predicted Mean C mineralization (µg C g^{-1} soil d^{-1}) over the 322 day incubation period (regression of means represented by blue line) for the two perennial monoculture cropping systems at the high fertility Molliosl (ARL, A–B) and moderate fertility Alfisol (KBS, C–D) sites. Shaded bands represent standard error from the mean. Each point represents the average value from the corresponding figures in Fig. S2.

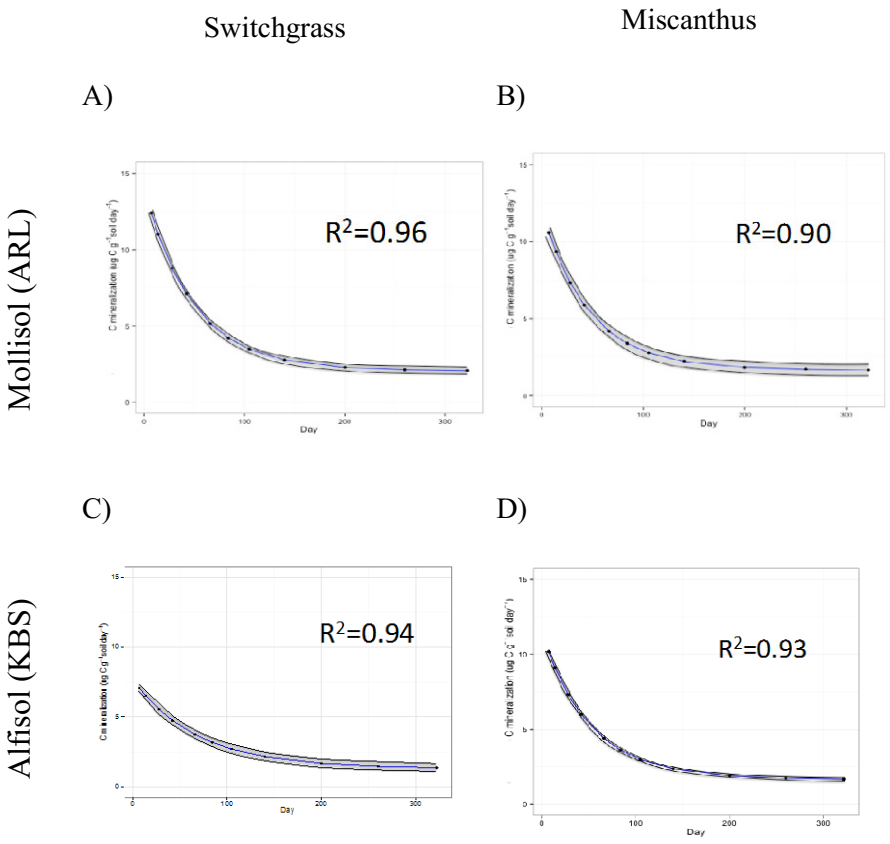

Figure S6. Predicted Mean C mineralization (µg C g^{-1} soil d^{-1}) over the 322 day incubation period (regression of means represented by blue line) for the four perennial diverse biofuel cropping systems at the high fertility Mollisol (ARL, A–D) and moderate fertility Alfisol (KBS, E–H) sites. Shaded bands represent standard error from the mean. Each point represents the average value from the corresponding figures in Fig. S3.

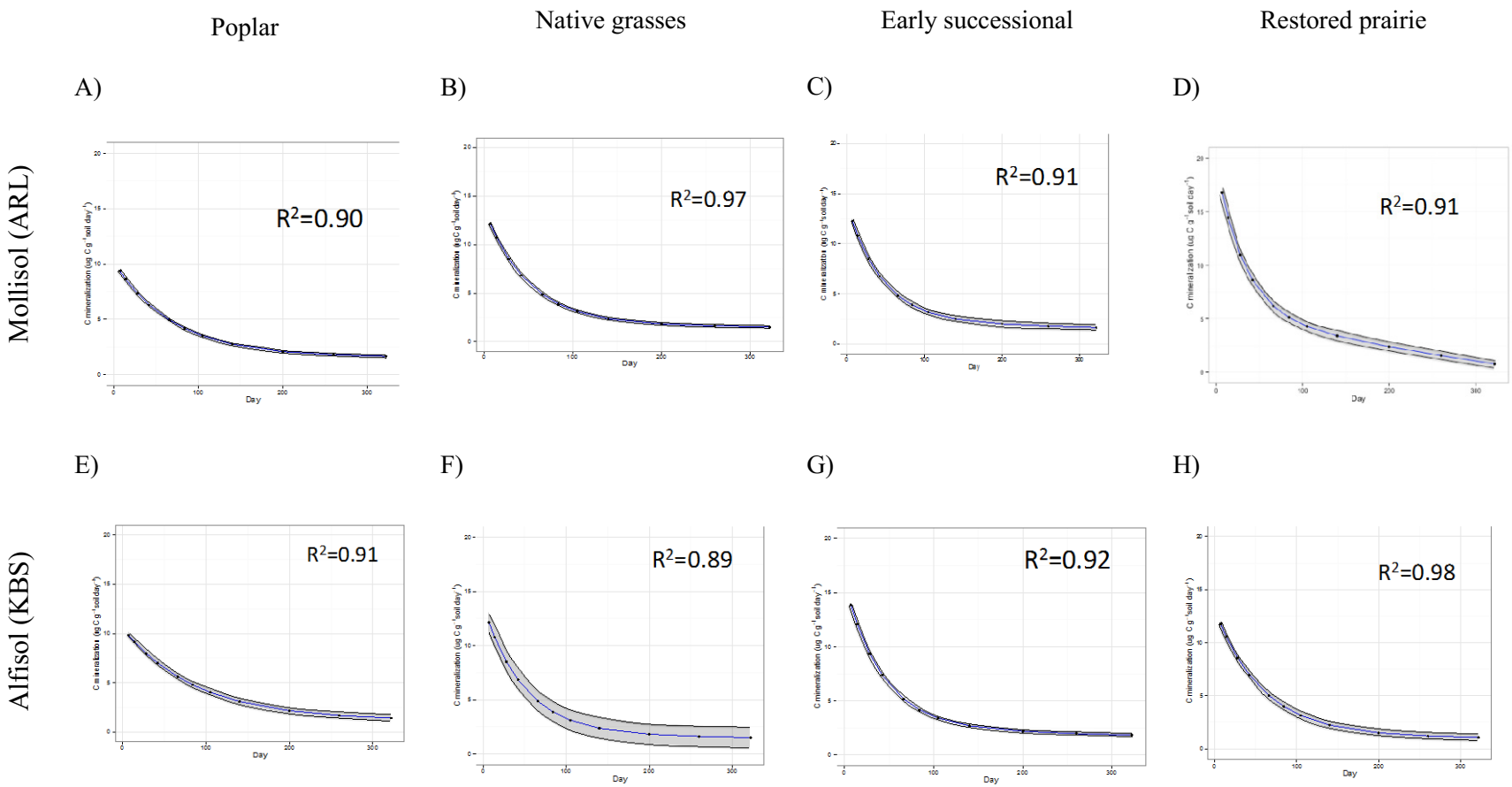

Table S1. Nonlinear model fit equations to regressions of mean measured C mineralization (μg C g^{-1} soil d^{-1}) over the 322 day incubation period for each of the ten biofuel cropping systems at the high fertility Mollisol (ARL) and moderate fertility Alfisol (KBS) sites.

Biofuel cropping system	Model fit to Equation $C_t = k_a (C_1 * e^{-ka*day}) + k_s (C_2 * e^{-ks*day}) + k_r (C_3 * e^{-kr*day})$
Annual	
Continuous corn	
Mollisol (ARL)	$C_t = 0.02\ (185.9 * e^{-0.02*day}) + 0.001\ (6176.6 * e^{-0.001*day}) + 0.0000083\ (15050 * e^{-0.0000083*day})$
Alfisol (KBS)	$C_t = 0.03\ (236.6 * e^{-0.03*day}) + 0.0009 (4779.2 * e^{-0.0009*day}) + 0.0000083\ (6695.8 * e^{-0.0000083*day})$
Corn-soy-canola	
Mollisol (ARL)	$C_t = 0.02\ (262 * e^{-0.02*day}) + 0.001\ (3916 * e^{-0.001*day}) + 0.0000083\ (15050 * e^{-0.0000083*day})$
Alfisol (KBS)	$C_t = 0.03\ (180.5 * e^{-0.03*day}) + 0.0003 (7486 * e^{-0.0003*day}) + 0.0000083\ (6695.8 * e^{-0.0000083*day})$
Soy-corn-canola	
Mollisol (ARL)	$C_t = 0.02\ (334.6 * e^{-0.02*day}) + 0.0008 (5136.3 * e^{-0.0008*day}) + 0.0000083\ (15050 * e^{-0.00000823*day})$
Alfisol (KBS)	$C_t = 0.02\ (339.5 * e^{-0.02*day}) + 0.0007 (4906.5 * e^{-0.0007*day}) + 0.0000083\ (6695.8 * e^{-0.00000823*day})$
Canola-corn-soy	
Mollisol (ARL)	$C_t = 0.02\ (374.9 * e^{-0.02*day}) + 0.0009 (5981 * e^{-0.0009*day}) + 0.0000083\ (15050 * e^{-0.00000823*day})$
Alfisol (KBS)	$C_t = 0.03\ (247.1 * e^{-0.03*day}) + 0.001 (5768.4 * e^{-0.001*day}) + 0.0000083\ (6695.8 * e^{-0.00000823*day})$
Perennial monoculture	
Switchgrass	
Mollisol (ARL)	$C_t = 0.03\ (322.6 * e^{-0.03*day}) + 0.001 (7026 * e^{-0.001*day}) + 0.0000083\ (15050 * e^{-0.00000823*day})$
Alfisol (KBS)	$C_t = 0.03\ (274.3 * e^{-0.03*day}) + 0.0009 (5015.2 * e^{-0.0009*day}) + 0.0000083\ (6695.8 * e^{-0.00000823*day})$
Miscanthus	

Mollisol (ARL) $C_t = 0.04(232.7*e^{-0.04*day}) +0.002(3383*e^{-0.002*day}) + 0.0000083\ (15050*e^{-0.00000823*day})$

Alfisol (KBS) $C_t = 0.03\ (299.3*e^{-0.03*day}) +0.001(5077.5*e^{-0.001*day}) + 0.0000083\ (6695.8*e^{-0.00000823*day})$

Perennial diverse

Poplar

Mollisol (ARL) $C_t = 0.02\ (423.4*e^{-0.02*day}) +0.0004(12425.7*e^{-0.0004*day}) + 0.0000083\ (15050*e^{-0.00000823*day})$

Alfisol (KBS) $C_t = 0.01\ (695.7*e^{-0.01*day}) +0.0003(9125.9*e^{-0.0003*day}) + 0.0000083\ (6695.8*e^{-0.00000823*day})$

Native grasses

Mollisol (ARL) $C_t = 0.03\ (630.7*e^{-0.03*day}) +0.0009(5387.4*e^{-0.0009*day}) + 0.0000083\ (15050*e^{-0.00000823*day})$

Alfisol (KBS) $C_t = 0.02\ (656*e^{-0.02*day}) +0.0006(6458.3*e^{-0.0006*day}) + 0.0000083\ (6695.8*e^{-0.00000823*day})$

Early successional

Mollisol (ARL) $C_t = 0.02\ (322.6*e^{-0.02*day}) +0.0008(11414*e^{-0.0008*day}) + 0.0000083\ (15050*e^{-0.00000823*day})$

Alfisol (KBS) $C_t = 0.03\ (500.8*e^{-0.03*day}) +0.0007(6904*e^{-0.0007*day}) + 0.0000083\ (6695.8*e^{-0.00000823*day})$

Restored prairie

Mollisol (ARL) $C_t = 0.03\ (630.7*e^{-0.03*day}) +0.0009(7268*e^{-0.0009*day}) + 0.0000083\ (15050*\ e^{-0.00000823day})$

Alfisol (KBS) $C_t = 0.03\ (638.2*e^{-0.03*day}) +0.0007(4715*e^{-0.0007*day}) + 0.0000083\ (6695.8*\ e^{-0.00000823*day})$

Table S2. Absolute values for active C, slow C, passive C, and total C for eight biofuel cropping systems at the high fertility Mollisol (ARL) site. Total C equals the sum of Active C, Slow C, and Passive C pools.

System	Active C (mg C g^{-1} soil)	Slow C (mg C g^{-1} soil)	Passive C (mg C g^{-1} soil)	Total C (mg C g^{-1} soil)
Continuous Corn	0.19 (0)^{c}	6.18 (1.8)^{b}	15.05*	21.4 (1.8)
Rotational Corn				
Corn-Soybean-Canola	0.26 (0.07)^{bc}	3.92 (0.9)^{c}	15.05*	19.2 (1.0)
Soybean-Corn-Canola	0.33 (0.1)^{bc}	5.13 (1.0)^{bc}	15.05*	20.5 (1.0)
Canola-Corn-Soybean	0.35 (0.02)^{bc}	5.98 (1.3)^{bc}	15.05*	21.4 (1.3)
Switchgrass	0.32 (0.04)^{bc}	7.03 (2.0)^{b}	15.05*	22.4 (2.0)
Miscanthus	0.23 (0.03)^{bc}	3.38 (1.0)^{c}	15.05*	18.7 (1.0)
Poplar	0.42 (0.06)^{b}	12.42 (1.8)^{a}	15.05*	27.9 (1.9)
Native Grasses	0.40 (0.02)^{b}	5.39 (0.3)^{c}	15.05*	20.8 (0.4)
Early Successional	0.37 (0.03)^{bc}	11.42 (0.7)^{a}	15.05*	26.8 (0.8)
Restored Prairie	0.63 (0.13)^{a}	7.27 (0.3)^{b}	15.05*	23.0 (0.4)

*Passive C values did not differ by treatment, thus only one value was used in the three-pool model per site.

Table S3. Absolute values for active C, slow C, passive C, and total C for eight biofuel cropping systems at the moderate fertility Alfisol (KBS) site. Total C is the sum of Active C, Slow C, and Passive C pools.

System	Active C (mg C g^{-1} soil)	Slow C (mg C g^{-1} soil)	Passive C (mg C g^{-1} soil)	Total C (mg C g^{-1} soil)
Continuous Corn	0.24 (0.07)[b]	4.78 (0.4)[b]	6.70[*]	11.71 (0.4)
Rotational Corn				
Corn-Soybean-Canola	0.19[t]	7.49[t]	6.70[*]	14.4[t]
Soybean-Corn-Canola	0.34 (0.09)[b]	4.90 (0.5)[b]	6.70[*]	11.9 (0.6)
Canola-Corn-Soybean	0.25 (0.01)[b]	5.77 (0.5)[b]	6.70[*]	12.7 (0.5)
Switchgrass	0.28 (0.03)[b]	5.02 (0.9)[b]	6.70[*]	12.0 (0.9)
Miscanthus	0.3 (0.03)[b]	5.08 (0.9)[b]	6.70[*]	12.1 (0.8)
Poplar	0.7 (0.22)[a]	9.13 (2.1)[a]	6.70[*]	16.5 (2.1)
Native Grasses	0.48 (0.19)[a]	6.46 (0.5)[ab]	6.70[*]	13.6 (0.5)
Early Successional	0.50 (0.06)[a]	6.90 (0.5)[ab]	6.70[*]	14.1 (0.5)
Restored Prairie	0.64 (0.05)[a]	4.72 (0.74)[b]	6.70[*]	12.1 (0.5)

*Passive C values did not differ by treatment, thus only one value was used in the three-pool model per site.
t represents (n=1).