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# Land use-land cover gradient demonstrates the importance of perennial grasslands with intact soils for building soil carbon in the fertile Mollisols of the North Central US

Gregg R. Sanford[a,*], Randall D. Jackson[a], Yichao Rui[b], Christopher J. Kucharik[a,c]

[a] Department of Agronomy, University of Wisconsin – Madison, 1575 Linden Drive, Madison, WI 53706, United States
[b] Rodale Institute, 611 Siegfriedale Road, Kutztown, PA 19530, United States
[c] Center for Sustainability and the Global Environment, Nelson Institute of Environmental Studies, 1710 University Ave, Madison, WI 53726, United States

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ABSTRACT

The impact of land use change and agricultural management on the cycling of soil organic carbon (SOC) is not well understood, limiting our ability to manage for, and accurately model, soil carbon changes at both local and regional scales. To address this issue, we combined long-term soil incubations with acid-hydrolysis and dry combustion to parse total SOC ($C_t$) into three operationally defined SOC pools (active, slow, and recalcitrant) from 9 long-term sites with varying land uses on current and former tallgrass prairie soil. Land uses represented a gradient of soil disturbance histories including remnant prairie, restored prairie, grazed pasture, annual crop rotations, and continuous maize. Dry combustion was used to estimate total carbon ($C_t$, physical), while acid hydrolysis of both the active ($C_a$) and slow ($C_s$) pools was used to estimate a recalcitrant carbon pool ($C_r$, chemical). Non-linear modeling of $CO_2$ efflux data from the long-term incubations was then used to estimate $C_a$, and the decomposition rates of both $C_a$ and $C_s$ ($k_a$ and $k_r$, biological). The size of the slow pools $C_s$ was then defined mathematically as $C_t$-($C_a + C_r$). Remnant prairie had the highest $C_t$, while cool-season pasture and a 35-y-old restored prairie had higher $C_t$ than the other agricultural systems. All agricultural systems, including pasture, had the highest fraction of $C_t$ as $C_r$ (~50%), whose mean residence time (MRT) in these soils is ≥500 years (Paul et al., 2001a) demonstrating that this fraction persists, while the more labile fractions were lost over the course of a few months ($C_a$) to a few decades ($C_s$) as a result of tillage-intensive agriculture. The two- to four-decade MRT time of $C_s$ indicated a pool likely to be more responsive to the 20 to 40 years of land-use practices used at some of the sites. The $C_s$ pool was largest in the remnant- and 35-y-old prairies indicating significant C accrual and stabilization compared to the agricultural ecosystems. Interestingly, the remnant prairie maintained the highest $C_a$ pool as well, demonstrating the strong connection between the quantity of fresh C inputs and the potential for long-term C stabilization and accrual. The accumulation of C in active (≈labile) pools as a first step toward long-term stabilization highlights the tenuous nature of early carbon gains, which can be quickly lost in response to climate change or poor management.

## 1. Introduction

Maintenance or accrual of organic carbon (SOC) in agricultural soils has important benefits for both farmers and society and is therefore considered a benchmark for agricultural sustainability (Wiesmeier et al., 2019). SOC is critical for maintaining soil structure, reducing erosion and nutrient pollution, and increasing water holding capacity and plant-available nutrients for food, feed, and fiber production (Lal et al., 2015; Paustian et al., 2016; Zomer et al., 2017). Further, SOC contains more C than the atmosphere and vegetation pools combined (Brady and Weil, 2008; Houghton, 2007; Lal, 2008), so it exerts a large influence on the global C budget (Bellamy et al., 2005). At present there is considerable interest in managing agricultural soils to serve as C sinks, with the goal of reversing historic carbon losses and mitigating rising levels of atmospheric $CO_2$ (Paustian et al., 2016; Sanderman et al., 2017). The North Central U.S. (IA, IL, IN, MI, MN, ND, NE, OH, SD, WI), where roughly three quarters of the nation's corn and soybeans are produced, has experienced some of the greatest SOC losses in the U.S. since the advent

* Corresponding author.
*E-mail address:* gsanford@wisc.edu (G.R. Sanford).

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of 19th Century agriculture (Sanderman et al., 2017; USDA-NASS, 2020). Annual cropping systems replaced much of what was tallgrass prairie in the region and owe much of their productivity to the fertile, carbon-rich Mollisols dominating the landscape (McCulley et al., 2005). Avoided conversion of extant perennial landscapes such as pasture, prairie, and savannah, which tend to have large stocks of SOC, is therefore considered a critical first step in proposed natural climate solutions (NCS, Griscom et al., 2017). Other NCS include enhancing current management practices to reduce soil disturbance, increase the amount of living cover in a system, and reintegrate livestock (Brewer and Gaudin, 2020; Griscom et al., 2017; Wiesner et al., 2020). This approach to fighting climate change is attractive in that it is relatively inexpensive and can be implemented using current agricultural technologies (Harden et al., 2017; Jackson et al., 2017; Lal, 2008). Despite historic losses, recent analysis suggests that cropland alone (~50% of the land area) accounts for ~20% of SOC in the coterminous U.S. (Guo et al., 2006; Kumar, 2015) and with agriculture, forestry, and other land use contributing roughly a tenth of global $CO_2$ emissions, in large part from liberated SOC, it is clear that soils have an important role to play in reversing this trend and mitigating climate change (United States Environmental Protection Agency, 2021). However, soil management can only be considered part of a serious climate mitigation strategy that must ultimately address fossil fuel combustion (Amundson and Biardeau, 2018; Schlesinger and Amundson, 2019).

Agricultural practices promoted for building SOC include fertilization (Nafziger and Dunker, 2011; Poffenbarger et al., 2017), conversion from conventional to reduced- or no-till management (Blanco-Canqui, 2021; Lal, 2015; Ogle et al., 2005), planting cover crops (Blanco-Canqui et al., 2015; Jian et al., 2020), expanding rotations to include perennials or increase diversity (Blanco-Canqui, 2016; Kibet et al., 2015; MacHmuller et al., 2015; Mosier et al., 2021; Poeplau, 2021; Sprunger et al., 2020; Yang et al., 2019), and reintegrating livestock (Brewer and Gaudin, 2020; Wiesner et al., 2020). Uncertainties remain however about the potential for C accrual in agricultural systems and there are many examples in the scientific literature of significant losses even under what are considered *best management practices* (Bellamy et al., 2005; Cates and Jackson, 2019; Keel et al., 2019; Sanderman et al., 2017; Sanford, 2014; Sanford et al., 2012; Steinmann et al., 2016; Szymanski et al., 2019). Maintaining or increasing SOC in light of global change requires a comprehensive understanding of the impacts that current and future agricultural practices have on this valuable resource. Data on changes in total SOC, at depth and over time are difficult to secure, but necessary for accurate assessment of SOC sequestration or loss (Sanderman and Baldock, 2010; Sanford et al., 2012). High variability and large background levels of SOC makes documenting SOC change over time highly uncertain (Necpalova et al., 2014). However, assessment of SOC pools and their turnover times can provide valuable information about agricultural practices that favor SOC loss or accrual long before direct measurement of change can be made (Leifeld and Kögel-Knabner, 2005; Sprunger and Robertson, 2018).

SOM is a complex continuum of plant-, animal-, and microbial-derived residues that differ in their mineralization rates and contribution to soil physical and chemical properties (McLauchlan and Hobbie, 2004; Paul et al., 2001b). While recent work indicates that the most stabilized SOC comes from microbial necromass (Liang et al., 2017), the SOC continuum is often conceptually or operationally defined by subdivision into fractions which vary in both size and turnover time (Cates et al., 2019; Paul et al., 2006; Six et al., 2002). Several fractionation methods have been employed to separate SOC into relatively labile and recalcitrant pools, based on physical or chemical soil properties (McLauchlan and Hobbie, 2004; Paul et al., 2006; Poirier et al., 2005; von Lutzow et al., 2007). The “acid hydrolysis-incubation (AHI)” method is one such approach that combines biological and chemical fractionation to estimate the size and decomposition rate of a rapidly mineralized or “active” C pool ($C_a$), a slowly mineralized C pool ($C_s$), and an older or “recalcitrant” C pool ($C_r$) (Collins et al., 2000; Paul et al., 2006, Paul et al., 1999). While chemical recalcitrance is no longer considered a primary mechanism providing long-term carbon stabilization, the residual non-hydrolysable carbon isolated via acid hydrolysis is reproducibly much older than the bulk soil carbon and provides a reasonable estimate of “stable” carbon ($C_r$) with which to constrain the non-linear three pool model used to estimate $C_a$ and $C_s$ (Collins et al., 2000; Paul et al., 2006, Paul et al., 2001a). It is also worth noting that despite laboratory artifacts which occur with all in-vitro methods of soil fractionation (e.g. aggregate disruption via soil sieving, microbial lysis, absence of autotrophic respiration, etc.), the AHI method has a long-history of effective use evaluating relative differences between agricultural systems (Collins et al., 2000; Paul et al., 2006, 2001a; Sanford and Kucharik, 2013).

Generally, the relatively labile fractions of SOC contribute to soil microbial activity, SOM decomposition, and nutrient cycling (Cambardella and Elliot, 1994; Cookson et al., 2005; Elliott, 1997), whereas more stable forms of SOC have longer residence times and exert greater influence on the physicochemical reactivity of soil (Baldock and Smernik, 2002; Krull et al., 2004; Schmidt et al., 2011). While the turnover times of these pools range from years to millennia, it is clear that all of these pools are decomposing and being recharged continuously (Haddix et al., 2020; Kuzyakov et al., 2019). Evaluating multiple pools with varying levels of persistence provides key insight about the mechanisms underpinning carbon accrual in agroecosystems as well as the sensitivity of carbon stocks to changes associated with management and climate.

Sanford et al. (2014; 2012) reported long-term SOC losses under agricultural best management practices on Mollisols in southern Wisconsin. In these studies, both warm- and cool-season perennial grasslands showed greater potential to stabilize and/or accrete SOC than annual grain and semi-perennial cropping systems. Other work has shown that more diverse grassland species assemblages have greater capacity to accumulate SOC (Liang et al., 2016; Yang et al., 2019; Zhu et al., 2020). Here, we used the AHI method to evaluate SOC dynamics on Mollisols of southern Wisconsin along a land use-land cover (LULC) gradient ranging from high-input, low-diversity cropping systems (i.e., continuous maize) to low-input, high-diversity grasslands (i.e., remnant tallgrass prairie). We hypothesized that 1) total soil organic carbon ($C_t$) and subsequent rates of $CO_2$ evolution would be greatest in perennial grasslands, 2) tillage would result in a greater proportion of $C_t$ in the $C_r$ pool as a result of oxidative losses from $C_a$ and $C_s$, and that conversely, 3) perennial grasslands or systems with limited tillage would have the highest proportion of $C_t$ in rapidly and slowly mineralized pools (i.e., $C_a$ and $C_s$).

## 2. Methods

### 2.1. Land use-land cover gradient

We assessed SOC dynamics from 8 LULC categories ranging from low-diversity/high-input annual cropping systems to high-diversity/low-input perennial grasslands selected based on 1) edaphic similarity (soil classification and climate), 2) age (at least 10 years since establishment), and 3) representativeness to ecosystems of the North Central United States (Tables 1 and 2). Five of the 8 LULCs were part of the Wisconsin Integrated Cropping Systems Trial (WICST) located at the University of Wisconsin-Madison’s Arlington Agricultural Research Station (ARL: 43°18′10″N, 89°20′43″W), two of the 8 LULCs were at the Wisconsin Crop Rotation Trial (WCRT, also at ARL and 1.6 km from WICST) including one shared with and LULC at WICST (see below), one was located on land managed by the Wisconsin Department of Natural Resources in Waunakee, WI (WW, 43°13′57″N, 89°26′22″W), which is 10.9 km south of ARL), and one was located at the Madison Audubon’s Goose Pond Prairie. Additional details for WICST and WCRT can be found in Posner et al. (1995) and Pedersen and Lauer (2002), respectively.

All soils were classified as Plano silt loam (Fine-silty, Mixed,

**Table 1**
LULC soil physical and chemical parameters. Numbers in parentheses equal one standard error of the mean.

| Site | Age (y) | LULC[†] | Management | n | USDA soil series[‡] | Texture | pH | SOM[§] | P[¶] | K[¶] | Ca[#] | Mg[#] | CEC[††] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | g kg$^{-1}$ | mg kg$^{-1}$ | | | | |
| WCRT | 27 | m-m | conventional | 3 | Plano | silt loam | 5.2 (0.1) | 31 (0.0) | 13 (0.7) | 112 (0.9) | 1271 (12) | 343 (6) | 9 (0.0) |
| | 27 | m-m | no-till | 3 | Plano | silt loam | 5.1 (0.1) | 35 (0.3) | 18 (0.9) | 142 (1.2) | 1163 (7) | 331 (4) | 8 (0.0) |
| WICST | 20 | m-m | conventional | 3 | Plano | silt loam | 6.1 (0.1) | 38 (0.3) | 62 (4.9) | 172 (8.1) | 1547 (16) | 429 (10) | 11 (0.0) |
| | 20 | m-s | no-till | 3 | Plano | silt loam | 6.3 (0.1) | 37 (1.2) | 46 (0.7) | 114 (1.2) | 1720 (33) | 507 (12) | 12 (0.3) |
| | 20 | m-a-A-A | conventional | 3 | Plano | silt loam | 6.7 (0.0) | 36 (0.6) | 62 (0.9) | 111 (2.1) | 1693 (141) | 535 (12) | 12 (0.6) |
| | 20 | pasture | rotational grazing | 3 | Plano | silt loam | 6.1 (0.1) | 42 (0.0) | 46 (0.3) | 121 (1.5) | 1545 (25) | 492 (3) | 11 (0.3) |
| | 11 | 11 yr. prairie restoration | periodic burns | 3 | Plano | silt loam | 6.5 (0.1) | 39 (0.3) | 49 (1.0) | 137 (2.8) | 1794 (93) | 492 (8) | 12 (0.7) |
| GP | 35 | 35 yr. prairie restoration | periodic burns | 3 | Plano | silt loam | 5.8 (0.1) | 40 (0.7) | 42 (0.3) | 100 (1.2) | 1680 (46) | 364 (10) | 10 (0.0) |
| WW | n/a | remnant prairie | historic grazing, and annual burning | 3 | Plano | silt loam | 5.5 (0.1) | 44 (0.6) | 8 (0.7) | 90 (1.2) | 1659 (28) | 405 (5) | 11 (0.3) |

[†] Abbreviations: a – first year alfalfa, A – established alfalfa hay, m – maize, s - soybean.
[‡] Plano silt loam: Fine-silty, Mixed Superactive, Mesic Typic Argiudolls; source: https://soils.usda.gov/technical/classification/osd/index.html.
[§] SOM = soil organic matter determined by weight loss on ignition.
[¶] Available P and K (Bray P1 extract).
[#] Exchangeable Ca and Mg (1 N $NH_4OAc$, pH 7.0).
[††] Calculated cation exchange capacity: CEC = $((Ca_{ppm} \div 200) + (Mg_{ppm} \div 122) + (K_{ppm} \div 391)) \times (5\ g\ sample \div 5\ g\ cc^{-1})$, source:http://uwlab.soils.wisc.edu/files/procedures/cation_exch_capacity.pdf.

**Table 2**
LULC site characteristics and management details.

| Site | Age (y) | LULC[†] | management | soil sampling | | | primary tillage equipment | N Inputs | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | n | Depth (cm) | Protocol | | 1st yr. avail. (kg ha$^{-1}$) | timing | source[‡] |
| WCRT | 27 | m-m | conventional | 72 | 0–15 | 4 field reps, 18 cores per. plot, homogenized | chisel plow | 196 | annual | F |
| | 27 | m-m | no-till | 72 | 0–15 | 4 field reps, 18 cores per. plot, homogenized | no-till | 196 | annual | F |
| WICST | 20 | m-m | conventional | 54 | 0–15 | 3 field reps, 18 cores per. plot, homogenized | chisel plow | 142 | annual | F |
| | 20 | m-s | no-till | 54 | 0–15 | maize phase only: 3 field reps, 18 cores per. plot, homogenized | no-till | 136 | maize phase | L, F |
| | 20 | m-a-A-A | conventional | 54 | 0–15 | maize phase only: 3 field reps, 18 cores per. plot, homogenized | chisel plow | 240 | pre and post maize | L, F, M |
| | 20 | pasture | rotational grazing | 54 | 0–15 | 3 field reps, 18 cores per. plot, homogenized | n/a | 52 | throughout season | M |
| | 11 | 11 yr. prairie restoration[§] | periodic burns | 54 | 0–15 | 3 field reps, 18 cores per. plot, homogenized | n/a | n/a | n/a | n/a |
| GP | 35 | 35 yr. prairie restoration[¶] | periodic burns | 48 | 0–15 | 12 cores from 4 random areas, homogenized | n/a | n/a | n/a | n/a |
| WW | n/a | remnant prairie[#] | historic grazing, annual burning[††] | 48 | 0–15 | 12 cores from 4 random areas, homogenized | n/a | n/a | n/a | n/a |

[†] Abbreviations: a – first year alfalfa, A – established alfalfa hay, m – maize, s – soybean.
[‡] F = fertilizer, L = legume, M = manure.
[§] Dominant native species included: big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans* [L.] nash), Canada wild rye (*Elymus canadensis* L.), and sawtooth sunflower (*Helianthus grosseserratus* M. Martens).
[¶] Dominant native species included: big bluestem, indiangrass, switchgrass (*Panicum virgatum* L.), purple coneflower (*Echinacea purpurea* [L.] Moench), goldenrod (*Solidago* spp. L.), and black-eyed susan (*Rudbeckia hirta* L).
[#] Dominant native species included: big bluestem, indiangrass, and needlegrass (*Achnatherum* spp. P. Beauv.).
[††] Prairie remnant grazed on and off until 2000 at which point management was taken over by the WI - Department of Natural Resources. Annual burning has occurred since 2000 to manage unwanted invasive species.

Superactive, Mesic Typic Argiudolls (Table 1). These are relatively deep (>1 m), well-drained Mollisols that formed under tallgrass prairie vegetation in loess deposits over calcareous glacial till. The 30-yr mean annual temperature and precipitation for this area were 6.9 °C and 869 mm, respectively (NOAA, 2018). The 8 LULCs evaluated were selected along a management intensity gradient that accounted for both the level of agricultural intervention and the application generally accepted soil conservation practices. From most intensively to least intensively managed these included: 1) tilled continuous maize (maize-WICST and maize-WCRT), 2) no-till continuous maize (NT maize-WCRT), 3) minimum-till maize-soybean (maize-soy-WICST), 4) maize with three years of alfalfa hay (forage-WICST), 5) rotationally grazed cool-season pasture (pasture-WICST), 6) 11-y-old tallgrass prairie restoration (11-y prairie-WICST), 7) 35-y-old tallgrass prairie restoration (35-y prairie-WW), and 8) remnant prairie (Rem-P-WW). A full description of management practices at each of the LULCs is presented in Table 2.

### 2.2. Soil sampling, processing, and analysis

We collected soil samples from each LULC in summer 2010 to a depth of 15 cm using a 2-cm diameter hand-held soil probe (see Table 2 for details). Soil samples from each LULC were thoroughly homogenized across field replicates or sampling areas (Table 2), sieved to 4 mm, picked free of all visible plant material, and stored at 4 °C until further processing. To minimize within-LULC variability all further analysis were pulled from these homogenized soils. Aliquots ($n = 3$) of each soil sample were analyzed for texture, nutrient content, and non-hydrolysable carbon (NHC). Soil texture was determined on three 50-g samples from each LULC using a standard hydrometer method (Peters, 2018). For standard agronomic nutrient analysis 100-g samples from each LULC were sent to the University of Wisconsin Soil and Plant Analysis Lab (SPAL) for determination of pH (1:1, soil:water), organic matter (weight loss-on-ignition, 360 °C), available P and K (Bray P1 extract), exchangeable Ca and Mg (1 N $NH_4OAc$, pH 7.0), and cation exchange capacity. NHC was determined for each sample ($n = 3$) by refluxing three 2-g samples of soil from each treatment in 20 ml of 6 M HCl at 115 °C for 16 h according to standard published protocols (Paul et al., 2006; Sollins et al., 1999). For SOC determination, aliquots ($n = 4$) from each LUCL were finely ground, weighed (8 to 10 mg), packed into a 5 × 9-mm tin capsule, and analyzed on a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy).We use total C interchangeably with SOC in this study because inorganic C in these surface soils is negligible (<0.05 g $kg^{-1}$, Paul et al. (2001a)).

### 2.3. Soil incubations to determine $CO_2$ fluxes

Samples were prepared for long term soil incubations (230 d) by packing sufficient field moist soils into a 100-ml specimen cup to reach a desired dry bulk density of 1.27 g $cc^{-1}$ (Sanford and Kucharik, 2013). Five specimen cups (replicates) were prepared for each LULC ($n = 35$). Soils were then wetted to 60% water filled pore space (WFPS) (Linn and Doran, 1984) based on their packed bulk density (1.27 g $cc^{-1}$) and an estimated particle density of 2.65 g $cc^{-1}$ (Campbell and Norman, 1998). Packed specimen cups were then placed in 950-ml glass canning jars, and 20 ml of deionized water was added to the bottom of each jar to maintain internal humidity. Vented metal lids (2 × 7-mm dia. hole, 2% of lid area) were placed on the jars and the soils were allowed to stabilize for 16 h in the dark at 22.2 °C prior to initial $CO_2$ flux measurements (Sanford and Kucharik, 2013). Air temperature was held constant at 22.2 °C for the duration of the soil incubations.

We measured soil $CO_2$ fluxes with a LI-820 infrared gas analyzer (IRGA) (LI-COR Biosciences, Lincoln, NE), which recorded $CO_2$ concentrations (μl $L^{-1}$) every 10 s over 10-min intervals. Flux rates were determined by fitting a simple linear regression model to the output data and then converting from $CO_2$ concentration change over time (μl $L^{-1}$ $s^{-1}$) to mass loss of C over time (μg C [g soil]$^{-1}$ $day^{-1}$). Following each IRGA reading, soil moisture was adjusted to 60% water-filled pore space. Vented lids were then re-attached to the incubation chambers and the jars were placed in the dark until the next IRGA reading. Readings were taken every few days for the first month and then approximately monthly until day-125 at which point readings increased to multiple events per month until the end of the experiment. This resulted in a total of 25 readings over 230 d. Soil moisture content was maintained within 1% of optimum for microbial activity (Linn and Doran, 1984) throughout the study by adding water weekly to each specimen cup to replace evaporative losses, irrespective of IRGA measurements.

### 2.4. Estimating SOC parameters

A three-pool constrained model (Eq. (1)) with first order kinetics was used to evaluate the size and decomposition rates of three SOC pools for each LULC (Eq. (1)) (Paul et al., 2001b).

$$C_{t(t)} = C_a e^{-k_a(t)} + C_s e^{-k_s(t)} + C_r e^{-k_r(t)} \tag{1}$$

In this model, $C_{t(t)} =$ is total SOC at time $t$; $C_a$, $C_s$, and $C_r$ represent the C mass in the active (i.e. rapidly mineralized), slowly mineralized, and recalcitrant (i.e. non-hydrolysable) fractions respectively; $k_a$, $k_s$, and $k_r$ are the decomposition rates of each fraction equal to the inverse of each pools mean residence time (MRT). SOC data estimated via dry combustion was used for $C_t$ at time = 0, and $C_r$ was estimated by 6 N HCl acid hydrolysis (section 2.2). Paul et al. (Paul et al., 2001a), evaluating Plano silt loam soils from UW-ARL under continuous maize management, reported an MRT for $C_t$ and $C_r$ of 485 and 2840 years respectively. As parameter estimates for $C_a$, $k_a$, and $k_s$, from non-linear regression modeling (see below) did not differ when either of these two values was used for the MRT of $C_r$, the more conservative value of 500 years was used to account for the diversity of LULCs evaluated. Both $C_r$ and $k_r$ serve to constrain the three-pool model to estimate the other model parameters. The first derivative of Equation (1) was used to estimate $C_a$, $k_a$, and $k_s$ via curve fitting of $CO_2$ flux data from each individual incubation chamber ($n = 5$ per treatment) using the NLIN procedure (METHOD = MARQUART) of SAS version 9.4 (Eq. (2)).

$$-\frac{dC}{dt} = C_a k_a e^{(-k_a t)} + C_s k_s e^{(-k_s t)} + C_r k_r e^{(-k_r t)} \tag{2}$$

The mean residence time (MRT) for each of the three pools was obtained via the inverse of the decomposition rate (1/k) scaled to field temperatures by multiplying the lab MRT by a $Q_{10}$ of 2.89 (Eq. (3)) based on the difference between laboratory temperature (labT = 22.2 °C) and mean annual temperature (MAT = 6.9 °C).

$$Q_{10} = \left(2^{\left(\frac{labT - MAT}{10}\right)}\right) \tag{3}$$

Finally, $C_s$ was estimated by subtracting $C_a$ and $C_r$ from SOC. See Fig. 1 for a schematic representation of the modeling and AHI process.

### 2.5. Statistical analyses

Non-linear regression model differences were evaluated via F-tests on model reduction (Eq. (4)).

$$F = \frac{\left(\frac{SSE_{(reduced)} - SSE_{(full)}}{df_{SSE(reduced)} - df_{SSE(full)}}\right)}{\left(\frac{SSE_{(full)}}{df_{SSE(full)}}\right)} \tag{4}$$

where, $SSE$ = sums of squares for error and $df$ = degrees of freedom. Numerator degrees of freedom for the F-test were calculated as the difference between the full and reduced model error degrees of freedom, and the denominator degrees of freedom for the F-test were taken from the error degrees of freedom from the full model. A Bonferroni

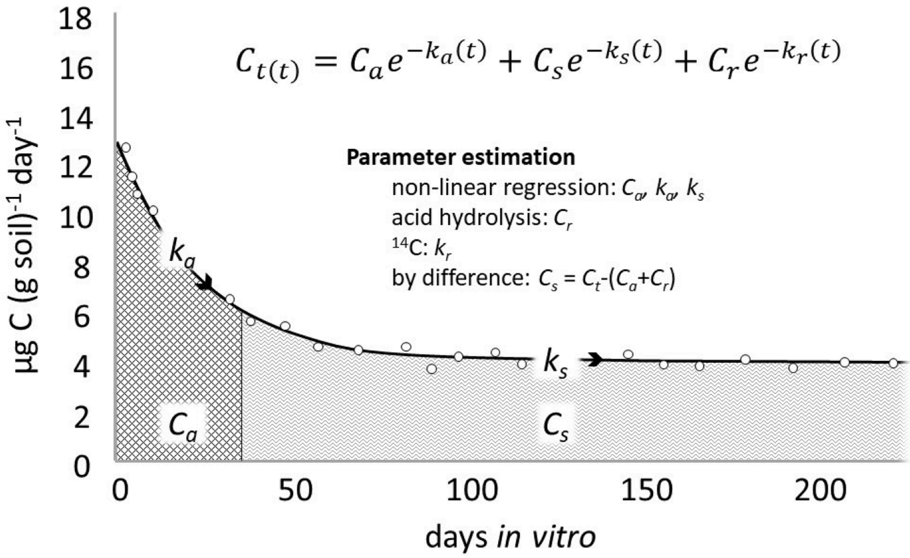


**Fig. 1.** Schematic depicting non-linear regression analysis of long-term soil incubation data used in conjunction with 6 N HCL acid hydrolysis and $^{14}$C dating to estimate three conceptual carbon pools ($C_a$, $C_s$, $C_r$) and their mean residence times ($1/k_a$, $1/k_s$, $1/k_r$).

correction was applied to all p-values to address the issue of multiple statistical comparisons. LULCs with statistically indistinguishable non-linear regression models were grouped together and analyzed using a single non-linear regression model for the final analysis (section 3.1). Asymptotic confidence limits (95%) provided by the NLIN Procedure in SAS v9.4 were used to compare parameter estimates for $C_a$, MRT-$C_a$, and MRT-$C_s$.

The MIXED procedure in SAS version 9.4 was used to analyze soil physical and chemical data. Each dependent variable was analyzed using a completely randomized design model structure. The resulting mixed effect model was.

$$y = \mu + R_i + T_j + \varepsilon_{ij} \tag{5}$$

where, $\mu$ = population mean, $R$ = random effect of the $i^{th}$ replicate (n = 3), $T$ = fixed effect of the $j^{th}$ LULC (n = 5), and $\varepsilon$ = the error term associated with the interaction of the $j^{th}$ replicate and $i^{th}$ LULC. Orthogonal contrasts were used to further investigate questions specific to our initial hypotheses.

## 3. Results

### 3.1. Model performance and groupings

Non-linear regression model comparisons of $CO_2$ flux data (see Eq. (4)) winnowed our original 8 independent LULCs into five distinct ($p < 0.01$) LULC groupings (LULCGs): 1) Remnant prairie (**REM**), 2) 35-year prairie (**35yP**), 3) pasture (**PAST**), 4) ‘conservation agriculture’ (**CA**), and 5) ‘conventional agriculture’ (**AG**) (Table 3 and Fig. 2). REM had the highest initial $CO_2$ respiration rate (16 µg C [g soil]$^{-1}$ day$^{-1}$) and longest interval (~100 day) between the start of the of incubation and when the $CO_2$ flux began to stabilize at around 4 µg C (g soil)$^{-1}$ day$^{-1}$. 35yP had the most distinct $CO_2$ respiration profile with a high initial flux rate (13 µg C [g soil]$^{-1}$ day$^{-1}$) that dropped rapidly to around 6 µg C (g soil)$^{-1}$ day$^{-1}$ between days 50 and 150 before dropping to 4 µg C (g soil)$^{-1}$ day$^{-1}$ by day 230. In CA, $CO_2$ flux rates ranged from 10 to 15 µg C (g soil)$^{-1}$ day$^{-1}$ at day 0 and began to stabilize at around 4 µg C (g soil)$^{-1}$ day$^{-1}$ by day 75. The non-linear model fit for the Pasture-WICST LULC, though visually similar, differed significantly ($p < 0.01$) from CA and was therefore kept separate from CA. In PAST, initial $CO_2$ flux rates were 2 to 4 µg C (g soil)$^{-1}$ day$^{-1}$ lower than those in CA, and stabilization at 4 µg C (g soil)$^{-1}$ day$^{-1}$ occurred by approximately day 50, 25 days earlier than observed for CA. AG displayed the greatest total variability throughout the 230 day incubation as well as the lowest average initial flux rate of 6 µg C (g soil)$^{-1}$ day$^{-1}$ which stabilized by day 50 around 4 µg C (g soil)$^{-1}$ day$^{-1}$, similar to PAST. Non-linear regression models for each LULCG (REM, 35yP, CA, PAST, and AG) are presented in Fig. 2. Although flux rates slowed 30 to 75% in the course of the 230-day incubation, it was unclear if $CO_2$ flux in all of the LULCGs had reached a long-term equilibrium by the end of the study. This clearly was not the case for 35yP in which $CO_2$ flux was not well explained by a three-pool model with first order kinetics (Fig. 2).

**Table 3**
LULCGs and their descriptions based on F-tests of non-linear model reduction (Eq. (4)), including the LULCs contained within each LULCG.

| LULCG | description | LULC |
|---|---|---|
| REM | remnant prairie | remnant prairie (WW) |
| 35yP | 35-year prairie restoration | 35 yr. prairie restoration (GP) |
| CA | conservation agriculture | no-till m-m (WCRT), 11 yr. prairie restoration (WICST) |
| PAST | pasture | pasture (WICST) |
| Ag | conventional agriculture | m-m (WCRT), m-m (WICST), m-s (WICST), m-a-A-A (WICST) |

### 3.2. Breakdown of C pools among LULCGs

Total SOC was highest in REM, second highest in 35yP and PAST, and lowest in CA and AG, which both had LULCs that included annual crops. The most labile pool ($C_a$) in REM was almost twice the size of the next highest grouping, which included all but AG. Likewise, the $C_s$ pool was highest in REM, while the $C_r$ pool was highest in CA, PAST, and AG (Table 4). Perhaps more informative than these total pool sizes were their relative contributions to total SOC and the estimated MRT for each. Again, REM had the highest portion of total SOC as $C_a$, but the MRT of REM was not significantly different than CA, PAST, and AG. Instead, a higher proportion of SOC was $C_s$ in REM and 35yP, but 35yP had the lowest MRT in $C_s$, which was highest in REM.

### 3.3. Labile carbon depleted in production agriculture

The size of the rapidly mineralized C pool ($C_a$) was small relative to SOC and quite variable, ranging from 0.6% SOC for REM to 0.3% SOC for 35yP and PAST. The MRT of $C_a$ was also quite variable but followed

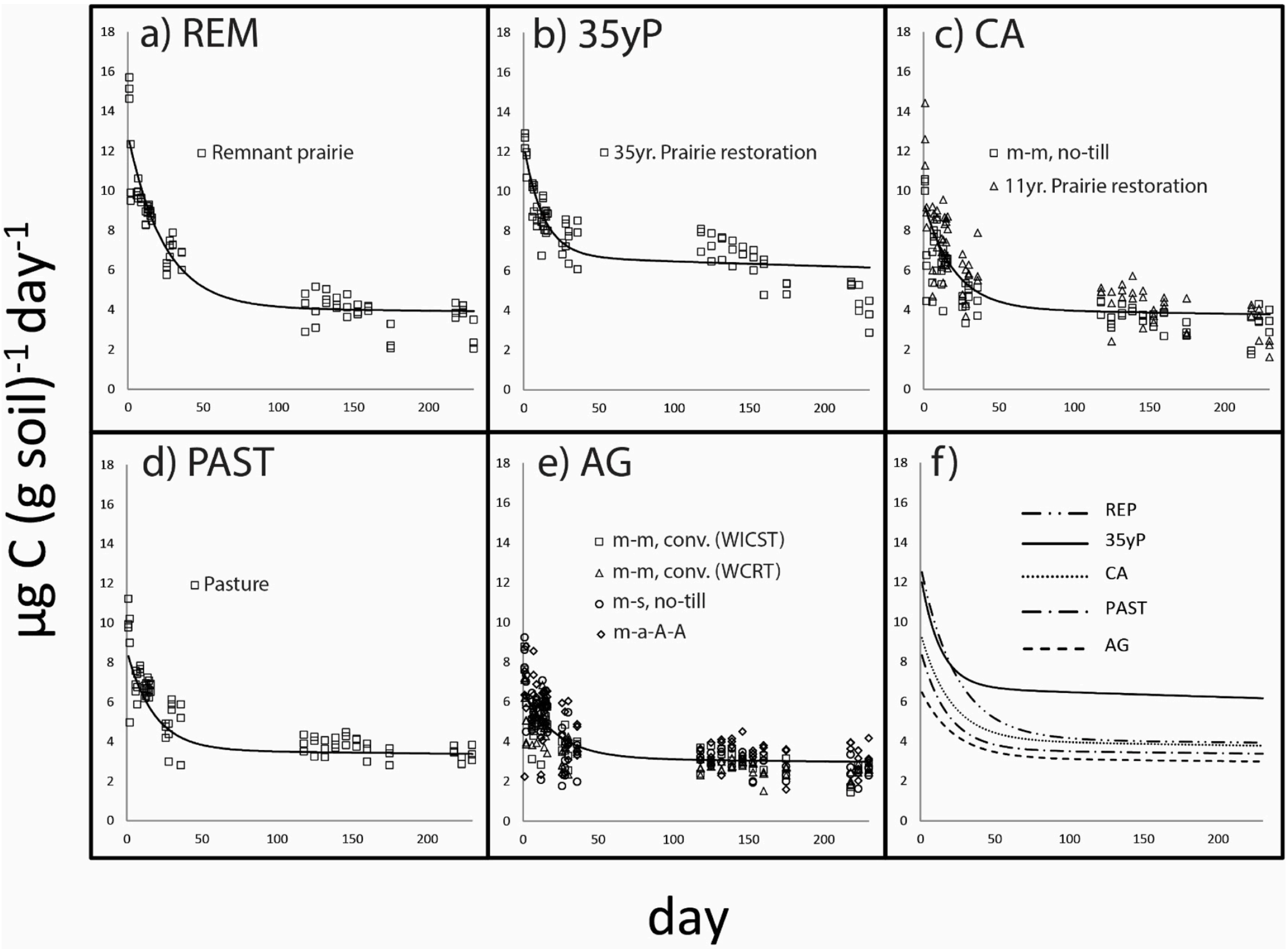


**Fig. 2.** Respiration data and non-linear regression models for the five distinct land use-land cover groupings (LULCGs). a) REM, b) 35yP, c) CA, d) PAST, e) AG, and f) the five LULCG models overlaid together.

**Table 4**
Parameter estimates for total soil organic carbon, carbon pools and their mean residence times for the five distinct land use-land cover groupings (LULCGs). Different letters within a given parameter indicate significance at α = 0.05.

| LULCG | description | n | SOC | $C_a$[†] | | | | $C_s$ | | | | $C_r$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | pool | | field MRT | | pool[‡] | | field MRT | | pool[§] | | field MRT[¶] |
| | | | g kg$^{-1}$ | g kg$^{-1}$ | %SOC[#] | day | CL[††] | g kg$^{-1}$ | %SOC | yr | CL | g kg$^{-1}$ | %SOC | yr |
| **REM** | remnant prairie | 3 | 34.2[a] | 0.21[a] | 0.6[a] | 69[a] | 58–87 | 22.9[a] | 67.0[a] | 44[a] | 40–49 | 11.1[b] | 32.4[b] | 500 |
| **35yP** | 35 year prairie restoration | 3 | 26.7[bc] | 0.07[b] | 0.3[c] | 37[b] | 28–55 | 17.0[b] | 63.5[a] | 21[d] | 20–22 | 9.7[b] | 36.2[b] | 500 |
| **CA** | conservation agriculture[‡‡] | 6 | 24.4[cd] | 0.11[b] | 0.5[b] | 58[ab] | 45–80 | 12.6[cd] | 51.6[b] | 26[c] | 24–29 | 11.7[b] | 47.9[a] | 500 |
| **PAST** | pasture | 3 | 29.4[b] | 0.09[b] | 0.3[c] | 52[ab] | 40–73 | 14.5[bc] | 49.4[b] | 32[b] | 29–35 | 14.8[a] | 50.3[a] | 500 |
| **AG** | conventional agriculture[§§] | 11 | 22.8[d] | 0.08[b] | 0.4[c] | 63[ab] | 20–83 | 11.3[d] | 49.6[b] | 31[b] | 29–33 | 11.4[b] | 50.0[a] | 500 |

† $C_a$ = rapidly mineralized carbon, $C_s$ = slowly mineralized carbon, and $C_r$ = non-hydrolysable carbon.
‡ $C_s$ pool size = SOC-$C_a$-$C_r$.
§ $C_r$ pool estimated via 6 N HCl hydrolysis.
¶ MRT was set conservatively at 500 yr based on $^{14}$C dates for SOC reported in Paul et al. (2001a).
\# %SOC = percent of total SOC accounted for in a given pool.
†† CL = 95% confidence limit.
‡‡ CA: NT maize-WCRT and 11-y prairie-WICST.
§§ AG: maize-WCRT, maize-WICST, maize-soy-WICST, forage-WICST.

the patterns in the pool size of $C_a$ very closely. Turnover of $C_a$ was slowest in REM and fastest in 35yP (Table 4). Despite the high parameter variability associated with $C_a$, the combined proportion of mineralized C ($C_a$ + $C_s$) relative to SOC followed the trends that we initially hypothesized. The highest proportion of mineralized C was associated with REM at 67.6% SOC followed by 35yP at 63.8% SOC and CA at 52.1% SOC. Mineralized C accounted for roughly 50% of SOC in both PAST and AG. Implicit in these results is that $C_r$ occupied a greater proportion of SOC in the agricultural systems (CA, PAST, and AG) than it did in the native prairie systems (REM and 35yP).

## 4. Discussion

### 4.1. Soil disturbance of primary importance to total SOC and distribution of SOC fractions

Little to no soil disturbance was associated with greater SOC quantity, but the more 'resistant' or 'passive' SOC ($C_r$) was a greater proportion of total SOC in the more highly disturbed systems (e.g., AG). This second point seems counterintuitive given the focus on finding ways through improved management (e.g. cover crops, perennials) to direct C to this 'stabilized' pool. Closer examination however shows that a higher proportion of $C_r$ in the disturbed systems reflects a higher degree of loss in the more active ($C_a$), and slowly cycling ($C_s$) carbon pools in ecosystems with recent soil disturbance. This is consistent with modeling exercises (e.g., the Century Model) in which passive soil C (i.e., $C_r$) occupies a greater proportion of total SOC in cultivated systems (Parton et. al., 1987). We know that the frequency and intensity of soil disturbance are significant drivers of SOC stocks (Tiefenbacher et al., 2021; West and Post, 2002), but these results make clear how much more important this factor is than vegetation quantity or quality in Mollisols. For example, within LULCGs, vegetation as morphologically and phenologically distinct as corn and tallgrass prairies had similar SOC stocks. Interestingly, previous studies have been unable to detect this proportional shift toward passive carbon (NHC) in tilled systems (see Paul et al., 2006), highlighting the sensitivity of the current experiment, and the importance of evaluating multiple land management practices while constraining edaphic variability as much as possible. In the systems where tillage had occurred in recent decades (i.e., all but REM and 35yP), about half of the total SOC ($C_t$) was C that remained after acid hydrolysis ($C_r$) indicating that historic tillage has likely led to oxidation of the more active fractions ($C_a$ and $C_s$), which occupy a large part of the SOC pool in the undisturbed remnant prairie. It appears that even in systems that have been undisturbed for decades (e.g. 35yP) these active fractions have yet to be sufficiently 're-charged' by new C inputs.

### 4.2. High amounts of C inputs important too

REM and 35yP only had about 1/3 of their SOC in $C_r$ while maintaining the highest overall SOC stocks, indicating the importance of C inputs to the system accumulating in more readily mineralizing forms (i.e. $C_a$ and $C_s$), which comprised almost 2/3 of the total SOC. The 'mineralizability' or 'lability' of these C pools is relative to the soil disturbance regimen. This point was emphasized to an even higher degree when examining the very active fraction of SOC ($C_a$) in the remnant prairie, which was about twice the size of the other treatments. These active and slowly mineralizing SOC pools are important contributors to the overall SOC stock but also highly sensitive to significant changes in land management. Avoiding the conversion of these perennial grasslands to intensive annual cropping of maize and soybean is critical to maintaining SOC in the fertile Mollisols of the North Central US.

In the current study our estimates of $C_a$ were<1% of SOC. These estimates are similar to those found by Schwendenmann and Pendall (2008) in tropical Oxisols (0.3 to 0.9% SOC) and of Haile-Mariam et al. (2000) in California Ultisols (0.7 to 0.9% SOC) but are lower than those reported by Collins et al. (2000) from agricultural Mollisols collected at UW-ARL (~2% SOC). This difference may be largely methodological in nature and associated with vented rather than unvented chambers and the use of an infrared gas analyzer (IRGA) rather than chemical capture of $CO_2$ (2 M NaOH) followed by titration (Collins et al., 2000; Sanford and Kucharik, 2013). The relatively large $C_a$ in the remnant prairie reflects millenia without soil disturbance coupled with large quantities of fine-root inputs and their microbially processed biproducts, including microbial necromass (Liang et al., 2017). Plots with any long-term tillage history (i.e., all but REM) had roughly equivalent $C_a$ concentrations and the generally lower quantity of $C_a$ in these categories, including the grassland systems, suggests that C dynamics in these systems largely reflect recent (decadal) agricultural history. $C_a$ is thought to consist of partially decomposed plant residues and non-occluded labile C from root and microbial biomass (Collins et al., 2000; Haile-Mariam et al., 2000; Paul et al., 2011). The long MRTs associated with $C_a$ and $C_s$ in REM, relative to the other LULCGs, may reflect the influence of fine root morphology and extensive mycorrhizal colonization on the stabilization of labile plant-derived C. The large surface area associated with fine root biomass increases the interaction of both fine roots and root hairs with soil micro-pores and micro-aggregates. Penetration of root hairs into such micro sites, where anoxic conditions prevail and which are otherwise inaccessible to microbial decomposers, may preferentially stabilize these stocks of root-derived C (Rasse et al., 2005). Alternatively, the greater surface area associated with fine root biomass may simply increase microbial access to root exudates and plant-derived C where it is consumed and eventually stabilized as microbial necromass.

While the size and relative percent of $C_a$ was small, the size of $C_r$ was quite large and accounted for 32 to 50% of SOC, consistent with the findings of others (Paul et al., 2001a). Recent conceptual and empirical advancements suggest that the most persistent C in the soil is formed as low molecular weight compounds become physically protected in organo-mineral associations (Kallenbach et al., 2016; Schmidt et al., 2011; Six et al., 2002). Therefore, compared to partially decomposed plant debris that are of larger size and less protected, $C_r$ is less vulnerable to soil disturbance. Jacobs et al. (2010) reported that $C_r$ occupied a greater proportion of SOC in a tilled agricultural system when compared to a minimally-tilled system. While recent additions of plant lignin can escape hydrolysis, increasing the size of $C_r$ in agricultural systems (Kögel-Knabner et al., 1994; Paul et al., 2006; Schwendenmann and Pendall, 2008), the lack of detectable differences between CA, PAST, and AG indicate that this was not a major factor influencing the size of $C_r$ in this study. Interestingly, the rotationally grazed pasture had significantly higher $C_r$ (14.8 g $kg^{-1}$) than all systems including REM. This may highlight the potential role of livestock grazing in creating efficient plant–microbe-soil associations, stimulating soil biology and microbial necromass, and promoting the formation of organo-mineral associations as others have found (Mosier et al., 2021; Rui et al., 2022; Wilson et al., 2018).

### 4.3. Carbon allocation below ground higher in grassland systems

The five LULCGs corresponded well with our initial hypothesis that $CO_2$ flux would be greatest among perennial grassland systems and lowest in the conventional agricultural systems. This hypothesis is supported by the finding of others that perennial grasslands typically have greater SOC stocks than conventional agricultural systems because of increased C inputs from fine-root biomass, root hairs, root exudates, the lack of removal of aboveground vegetation, and the absence of physical soil disturbance (DeLuca and Zabinski, 2011; Guzman and Al-Kaisi, 2010). For example, Jelinski et al. (2011) reported that the annual belowground net primary production of a remnant tallgrass prairie in southern Wisconsin averaged 5.7 Mg $ha^{-1}$ $yr^{-1}$: more than twice that of an adjacent 11-yr-old prairie restoration (2.8 Mg $ha^{-1}$ $yr^{-1}$) or a nearby soybean field (2.3 Mg $ha^{-1}$ $yr^{-1}$). Tufekcioglu et al. (1998), working in central Iowa, found that live fine-root biomass in perennial cool season pastures exceeded 6 Mg $ha^{-1}$, while in both maize and soybean systems, fine root biomass was $<$ 2.3 Mg $ha^{-1}$ in the surface 35 cm. Cahill et al. (2009) evaluated root production in two 16-year-old grassland systems (one C3 and one C4 system) and an annual grain rotation in southern Wisconsin. They estimated total root biomass (0 to 50 cm) for the C4 and C3 grass systems at 6.6 and 4.8 Mg $ha^{-1}$, respectively, while total root biomass in the annual system was far lower at 0.7 Mg $ha^{-1}$. Finally, Kucharik et al. (2006) reported total root biomass (0 to 30 cm) numbers as high as 30.3 and 21.5 Mg $ha^{-1}$ for a remnant and 65-year prairie restoration on poorly drained soils in southern Wisconsin.

In agreement with our study, Yoo et al. (2006) found that mean $CO_2$ mineralization rates were significantly greater from incubated prairie soil than from cultivated soil (72 vs. 47 μg $CO_2$ $g^{-1}$ soil $d^{-1}$, respectively) as part of a 12-day incubation study using soils from adjacent cultivated and native prairie sites in central Illinois. They attributed this to greater C substrate content in the prairie system as was the case in our study. Interestingly, four of the five groupings in our study (REM, 35yP, CA, and PAST) fell along a continuum of management intensity within perennial grass systems. This suggests that in systems with similar below ground C inputs (quantity and quality), historical land use (e.g. tillage) leaves a long-term signature that persists for decades.

We did not detect significant differences between the conventional agricultural systems despite their range in management practices (i.e., grain, forage, CT, NT). In a similar incubation study on Alfisols in southern Michigan, Paul et al. (1999) were unable to detect significant differences in C mineralization amongst the five agricultural systems they evaluated (ranging from CT maize-soybean to alfalfa). However, they did find that these systems differed from hybrid poplar and two successional plant communities (never tilled and historically tilled) in which cumulative $CO_2$ flux was much greater than it was in production agricultural systems. In the current study, the LULCs in AG differed substantially from the NT continuous maize (in CA), which displayed $CO_2$ flux dynamics similar to the 11-year prairie restoration, in that they were periodically tilled or returned less crop residue to the soil. These results highlight the sensitivity of this method and suggest that in some situations where independent decomposition models might be expected (as in AG), a single model of soil C dynamics may suffice. Alternatively, as was the case here, grassland systems of differing age and management intensity may require more system specific consideration.

### 4.4. Pros and cons of AHI method

The AHI method has been used to compare the relative distribution and kinetics of SOM in agricultural, forest and grassland systems (Collins et al., 2000; Fortuna et al., 2003; Haile-Mariam et al., 2000; Paul et al., 2001b; Paul et al., 2001a). The work of Paul et al. (1999) and Paul et al. (2006) demonstrated the value and applicability of using parameter estimates obtained via the AHI method to improve biogeochemical model output. Others have also demonstrated the efficacy of using soil mineralization rates and chemical isolates of stabilized C to improve biogeochemical modeling. Scharnagl et al. (2010) found that C mineralization rates obtained during soil incubations provided sufficient information to reliably estimate all C pools in the ROTHC model. Furthermore, Juston et al. (2010) demonstrated that even rough estimates of an "inert" SOM pool, like those obtained via chemical isolates (e.g., acid hydrolysis), were quite valuable at reducing uncertainties in the Introductory Carbon Balance Model (ICBM). Collins et al. (2000) concluded that sufficient interactive effects with climate, parent material, and soil depth were found that predictive biogeochemical models used for decision making cannot rely on the generalizations about SOM dynamics that are present in most extant models. Rather, they suggested that such models require analytically determined factors, such as those defined by the AHI method, for at least major subdivisions of the soils being studied. Our findings support these conclusions and indicate that even when similar agroecosystems share the commonalities of climate and parent material, site specific model parameters may be required. This may be due in part to emergent qualities associated with plant-soil or plant–microbe interactions that are otherwise unaccounted for. Additional work to evaluate similar agroecosystems that are geographically proximal and share the same soil characteristics will help to elucidate the degree to which model parameters require finer resolution than that of major soil subdivisions. Further modeling work is also required to evaluate the utility of such site-specific parameter estimates.

The AHI method is not without potential drawbacks. Bruun and Luxhoi (2006) argued that fitting a two pool [or three pool] model to $CO_2$ flux data obtained from soil incubations will only provide meaningful pools of SOM if these distinct pools actually exist. In a similar vein it is important to remember that all methods to isolate soil fractions in the lab come with their potential artifacts. For AHI these including the impact of soil processing (e.g. sieving) on soil structure and microbial community composition, as well as the complication of estimating heterotrophic $CO_2$ fluxes in an artificial environment devoid of interactions between heterotrophs and autotrophs. The use of acid-hydrolysis to isolate a stable SOM pool can also be problematic in that; 1) recent plant materials may persist in the non-hydrolysable SOC fraction, 2) the size of $C_r$ can, in some instances, respond rapidly to land use change, and 3) acid-hydrolysis places undue emphasis on chemical recalcitrance as the primary mode of SOC stabilization. Schwendenmann and Pendall (2008), Kögel-Knabner et al. (1994), Collins et al. (2000), and others have demonstrated that $C_r$ may include recent carbon additions from plant residues. In addition to this discrepancy, Paul et al. (2006) found that $C_r$ is more dynamic than would be expected based on its $^{14}C$ age. Both Dungait et al. (2012) and Zakem et al. (2021) demonstrated that SOC preservation is primarily a factor of substrate accessibility, and not chemical recalcitrance, although some degree of chemical recalcitrance as a C stabilization factor in soils cannot be ruled out.

Despite these potential complications, the AHI method has proven useful in evaluating relative SOM dynamics between diverse agricultural systems as well as improving the predictive capacity of soil biogeochemical models. Paul et al. (2006) conducted a literature review to evaluate the utility of the AHI method and concluded that the method yielded reproducible and sensitive pools of SOC with kinetics validated using $^{13}C$ and $^{14}C$ markers. Furthermore, although $C_r$ can be biased by the inclusion of fresh plant and microbial biomass, $^{14}C$ dating has confirmed its great age relative to bulk SOC (Paul et al., 2001a).

Parameter estimates obtained via the AHI method correspond well with the active, slow, and passive C pools used in biogeochemical models such as CENTURY (Parton et al., 1987) and ROTHC (Coleman and Jenkinson, 1996). They can therefore be used to improve regional SOC modeling efforts by tailoring belowground C dynamics to fit edaphic conditions and/or specific agroecosystems (Collins et al., 2000; Haile-Mariam et al., 2000). Using the System Approach to Land Use Sustainability (SALUS) model Paul et al. (1999) report that parameterization with SOM pool estimates obtained via the AHI method resulted in $CO_2$-C fluxes that agreed well with field data. They found the greatest agreement between SALUS and field $CO_2$ flux during periods where no living crop was in the field because root respiration was absent. These results were consistent with those of Paul et al. (2006) who showed that parameterizing the DAYCENT model using estimates of $C_a$, $C_s$, and $C_r$, obtained from the AHI method provided $CO_2$ evolution rates that were well correlated with field $CO_2$ measurements.

## 5. Conclusions

Soil organic C dynamics differed across 5 land use-land cover groups (LULCGs), but surprisingly were not different across agricultural systems typical of the North Central U.S. In contrast, perennial grasslands with subtly different land cover and management histories exhibited distinct SOC dynamics. The estimates obtained from the constrained three pool model fit to our $CO_2$ flux data strongly supported our initial hypotheses that tillage would result in a loss of SOC from active and slow cycling carbon pools ($C_a$ and $C_s$) as a result of oxidative loss, leaving a relatively greater proportion of SOC in the more stable and presumably older $C_r$. While NT continuous maize demonstrated SOC dynamics similar to an 11-year prairie restoration and other perennial grass systems, it was the remnant prairie system (REM) that supported substantial C allocation to both the rapidly mineralized and slowly mineralized C pool. These results combined with the long MRTs associated with REM highlight both the potential sensitivity of this and other grassland systems to future disturbance (management or climate) and the importance of perennial grassland systems in stabilizing belowground additions of labile SOC.

Regionally specific estimates of C pools and their kinetics from diverse agroecosystems have been shown to improve biogeochemical modeling efforts. The AHI method should prove valuable in efforts to better understand how changes in climate and land management will affect current and future soil C stocks across diverse agricultural landscapes.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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