Soil microbial respiration and carbon turnover under perennial and annual biofuel crops in two agricultural soils

By

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1. Abstract

Bioenergy crops have the potential to provide a low carbon-intensive alternative to fossil fuels. More than a century of agricultural research has shown that conventional cropping systems can reduce soil organic matter (SOM) reservoirs, resulting in long-term soil nutrient loss and carbon (C) release to the atmosphere. In the face of climate change and other human disruptions to biogeochemical cycles, identifying biofuel crops that can maintain or enhance soil resources is desirable for the sustainable production of bioenergy. The objective of my research was to compare the effects of four biofuel crop treatments on SOM dynamics in two agricultural soils: Mollisols at Arlington Agricultural Research Station (ARL) in Wisconsin and Alfisols at Kellogg Biological Station (KBS) in Michigan, USA. I used fresh soils collected in 2013 and archived soils collected in 2008 to measure differences among biofuel crops after 5 years of management. Archived soils collected at the time of field treatment establishment provide the opportunity to understand the effects of agricultural management on soil C dynamics over time. Using a one-year-long laboratory soil incubation coupled with a regression model, I separated soils into three SOM pools and their corresponding turnover times. I found that the total amount of soil C respiration in surface soils differed among biofuel crop types and was positively correlated with root biomass. Total soil C respiration increased in the following order: mixed species perennials > monoculture perennials > monoculture annuals. The distribution of C among SOM fractions varied between the two soil types, with greater C content associated with the active fraction in the coarser textured-Alfisol and greater C content associated with the slow-cycling fraction in the Mollisols with high clay content. The active pool (C_a), or biologically

available C, was more sensitive to cropping treatment in soils with higher clay content and decreased at both sites from 2008 to 2013. Changes in the C_s, which is theorized to represent SOM that is not rapidly mineralized by microbes, most likely because of occlusion within soil aggregates or absorption to soil particles, indicated an opposite trend, with greater sensitivity in soils with greater sand content. From 2008 to 2013 the change in the C_s among biofuel crops differed, with gains in the C_s in the native prairie and poplar soils and losses in the C_s in the switchgrass and corn soils. Differences in the response of soil C pools to biofuel treatments between our sites suggest that the C sequestration potential of bioenergy crops may differ depending on what crops are grown and on what soil type. Bioenergy crop land-use change affects soil C dynamics, with implications for assessing C costs associated with biofuel production. Monitoring bioavailable C pools may provide an earlier indicator of change in response to agricultural management than bulk soil C stocks.

2. Introduction

The global production of bioenergy crops has increased five-fold from 2000 to 2010 in response to policies targeting non-fossil energy sources as a means to mitigate rising carbon (C) concentrations in the atmosphere (Davis et al. 2011; Anderson-Teixeira et al. 2013; Warner et al. 2013). More than a century of agricultural research has shown that conventional cropping systems typically reduce C stored in soil organic matter (SOM) reservoirs (Lal et al. 1998; Kucharik et al. 2001; Don et al. 2011). Conversion of forests and grasslands to agricultural use and cultivation of croplands is the second largest source of anthropogenic C emissions after fossil fuel combustion and industrial

sources (Houghton et al. 2012). With increased demand for biofuels, and the conversion of existing and new agricultural lands to bioenergy crops, identifying crops that can maintain or enhance soil resources is desirable for the sustainable production of bioenergy (Anderson-Teixeira et al. 2009).

In agricultural systems where aboveground biomass is harvested, the fate of more long-lived C pools in soil becomes important for determining ecosystem C balance. Crop effects on belowground C dynamics vary with plant species and management (Tilman et al. 2007; Lange et al. 2015; Tiemann et al. 2015). Crop selection and rotation practices influence the quantity and chemistry of C inputs to the soil via leaf litter and roots (Neff and Hooper 2002; Don et al. 2011). Carbon allocation to either aboveground production of shoots or belowground roots, differ between annual and perennial cropping systems (Bolinder et al. 2002). Conversion of annual agricultural crops to perennials crops generally results in soil C gain attributed to increased root-associated C (Lemus and Lal 2005; Anderson-Teixeira et al. 2013; Teimann and Grandy 2014). Agricultural management has been shown to influence C dynamics and several cropping systems have shown the potential to increase soil C stocks (West and Post 2002; Jokela et al. 2011; Stockmann et al. 2013). Soils' capacity to store C is influenced by abiotic factors including climate and soil texture (Kleber et al. 2007; Sierra et al. 2011), thus crops may respond differently across geographic regions.

The response of soil C stocks to changes in plant inputs and agricultural management is often confounded by the size and heterogeneity of the 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 biochemical and physical mechanisms

that protect organic matter from decomposers (Collins et al. 2000, Sierra et al. 2011, Torn et al. 2013). A number of approaches have been developed to separate soil C into functionally distinct pools based on chemical structure, size, bioavailability, or location in the soil matrix, these include aggregate, density, and biological fractionation. These fractionation methods have revealed changes in soil C dynamics by isolating SOM pools that are more sensitive than the bulk soil C stock (Christensen 2001; Marin-Spiotta et al. 2008, 2009; Wagai et al. 2009; Schrumpf et al. 2013).

In the biological fractionation method, modeling of changes in microbial respiration rates over a long-term incubation are used to isolate soil into two pools, the active pool and the slow pool (Paul 2001b). The active pool 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 slow pool cycles on a decadal time scale and is theorized to represent SOM 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). A third pool, the resistant pool, isolated by acid hydrolysis, is used to constrain the model and represent that C which is biochemically inaccessible to decomposers and has longer turnover times than can be captured during laboratory incubations. This three-pool SOM model is the basis for many biogeochemical models used to predict the effects of climate and land-use change on terrestrial C dynamics (Parton et al. 1993; Sierra et al. 2012). Whereas recent literature raises questions about the ecological function of the acid-resistant pool (Paul et al. 2006; Kleber and Johnson 2010), biological fractionation through lab incubations is well suited for measuring differences in the size of the C pool

readily available for microbial decomposition. This bioavailable C which may be a better indication of management effects on annual to decadal time scales than the bulk soil C stock (Collins et al. 2000; Paul et al. 2001a; Conant et al. 2011).

This study was designed to understand the effects of different biofuel cropping systems, in particular annual and perennial crops, single and multiple species crops, on SOM pools. I asked the following research questions: **1) What are the effects of biofuel crop treatments on soil organic C dynamics? 2) How do different crop treatments affect C losses by microbial respiration? 3) Do these treatment effects vary by soil type?** I hypothesized that **1)** ***the size and contribution of the active pool to total soil C would differ among crop treatments due to differences in root biomass inputs; 2) microbial respiration would be greater under treatments with greater root inputs and greater microbial biomass; 3) and that biofuel crops grown on different soils would accumulate C in pools with different turnover rates, which may affect the long-term trajectory of C between sites. Specifically, I hypothesized that soils with coarser texture would accumulate C in the active pool, whereas soils with greater clay content would have more C in pools with slower turnover times due to greater stabilization in soil aggregates and minerals with greater surface area.***

My research takes advantage of the Great Lakes Bioenergy Research Center biofuel cropping field trials established on fine-textured Mollisols at Arlington Research Station in Wisconsin and on coarse-textured Alfisols at Kellogg Biological Station in Michigan. I used a year-long incubation of soils collected at the start of the field experiments and 5 years later to test for the effects of different crops on soil microbial respiration rates and applied a biological fractionation approach to isolate SOM pools with different turnover

times. Understanding how belowground C dynamics vary among biofuel crops and are influenced by abiotic factors will provide a more comprehensive assessment of the C costs of biofuel production.

3. Methods

3.1 Site Description

The Great Lakes Bioenergy Research Center's (GLBRC)'s biofuel cropping system experiment (BCSE) was established in 2008 at the University of Wisconsin - Madison's Arlington Agricultural Research Station (ARL) in Arlington, Wisconsin (43°18'N, 89°21'W) and at Michigan State University's Kellogg Biological Station (KBS) in Hickory Corners, Michigan (42°24'N, 85°24'W). The soils at ARS are Mollisols in the soil series Plano silt-loam (Fine-silty, Mixed, Superactive, Mesic Typic Argiudolls) and are characterized by a dark A horizon, which is rich in organic matter. Historically these soils formed under tall grass prairies on post-glacial loess deposits (Sanford and Kucharik 2013). The soils at KBS are Alfisols in the soil series Kalamazoo loam (Fine-Loamy, Mixed, Semiactive, Mesic Typic Hapludalfs) (Paul et al. 1999). Soil characteristics and nutrient concentrations differ at the two sites (Table 1) and by depth and crop treatment (Table 2).

The GLBRC's BCSEs in Wisconsin (Figure 1) and Michigan (Figure 2) were established following a randomized complete block design. The sites are comprised of five replicate blocks, each containing one 27.4 by 42.7 m plot of each of ten cropping systems. The research presented here is focused on three replicate blocks under the

following four cropping systems: continuous corn, switchgrass, poplar, and native prairie at KBS and ARL.

Prior to establishment of the BCSE, blocks B1, B2, and B3 were under the same land use. At ARL, the three blocks had the same land use history, corn-soybean annual rotation, since 2000, and were converted to a mix of alfalfa and orchard grass in 2005. At KBS, the sites were under the same management since 1989 and were seeded in alfalfa from 2002 to 2007 (Roley, S., personal communication). There is no remaining alfalfa cover at KBS; however, north-south alleyways remain in an alfalfa/orchard grass cover at ARL. In preparation for the establishment of the BCSE treatments, blocks B1, B2, and B3 were tilled in the spring of 2008 to remove previous crop residues. After successful establishment of field treatments, no-till agricultural practices were implemented and none of the treatments have been tilled since site preparation.

All crop treatments were established between May and June of 2008. Plant hybrids were selected to maximize productivity and mirror local farming practices. At both sites, all hybrids planted, with the exception of corn, were the same. Corn hybrids differed between the two sites due to varying needs of advanced traits (e.g., herbicide and insect resistance) and differences in the length of the growing season. Corn plots at KBS were annually seeded with Dekalb52-59. Corn plots at ARL were planted with Dekalb52-59 in 2008, 2010, and 2011, and with Pioneer 35F40 in 2009 and 2012, and with FS53TV4 in 2013. Switchgrass, *Panicum virgatum L.*, plots were seeded with the cultivar “cave-in-rock”. Poplar plots were hand planted with NM-6 hybrid poplar whips, *Populus nigra* x *Populus maximowiczii* (Simmons and Sanford 2015). The native prairie plots were planted with a mixture of six grasses, three leguminous forbs, andnine non-

leguminous forbs including big bluestem (*Andropogon gerardii*), Canada wild rye (*Elymus canadensis*), Indiangrass (*Sorghastrum nutans*), Junegrass (*Koeleria cristata*), little bluestem (*Schizachyrium scoparium*), switchgrass (*Panicum virgatum*, "Southlow"), roundhead bushclover (*Lespedeza capitata* Michx.), showy tick-trefoil (*Desmodium canadense*), white wild indigo (*Baptisia leucantha*), black-eyed susan (*Rudbeckia hirta*), butterfly milkweed (*Asclepias tuberosa*), cup plant (*Silphium perfoliatum*), Canadian meadow anemone (*Aneomone canadensis*), New England aster (*Symphyotrichum novae-angliae*), pinnate prairie coneflower (*Ratibida pinnata*), showy goldenrod (*Solidago speciosa*), stiff goldenrod (*Solidago rigida*), and wild bergamot (*Monarda fistulosa*) (Sanford et al. *in review*).

A region-specific crop management philosophy 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 (as outlined by MSU and UW extension) and climate considerations. Fertilizer type and amounts applied to each cropping system were based on target biomass yield goals. Corn plots received annual applications of potash, diammonium phosphate, or liquid nitrogen, as determined by yearly soil testing of nitrogen, phosphorus, and potassium. The perennial systems, not including poplar, received fertilizer treatments of ammonium nitrate starting in 2010 once the perennial target crops had been established. Poplars received a single ammonium-nitrate fertilizer application in 2010 (Sanford et al. *in review*).

Similar to fertilizer treatments, herbicide applications varied by site, treatment and year, based on local agronomic practices. Herbicide treatments included a burn down, where all weeds are killed before planting of crops; a pre-emergent application, where

weeds are killed at approximately the same time as planting; and a post-emergent application, where specific weeds, usually broadleaves or grasses, are targeted and killed, usually after the crops have been planted but prior to rapid aboveground biomass production. All plots at both field sites received a burn down herbicide treatment prior to BCSE establishment. The continuous corn treatments received an annual post-emergent herbicide application and burn down and if needed an annual pre-emergent herbicide application. The perennial systems received an herbicide treatment on an as needed basis, depending upon the weed assemblage present (Simmons and Sanford 2015).

3.2 Experimental Design, Soil Collection, and Archive Protocol

I analyzed soils collected from two depths (0-10 and 25-50 cm) from three replicate blocks (B1, B2, and B3) under four cropping treatments (continuous corn, switchgrass, poplar, and native prairie), at two sites (ARL and KBS) over two time points (2008 and 2013), yielding a total of 96 soil samples. I also collected and analyzed soils from the alleyways along the three blocks of the Arlington sites, which were still seeded in alfalfa/orchard grass as a pre-treatment control. The alleyways have remained in alfalfa/orchard grass cover since 2005 and because none of the plots since conversion to the BCSE remained in alfalfa/ orchard grass cover, the alleyways provide the best control available. This resulted in a total of 102 soil samples.

Three sampling stations for the collection of soils were established throughout the plots in a stratified, quadrat sampling design. Soil samples were collected from these sampling stations and homogenized by plot for blocks 1, 2, and 3. Soil cores in 2008 were collected by a field crew in the summer at KBS and in the summer/fall at ARS and

archived for future use. In 2013, soil cores were collected in the late fall at both sites. A Giddings (model #15-TS GSRTS, Giddings Machine Company; Windsor, CO) soil probe equipped with a 48" (121.92 cm) long, 3.5" (8.89 cm) diameter probe was used to collect samples at ARL and a Geoprobe (model 540MT, Geoprobe Systems; Salina, Kansas) with a smaller diameter of 3" (7.62 cm) was used at KBS. At both field sites, soils were collected via a probe with a plastic sleeve insert. Soil cores were refrigerated until processed for bulk density and then air dried. After drying, soils were sieved to 2 mm and visible roots and plant material were removed by hand. Soils collected in 2008 were oven-dried between 50 and 60 °C and stored in glass jars in a cool, dry facility between 2008 and the time of this study. Following processing, soils collected in 2013 were air-dried prior to analysis.

3.3 Soil Carbon and Nitrogen Stocks

To determine soil bulk density, refrigerated cores were cut at specific intervals, 0-10, 10-25, 25-50, and 50-100 cm. Soils were oven dried at 105 °C for 24 hours to determine soil moisture and cylinder core volumes were used to determine bulk density.

For elemental analysis, subsamples of the 2008 and 2013 soils were finely ground using a SpexMill-8000D (SPEX SamplePrep; Metuchen, NJ) ball grinder and analyzed on a Flash 2000 carbon and nitrogen combustion elemental analyzer (CE Elantech, Lakewood, NJ) at the University of Wisconsin - Madison. All samples were run in duplicate with < 10 % replicate error for total C and <15 % for total N and aspartic acid as a standard. No presence of inorganic C was detected by an acid effervescence test,

hence total C represents organic C. Soil C concentrations (%) were converted to stocks using bulk density and measurement depth.

Management practices and biotic factors may result in changes to bulk density, which could mask changes in soil C stocks (Don et al. 2011; Ellert and Bettany 1995). To compare soil C stocks over time and between treatments, 2013 values were corrected using an equation modified for equivalent soil mass comparison from Ellert and Bettany (1995). The equivalent soil mass (ESM) for the 2008 samples and the soil mass of the 2013 samples were determined following Equations 1and 2, respectively:

$$ESM_{2008} = bd_{2008} \times vol_{2008} \quad \text{(Equation 1)}$$

where: ESM_{2008} = equivalent soil mass of the 2008 sample (g), bd_{2008} = bulk density of 2008 sample (g cm^{-3}), and vol_{2008} = lower depth of soil collection - upper depth of soil collection in 2008 (cm^3).

$$SM_{2013} = bd_{2013} \times d_{2013} \quad \text{(Equation 2)}$$

where: SM_{2013} = soil mass of the 2013 sample (g), bd_{2013} = bulk density of 2013 sample (g cm^{-3}), and vol_{2013} = lower depth of soil collection – upper depth of soil collection in 2013 (cm^3).

These two values were then used to calculate V_{excess}; this is the volume that is subtracted from the 2013 soil collection volume (Equation 3) to calculate a corrected soil volume to adjust the 2013 soil C or N stocks:

$$V_{excess} = (ESM_{2008} - SM_{2013})/ bd_{2013} \quad \text{(Equation 3)}$$

where: ESM_{2008} = equivalent soil mass of the 2008 sample (g), SM_{2013} = soil mass of the 2013 sample (g), bd_{2013} = bulk density of the 2013 sample (g cm^{-3})

This volume, V_{excess}, was then subtracted from the 2013 collection volume for each sample to calculate an adjusted volume (Equation 4).

$$V_{corrected} = (vol_{2013} - V_{excess}) \quad \text{(Equation 4)}$$

Where: vol_{2013} = lower depth of soil collection – upper depth of soil collection in 2013 (cm^3), V_{excess} = ESM corrected volume difference between 2008 and 2013 sample (cm^3)

This new volume, $V_{corrected}$, was then used in place of the 2013 collection volume to calculate total C stocks (Equation 5) and total nitrogen stocks (Equation 6). These corrected stocks were used to determine the effect of year and treatment.

$$\text{2013 ESM Corrected C Stock} = \text{pct } C_{2013} \times bd_{2013} \times (V_{corrected}) \quad \text{(Equation 5)}$$

$$\text{2013 ESM Corrected N Stock} = \text{pct } N_{2013} \times bd_{2013} \times (V_{corrected}) \quad \text{(Equation 6)}$$

Where: pct C_{2013} = percent C of sample in 2013 (%), pct N_{2013} = percent nitrogen of sample in 2013 (%), bd_{2013} = bulk density of sample in 2013(g cm^{-3}), $V_{corrected}$ = 2013 corrected volume (cm^3)

3.4 Soil Microbial Respiration

To understand how different crop treatments affect microbial respiration, I performed a 365-day laboratory incubation to measure CO_2 production from the 102 unique soil samples collected from the two depths, two sites, two years, four treatments and three replicate blocks, plus the alfalfa alleyways. All soils were air dried and sieved to 2 mm as described above and split into four soil incubation replicates. For each lab replicate, 25 g of soil was placed into a specimen cup and DI water was added until the soils reached 60 % water filled pore space, which has been determined to be optimal for microbial activity (Linn and Duran 1984). The specimen cups with the soil were placed in pint size mason jars and sealed with a lid containing two, 0.635 cm holes to allow for venting. The venting of the chambers allowed for near ambient conditions while maintaining the soils' moisture between weekly wettings (Sanford and Kucharik 2013). The jars were stored in an unlit cabinet during the incubation to inhibit autotrophic respiration in between collection time points.

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 to represent t_0 and 60 minutes later another 10 ml volume was collected (t_{60}). The collected air was injected and stored in an overcharged evacuated 5.9 ml glass exetainer (Labco Limited; Buckinghamshire, UK) vial until analysis. Air samples were analyzed for CO_2 concentrations on a gas chromatography (GC) with an infra-red gas analyzer for CO_2 (LI-COR LI-820 CO_2 Analyzer; Lincoln, NE) and equipped with an autosampler. The gas chromatograph was calibrated before each run using 15 to 25 laboratory standards (400 ppm CO_2). Average coefficients of variation across all measurement dates for these standards were 0.023.

Soil incubation respiration collections occurred every other day for the first week, twice weekly for the following three weeks, once weekly for the subsequent four weeks, then once every three to four weeks thereafter for the duration of one year. Respiration data was collected more often at the onset of the incubation because there is a greater change in CO_2 respiration at this time.

Total soil C respiration over 365-days was calculated to represent the amount of CO_2-C respired during collection throughout the incubation study. To calculate total soil C respiration, each lab replicates' estimated parameters from modeling (see below) and calculated parameters, were used with t set to 365 days in Equation 7.

3.5. Modeling Carbon Pool Sizes

C flux data was used to create a three-pool regression model with first order kinetics to determine total soil C at time t ($C_{t(t)}$). This model defines the size and decomposition rate of three operationally distinct C pools (Paul et al. 2001b; Sanford and Kucharik 2013):

$$C_{t(t)} = C_a e^{-ka(t)} + C_s e^{-ks(t)} + C_r e^{-kt(t)} \quad \text{(Equation 7)}$$

In this equation the total soil C at time t is: $C_{t(t)}$. The active pool, slow pool, and resistant pool sizes and decomposition rates are, respectively, C_a, C_s, and C_r, and k_a, k_s, and k_r.

The resistant pool is used to constrain the model and it has been found that if the mean residence time (MRT) is greater than 350 years there will be no effect on the modeled parameters (2001a). Previous research estimating the age of the resistant pool at

ARL and KBS by Paul et al (2001a, 2001b) found the MRT to be between 485 and 4,400 years depending on depth. Here I used an assumed field MRT of 500 years at both sites to constrain the model. The assumed field MRT was then adjusted to a laboratory resistant pool decomposition rate using a Q_{10} of $2^{(IncTemp - FieldMAT)/10}$ (Paul 2001b). The incubation room temperature was monitored in the lab and an average temperature (21.72 °C) was used for the calculations. The MAT at ARL is 7.6 °C and the MAT at KBS is 9 °C.

I used published resistant pool values for both sites based on previous research that used acid hydrolysis to quantify the proportion of soil C in the acid-resistant pool (Paul et al. 2001a). These values were: for ARL: 50 % (0-10 cm) and 35 % (25-50 cm) and for KBS, 45 % (0-10 cm) and 31 % (25-50 cm) (Paul et al. 2001a). The calculated C_r and K_r and the daily CO_2 flux rate were then used in the regression analysis to model C_a, C_s, K_a, and K_s for each incubation jar using the NLIN procedure (METHOD = MARQUART) of SAS version 9.3.

3.6 Crop Treatment Productivity Data

Annual soil and crop data was collected from all cropping treatments at all blocks at both sites by the GLBRC. Root biomass measurements varied by crop due to differences in rooting characteristics. Perennial crop, fine root production to 13 cm below the surface was determined by root in-growth cores. Annual crop root biomass analysis varied by site. At ARL all plants in 1 m^2 were excavated whereas at KBS one plant was completely excavated. Roots were washed, sieved, dried at 60 °C for 48 hours, then weighed (Oates and Sippel 2008). Corn root biomass data was collected at both

sites and in both years (Robertson et al. 2015c), switchgrass root biomass was collected at ARL to 15 cm in 2008 and to 13 cm in 2013 and at KBS to 13 cm in 2013. Poplar root biomass was collected to 13 cm at ARL in 2013 and at KBS to 25 cm in 2008 and to 13 cm in 2013. Prairie root biomass data was collected at ARL to 15 cm in 2008, to 13 cm in 2013 and at KBS to 13 cm in 2013 (Robertson et al. 2015a, 2015b). Root biomass values collected to either 13 cm or 15 cm were used to represent root biomass from 0-10 cm.

Additional sampling of perennial subsurface standing biomass was done in 2013 by one core to 1 m depth per plot (Oates and Sippel 2008). Subsurface root biomass was collected at both sites from 0-10 cm and 25-50 cm in 2013 for switchgrass and native prairie treatments (Robertson et al. 2015a).

3.7 Statistical Analyses

The effects of crop treatment and the effects of year on C pool sizes and MRT, soil bulk density, and C and N stocks at each site and depth interval were tested using a one-way ANOVA standard least squares test, followed by a post-hoc Tukey's honest significance difference test on JMP Pro 11.0.0 statistical software. Treatment was analyzed as a fixed effect and block was nested within treatment and analyzed as a random effect. Year was analyzed as a fixed effect and was analyzed separately, also using a one-way ANOVA standard least squares test. Significance was determined at $\alpha = 0.05$, unless otherwise specified.

4. Results

4.1 Soil Respiration

Total cumulative respiration over the 365-day incubation differed among biofuel crop treatments and ranged from 0.65 to 4.8 g CO_2-C kg $soil^{-1}$, with an average of 2.5 g CO_2-C kg $soil^{-1}$. The total amount of C respired averaged 15.3 % of the initial bulk soil C pool at ARL and 24.7 % at KBS. Surface soil total respiration differed among crop treatments with the same trends seen at both ARL and KBS (Table 2). Surface soils (0-10 cm) collected in 2013 from ARL under native prairie (4.2 ± 0.4 g CO_2-C kg $soil^{-1}$) and alfalfa (4.2 ± 0.1 g CO_2-C kg $soil^{-1}$) respired more C than switchgrass (3.1 ± 0.4 g CO_2-C kg $soil^{-1}$) and poplar (3.0 ± 0.5 g CO_2-C kg $soil^{-1}$) soils; and these respired more than corn (2.4 ± 0.2 g CO_2-C kg $soil^{-1}$) ($p = 0.001$). Similarly, soils from 2013 at KBS respired more under native prairie (3.3 ± 0.4 g CO_2-C kg $soil^{-1}$) and poplar (3.2 ± 0.2 g CO_2-C kg $soil^{-1}$) than corn (2.3 ± 0.4 g CO_2-C kg $soil^{-1}$) ($p = 0.09$).

At depth, 25-50 cm, total soil C respiration did not differ among crop treatments at either site, yet, the amount of C respired as a proportion of the initial bulk soil C pool did differ among biofuel crops at ARL (Table 3). Deeper soils showed a similar trend to surface soils, with native prairie respiring a greater percent of total soil C (22.5 ± 0.0 %) compared to corn (10.1 ± 1.3 %).

Total cumulative respiration (g CO_2-C kg $soil^{-1}$) was positively correlated with root biomass (g m^2) of surface soils pooled for 2008 and 2013 at ARL ($p = 0.09$) (Figure 1A) and at KBS in 2013 ($p = 0.056$) (Figure 1B).

Changes from 2008 to 2013

Generally, cumulative respiration over the 365-day incubation and the total amount of soil C respiration as a proportion of the initial bulk soil C pool decreased from 2008 to 2013 (Table 2 and 3). At ARL, total soil C respiration decreased in surface soils under corn by 22.3 % ($p < 0.0001$), switchgrass by 11.0 % ($p = 0.04$), and poplar by 10.6 % ($p = 0.08$). Similarly, at KBS, total soil C respiration in surface soils decreased under corn by 26.8 % ($p < 0.001$) and under switchgrass by 14.5 % ($p = 0.004$). The only change in cumulative respiration at depth was at KBS, where it increased under native prairie by 95.9 % ($p = 0.005$).

Cumulative respiration as a proportion of the initial bulk soil C pool decreased from 2008 to 2013 at both sites and at both depths under biofuel crops (Table 3). At ARL, total soil C respiration as a proportion of the initial bulk soil C pool decreased from 2008 to 2013 in surface soils under corn by 2.9 % ($p = 0.0006$) and poplar by 2.0 % ($p = 0.01$) and in soils at depth under corn by 2.3 % ($p = 0.06$). At KBS, the total soil C respiration as a proportion of the initial bulk C pool decreased in surface soils under corn by 5.9 % ($p = 0.001$) and poplar by 3.3 % ($p = 0.01$) and in soils at depth, decreased under poplar by 4.7 % ($p = 0.03$) and native prairie by 8.9 % ($p = 0.03$).

4.2 Modeled Soil Carbon Pools

The active pool (C_a) was calculated using the three-pool regression model and is primarily driven by the CO_2 flux, whereas the slow pool (C_s), is primarily driven by estimates of the size of the resistant pool fraction (see methods section 4.5). Here I report results for both raw mean size of the pools as estimated by the model as well as the distribution of total soil C in the active pool (size of active pool standardized by size of

total soil C pool for each sample, given as the percent C_a). The active and slow pool decomposition rate was converted to field MRT using mean annual field temperatures and mean laboratory incubation temperature.

4.2.1 Active pool

The size of the active pool (C_a) and C_a as a fraction of total soil C stocks did not differ among biofuel crops at either depth in either year at either site. The percent contribution of the C_a to the bulk soil C pool ranged from 0.51 % in soils from 25-50 cm under corn at ARL in 2013 (Table 5) to 10.48 % in soils from 25-50 cm under corn at KBS in 2008 (Table 6), and had an overall average of 2.6 %. Additionally, the percent contribution of the C_a to the bulk soil C pool differed among sites in soils from 0-10 cm ($p = 0.0005$) and 25-50 cm ($p = 0.0001$).

Changes from 2008 to 2013

The C_a in surface soils decreased at ARL under all treatments from 2008 to 2013 (Table 8a). The C_a decreased under corn by 43% ($p = 0.01$), switchgrass by 57 % ($p < 0.0001$), poplar by 51 % ($p = 0.005$), and native prairie by 31 % ($p < 0.0001$). Similarly, at ARL the C_a fraction of SOC in surface soils decreased under all treatments from 2008 to 2013. The C_a fraction of SOC decreased under corn by 0.5 % ($p = 0.0162$), switchgrass by 0.8 % ($p < 0.0001$), poplar by 0.7 % ($p = 0.007$), and native prairie by 0.5 % ($p < 0.0001$). In soils at depth at ARL, neither the C_a nor the C_a fraction of SOC differed among crop treatments (Table 8a).

At KBS the C_a in surface soil decreased under switchgrass by 50 % ($p = 0.003$) and increased in soils at depth by 462 % ($p = 0.02$) (Table 8b). Similarly, the C_a as a

fraction of the bulk C pool decreased in surface soils under switchgrass by 0.8 % ($p = 0.002$) and increased at depth by 4.8 % ($p = 0.07$) (Table 8b).

4.2.2 Active pool mean residence time (MRT)

The field-based MRT of C_a ranged from 1.7 days to 227 days, with an average of 23.8 days at ARL and 104 days at KBS. The MRT of the C_a differed among crop treatments in surface soils at ARL in 2013 ($p = 0.005$) (Table 5) and at KBS in 2008 ($p = 0.08$) (Table 6). At ARL the C_a MRT of alfalfa (4.6 ± 0.7 days) was shorter than that of switchgrass (9.1 ± 5.8 days) and corn (19.9 ± 25.2 days). At KBS the C_a MRT of corn (3.7 ± 0.9 days) and native prairie (3.8 ± 0.7 days) was shorter than under switchgrass (5.5 ± 2.0 days). MRT of the C_a did not differ among crop treatments at depth in either year at either site (Tables 4-7).

Changes from 2008 to 2013

At ARL the C_a MRT of surface soils increased from 2008 to 2013 under corn by 272 % ($p = 0.003$) and under switchgrass by 71 % ($p = 0.06$) (Table 8a). At depth, the MRT increased in the switchgrass treatments by 413 % ($p = 0.05$) and in the native prairie treatments by 50 % ($p = 0.08$) and decreased in the poplar by 98 % ($p = 0.003$) (Table 8a).

At KBS the C_a MRT of surface soils did not change from 2008 to 2013 under any of the four biofuel crops (Table 8b). At depth, the C_a MRT increased in the switchgrass treatments by 575 % ($p = 0.05$) (Table 8b).

4.2.3 Slow pool

The percent contribution of the C_s to the initial bulk soil C pool ranged from 47 % in surface soils to 80 % in soils at depth, with an average pool size of 9.3 g C/ kg soil at ARL and 5.3 g C/ kg soil at KBS. The percent contribution of the C_s to the bulk soil C pool differed among sites in soils from 0-10 cm ($p = < 0.0001$), however, not at depth. The size of the C_s differed among crop treatments in surface soils at ARL in 2013 ($p = 0.04$) (Table 5) and in soils at depth at KBS in 2008 ($p = 0.01$) (Table 6). The contribution of the C_s to bulk soil C is determined by changes in the C_a pool and a fixed C_r pool, hence, changes in C_s as a proportion of total soil C follow the same trends as the C_a fraction and are not further described.

In 2013, at ARL, the mean size of the C_s in surface soils (0-10 cm) under poplar (12.5 ± 0.02 g C kg soil^{-1}) was greater than that under switchgrass (10.8 ± 0.01 g C kg soil^{-1}). The size of the C_s did not differ at depth in either year at ARL (Table 4 and 5).

In 2008, at KBS, the mean size of the C_s in soils at depth, 25-50 cm, under poplar (3.4 ± 0.2 g C kg soil^{-1}) was greater than under corn (1.7 ± 0.2 g C kg soil^{-1}) and native prairie (1.5 ± 0.00 g C kg soil^{-1}) treatments (Table 6). The size of the C_s did not differ in surface soils in either year at KBS (Table 6 and 7).

Changes from 2008 to 2013

Between both sites, the response of the C_s MRT varied among biofuel crops from 2008 to 2013. At ARL in soils from 0-10 cm, the size of the C_s increased under native prairie by 6.2 % ($p = 0.0009$) (Table 8a) and the contribution of the C_s pool to initial bulk soil C increased by 0.6 %. The C_s size did not change from 2008 to 2013 in soils at depth at ARL (Table 8a).

At KBS in soils from 0-10 cm, the C_s decreased under corn by 5.2 % ($p = 0.03$) and under switchgrass by 6.9 % ($p = 0.05$), and conversely increased under poplar by 15.9 % ($p = 0.08$) (Table 8b). The percent change of the C_s as a proportion of bulk soil C decreased under corn by 0.2 % and under native prairie by 0.7 %, while it increased under switchgrass by 1.4 % (Table 8b). At depth, the size of the C_s and its contribution to bulk soil C increased under poplar by 28.5 % and 1.7 %, respectively, and under native prairie by 166.5 % and 3.9 % (Table 8b).

4.2.4 Slow pool mean residence time (MRT)

The mean field C_s MRT in soils at ARL was 10.5 ± 1.2 years and at KBS was 6.9 ± 1.2 years and between the two sites ranged from 3.8 to 32.7 years. The C_s MRT differed among crop treatments in surface soils at ARL in both 2008 ($p = 0.01$) (Table 4) and 2013 ($p < 0.0001$) (Table 5) and in soils at depth in 2013 ($p = 0.04$) (Table 5). The C_s MRT did not differ among crop treatments at KBS (Table 6 and 7).

In surface soils at ARL in 2008 the C_s MRT of native prairie (6.5 ± 0.4 years) was shorter than the MRT of switchgrass (8.1 ± 1.1 years) and corn (9.3 ± 0.9 years) (Table 4). The C_s MRT did not differ among biofuel crops in soils at depth in 2008 at ARL (Table 4).

In surface soils at ARL in 2013, the C_s MRT of native prairie (6.5 ± 0.9 days) and alfalfa (6.6 ± 0.3 days) was shorter than the C_s MRT of switchgrass (8.3 ± 1.3 days), which was shorter than the C_s MRT of poplar (12.1 ± 3.1 days) and corn (11.9 ± 1.0 days). At depth at ARL, the C_s MRT of native prairie (6.5 ± 0.00 days) was shorter than that of alfalfa (18.0 ± 8.6 days) and corn (18.7 ± 6.2 days).

Changes from 2008 to 2013

At both ARL and KBS the C_s MRT of surface soils increased from 2008 to 2013 under corn by 10.8 % ($p = 0.002$) and 355 % ($p = 0.005$) respectively, and poplar by 258 % ($p = 0.08$) and 38 % ($p = 0.02$), respectively (Table 8a and 8b). The C_s MRT did not change from 2008 to 2013 under any other crops in soils from 25-50 cm at either site.

4.3 Soil Carbon Stocks

Soil bulk density differed among crop treatments at both sites and from 2008 to 2013 (Appendix Table 1), thus all SOC stocks were corrected for differences in bulk density using an equivalent soil mass approach (see Methods section 4.3). Generally, soil organic carbon (SOC) stocks were greater at ARL than KBS.

The biofuel crop treatments did not differ in SOC (Appendix Table 2) stocks at 0-10 cm at either ARL or KBS in 2008, as expected considering all plots were under the same land use prior to the establishment of the field crop treatment experiments. Plots at KBS did show some differences in 2008 stocks for the 25-50 cm depth. Mean SOC stocks of poplar treatments (2.3 ± 0.2 Mg C ha^{-1}) were greater than under corn (2.1 ± 0.09 Mg C ha^{-1}) and native prairie (2.0 ± 0.03 Mg C ha^{-1}) ($p = 0.01$; Appendix Table 2).

Five years after the establishment of the biofuel treatments, in 2013, only surface soils (0-10 cm) from ARL showed a significant cropping treatment effect ($p = 0.06$) on SOC stocks. Poplar had more surface SOC (3.0 ± 0.6 Mg C ha^{-1}) than native prairie (2.2 ± 0.17 Mg C ha^{-1}) and alfalfa (2.1 ± 0.02 Mg C ha^{-1}). In 2013, SOC under the four biofuel crops did not differ at KBS in soils from 0-10 cm or at either site in soils from 25-50 cm.

Changes from 2008 to 2013

At ARL, surface SOC stocks decreased in the native prairie treatments from 2008 to 2013 ($p = 0.01$) (Figure 2). At KBS from 0-10 cm, SOC stocks decreased in the corn, switchgrass, and native prairie treatments ($p = 0.001$, 0.002, and <0.0001 respectively) (Figure 2). At depth, there were no significant changes in SOC stocks from 2008 to 2013 within any treatments (Figure 2).

5. Discussion

5.1 Total soil C respiration differed among biofuel crops

Soil microbial respiration, a proxy for microbial activity and bioavailability of soil C, differed among perennial and annual crops after 5 years of experimental biofuel production on former agricultural lands. Total soil C respiration during a one-year long laboratory incubation of soils collected under four biofuel cropping treatments differed at our two field sites, Arlington Agricultural Research Station (ARL), WI and Kellogg Biological Station (KBS), MI. Generally, mixed species perennials lost more C through microbial respiration than monoculture annual crops, which could be explained by greater root inputs associated with perennial crops. I hypothesized that microbial respiration would be greater under biofuel crops with greater root and microbial biomass. Regression analysis of total soil C respiration and root biomass at both sites suggest increased microbial activity with increasing root biomass.

Belowground litter inputs are a dominant source of plant contributions to SOM (Rasse et al. 2005), hence differences in rooting systems are expected to affect soil C dynamics. Annual and perennial crops differ in their rooting structure. Annual crops

invest proportionally more resources into producing aboveground biomass than roots, whereas perennial crops do the opposite, which results in a smaller shoot to root ratio in the latter (Bolinder et al. 2002). In addition to providing additional belowground C, increased root biomass leads to soil aggregation formation and stabilization, which is an important mechanism of SOM protection from decomposition (Tisdall and Oades 1982; Jastrow 1998; Ontl et al. 2015). While the GLBRC field trials were not designed for the purpose of testing effects of increasing crop species diversity on C, our findings of greater 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 and perenniality and soil microbial activity (McDaniel 2014; Tiemann and Grandy 2014; Tiemann et al. 2015).

Results from the laboratory incubations are also consistent with field data. Field, surface CO_2 fluxes at ARL were greater for prairie systems than corn, and this result was attributed to greater microbial biomass in the former (Brye et al. 2002). Similarly at KBS, field CO_2 fluxes were correlated with total C inputs, but additional relationships with microbial biomass were not tested (Paul et al. 1999). While we do not currently have microbial biomass data for our sites, a previous study of southern Wisconsin agricultural sites reported microbial biomass increased in the order: corn < switchgrass < native prairie (Liang et al. 2012), consistent with our findings. Further analysis of samples collected at the start and end of our year-long incubation for phospholipid fatty acid analysis (PLFA) will provide an estimate of active microbial biomass and differences in community composition during the incubation. This data will allow us to test for relationships between root biomass, microbial biomass, and respiration.

At ARL, soil respiration differed among biofuel crops in 2008, with greater amounts of C respired from native prairie soils; these initial differences were accentuated after 5 years of management. The field plots were selected to reduce initial variability among cropping treatments, and my results suggest few differences in soil C stocks in 2008 (refer to Table in Appendix). At ARL, soil sampling occurred 3-6 months after establishment of the field trials. Microbial communities are known to respond on very short timescales to changes in plant inputs and soil biotic and abiotic conditions (Herzberger et al. 2014; Smith 2013; Smith et al. 2015) as may occur with agricultural management. More immediate soil sampling after crop planting or before the experimental treatments were established may have provided a more accurate time 0 snapshot. At KBS, there were no initial differences among treatments, which may be attributed to more immediate soil sampling, 1-2 months after establishment of the field trials, and a slow establishment of perennial cropping systems. However, after 5 years, biofuel crops at KBS did differ in the amount of total soil C respiration, following the same trends as in ARL, confirming that the observed differences in 2013 were due to treatment effects, and not confounding variability among the field plots at the start of the experiment.

5.2 Biofuel crops lost C associated with the active pool after 5-years

Carbon costs or benefits associated with the production of bioenergy crops are integral in determining if biofuels are sustainable. The active pool (C_a) has the potential to be managed for C accrual, as it responds rapidly to changes in plant inputs and agricultural management; however, all experimental biofuel crop treatments lost C

associated with the C_a in surface soils at ARL from 2008 to 2013. At KBS, C_a pools showed much smaller losses. There was no difference in the size and contribution of the C_a to total soil C among crop treatments as hypothesized.

Fractionation methods that attempt to divide bulk soil C stocks into pools with different characteristics, result in C pools that are operationally defined (Crow et al. 2007). In the biological fractionation approach I used here, the C_a is defined as that C which is readily accessible to microbes, and measured through C mineralization rates (Paul et al. 2001a, 2001b). Functionally, this pool likely consists of both soluble C (or dissolved organic C), including leaf leachates, root and microbial exudates, and particulate organic matter (POM), composed of above and belowground plant litter (Kaiser et al. 2010). Decreases in the surface soil C_a pool after 5 years of biofuel cropping, irrespective of crop type, may be due to a number of reasons.

Many studies have reported changes in the C_a pool in response to agricultural management that alters soil structure and plant inputs. In this study, the sites were plowed during crop establishment to prepare the fields for planting; thereafter, no-till practices were implemented. Tillage results in soil aggregate disturbance (Six and Paustian 2013), which exposes previously protected C to decomposers and results in soil C losses, both from microbial respiration and leaching (Davidson and Ackerman 1993; Six et al. 2000; Angers and Eriksen-Hamel 2008). Decreases in the C_a pool during the 5-year field trials may be the result of reduced field disturbance due to no-till practices, resulting in less bioavailable active C as new plant inputs are incorporated into soil aggregates.

Additionally, decreased active C may be a result of reduced aboveground C inputs in these agricultural systems. Aboveground biomass is harvested annually at the field sites, reducing the amount of C inputs to the soil. Other studies have reported management- or land-change-induced alterations in the relative size of physically-unprotected and bioavailable C pools, as determined by a number of soil fractionation methods (Alvarez et al. 1998; John et al. 2005; Marin-Spiotta et al. 2009; Jokela 2011).

Whereas bulk soil C stocks showed few responses to the different biofuel crop treatments after 5 years of management, the C_a was more sensitive and served as an indicator of changes in the distribution of SOC among pools with different turnover times. Using a field method for evaluating the operationally defined C_a, Weil et al. (2003) found that the active C pool was more sensitive to management than the total C pool and that the dynamics of C_a are more closely related to soil properties than total C. In surface soils, the C_a decreased under all biofuel crops at ARL whereas the total soil C pool only decreased under native prairie, which supports the concept of increased sensitivity of the C_a, compared to bulk soil. The proportion of bulk soil C in the active pool in this study was estimated at 1.4 % of the bulk soil C pool in surface soils (0-10 cm) and 3.9 % of bulk soil C in soils at depth (25-50 cm). These values are similar to the 1.8 to 3.7 % reported by Sanford and Kucharik (2013), who used a similar three-pool model, for soils (0-25 cm) from similar agroecosystems in Wisconsin.

Differing response of soil C pools to similar agricultural management at ARL and KBS may be explained by differences in soil properties between the sites. Unlike at ARL where the C_a decreased under all biofuel crop treatments, there were minimal changes to the C_a at KBS. Under switchgrass, the C_a decreased in surface soils and increased at

depth. Increased sensitivity of the C_a in soils at ARL compared to KBS may be attributed to differences in soil texture, which influences a key mechanism of SOM stabilization, through the formation of soil aggregates and the sorption of C to mineral surfaces (Hassink 1997; Six et al. 2004; Stockmann et al. 2013). Clay content has been shown to affect SOM dynamics and increasing clay content is correlated with increased protection from microbial decomposition (Paul 1984, Six et al. 2002). Mineral sorption is an important mechanism for C stabilization in agricultural systems (Lagomarsino et al. 2012) and clay content is associated with microaggregate formation (Oades 1984). The ARL site is characterized as a silt loam with ~65 % silt, ~ 25 % clay, and ~5 % sand, whereas the KBS site is characterized as a sandy loam with ~65 % sand, ~30 % silt, and ~5 % clay (Table 1). Reduced clay content in the soils at KBS compared to ARL can explain observed greater amounts of total soil C in the active pool at KBS (1.84 %) than at ARL (1 %). My results suggest the biologically available active C may be more sensitive to changes in land use in soils with increased clay content.

5.3 Biofuel cropping systems affected changes in the slow C pool

Modeled estimates of the slow C pool showed more variable response of this SOM fraction to 5 years of biofuel agricultural systems. Specifically, the C_s decreased from 2008 to 2013 under corn and switchgrass and increased under poplar and native prairie in surface soils. Deeper soils (25-50 cm) showed an increase in the C_s under poplar and native prairie at KBS. These patterns are consistent with gains for mixed species perennial crops and losses for monoculture perennials and monoculture annuals. Greater variable response of the C_s at KBS may suggest that while the C_a may be more

sensitive than the total C pool to management change in high clay soils, the C_s may be more sensitive to management changes in sandy soils. This suggests that soil C stabilization via physical protection is more sensitive to crop treatment in sandy loams compared to silt loams because with reduced soil minerals and soil surface area associated with sandy soils, there is reduced protection to decomposition.

In this study we found the C_s to represent 47 to 80 % of the bulk soil C pool, similar to the size found in other agricultural studies (Paul et al. 1999). Given the size of this pool, the response of the C_s fraction to changes in land management has implications for overall soil C stock losses and gains. The C_s cycles on average on a decadal time scale and is theorized to represent SOM 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). Roots contribute to aggregate formation which results in increased physically protected C (Tisdall and Oades 1982; Jastrow 1998; Ontl et al. 2015). Root biomass variability among annual and perennial crops, and the tendency of root biomass to increase with increasing plant species diversity, suggests soils' ability to form aggregates and protect C will vary among crops (Bolinder et al. 2002; Tiemann and Grandy 2014).

In a separate study of biofuel field trials at KBS, increasing plant species rotational diversity resulted in changes in the microbial community composition, aggregate formation, and increases in soil C (Tiemann et al. 2015). This finding was again supported by research in a grassland experiment where increased plant diversity resulted in increased microbial activity and C storage (Lange et al. 2015). Increased C

associated with the slow pool under native prairie and losses under switchgrass and corn in our study support these trends.

Poplar, a woody perennial species, harvested following the sixth growing season, may have acted as an intermediary along the continuum from multi-species perennials to single species perennials. While C associated with the slow pool decreased under switchgrass, a monoculture perennial, C associated with the slow pool increased under poplar. Since poplar was not harvested annually a mix of forbs and grasses comprised the understory of these plots. This understory may have acted similar to the high diverse perennial plots, increasing root biomass, soil aggregation, and the stabilization of C.

The modeling approach used in this study may underestimate changes in the size of the C_s pool by not accounting for potential changes in the resistant pool (C_r) during the field trials. The laboratory soil incubation approach calculated C_a by measuring CO_2 fluxes over one year, whereas the C_s was mathematically calculated by subtracting the C_a and the C_r from the bulk SOC pool (Paul et al. 2001a, 2001b; Sanford and Kucharik 2013). The size of the resistant pool, C_r, was estimated from published values using acid hydrolysis on similar soils and agricultural systems and a fixed value per site and soil depth was used to constrain our model (Paul 2001a, 2001b). The C_r, which is characterized by turnover times spanning from centuries to 1000s of years, is expected to be less sensitive to changes in management, yet some studies have reported rapid response of this SOM fraction to land use change. For example, Butman et al. (2014) estimated that soil disturbance in agricultural systems could result in the mobilization of old (>1,000 years old) SOM. Paul et al. (2006) found that the non-hydolyzable fraction, generally old C with a MRT of > 1,000 years, can be lost or gained in a short time frame

compared to the non-hydrolyzable fraction (Paul et al. 2006). These findings suggest that the resistant pool size and turnover rate may have changed in response to biofuel cropping treatments even in the short 5-year time span between the collection of our soils. Current research being conducted at the same sites and using a different SOM fractionation approach (A. Von Haden, personal communication) will help constrain the size and turnover time of the slow and resistant pools. Overall this effort will allow for further understanding of how biofuel crop treatments and management affect soil C dynamics.

To further address how biofuel crop treatments may have altered soil C turnover, future work will include ^{14}C radiocarbon analysis of bulk soils and of respired CO_2 during the incubations. This data will allow us to better constrain the age of C being accessed by microbes and of the residual C pools. Additionally, phospholipid fatty acid analysis (PLFA) will provide an estimate of active microbial biomass, which will allow us to test the relationship between microbial respiration, microbial biomass, and pool sizes.

6. Conclusions

Bioenergy crops differed in their effect on soil microbial respiration and the size and mean residence time of soil C fractions. Mixed species perennials respired more soil C and had greater root biomass than monoculture annual crops, suggesting differences in root inputs influenced microbial activity and the loss of bioavailable C. Biofuel crop production and management resulted in the loss of active C after five years, possibly due to decreased total plant C inputs through harvest of aboveground biomass. The slow

cycling pool showed a varied response to biofuel crops, with gains in the native prairie and poplar soils and losses in the switchgrass and corn soils. These results are consistent with increasing C protection via aggregate formation due to increased root biomass associated with perennials and greater plant species diversity (Tiemann and Grandy 2014; Tiemann et al. 2015).

Although changes in the total C respired and C pools were detected on a 5-year timescale, soil C monitoring needs to be continued over longer time scales to properly assess the influence of management practices and crop treatment because the processes underlying mechanisms of SOM formation and destabilization and a return to an equilibrium state are slow (Gollany et al. 2011). My research suggests that the C_a may be more sensitive than the total C pool in high clay content soils while the C_s may be more sensitive than the total C pool in sandy soils. To holistically evaluate changes in soil C dynamics, it is necessary to separate soil into functionally distinct C pools through fractionation techniques such as soil incubations employed by this study. Carbon dynamics differ among biofuel crops and their impacts vary among soil type, monitoring of soil C respiration and soil C pools will provide an early indicator of management and land use change impacts.

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8. Tables

Table 1: Arlington Agricultural Research Station (ARL), WI and Kellogg Biological Station (KBS), MI site differences including mean annual temperature (ºC), mean annual precipitation (mm), soil classification, and soil texture.

Site Differences between Arlington Agricultural Research Station (ARL), WI and Kellogg Biological Station (KBS), MI

Site	ARL	KBS
Mean annual temperature (°C)	7.4	9.8
Mean annual precipitation (mm)	833	986
Soil Order	Mollisols	Alfisols
Soil Series	Plano silt-loam	Kalamazoo loam
Soil Classification	Fine-silty, Mixed, Superactive, Mesic Typic Argiudolls	Fine-Loamy, Mixed, Semiactive, Mesic Typic Hapludalfs
% Sand		
Corn, 0-10cm	6.3	63.3
Corn, 25-50cm	3.7	78.3
Switchgrass, 0-10cm	8.7	63.3
Switchgrass, 25-50cm	7.7	72.5
Poplar, 0-10cm	6.7	63.3
Poplar, 25-50cm	6.0	59.2
Native Prairie, 0-10cm	8.7	65.0
Native Prairie, 25-50cm	4.3	75.8
% Silt		
Corn, 0-10cm	68.7	30.8
Corn, 25-50cm	66.7	10.8
Switchgrass, 0-10cm	67.0	29.2
Switchgrass, 25-50cm	62.3	15.8
Poplar, 0-10cm	67.7	31.7
Poplar, 25-50cm	65.0	31.7
Native Prairie, 0-10cm	66.0	30.0
Native Prairie, 25-50cm	63.7	13.3
% Clay		
Corn, 0-10cm	25.3	5.8
Corn, 25-50cm	29.3	10.8
Switchgrass, 0-10cm	24.3	7.5
Switchgrass, 25-50cm	30.3	11.7
Poplar, 0-10cm	25.7	5.0
Poplar, 25-50cm	29.0	9.2
Native Prairie, 0-10cm	25.0	5.0
Native Prairie, 25-50cm	32.0	10.8

Table 2. Mean and standard error of cumulative respiration (g CO_2-C kg $soil^{-1}$) at Arlington Agricultural Research Station (ARL), WI and Kellogg Biological Station (KBS), MI in soils from 0-10 cm and 25-50 cm. Alfalfa soil samples and data were only collected at ARL in 2013 (see methods 4.2). Lower case letters indicate significant within year differences among treatments ($p < 0.05$); upper case letters indicate significant within treatment differences from 2008 to 2013 ($p < 0.05$); letters followed by an asterisk (*) indicate marginal significance ($p < 0.1$).

Cumulative Respiration (g CO_2-C kg $soil^{-1}$)

		Arlington Agricultural Research Station, WI		**Kellogg Biological Station, MI**	
Treatment	**Depth (cm)**	**2008**	**2013**	**2008**	**2013**
corn	0-10	3.0 ± 0.2; Ab	2.3 ± 0.2; Bc	3.1 ± 0.3; A	2.3 ± 0.4; Bb
switchgrass	0-10	3.4 ± 0.3; Ab	3.1 ± 0.4; Bb	2.9 ± 0.2; A	2.5 ± 0.2; Bab
poplar	0-10	3.4 ± 0.2; A*b	3.0 ± 0.5; B*b	3.2 ± 0.3	3.2 ± 0.2; a
prairie	0-10	4.0 ± 0.2; a	4.2 ± 0.4; a	2.9 ± 0.3	3.3 ± 0.4; a
alfalfa	0-10	--	4.2 ± 0.1; a	--	--
corn	25-50	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	1.1 ± 0.5
switchgrass	25-50	1.3 ± 0.3	1.3 ± 0.4	1.0 ± 0.3	1.2 ± 0.2
poplar	25-50	1.4 ± 0.3	2.0 ± 0.1	1.2 ± 0.2	1.2 ± 0.2
prairie	25-50	1.6 ± 0.2	1.7 ± 0.0	0.9 ± 0.0; B	1.8 ± 0.2; A
alfalfa	25-50	--	1.2 ± 0.3	--	--

Table 3. Mean and standard error of total soil C respiration as a proportion of bulk soil C (%) at Arlington Agricultural Research Station (ARL), WI and Kellogg Biological Station (KBS), MI in soils from 0-10 cm and 25-50 cm. Alfalfa soil samples and data were only collected at ARL in 2013 (see methods 4.2). Lower case letters indicate significant within year differences among treatments ($p < 0.05$); upper case letters indicate significant within treatment differences from 2008 to 2013 ($p < 0.05$); letters followed by an asterisk (*) indicate marginal significance ($p < 0.1$).

Total soil C respiration as a proportion of bulk soil C (%)

		Arlington Agricultural Research Station, WI		Kellogg Biological Station, MI	
Treatment	**Depth (cm)**	**2008**	**2013**	**2008**	**2013**
corn	0-10	13.9 ± 1.1; Ab	11.0 ± 0.9; Bc	25.5 ± 2.7; A	19.6 ± 3.2; B
switchgrass	0-10	15.7 ± 1.5; a,b	14.7 ± 1.9; b	23.4 ± 1.5	21.8 ± 2.2
poplar	0-10	13.2 ± 0.9; Ab	11.2 ± 1.9; Bc	24.1 ± 2.7; A	20.8 ± 1.4; B
prairie	0-10	18.0 ± 0.7; a	17.9 ± 1.8; a	24.1 ± 2.6	25.9 ± 3.1
alfalfa	0-10	--	17.5 ± 0.5; a,b	--	--
corn	25-50	12.4 ± 1.3; A*	10.1 ± 1.3; B*b*	33.1 ± 5.9	29.5 ± 7.1
switchgrass	25-50	18.3 ± 4.0	16.7 ± 5.0; a,b*	33.5 ± 9.3	26.6 ± 6.2
poplar	25-50	13.3 ± 4.6	18.0 ± 0.6; a,b*	23.3 ± 4.4; A	18.6 ± 2.7; B
prairie	25-50	20.6 ± 2.9	22.5 ± 0.0; a*	37.0 ± 1.1; A	28.1 ± 3.0; B
alfalfa	25-50	--	11.5 ± 4.0; a,b*	--	--

Table 4. 2008 Arlington Agricultural Research Station, WI mean ($\overline{X}$) and standard error (SE) of SOC (g kg^{-1}) and parameter estimates of the active pool (C_a, g kg^{-1}) and the slow pool (C_s, g kg^{-1}) and their corresponding decomposition rates (K_a, day^{-1}; K_s, day^{-1}). Decomposition rates converted to field mean residence time (MRT) using $Q_{10} = 2^{[(MIT-MFT)/10]}$, where MFT = mean annual field temperatures and MIT = mean incubation temperature. Parameter estimates calculated and constrained by resistant pool, which was determined using SOC values and percent resistant, previously determined by Paul et al. (2001a; 2001b), and an assumed field MRT of 500 years.

2008, Arlington Agricultural Research Station, WI															
				Active Pool (C_a)						Slow Pool (C_s)					
		SOC (g kg^{-1})		C_a Parameter Estimate (g kg^{-1})			K_a (day^{-1})	MRT (day)		C_s Parameter Estimate (g kg^{-1})			K_s (day^{-1})	MRT (year)	
Treatment	Depth (cm)	$\overline{X}$	SE	$\overline{X}$	SE	% of SOC	$\overline{X}$	$\overline{X}$	SE	$\overline{X}$	SE	% of SOC	$\overline{X}$	$\overline{X}$	SE
Corn	0-10	23.0	1.2	0.27	0.04	1.2	0.5	5.3	0.7	10.79	0.04	47.0	0.0008	9.3	0.9
Switchgrass	0-10	22.2	0.8	0.34	0.01	1.5	0.5	5.3	0.4	10.76	0.01	48.5	0.0009	8.1	1.1
Poplar	0-10	25.8	0.9	0.35	0.02	1.4	0.5	5.8	0.9	12.46	0.02	48.3	0.0007	9.9	0.9
Prairie	0-10	22.5	0.3	0.31	0.03	1.4	0.5	5.4	1.1	10.92	0.03	48.5	0.0011	6.5	0.4
Corn	25-50	9.6	0.9	0.08	0.03	0.9	0.3	15.5	7.7	5.95	0.03	62.2	0.0005	14.0	1.8
Switchgrass	25-50	7.3	0.4	0.09	0.01	1.2	0.4	22.6	6.8	4.47	0.01	61.0	0.0008	9.1	2.4
Poplar	25-50	12.7	2.4	0.23	0.16	1.8	0.1	71.1	78.3	10.15	0.16	80.2	0.0006	32.7	1.9
Prairie	25-50	7.8	0.1	0.46	0.16	5.9	0.3	139.8	36.4	4.54	0.16	58.1	0.0008	12.7	2.6

Table 5. 2013 Arlington Agricultural Research Station, WI mean ($\overline{X}$) and standard error (SE) of SOC (g kg^{-1}) and parameter estimates of the active pool (C_a, g kg^{-1}) and the slow pool (C_s, g kg^{-1}) and their corresponding decomposition rates (K_a, day^{-1}; K_s, day^{-1}). Decomposition rates converted to field mean residence time (MRT) using $Q_{10} = 2^{[(MIT-MFT)/10]}$, where MFT = mean annual field temperatures and MIT = mean incubation temperature. Parameter estimates calculated and constrained by resistant pool, which was determined using SOC values and percent resistant, previously determined by Paul et al. (2001a; 2001b), and an assumed field MRT of 500 years.

2013, Arlington Agricultural Research Station, WI															
				Active Pool (C_a)						Slow Pool (C_s)					
		SOC (g kg^{-1})		C_a Parameter Estimate (g kg^{-1})			K_a (day^{-1})	MRT (day)		C_s Parameter Estimate (g kg^{-1})			K_s (day^{-1})	MRT (year)	
Treatment	Depth (cm)	$\overline{X}$	SE	$\overline{X}$	SE	% of SOC	$\overline{X}$	$\overline{X}$	SE	$\overline{X}$	SE	% of SOC	$\overline{X}$	$\overline{X}$	SE
Corn	0-10	21.62	0.68	0.15	0.14	0.71	0.29	19.86	25.20	10.55	0.14	48.80	0.0006	11.88	1.02
Switchgrass	0-10	20.85	0.77	0.15	0.03	0.70	0.38	9.05	5.76	10.28	0.03	49.30	0.0009	8.28	1.31
Poplar	0-10	26.51	0.68	0.17	0.14	0.64	0.60	12.59	23.81	13.21	0.14	49.83	0.0006	12.06	3.08
Prairie	0-10	23.62	0.18	0.21	0.03	0.90	0.46	6.38	2.18	11.60	0.03	49.10	0.0011	6.54	0.89
Alfalfa	0-10	24.39	0.45	0.15	0.01	0.62	0.59	4.61	0.73	11.95	0.01	49.00	0.0011	6.55	0.28
Corn	25-50	10.09	0.47	0.05	0.05	0.51	0.60	22.11	37.57	6.66	0.05	65.99	0.0004	18.71	6.15
Switchgrass	25-50	8.00	0.02	0.17	0.16	2.15	0.04	116.23	65.85	5.03	0.16	62.85	0.0007	13.02	6.48
Poplar	25-50	11.26	1.53	0.34	0.08	3.01	2.91	1.68	0.03	7.07	0.08	62.81	0.0007	9.93	0.04
Prairie	25-50	7.68	0.20	0.04	0.00	0.58	0.04	69.66	0.00	4.95	0.00	64.42	0.0011	6.48	0.00
Alfalfa	25-50	9.88	1.79	0.12	0.18	1.22	0.26	44.19	82.24	7.43	0.18	75.28	0.0005	17.95	8.55

Table 6. 2008 Kellogg Biological Station, MI mean ($\overline{X}$) and standard error (SE) of SOC (g kg^{-1}) and parameter estimates of the active pool (C_a, g kg^{-1}) and the slow pool (C_s, g kg^{-1}) and their corresponding decomposition rates (K_a, day^{-1}; K_s, day^{-1}). Decomposition rates converted to field mean residence time (MRT) using $Q_{10} = 2^{[(MIT-MFT)/10]}$, where MFT = mean annual field temperatures and MIT = mean incubation temperature. Parameter estimates calculated and constrained by resistant pool, which was determined using SOC values and percent resistant, previously determined by Paul et al. (2001a; 2001b), and an assumed field MRT of 500 years.

2008, Kellogg Biological Station, MI															
				Active Pool (C_a)						Slow Pool (C_s)					
		SOC (g kg^{-1})		C_a Parameter Estimate (g kg^{-1})			K_a (day^{-1})	MRT (day)		C_s Parameter Estimate (g kg^{-1})			K_s (day^{-1})	MRT (year)	
Treatment	Depth (cm)	$\overline{X}$	SE	$\overline{X}$	SE	% of SOC	$\overline{X}$	$\overline{X}$	SE	$\overline{X}$	SE	% of SOC	$\overline{X}$	$\overline{X}$	SE
Corn	0-10	12.28	0.11	0.23	0.05	1.85	0.71	3.68	0.87	6.54	0.05	53.29	0.0016	4.24	0.63
Switchgrass	0-10	12.57	0.31	0.23	0.04	1.80	0.48	5.53	1.96	6.73	0.04	53.53	0.0014	4.88	0.41
Poplar	0-10	13.66	0.47	0.19	0.06	1.42	0.62	4.09	0.86	7.20	0.06	52.68	0.0015	4.57	0.67
Prairie	0-10	12.08	0.00	0.19	0.04	1.61	0.67	3.75	0.68	6.46	0.04	53.47	0.0015	4.59	0.74
Corn	25-50	2.94	0.26	0.31	0.20	10.48	0.03	143.94	76.34	1.66	0.20	56.53	0.0013	6.24	4.82
Switchgrass	25-50	3.37	0.22	0.05	0.02	1.44	0.13	24.74	23.40	2.14	0.02	63.64	0.0019	5.43	4.90
Poplar	25-50	5.18	0.29	0.27	0.18	5.12	0.12	63.76	36.67	3.41	0.18	65.70	0.0009	12.52	13.72
Prairie	25-50	2.56	0.13	0.18	0.00	7.09	0.55	75.76	0.18	1.54	0.00	60.07	0.0018	3.77	0.16

Table 7. 2013 Kellogg Biological Station, MI mean ($\overline{X}$) and standard error (SE) of SOC (g kg^{-1}) and parameter estimates of the active pool (C_a, g kg^{-1}) and the slow pool (C_s, g kg^{-1}) and their corresponding decomposition rates (K_a, day^{-1}; K_s, day^{-1}). Decomposition rates converted to field mean residence time (MRT) using $Q_{10} = 2^{[(MIT-MFT)/10]}$, where MFT = mean annual field temperatures and MIT = mean incubation temperature. Parameter estimates calculated and constrained by resistant pool, which was determined using SOC values and percent resistant, previously determined by Paul et al. (2001a; 2001b), and an assumed field MRT of 500 years.

2013, Kellogg Biological Station, MI															
				Active Pool (C_a)						Slow Pool (C_s)					
		SOC (g kg^{-1})		C_a Parameter Estimate (g kg^{-1})			K_a (day^{-1})	MRT (day)		C_s Parameter Estimate (g kg^{-1})			K_s (day^{-1})	MRT (year)	
Treatment	Depth (cm)	$\overline{X}$	SE	$\overline{X}$	SE	% of SOC	$\overline{X}$	$\overline{X}$	SE	$\overline{X}$	SE	% of SOC	$\overline{X}$	$\overline{X}$	SE
Corn	0-10	11.68	0.26	0.29	0.32	2.48	0.55	45.66	59.51	6.20	0.32	53.11	0.0011	7.15	2.89
Switchgrass	0-10	11.41	0.39	0.11	0.03	0.99	0.39	9.19	9.18	6.26	0.03	54.89	0.0013	5.23	0.73
Poplar	0-10	16.00	1.04	0.26	0.26	1.63	0.58	20.35	40.15	8.34	0.26	52.15	0.0012	5.81	0.92
Prairie	0-10	12.47	0.35	0.37	0.45	2.97	0.51	43.52	78.14	6.58	0.45	52.79	0.0016	5.62	3.32
Corn	25-50	4.39	0.90	0.45	0.59	10.18	0.40	225.90	234.27	2.59	0.59	59.05	0.0010	14.09	20.06
Switchgrass	25-50	4.36	0.96	0.27	0.16	6.26	0.03	159.46	125.30	3.02	0.16	69.22	0.0011	7.10	3.12
Poplar	25-50	6.49	0.77	0.17	0.18	2.55	0.54	69.10	96.41	4.38	0.18	67.42	0.0007	9.81	2.73
Prairie	25-50	6.40	0.00	0.33	0.27	5.23	0.62	70.62	66.18	4.09	0.27	64.00	0.0012	7.52	3.31

Table 8. Change in SOC concentrations (g kg^{-1}) and percent of pools as a fraction of SOC from 2008 to 2013 A) At Arlington Agricultural Research Station, WI. B) At Kellogg Biological Station, MI.

A)

Percent Change in Pool Sizes from 2008 to 2013 at Arlington Agricultural Research Station, WI

		SOC	Active Pool			Slow Pool		
			C_a		MRT	C_s		MRT
Treatment	Depth (cm)	% Change	% Change in C_a (g kg^{-1})	% Change as a fraction of SOC	% Change	% Change in C_s (g kg^{-1})	% Change as a fraction of SOC	% Change
Corn	0-10	-5.9	-43.3	0.5	271.9	-2.2	1.8	10.8
Switchgrass	0-10	-6.1	-56.9	0.1	71.4	-4.5	0.8	18.3
Poplar	0-10	2.6	-51.3	0.5	117.0	6.0	1.6	257.8
Prairie	0-10	4.9	-30.9	0.0	17.4	6.2	0.6	132.9
Corn	25-50	5.4	-39.2	0.2	42.2	11.9	3.8	234.9
Switchgrass	25-50	9.1	101.2	1.8	413.2	12.4	1.9	173.8
Poplar	25-50	-11.1	49.1	-0.5	-97.6	-30.3	-17.4	-98.1
Prairie	25-50	-1.9	-90.4	-2.0	-50.2	8.9	6.4	-100.0

B)

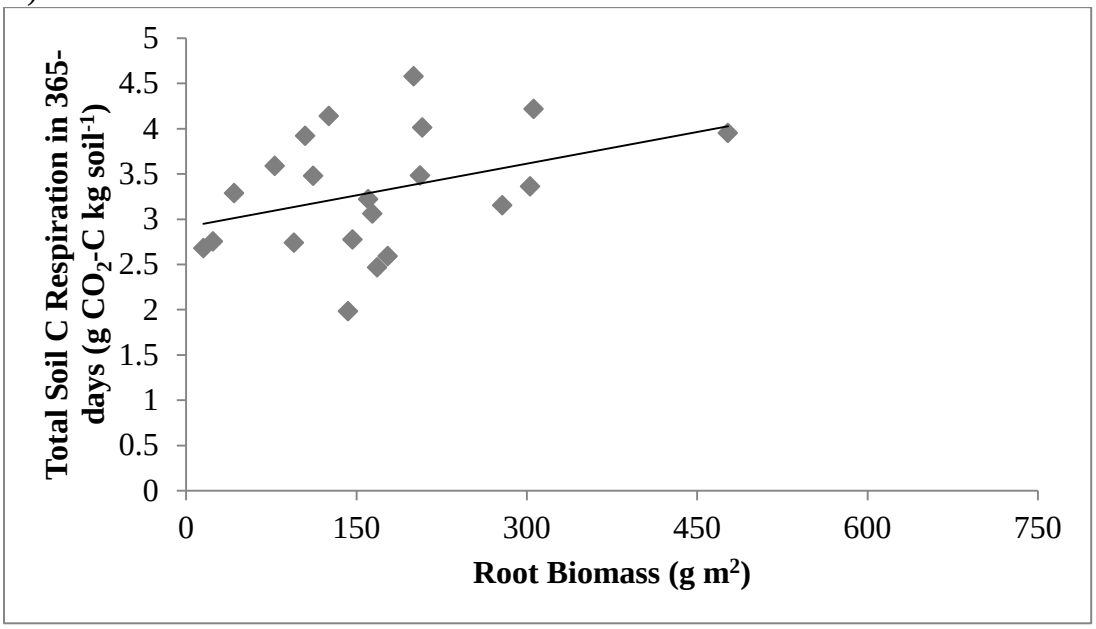
Percent Change in Pool Sizes from 2008 to 2013 at Kellogg Biological Station, MI

		SOC	Active Pool			Slow Pool		
			C_a		MRT	C_s		MRT
Treatment	Depth (cm)	% Change	% Change in C_a (g kg^{-1})	% Change as a fraction of SOC	% Change	% Change in C_s (g kg^{-1})	% Change as a fraction of SOC	% Change
Corn	0-10	-4.8	27.6	2.3	1140.4	-5.2	-0.2	355.2
Switchgrass	0-10	-9.3	-50.2	0.0	66.2	-6.9	1.4	79.8
Poplar	0-10	17.1	33.8	1.2	396.9	15.9	-0.5	38.0
Prairie	0-10	3.2	90.5	3.2	1060.3	1.9	-0.7	350.8
Corn	25-50	49.1	44.7	6.7	56.9	55.7	2.5	316.6
Switchgrass	25-50	29.4	461.7	3.0	544.5	40.8	5.6	-36.3
Poplar	25-50	25.2	-37.7	-0.7	8.4	28.5	1.7	-80.1
Prairie	25-50	150.1	84.6	4.1	-6.8	166.5	3.9	1945.5

9. Figures

Figure 1. Total soil C respiration (g CO_2-C kg $soil^{-1}$) by root biomass (g m^2) in surface soils. A) In 2008 and 2013 at Arlington Agricultural Research Station (ARL), WI (r^2 = 0.14 and p = 0.09). Note: poplar root biomass was not included in this analysis because it was not collected in 2008 B) In 2013 at Kellogg Biological Station (KBS), MI (r^2 = 0.32 and p = 0.056).

A)



B)

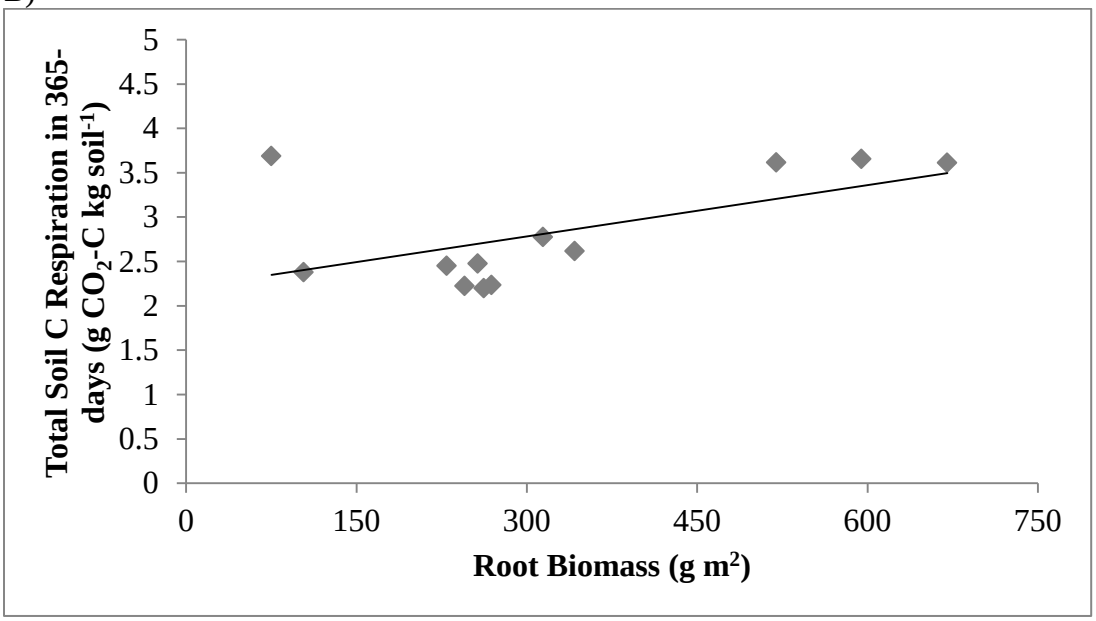


Figure 2. Changes in C Stocks (Mg C ha^{-1}) in soils from 0-10 cm and 25-50 cm at Arlington Agricultural Research Station, WI and Kellogg Biological Station, MI. Bars marked with an asterisk indicate a significant difference ($p < 0.05$).

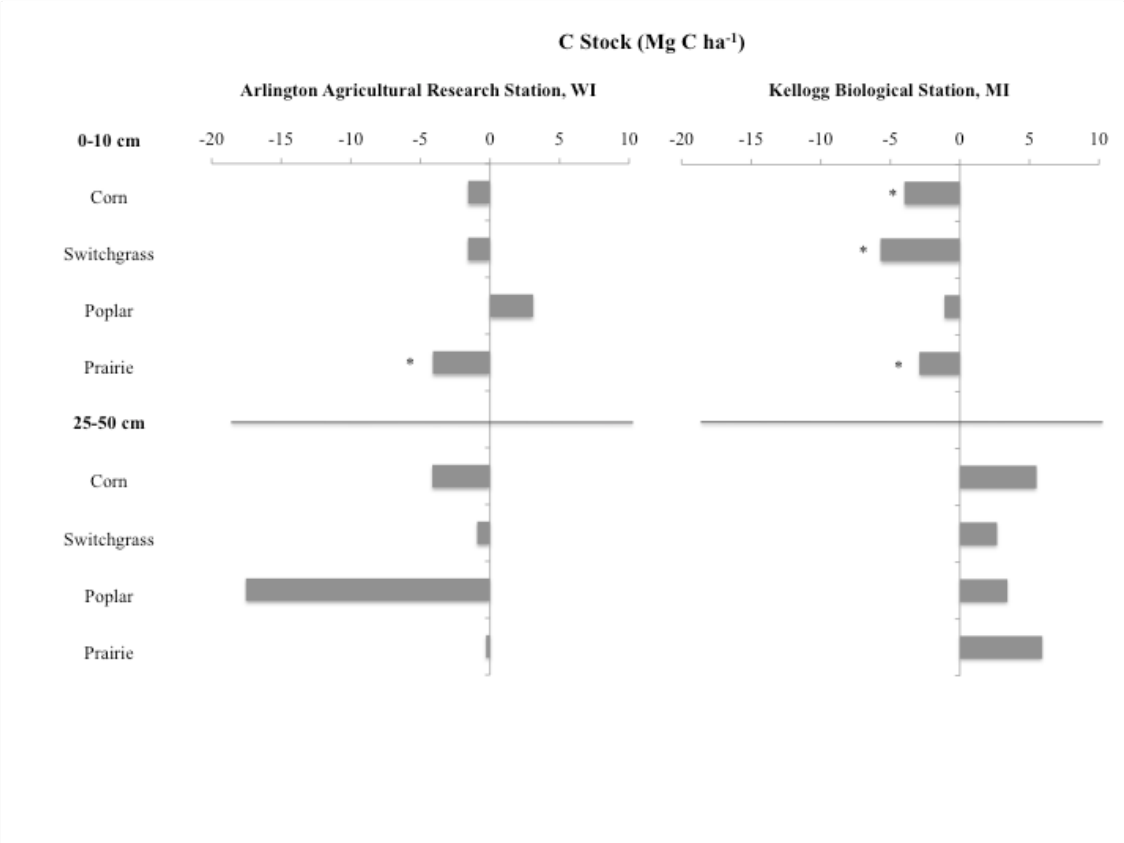


Figure 3. Directional change from 2008 to 2013 of treatments: active pool (C_a; g C kg soil^{-1}), slow pool (C_s; g C kg soil^{-1}), active pool mean residence time (MRT; day), slow pool mean residence time (MRT; year), cumulative respiration (g C kg soil^{-1}) at Arlington Agricultural Research Station (ARL), WI in soils from 0-10 cm and 25-50 cm. Arrows indicate significant within treatment differences ($p < 0.05$); an asterisk (*) indicates marginal significance ($p < 0.01$).

Conceptual soil pool dynamics from 2008 to 2013 at Arlington Agricultural Research Station, WI

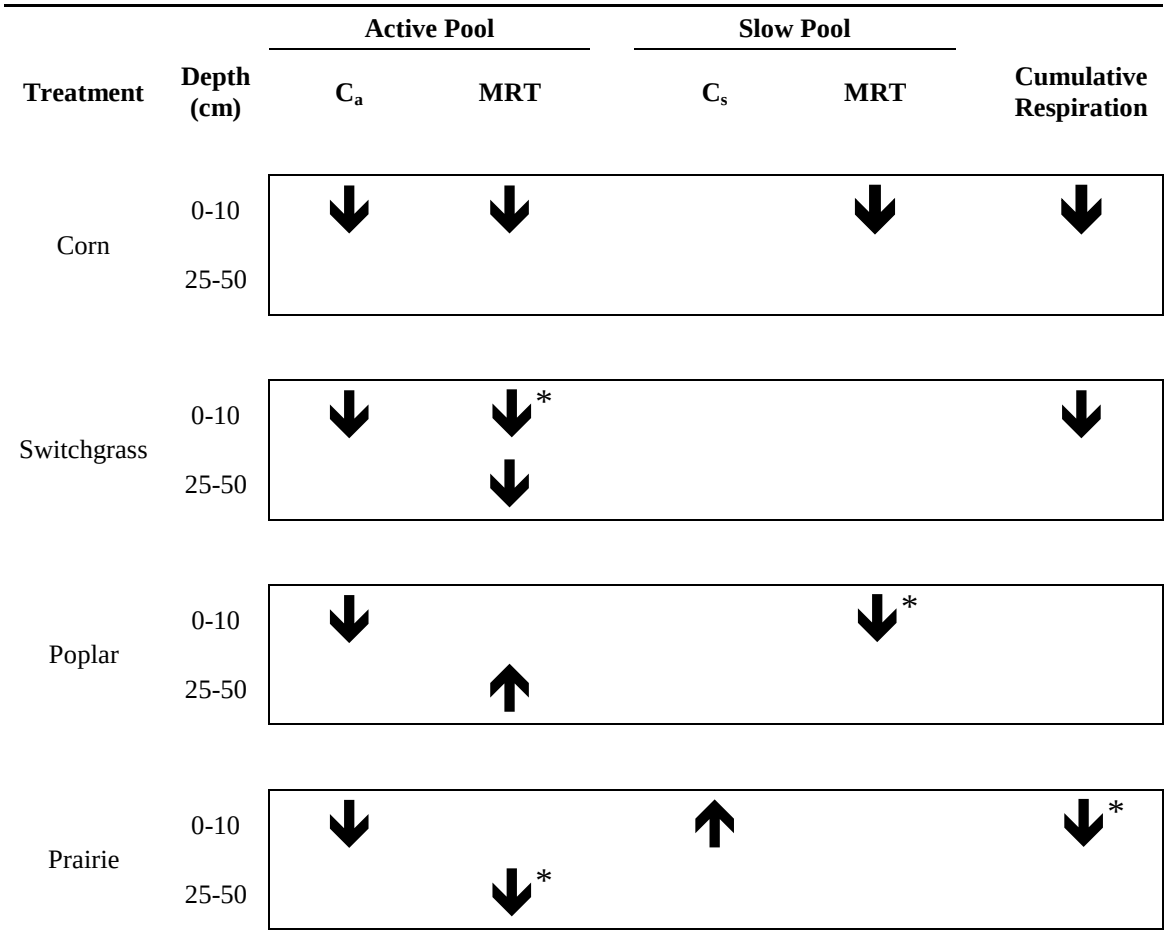
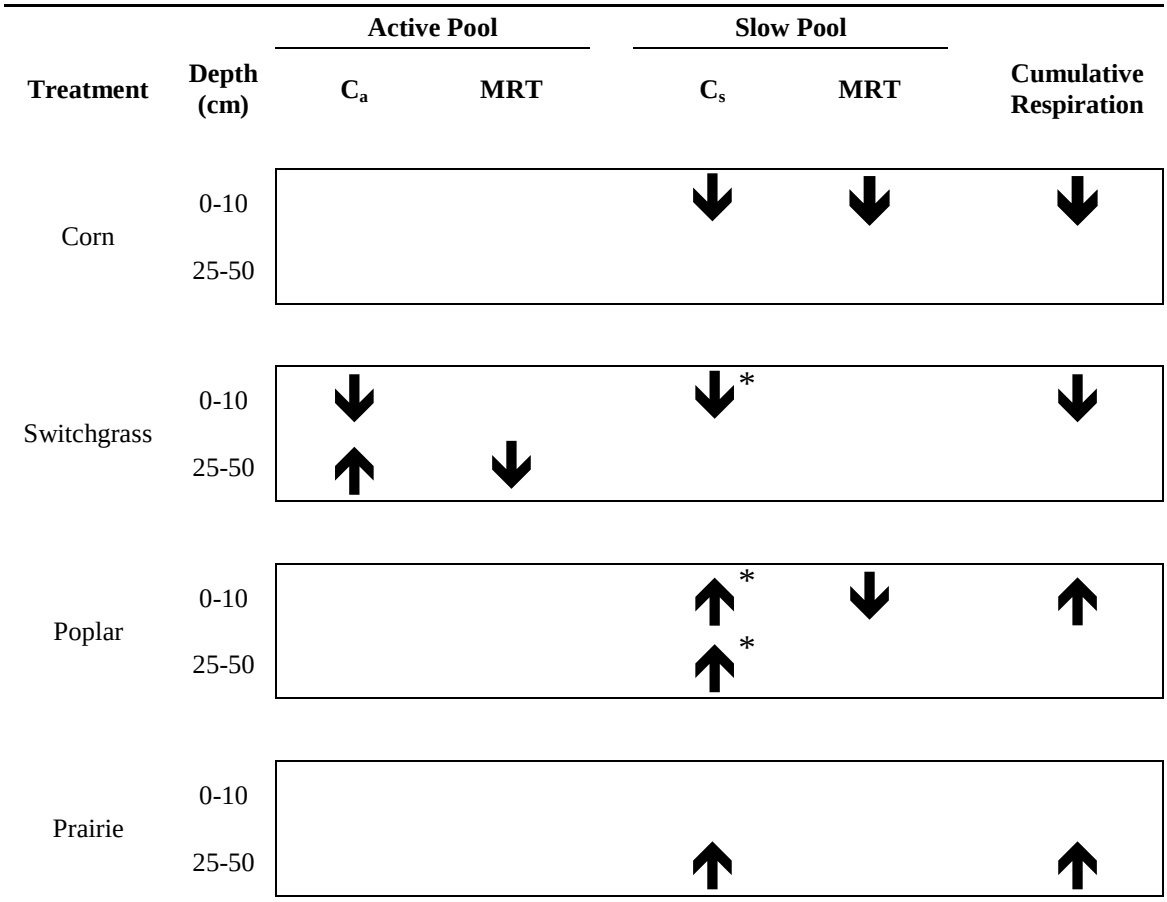


Figure 4. Directional change from 2008 to 2013 of treatments: active pool (C_a; g C kg soil^{-1}), slow pool (C_s; g C kg soil^{-1}), active pool mean residence time (MRT; day), slow pool mean residence time (MRT; year), cumulative respiration (g C kg soil^{-1}) at Kellogg Biological Station (KBS), MI in soils from 0-10 cm and 25-50 cm. Arrows indicate significant within treatment differences ($p < 0.05$); an asterisk (*) indicates marginal significance ($p < 0.01$).

Conceptual soil pool dynamics from 2008 to 2013 at Kellogg Biological Station, MI



10. Appendix

10.1 Appendix Supplementary Text

10.1.1 Bulk density

Crop treatment had a significant effect on bulk density in surface soils (0-10 cm) in both years, 2008 and 2013, and at both sites, ARL and KBS (Appendix Table 1). At ARL in surface samples from 2008, the mean bulk density of native prairie plots (1.4 ± 0.02 g cm^{-3}) was greater ($p = 0.02$) than the mean bulk density of the poplar plots (0.96 ± 0.14 g cm^{-3}). Switchgrass and corn plots did not differ from the native prairie and poplar. At ARL in 2013, the mean bulk density of switchgrass plots (1.3 ± 0.07 g cm^{-3}) was significantly greater than that of native prairie (1.2 ± 0.04 g cm^{-3}), alfalfa (1.1 ± 0.02 g cm^{-3}), and poplar (1.1 ± 0.03 g cm^{-3}) treatments. Mean soil bulk density under corn (1.28 ± 0.03 g cm^{-3}) was significantly greater than under alfalfa and poplar. At KBS in 2008, the mean bulk density of the poplar plots (1.51 ± 0.05 g cm^{-3}) were significantly less than the means of all other treatments. At KBS in 2013, the mean bulk density of the corn plots (1.44 ± 0.04 g cm^{-3}) were greater compared to the mean bulk density of poplar treatments (1.24 ± 0.05 g cm^{-3}); the mean bulk density of the native prairie and switchgrass treatments did not differ from the corn and poplar treatments.

At 25-50 cm, bulk density did not differ in 2008 at ARL, in 2013 at ARL, or in 2008 at KBS (Appendix Table 1). Crop treatment had a marginally significant effect ($p = 0.08$) on soil bulk density at KBS in 2013, with poplar having lower values (1.6 ± 0.06 g cm^{-3}) than the corn (1.7 ± 0.01 g cm^{-3}), switchgrass (1.8 ± 0.02 g cm^{-3}), and native prairie (1.7 ± 0.04 g cm^{-3}) (Appendix Table 1).

Changes from 2008 to 2013

In ARL, from 2008 to 2013 at 0-10 cm, bulk density decreased in the native prairie treatment ($p = 0.001$) (Appendix Table 1). In ARL, from 2008 to 2013, bulk density (25-50 cm) decreased under switchgrass ($p = 0.02$) (Appendix Table 1). At KBS, from 2008 to 2013 at 0-10 cm, bulk density significantly decreased among all treatments (Appendix Table 1). At depth at KBS, from 2008 to 2013, bulk density significantly decreased under both corn and switchgrass (Appendix Table 1). General trends at both sites showed decreasing bulk density from 2008 to 2013 under all treatments at both depths.

10.2 Appendix Tables

Appendix Table 1. Bulk density (g cm^{-3}) mean and standard error of treatments at Arlington Agricultural Research Station (ARL), WI and Kellogg Biological Station (KBS), MI in soils from 0-10 cm and 25-50 cm in year 2008 and 2013. Alfalfa soil samples and data were only collected at ARL in 2013 (see methods 4.2). Lower case letters indicate significant within year differences among treatments ($p < 0.05$); upper case letters indicate significant within treatment differences from 2008 to 2013 ($p < 0.05$); letters followed by an asterisk (*) indicate marginal significance ($p < 0.1$).

		Bulk Density (g cm^{-3})			
		Arlington Agricultural Research Station, WI		**Kellogg Biological Station, MI**	
Treatment	**Depth (cm)**	**2008**	**2013**	**2008**	**2013**
corn	0-10	1.32 ± 0.06; ab	1.23 ± 0.03; ab	1.70 ± 0.05; Aa	1.44 ± 0.04; Ba
switchgrass	0-10	1.31 ± 0.07; ab	1.31 ± 0.04; a	1.71 ± 0.02; Aa	1.38 ± 0.02; Bab
poplar	0-10	0.96 ± 0.14; b	1.05 ± 0.03; c	1.51 ± 0.05; Ab	1.24 ± 0.05; Bb
prairie	0-10	1.41 ± 0.02; Aa	1.17 ± 0.02; Bbc	1.69 ± 0.02; Aa	1.39 ± 0.03; Bab
alfalfa	0-10	--	1.10 ± 0.02; c	--	--
corn	25-50	1.45 ± 0.03	1.32 ± 0.04	1.86 ± 0.01; A	1.72 ± 0.01; Ba*
switchgrass	25-50	1.44 ± 0.02; A	1.36 ± 0.01; B	1.84 ± 0.01; A	1.76 ± 0.02; Ba*
poplar	25-50	1.35 ± 0.03	1.26 ± 0.04	1.72 ± 0.06	1.60 ± 0.06; b*
prairie	25-50	1.30 ± 0.18	1.32 ± 0.01	1.80 ± 0.08	1.73 ± 0.04; a*
alfalfa	25-50	--	1.28 ± 0.04	--	--

Appendix Table 2. Mean and standard error of C Stock (Mg C ha^{-1}) in soils from 0-10 cm and 25-50 cm in 2008 at Arlington Agricultural Research Station (ARL), WI and Kellogg Biological Station (KBS), MI. Lower case letters indicate significant within site differences among treatments ($p < 0.05$).

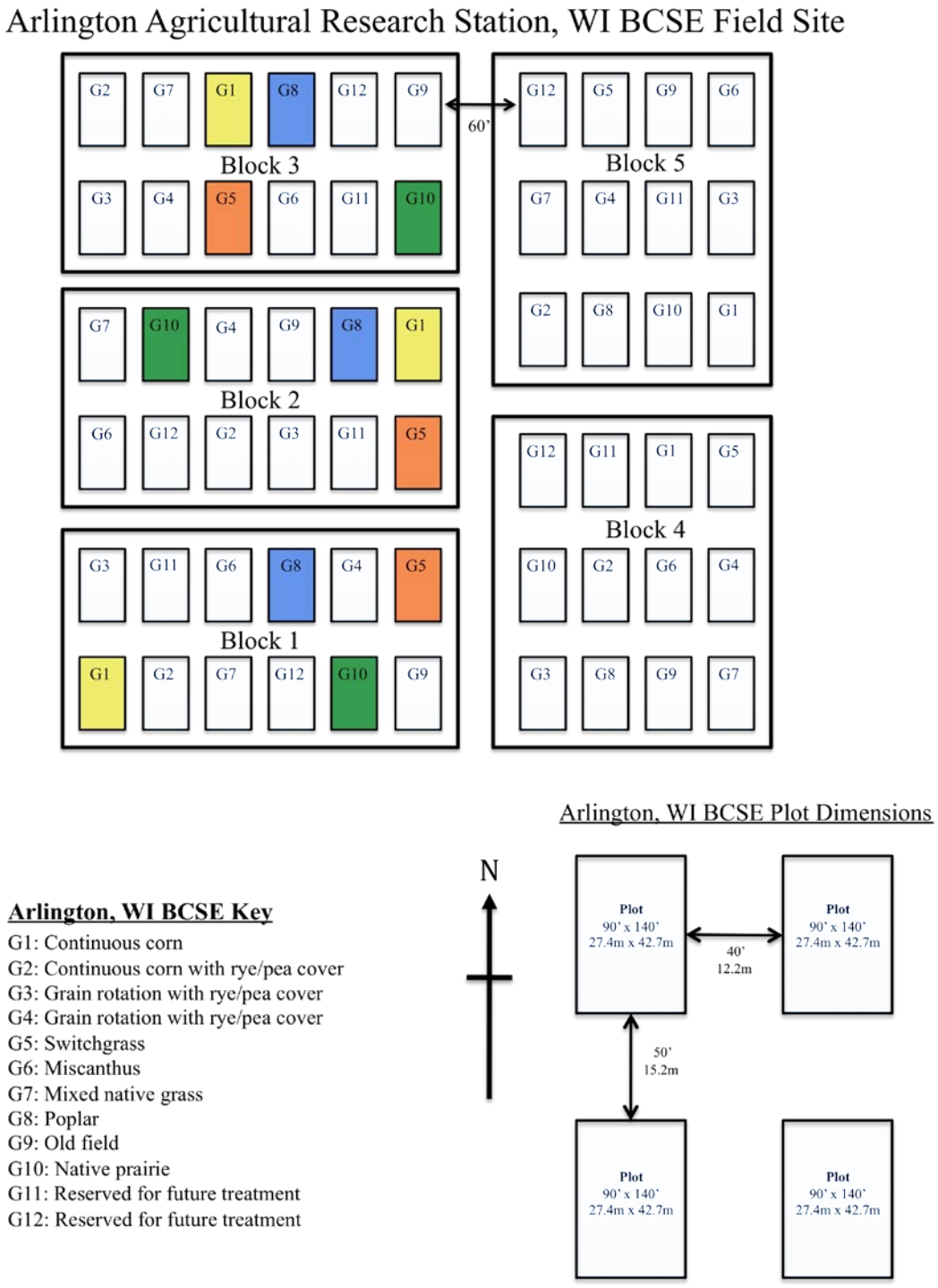
		C Stocks (Mg C ha^{-1})	
		Arlington Agricultural Research Station, WI	**Kellogg Biological Station, MI**
Treatment	**Depth (cm)**	$\overline{X}$ **± SE**	$\overline{X}$ **± SE**
corn	0-10	28.9 ± 2.4	20.9 ± 0.9
switchgrass	0-10	28.8 ± 0.7	21.6 ± 1.3
poplar	0-10	25.0 ± 5.1	20.4 ± 2.1
prairie	0-10	31.7 ± 0.7	20.4 ± 0.3
corn	25-50	33.3 ± 5.2	13.2 ± 1.6; b
switchgrass	25-50	25.3 ± 2.9	14.6 ± 2.0; ab
poplar	25-50	53.0 ± 19.6	22.7 ± 2.0; a
prairie	25-50	25.2 ± 4.1	11.2 ± 0.8; b

Appendix Table 3. Mean and standard error of N Stock (Mg N ha^{-1}) in soils from 0-10 cm and 25-50 cm in 2008 at Arlington Agricultural Research Station (ARL), WI and Kellogg Biological Station (KBS), MI. Lower case letters indicate significant within site differences among treatments ($p < 0.05$).

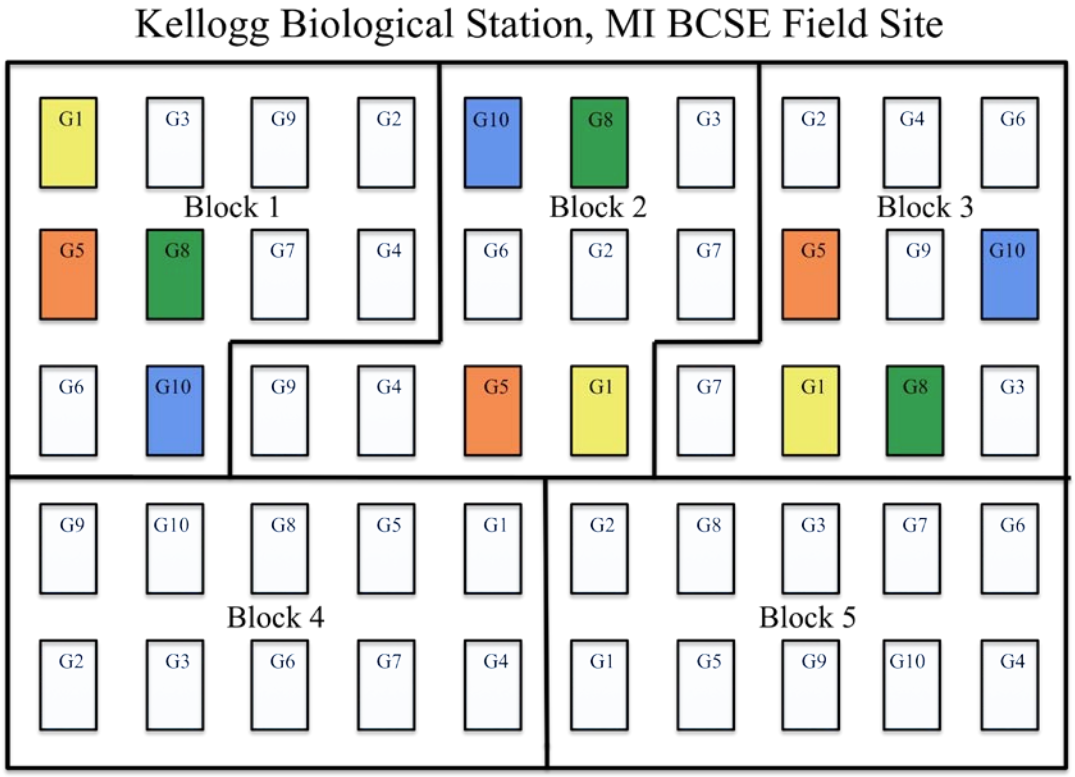
		N Stocks (Mg N ha^{-1})	
		Arlington Agricultural Research Station, WI	**Kellogg Biological Station, MI**
Treatment	**Depth (cm)**	$\overline{X}$ **± SE**	$\overline{X}$ **± SE**
corn	0-10	3.6 ± 1.1	2.1 ± 0.1
switchgrass	0-10	2.6 ± 0.0	2.3 ± 0.2
poplar	0-10	2.2 ± 0.4	2.1 ± 0.2
prairie	0-10	2.8 ± 0.1	2.2 ± 0.1
corn	25-50	6.7 ± 2.2	1.3 ± 0.1; b
switchgrass	25-50	7.0 ± 2.2	1.5 ± 0.1; b
poplar	25-50	7.0 ± 2.4	2.5 ± 0.2; a
prairie	25-50	4.0 ± 1.2	1.2 ± 0.1; b

10.3 Appendix Figures

Appendix Figure 1. Arlington Agricultural Research Station (ARL), WI field design and layout

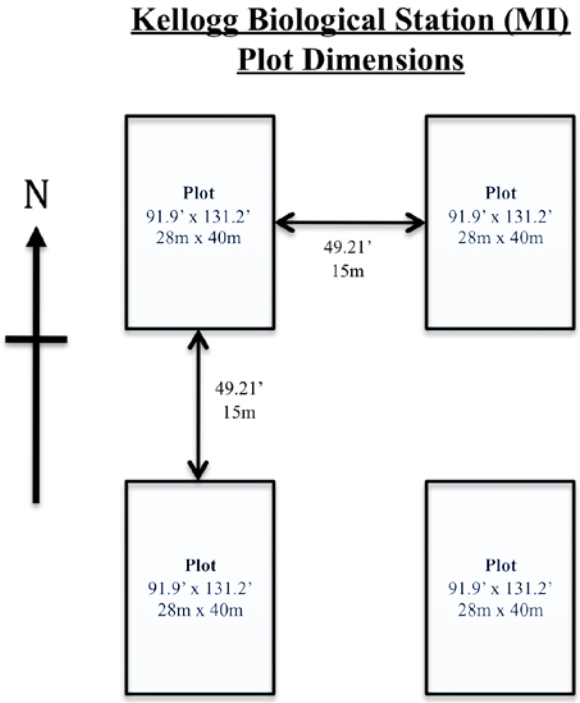


Appendix Figure 2. Kellogg Biological Stations (KBS), MI field design and layout



Kellogg Biological Station (MI) Key

G1: Continuous corn
G2: Continuous corn with rye/pea cover
G3: Grain rotation with rye/pea cover
G4: Grain rotation with rye/pea cover
G5: Switchgrass
G6: Miscanthus
G7: Mixed native grass
G8: Poplar
G9: Old field
G10: Native prairie



Appendix Figure 3. Changes in N Stocks (Mg N ha^{-1}) in soils from 0-10 cm and 25-50 cm at Arlington Agricultural Research Station, WI and Kellogg Biological Station, MI. Bars marked with an asterisk indicate a significant difference ($p < 0.05$), bars marked with two asterisks indicate a marginally significant difference ($p < 0.1$).