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Disciplines

Agricultural Science | Agronomy and Crop Sciences | Soil Science

PHYSICO-CHEMICAL ORGANIC MATTER STABILIZATION ACROSS A

RESTORED GRASSLAND CHRONOSEQUENCE

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Abstract

In reconstructed grasslands, soil organic matter (SOM) is the largest CO_2 and reactive N sink but SOM gains after reconstruction rarely achieve precultivation levels. Through a chronosequence of reconstructed grasslands 1 to 21 yr after establishment, we explored which physicochemical mechanisms protect accumulated soil organic C (SOC) and N from mineralization. After 21 yr, total SOC and soil N concentrations increased by 32 and 23%. The SOC concentration was within 5% of a new equilibrium but was 64% of a never-cultivated remnant. Chemically stabilized C and N pools on free silt and clay surfaces increased with time. Coarse particulate organic matter C increased with time but accounted for <12% of SOC. Microaggregate-stabilized SOM did not change. The positive linear relationship between total SOC and free silt and clay C indicates that 21 yr after establishment, reconstructions have unsatisfied capacity for further SOM storage, despite proximity to a new SOC equilibrium. The accumulated C and N associated with free silt and clay suggest that ammonium oxalate-extractable Fe (AmOx-Fe) and polyvalent cation concentrations could be correlated with total C and N stocks. These promote SOM stabilization and were possibly affected by human activity before reconstruction. However, AmOx-Fe and polyvalent cation concentrations were not associated with total SOC or soil N and could not explain the slowing SOM accumulation. Regardless of time since reconstruction, AmOx-Fe was highly concentrated on microaggregate surfaces compared with other fractions and was positively associated with microaggregate C and N, suggesting a link between Fe and microaggregate stabilization.

Core Ideas

- Twenty-one years after cropland-to-grassland conversion, soil organic C (SOC) was within 5% of a new equilibrium but remained 64% of a never-cultivated remnant.

- Mineral-associated and free particulate organic matter pools increased with time but microaggregate pools did not.

- Ammonium oxalate-extractable Fe was highly concentrated on microaggregate silt and clay.

- Polyvalent cations and ammonium oxalate-extractable Fe were not correlated to total SOC or soil N.

Abbreviations

AmOx-Fe   ammonium oxalate extractable Fe

CPOM   coarse particulate organic matter

free SC   silt and clay outside of microaggregates

oPOM   occluded particulate organic matter

SOC   soil organic C

SOM   soil organic matter

Introduction

Uncultivated grassland ecosystems possess much larger amounts of soil C and N than arable lands in similar environments. Uncultivated remnant prairies (native grasslands in the US Midwest) average 48% higher C concentrations than cultivated areas (DeLuca and Zabinski, 2011). In Iowa, it is likely that cultivation released significant amounts of C because 12,000 ha of remnant prairies survive today out of an estimated 12.5 million before cultivation (Samson and Knopf, 1994).

As the consequences of climate change have become increasingly apparent, one approach to reduce atmospheric greenhouse gas concentration is to sequester CO_2 in SOC. Reconstruction of native prairies can increase SOM as well as sequester CO_2 in SOC. As such, prairie reconstruction would be considered a greenhouse gas "mitigation activity" by the Intergovernmental Panel on Climate Change (Smith et al., 2014).

In reconstructed prairies, net primary productivity, the largest organic matter input to soil, matches older reconstructions within several years (Hansen and Gibson, 2014; Matamala et al., 2008; O'Brien and Jastrow, 2013). However, despite their similar net primary productivity, soil C and N stocks in reconstructed prairies rarely return to the levels observed in uncultivated remnant prairies. Kucharik et al. (2006) observed that SOC in a Wisconsin reconstruction remained 52% lower than a paired remnant after more than half a century after reconstruction. Other studies have also highlighted a gap between remnant and reconstruction SOC stocks, with many estimating that SOC levels in prairie reconstructions could take centuries to match remnant counterparts (Matamala et al., 2008; O'Brien and Jastrow, 2013). Most of

these time extrapolations come after fitting reconstructed prairie data with the rise to maximum functions, with C and N stocks from a nearby remnant site being used as the maximal sink potential (Matamala et al., 2008; Rosenzweig et al., 2016).

The lack of SOC gain in reconstructions, despite net primary productivity recovery, has been attributed to microbial diversity and abundance (Fierer et al., 2013; Wilson et al., 2009), plant functional group diversity (Kindscher and Tieszen, 1998), and artificial subsurface drainage (DeLuca and Zabinski, 2011). Soil macrofauna, particularly the presence or absence of certain earthworms, can also influence SOM decomposition in many ecosystems (Hendrix and Bohlen, 2002).

Could the differences in SOC between remnant and reconstructed prairies lie in how plant C inputs are stored within the soil? Soil C and N can be stabilized or protected from mineralization by a variety of mechanisms. Some of these mechanisms have a finite capacity for C and N stabilization, and land use management is known to affect how much of this stabilization capacity is realized in a given soil (West and Six, 2007). Moreover, land-use management may have long-term legacy effects on the mechanisms of stabilization (Poffenbarger et al., 2018)

Conceptually, soil C and N stabilization processes are divided by function, which can be partitioned between chemical protection on silt and clay surfaces and physical occlusion within aggregates of mineral particles (Lützow et al., 2006). The biochemical recalcitrance of organic matter can be associated with these processes (Filley et al., 2008) but remains controversial (Dungait et al., 2012). Among these pathways, chemical bonding with silt and clay particles

accounts for the vast majority of stabilized SOM in fine-textured soils (Hassink, 1997; Plaza et al., 2013; Six et al., 2004). In prairie reconstructions, O'Brien and Jastrow (2013) postulated that physical and chemical stabilizing mechanisms are closely interlinked, with each undergoing several periods of growth and stagnation as the reconstructed prairie ages and adds more organic material.

A number of factors can control the capacity for physical and chemical stabilization of SOM. Polyvalent cations such as Ca and Mg enhance chemical bonding between organic matter and clay surfaces through "bridging" the negatively charged regions of both (Kahle et al., 2002; Kleber et al., 2007; Muneer and Oades, 1989). Although soil is considered to have a finite capacity for SOM storage because of the surface area of silt and clay particles (Hassink, 1997), polyvalent cation concentrations could theoretically change as arable land transitions into prairie. Cessation of tillage, acidifying fertilizers, liming, and biomass harvest all can influence polyvalent cation chemistry or distribution (Barak et al., 1997; Gransee and Führs, 2013; Hussain et al., 1999; Laird and Chang, 2013).

In addition to cations, certain oxides, particularly short-range ordered Fe species, can augment both physical aggregation and mineral-associated bonding (Oades and Waters, 1991; Six et al., 2004). However, perhaps because of the relatively low short-range ordered metal concentrations in Mollisols compared with other soil orders such as Oxisols, few studies, if any, have examined this chemical pathway. Regardless, several conceptual SOM models have recognized the potential importance of short-range ordered Fe as a binding agent, particularly in microaggregates (Regelink et al., 2015). Although the evidence is less clear, short-range ordered Al may enhance stability in some tropical soil aggregates (Igwe et al., 2009). As

reconstructed prairies become established, changes to redox potential or pH could affect Fe

dynamics (Gotoh and Patrick, 1974). Increases in soil surface Fe during a no-till transition have

also been observed in Mollisols and may be dependent on organic matter or pH changes

(Obour et al., 2017). Regardless, we would expect the majority of short-ranged ordered Fe or Al

within soils to be set by mineralogy rather than human activity.

By using a unique 21-yr chronosequence of replicated grassland reconstructions, our

goal was to examine which SOM pools accumulate after prairie establishment from cropland.

We hypothesized that total C and N would increase over time, including both chemically and

physically protected pools. We also expected to observe positive correlations between

physicochemical stabilized C and N, and short-range oxides as well as polyvalent cations. Our

reasoning was based on the well-known importance of fine mineral particles for SOM

stabilization capacity (Hassink, 1997), as well as recent research that demonstrates the role of

Fe (and to a lesser extent Al) as important drivers of chemical SOM stabilization capacity (Igwe

et al., 2009; Regelink et al., 2015; Silva et al., 2015; Wade et al., 2018). Unlike silt and clay,

polyvalent cations and reactive metal oxides could be increased in surface horizons through

human action, possibly increasing stabilization capacity and closing the gap between SOM in

reconstructed and remnant prairies.

Materials and Methods

Study Site

Nineteen tallgrass prairie reconstructions were nonrandomly selected at the Neal Smith

Wildlife Refuge near Prairie City, IA (41°33´30.5˝N, 93°16´48.2˝W). Located in the Walnut Creek

watershed with a humid continental climate, much of the landscape consisted of pre-Illinoian

glacial till underneath loess-capped hills (Palmer et al., 2014; Schilling et al., 2009). Before Neal

Smith Wildlife Refuge was established in the early 1990s, nearly 70% of the Walnut Creek

watershed was under row crops (Schilling and Drobney, 2014). Both managed pasture and row

crop cultivation were widely practiced in the area since at least 1860 (Winters, 1974). This study

attempted to maximize similarity in landscape position, soil texture, and slope. Reconstructions

within the chronosequence ranged in the time since establishment from 1 to 21 yr, with

unbalanced replication of individual reconstructions across years (Supplemental File S1).

Because of the unreliable sources of local prairie seed germplasm, which was gathered from

nearby uncultivated prairies or harvested on-site at Neal Smith Wildlife Refuge, the seed

mixtures differed somewhat each year (Neal Smith Wildlife Refuge, 2015; Supplemental File

S1). Before reconstruction, the sampled sites were leased as cropland to local farmers. As the

leases expired, the cropland was reconstructed to prairie. This continuous expiration of leases

and reconstruction of prairies provided a unique replicated chronosequence. As a condition of

cropland leases, renters were mandated to adopt no-till practices and a 2-yr crop rotation of

corn (Zea mays L.) and soybean [Glycine max (L.) Merr.], along with other restrictions (Schilling

and Thompson, 2000). Notable restrictions included a maximum yearly addition of 112 kg N

fertilizer ha–1 on corn and a ban on pre-emergent herbicides, with other nutrient and lime

amendments applied prescriptively (Schilling and Thompson, 2000). Because of seed

constraints at Neal Smith Wildlife Refuge, cropland leases with producers were typically

renewed for approximately 3 to 5 yr before prairie was sown (C. Dykstra, personal

communication, 2018). After establishment, the reconstructions are periodically burned every 1

to 3 yr in the spring, depending on the weather conditions. Aside from burning, other techniques to control invasive plant species included mowing and clipping, hand pulling, and herbicide spot-treatments (Neal Smith Wildlife Refuge, 2015).

All reconstructions were located on the soil series Otley (moderately well drained; fine, smectitic, mesic Oxyaquic Argiudolls), Mahaska (somewhat poorly drained; fine, smectitic, mesic Aquertic Argiudolls), or Tama (well drained; fine-silty, mixed, superactive, mesic Typic Argiudolls). Otley soil was present on 11 of the 19 prairie reconstruction sites, with Mahaska and Tama soil splitting the remaining eight locations. All soil series sampled possessed similar epipedons and were mainly distinguished by their subsoil properties. Within each of the 19 reconstructions, a sampling area was located on an upland location with a slope between 0 and 5% to minimize any confounding effects caused by deposition or erosion of silt and clay.

Soil Properties

In November 2015, plots (0.33 m by 0.33 m) were sampled six times to a depth of 15 cm with a 2.5-cm diameter soil probe. Composite samples of six cores were passed through an 8-mm sieve to preserve aggregates while ensuring no stones were included as soil. A shallow sampling depth was chosen to magnify possible differences in SOC storage between older and newer reconstructions (Kravchenko and Robertson, 2011; Six et al., 2000). Surface residue and plant material were removed before all soil sampling. The moist soil was then placed in a 4°C cooler for later use. A 30 g air-dried subsample was analyzed for texture (Kettler et al., 2001) and another 20 g air-dried sample was analyzed via dry combustion for total C and N. The pH

was measured with 10 g of moist soil mixed with 10 mL of deionized water. Bulk density was recorded via the core method on May 2016 using seven 2.5 by 15 cm cores.

Soil Physical Fractionation

We used a physical fractionation procedure modified from Six et al. (2004) to separate relatively labile and stable pools of C and N. Approximately 40 g of moist, sieved soil (8-mm sieve) from the November 2015 sampling was slaked overnight then added to a custom-built mechanical shaking device along with 50 metal beads and running distilled water. The long slaking period was first used by Gentile et al. (2010) and was intended to ensure that only water-stable microaggregates survived processing in high clay soil and to minimize the survival of larger soil aggregates, which are not considered a stable pool of C or N (Denef et al., 2001). A 250-µm sieve served to collect coarse particulate organic matter (CPOM). Any material that passed through the 250-µm sieve was then subsequently filtered with a 53-µm sieve. The process continued until the solution coming out of the microaggregate isolator was free of visible materials. Material that passed through the 53-µm sieve, considered as free silt and clay outside of microaggregates (free SC), was centrifuged at 1000×g (2069 rpm) for 30 min and excess water was decanted until the fraction could be placed in an aluminum pan. All fractions were dried at 105°C and homogenized with a ceramic mortar and pestle. The higher temperature was chosen to ensure timely drying of the fine soil and to minimize the effects of hydroscopic water bound to clay.

Soil retained on the 53-μm sieve was then set aside to remove any unprotected low-density plant material outside of the microaggregate fraction. A 30-mL sodium polytungstate [$Na_6(H_2W_{12}O_{40})$] solution with a density of 1.85 g cm–3 was used for the separation procedure. The mixture of the 53- to 250-μm fraction and the $Na_6(H_2W_{12}O_{40})$ was inverted several times in a 50-mL tube then placed in a centrifuge at 1000× g for 30 min. Afterwards, the floating material was suctioned onto a 20-μm nylon filter, dried at 105°C, homogenized with a mortar and pestle, and added to the CPOM fraction. The remaining material not removed by vacuum was subsequently rinsed by an addition of 30 mL distilled water to remove any remaining $Na_6(H_2W_{12}O_{40})$. After centrifuging at 1000× g for 30 min, the extra $Na_6(H_2W_{12}O_{40})$ and distilled water solution was decanted and the samples were prepared immediately for occluded particulate organic matter (oPOM) separation. Additional dilutions were not used because of the possibility of lower density oPOM being lost through decanting.

Fifteen mL of 0.5% sodium hexametaphosphate [$(NaPO_3)_6$] was added after density floatation. Ten glass beads were also added and samples were placed on a reciprocating shaker (Model 6010, Eberbach, St. Belleville, MI) for 18 h. After shaking, microaggregate silt and clay and oPOM were separated with distilled water and a 53-μm sieve. Material that passed through the sieve was considered to be microaggregate silt and clay and the rest to be oPOM. Both fractions were oven-dried at 105°C and homogenized with a ceramic mortar and pestle. Pools considered stable (i.e., protected) consisted of free SC, microaggregate silt and clay, and oPOM. Coarse particulate organic matter was considered to be a labile (i.e., unprotected) pool.

Chemical analyses

After physical fractionation, all silt and clay and particulate organic matter fractions were analyzed in duplicate for total C and N by dry combustion. Total SOC and soil N were defined as the sum of all C and N fractions, respectively. Carbon and N losses from the physical fractionation procedure were determined by subtracting total SOC and soil N from the C and N concentrations found in 8-mm sieved whole soil. The difference between the whole-soil measurements and the sum of the fractions was 11% and 2% for C and N, respectively. Carbon losses from intensive physical fractionation have not been studied well but another study that used an identical fractionation procedure had a similar C loss (Sleutel et al., 2006). Carbon and N losses from the fractionation were found to have no significant relationship with the age of the reconstructed prairie.

Estimates for short-range ordered Fe and Al were based on values from ammonium oxalate [$(NH_4)_2C_2O_4$] extraction. The procedure was adapted from Jackson et al. (1986). Soil fractions (free SC, microaggregate silt and clay, and whole soil) with a known moisture content were immersed in 35 mL of acidified 0.2M ammonium oxalate and placed in a 50-mL centrifuge tube wrapped in aluminum foil. The tubes were shaken in the dark for 4 h then filtered with 8-µm Whatman Grade 2 filter paper (GE Healthcare Lifesciences, Chicago, IL) and measured for Fe and Al via inductively coupled plasma mass spectrometry.

Polyvalent cation concentrations were measured for two soil fractions: free SC and whole soil. The microaggregate silt and clay fraction was not analyzed because of the limited amounts of material. The procedure for whole soil cation extraction was adapted from Warncke

and Brown (1998). Twenty mL of 1 M ammonium acetate ($NH_4CH_3CO_2$) solution at a pH of 7.0 and 2 g of moist whole-soil were combined and shaken for 30 min then filtered with 8-μm Whatman Grade 2 paper (GE Healthcare Lifesciences). The extract was measured for Ca and Mg via inductively coupled plasma mass spectrometry. Cations bound to free SC were measured by sending dried material to Iowa State University Soil and Plant Analysis Laboratory for inductively coupled plasma mass spectrometry determination of Ca and Mg.

Statistical Analysis

Statistical tests were performed with SAS version 9.4 (SAS Institute, Cary, NC) as well as SigmaPlot version 11.0 (Systat Software, Chicago, IL) software. Possible relationships among short-range ordered Fe or Al, Ca or Mg, soil texture, pH, prairie reconstruction age, N, or C values were analyzed with linear and nonlinear models. To determine evidence of possible C saturation, total SOC was compared with each stable C pool (free SC, microaggregate silt and clay, and oPOM) as per Stewart et al. (2008b). If a stable pool displayed an asymptotic relationship with total SOC such that the SOC concentration continues to increase but the stable pool C concentration does not, that pool can be considered C-saturated. Best fit lines were chosen based on the lowest sum of squares error. Mean comparisons and ANOVA between intervals were conducted with Duncan's new multiple range test and the SAS GLM procedure, respectively.

Results and Discussion

Soil Properties

Soil texture was not correlated with reconstruction age, with the mean concentrations (± SE) of silt at 64.32% (±0.95%) and clay at 32.01% (±0.98%) **(Table 1)**. The pH also did not differ across the chronosequence, with an average of 5.84 (±0.096) **(Table 1)**. There was no statistically significant relationship between bulk density and time since reconstruction; mean bulk density was 1.24 g cm–3 (±0.011) **(Table 1)**.

Table 1 Soil properties for 19 plots sampled from 0-15 cm.

Property	Minimum	Maximum	Median	Mean
Sand, g kg-1	31.2	46.0	35.8	36.8
Silt, g kg-1	541.0	757.0	648.0	643.0
Clay, g kg-1	197.0	428.0	317.0	320.0
Soil organic C, g C kg-1 soil	12.3	27.8	19.2	19.4
Soil N, g N kg-1 soil	1.26	2.31	1.81	1.75
Soil pH, 1:1	5.29	6.91	5.76	5.84
Cation exchange capacity, cmolc kg-1	16.1	21.3	18.4	18.9
Soil bulk density, g cm-3	1.16	1.35	1.23	1.24
Ca, mg Ca kg-1 soil	2388.0	3436.0	2727.0	2799.0
Magnesium, mg Mg kg-1 soil	356.6	672.0	507.0	508.6
Ammonium oxalate-extractable Fe, mg Fe kg-1 soil	2565.0	3714.0	3404.0	3241.0
Ammonium oxalate-extractable Al, mg Al kg-1 soil	912.0	1409.0	1161.0	1180.0

Soil Carbon and Nitrogen Pools

After 21 yr since prairie reconstruction, total SOC concentrations approached equilibrium **(Fig. 1A)**. The mean C concentration in the oldest group of reconstructions (21 yr) was 5% below the modeled equilibrium C concentration of 20.95 g C kg–1 **(reference line in Fig. 1A)**. Despite the age of these oldest reconstructions and the proximity of SOC concentrations to the modeled equilibrium, total SOC was only 64% that of a prairie remnant 95 km away with similar soil texture (Guzman and Al-Kaisi, 2010), suggesting that the equilibrium C observed did not represent effective C saturation (Stewart et al., 2008b). In contrast, total soil N, which increased from an average of 1.48 g N kg–1 soil in the youngest reconstructions to 1.82 g N kg–1 soil in the oldest, was found to be positively linearly correlated with time **(Fig. 1B)**. The differing C and N responses to time since reconstruction explain the lack of correlation between the ratio of soil C to N and the age of the reconstructed grassland **(Fig. 1C)**.
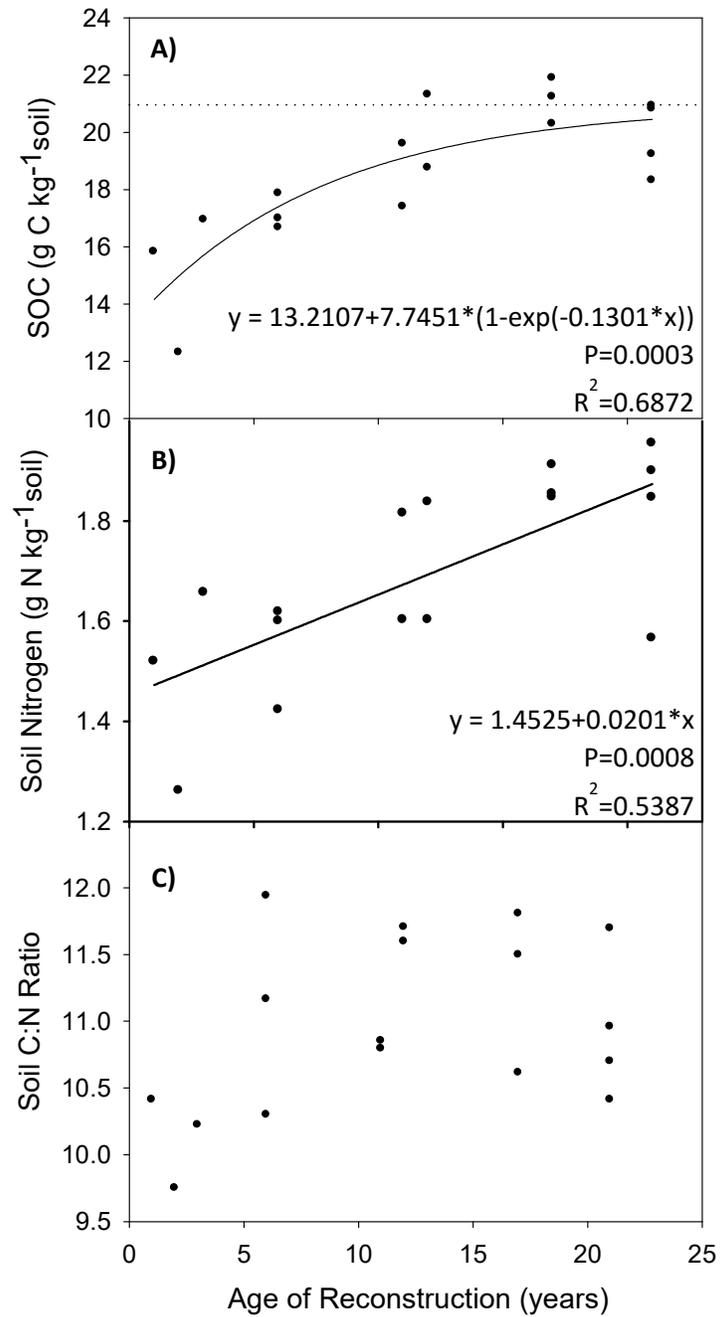
Figure 1 Total soil organic C (SOC) **(A)**, total soil N **(B)**, and soil C/N ratio **(C)** against time since land use change from a corn–soybean crop rotation to prairie reconstruction. The horizontal dotted line in **(A)** represents the asymptote derived from the rise to maximum function. All data points were sampled from 0 to 15 cm.

Only free SC and CPOM had statistically significant relationships with prairie reconstruction age. Coarse particulate organic matter C was positively associated with time since reconstruction, but there was no relationship between CPOM N and time **(Fig. 2A)**. Young reconstructed prairies, though variable, possessed approximately one-third of the CPOM C concentration (0.72 g C kg–1 soil) of the oldest reconstructed prairies (1.95 g C kg–1 soil).
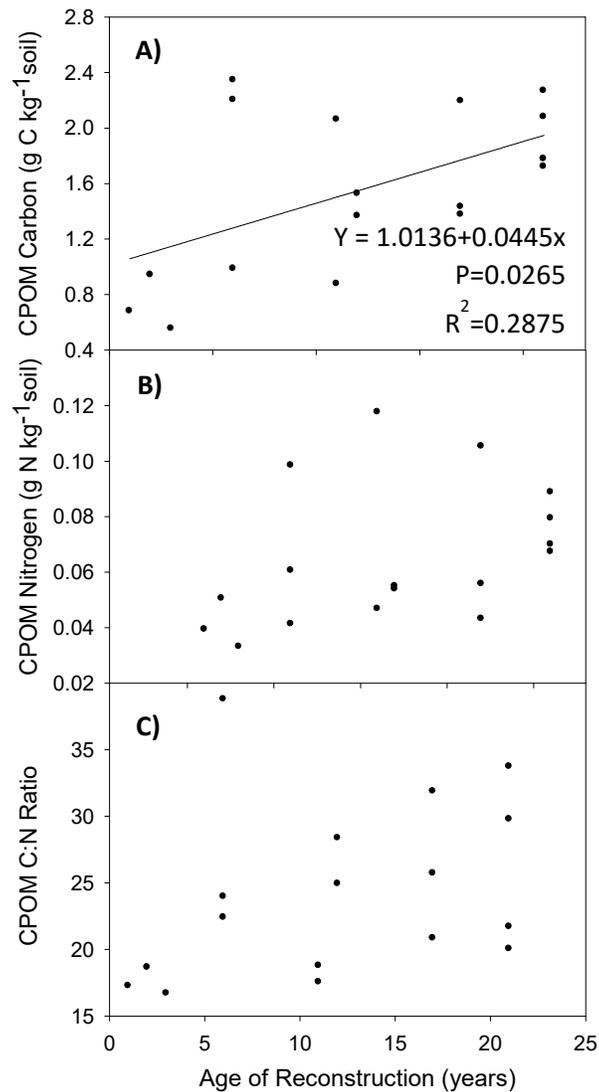

Figure 2 Carbon **(A)**, N **(B)**, and C/N ratio **(C)** of coarse particulate organic matter (CPOM) against time since land use change from a corn–soybean crop rotation to prairie reconstruction. All data points were sampled from 0 to 15 cm.

Unlike CPOM, both C and N concentrations in free SC were linearly correlated with time since reconstruction **(Fig. 3A, B)**. In addition, a strong (R^2 = 0.82) positive linear relationship was observed between free SC and total SOC, indicating unsatisfied capacity for further C storage in free SC **(Fig. 4A)**. The amount of clay in the mineral fraction can be used to estimate the maximum C holding capacity of the soil (Hassink, 1997). The linear relationship with SOC indicates that free SC has an unsatisfied C storage potential (Stewart et al., 2008b), despite total SOC approaching a near-equilibrium over time **(Fig. 1A)**. If free SC was effectively C-saturated, we would expect to see no increase in free SC C concentration, even with increasing SOC levels. In such a case, additional organic matter inputs would be stored in other SOC pools (Castellano et al., 2015, Stewart et al., 2008b). This relationship would display an exponential rise to maximum instead of the linear relationship observed in our data **(Fig. 4A)**.
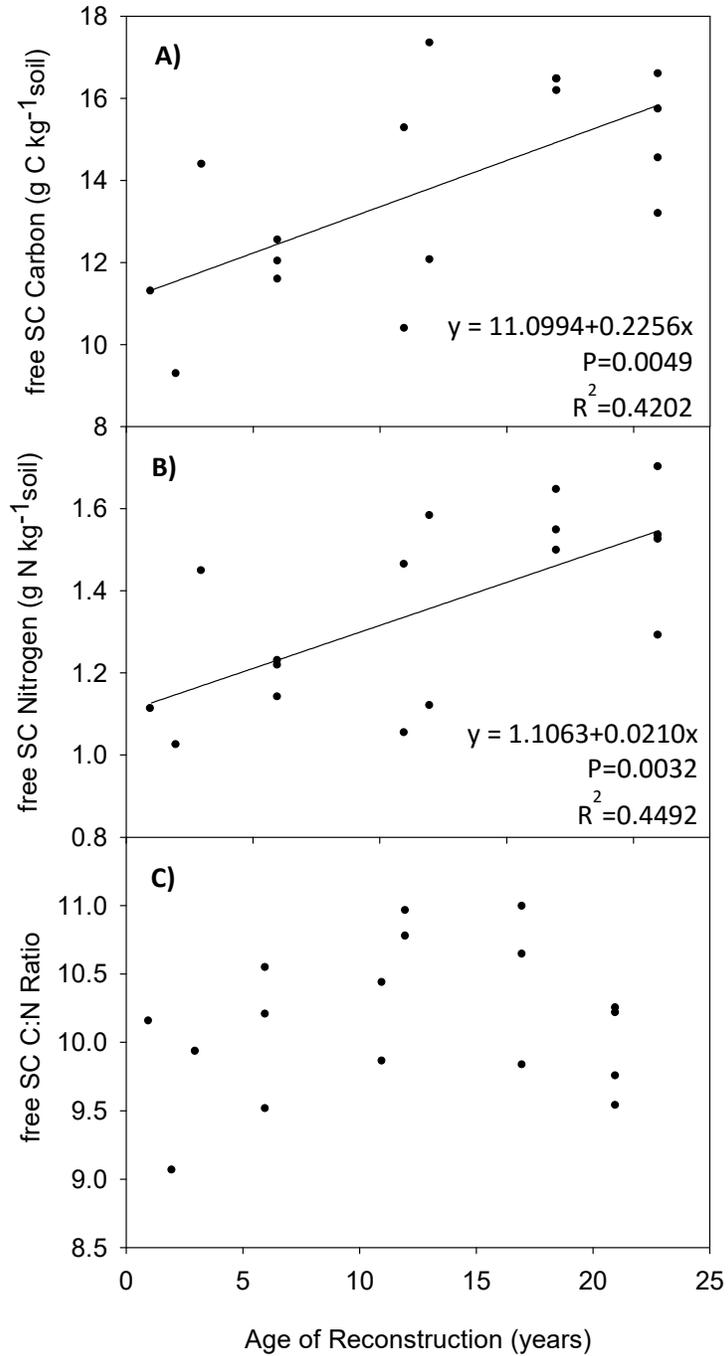
Figure 3 Carbon **(A)**, N **(B)**, and C/N ratio **(C)** of silt and clay outside of microaggregates (free SC) plotted against time since land use change from a corn–soybean crop rotation to prairie reconstruction. All data represent samples from 0 to 15 cm.
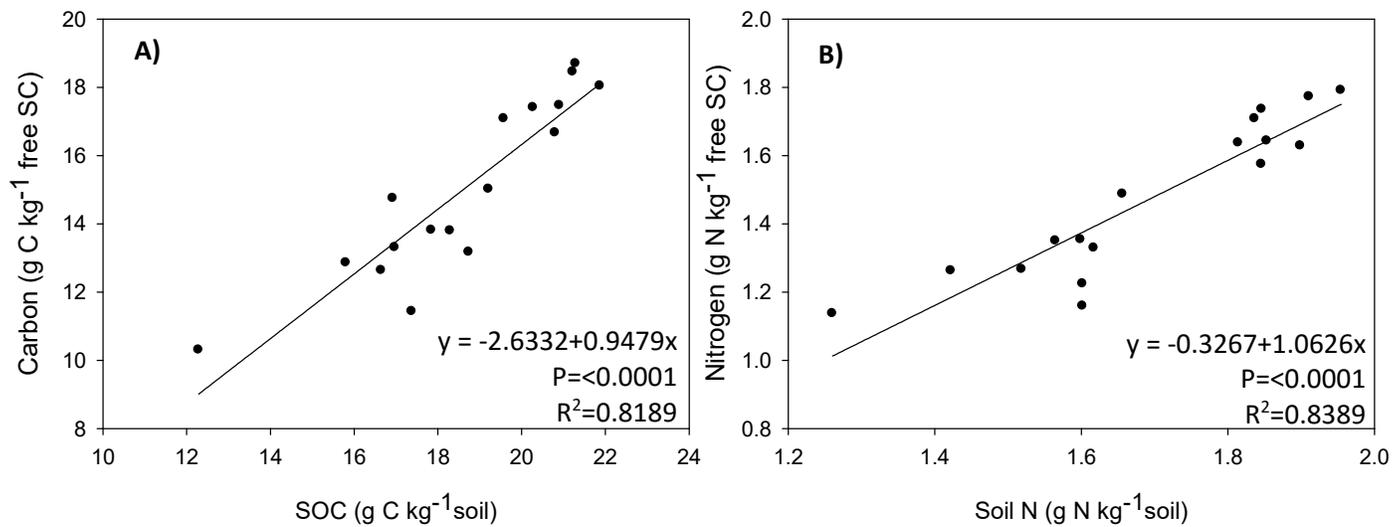
Figure 4 Silt and clay outside of microaggregates (i.e., free SC) C **(A)** and N **(B)** concentrations plotted against total soil organic C (SOC) and total soil N. All data points were sampled from 0 to 15 cm.

The observed total SOC and soil N gains over time are consistent with the hypothesis that organic matter gains under reconstructed prairie chiefly occur in the chemically stabilized free SC pool, which, on average, accounted for 88% of soil mass **(Fig. 5)**.
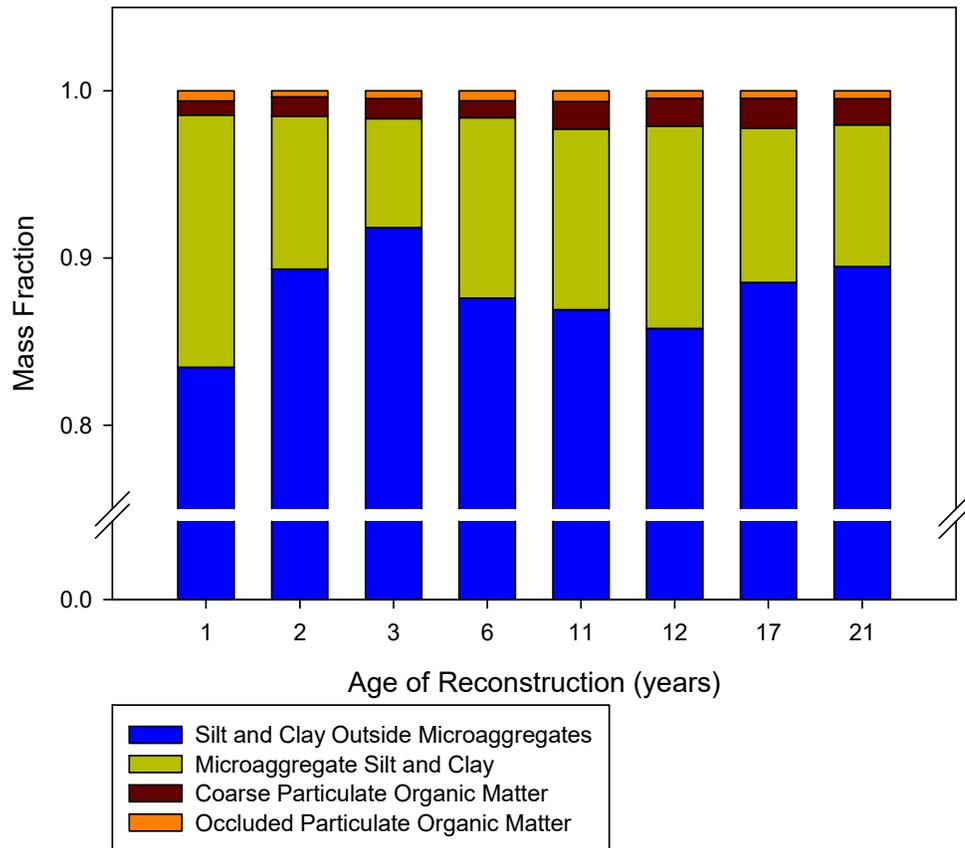

Figure 5 Dry mass fraction distribution compared to the age of the reconstructed prairie.

Contrary to our initial hypothesis, physically protected fractions, such as oPOM and microaggregate silt and clay, did not change with regard to C, N, or mass since grassland reconstruction. The lack of an increase in microaggregate-protected C and N concentrations over time contrasts with the conceptual model proposed by Six et al. (2000), in which no-till environments and decreases in disturbance favor microaggregate accumulation and slow labile particulate organic matter decomposition. Several studies support the conceptual model regarding tillage effects on arable lands (Bossuyt et al., 2002; Denef et al., 2004; Mikha and Rice, 2004; Six et al., 2004). However, there are a few studies that examine microaggregate changes from no-till to native prairie.

One explanation for the lack of increase in microaggregates is that the croplands in our study were managed without tillage for 3 to 5 yr prior to prairie reconstruction. Thus any gain of microaggregates from cessation of tillage may have occurred during the time when the land was under corn or soybean cultivation. As per Six et al. (2002), in the absence of disturbance, the maximum amount of microaggregates in soil is limited by the percentage and mineralogy of clay, which is essentially independent of time. Other biotic factors that could explain the lack of observed microaggregate accumulation, such as microbial or fungal composition (Six et al., 2006) and fine root biomass (Jastrow et al., 1998), were not examined in this study but could be important.

Soil Organic Matter Stabilization and Metal Oxides

No statistically significant relationships were found between any of the measured soil C

and N pools and ammonium oxalate-extractable Al. Ammonium oxalate-extractable Fe in free

SC, microaggregate silt and clay, and 8-mm sieved whole soil displayed no significant

relationship with pH or soil texture. The AmOx-Fe concentrations were similar in free SC (3310

mg Fe kg–1 free SC ± 121.7 mg) and whole soil (3241 mg Fe kg–1 soil ± 82.15 mg) fractions.

However, the concentration of AmOx-Fe in the microaggregate silt and clay fraction (6740 mg

Fe kg–1 microaggregate ± 399.2 mg) was approximately 100% greater than AmOx-Fe in both

free SC and whole soil. As expected, the age of the reconstruction had no relationship with

AmOx-Fe in any fraction.

However, AmOx-Fe appears to play an important role in microaggregation. The

concentration of AmOx-Fe in microaggregate silt and clay displayed significant rise-to-maximum

relationships with microaggregate silt and clay C and N concentrations **(Fig. 6A, B)**. In addition,

there was a positive linear correlation between microaggregate silt and clay AmOx-Fe

concentration and microaggregate silt and clay C to N ratio **(Fig. 6C)**.
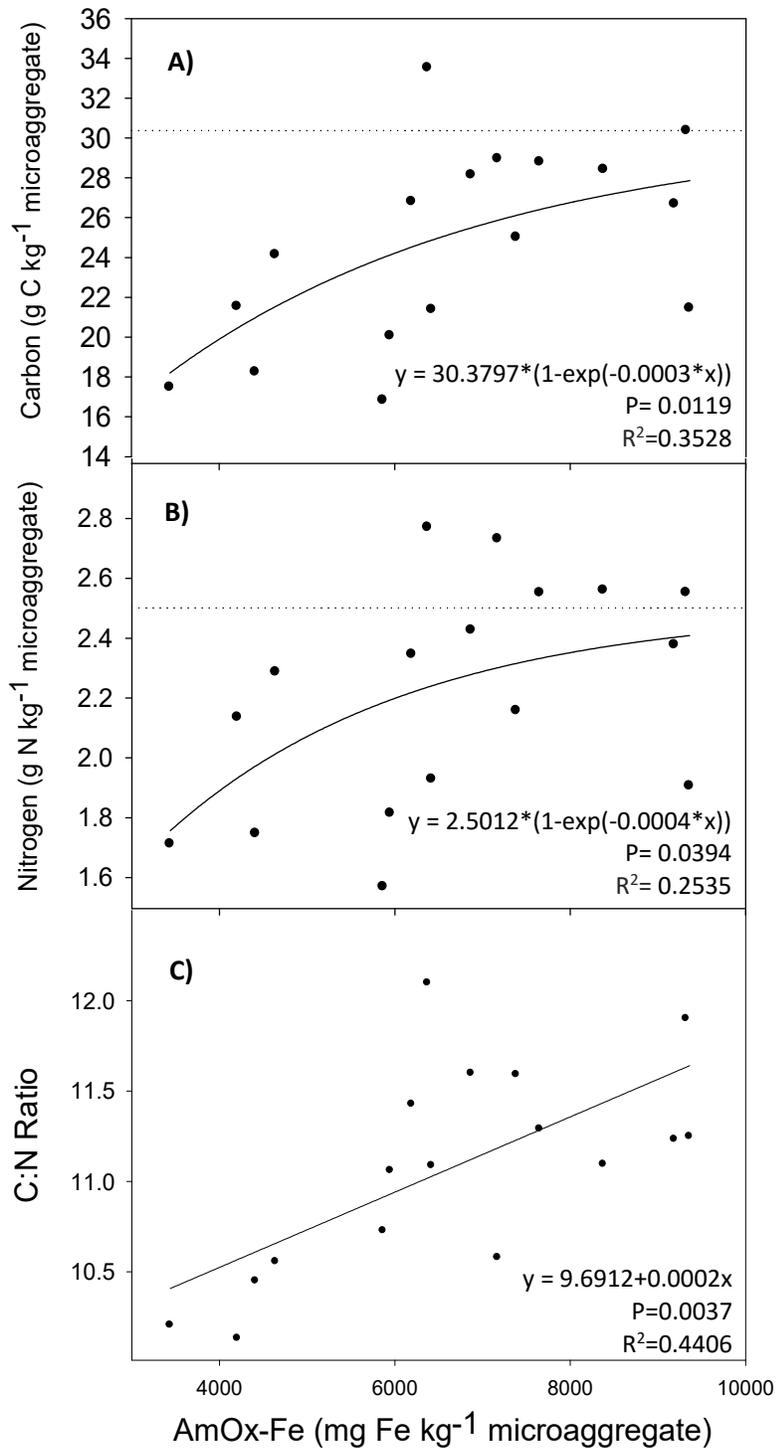
Figure 6 Microaggregate silt and clay C **(A)**, N **(B)**, and C/N ratio **(C)** plotted against microaggregate silt and clay ammonium oxalate-extractable Fe. The horizontal dotted line in A and B represents the asymptote derived from the respective rise to maximum function.

These significant positive relationships among microaggregate AmOx-Fe and microaggregate silt and clay C to N ratios, C, and N concentrations **(Fig. 6)** suggest a biochemical shift in the type of organic materials that are stabilized on these microaggregate silt and clay surfaces, whereas a rise-to-maximum relationship suggests an upper limit of short-range ordered Fe influence. Iron oxides can offer multiple strong SOM bonding mechanisms that can selectively bond with C-rich aromatic material rather than the organic matter with lower C/N ratios favored by silt and clay particles (Hall et al., 2016; Han et al., 2016). According to Silva et al. (2015), organically bound Fe was integral to stable microaggregate formation in a tropical soil chronosequence. Indeed, evidence of dense Fe content was apparent in the Mollisol microaggregates at our sites. The AmOx-Fe concentrations on microaggregate silt and clay were 108 and 104% higher than either 8-mm sieved whole soil or free SC AmOx-Fe concentrations, respectively. However, AmOx-Fe concentration in whole soil, free SC, and microaggregate silt and clay had no correlation with total SOC and soil N. This indicates that the effects of AmOx-Fe in our prairie soils, although present in microaggregates, are not strong enough to shift protected SOM toward remnant levels. Although this finding is contrary to our initial assumptions that greater AmOx-Fe concentrations in the soil would correspond to higher total SOC and soil N, the microaggregate dynamics of AmOx-Fe within Mollisols appears worthy of further study.

Polyvalent cations also could not explain the lack of C recovery: Ca bound to free SC may have increased over time but total SOC still was close to equilibrium. Calcium and Mg concentrations in the 8-mm sieved whole soil and the free SC fraction were not associated with C, N, or the C/N ratio of any soil fraction. We can reject our hypothesis that these polyvalent

cations were related to any C or N gains across the reconstructed prairie chronosequence. At

our sites, the large range of extracted Ca from free SC (6780–27, 740 mg Ca kg–1 free SC) also

cast doubt on any increased protection of C or N. Other experiments, which analyzed Ca

additions to soils, noted no statistical relationship with N mineralization (Tix et al., 2006) or

SOM stabilization (Stewart et al., 2008a). The increase in Ca bound to free SC over time

suggests cation transport from the subsoil via roots. Similar observations in forest stands

(Dijkstra and Smits, 2002) as well as grasslands (Jobbágy and Jackson, 2004) support this "Ca

pumping" or "nutrient uplift" hypothesis.

Conclusion

Our observations suggest that after conversion of no-till corn–soybean cropland to

prairie, C and N concentrations in free SC increase but C and N concentrations on

microaggregate-protected pools remain unchanged. The oldest reconstructed prairies showed

signs of reaching a new total SOC equilibrium, although these older reconstructions contained

only 64% of the SOC present in a nearby remnant prairie. In addition, these older

reconstructions appear to have ample room for additional C or N storage on free SC **(Fig. 4A, B)**.

Even with the large mineral surface area and total mass fraction provided by free SC, the

amount of polyvalent cations and short-range ordered metals had no significant effect on total

SOC or soil N and thus could not explain the apparent re-equilibration of total SOC after 21 yr

under tallgrass prairie. However, unrelated to the effect of reconstruction age, estimates of

short-range ordered Fe concentrations were twice as high on microaggregate silt and clay

surfaces than in other fractions and had significant positive associations with microaggregate

silt and clay C and N. Future physicochemical fractionation studies on Mollisols should measure short-range ordered Fe to see if similar relationships exist outside of our site.

If reconstructed prairies are to be used as C sinks through land use change (Smith et al., 2014), we must first understand why SOM accumulation stagnates below the SOM levels of nearby remnant prairies. Only then can we create a C-neutral solution to increase reconstructed prairie SOM levels to historical maxima. Notably, there are few manipulative experiments of soils from prairie reconstructions. Experimental rather than observational research may be needed to explain the causes of SOM stagnation and identify solutions. These experiments could focus on why neither the amount nor the concentration of SOM in microaggregates increased with time since reconstruction, as well as strategies to fill the unrealized storage capacity of the free SC pool. It is likely that both differences in biological communities (e.g., mesofauna) and environment (e.g., artificial drainage) contribute to the lack of complete SOM recovery.

References

Barak, P., B. O. Jobe, A. R. Krueger, L. A. Peterson, and D. A. Laird. 1997. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. Plant and Soil 197(1):61-69.

Bingham, A. H., and M. F. Cotrufo. 2016. Organic nitrogen storage in mineral soil: Implications for policy and management. Science of the Total Environment 551:116-126.

Bossuyt, H., J. Six, and P. F. Hendrix. 2002. Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. Soil Science Society of America Journal 66(6):1965-1973.

Castellano, M. J., K. E. Mueller, D. C. Olk, J. E. Sawyer, and J. Six. 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Global change biology, 21(9):3200-3209.

DeLuca, T. H., and C. A. Zabinski. 2011. Prairie ecosystems and the carbon problem. Frontiers in Ecology and the Environment 9(7):407-413.

Denef, K., J. Six, R. Merckx, and K. Paustian. 2004. Carbon sequestration in microaggregates of no-tillage soils with different clay mineralogy. Soil Science Society of America Journal 68(6):1935-1944.

Denef, K., J. Six, K. Paustian, and R. Merckx. 2001. Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry–wet cycles. Soil Biology and Biochemistry 33(15):2145-2153.

Dijkstra, F. A., and M. M. Smits. 2002. Tree species effects on calcium cycling: the role of calcium uptake in deep soils. Ecosystems 5(4):385-398.

Dungait, J. A., D. W. Hopkins, A. S. Gregory, and A. P. Whitmore. 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18(6):1781-1796.

Fierer, N., J. Ladau, J. C. Clemente, J. W. Leff, S. M. Owens, K. S. Pollard, R. Knight, J. A. Gilbert, and R. L. McCulley. 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342(6158):621-624.

Filley, T. R., T. W. Boutton, J. D. Liao, J. D. Jastrow, and D. E. Gamblin. 2008. Chemical changes to nonaggregated particulate soil organic matter following grassland-to-woodland transition in a subtropical savanna. Journal of Geophysical Research-BiogeSCiences 113:G03009.

Gentile, R., B. Vanlauwe, A. Kavoo, P. Chivenge, and J. Six. 2010. Residue quality and N fertilizer do not influence aggregate stabilization of C and N in two tropical soils with contrasting texture. Nutrient Cycling in Agroecosystems, 88(1): 121-131.

Gotoh, S., and W. H. Patrick. 1974. Transformation of iron in a waterlogged soil as influenced by redox potential and pH. Soil Science Society of America Journal 38(1):66-71.

Gransee, A., and H. Führs. 2013. Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. Plant and Soil 368(1-2):5-21.

Guzman, J. G., and M. M. Al-Kaisi. 2010. Soil carbon dynamics and carbon budget of newly reconstructed tall-grass prairies in south central Iowa. Journal of Environmental Quality 39(1):136-146.

Hall, S. J., W. L. Silver, V. I. Timokhin, and K. E. Hammel. 2016. Iron addition to soil specifically stabilized lignin. Soil Biology and Biochemistry 98:95-98.

Han, L., K. Sun, J. Jin, and B. Xing. 2016. Some concepts of soil organic carbon characteristics and mineral interaction from a review of literature. Soil Biology and Biochemistry 94:107-121.

Hansen, M. J., and D. J. Gibson. 2014. Use of multiple criteria in an ecological assessment of a prairie restoration chronosequence. Applied Vegetation Science 17(1):63-73.

Hassink, J. 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant and Soil 191(1):77-87.

Hendrix, P. F., and P. J. Bohlen. 2002. Exotic Earthworm Invasions in North America: Ecological and Policy Implications. BioScience 52(9):801-811.

Hussain, I., K. R. Olson, and S. A. Ebelhar. 1999. Long-term tillage effects on soil chemical properties and organic matter fractions. Soil Science Society of America Journal 63(5):1335-1341.

Igwe, C.A., M. Zarei, and K. Stahr. 2009. Colloidal stability in some tropical soils of southeastern Nigeria as affected by iron and aluminium oxides. Catena 77(3): 232-237.

Jackson, M.L., C. H. Lim, and L.W. Zelazny. 1986. Oxides, Hydroxides and Aluminosilicates. In Klute, A., Ed., Methods of Soil Analysis, Part 1, Physical and Mineralogical Methods, 2nd Edition, Agronomy Monogram 9, ASA and SSSA, Madison, 101-150.

Jastrow, J. D., R. M. Miller, and J. Lussenhop. 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biology and Biochemistry 30(7): 905-916.

Jobbágy, E. G., and R. B. Jackson. 2004. The uplift of soil nutrients by plants: biogeochemical consequences across scales. Ecology 85(9):2380-2389.

Kahle, M., M. Kleber, and R. Jahn. 2002. Predicting carbon content in illitic clay fractions from surface area, cation exchange capacity and dithionite-extractable iron. European Journal of Soil Science, 53(4):639-644.

Kettler, T. A., J. W. Doran, and T. L. Gilbert. 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. Soil Science Society of America Journal 65(3): 849-852.

Kindscher, K., and L. L. Tieszen. 1998. Floristic and soil organic matter changes after five and thirty-five years of native tallgrass prairie restoration. Restoration Ecology 6(2):181-196.

Kleber, M., P. Sollins, and R. Sutton. 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry, 85(1): 9-24.

Kravchenko, A. N., and G. P. Robertson. 2011. Whole-profile soil carbon stocks: The danger of assuming too much from analyses of too little. Soil Science Society of America Journal 75(1):235-240.

Kucharik, C. J., N. J. Fayram, and K. N. Cahill. 2006. A paired study of prairie carbon stocks, fluxes, and phenology: comparing the world's oldest prairie restoration with an adjacent remnant. Global Change Biology 12(1):122-139.

Laird, D. A., and C. W. Chang. 2013. Long-term impacts of residue harvesting on soil quality. Soil and Tillage Research 134:33-40.

Lützow, M. V., I. Kögel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner, and H. Flessa. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions–a review. European Journal of Soil Science 57(4):426-445.

Matamala, R., J. D. Jastrow, R. M. Miller, and C. T. Garten. 2008. Temporal changes in C and N stocks of restored prairie: implications for C sequestration strategies. Ecological Applications 18(6):1470-1488.

Mikha, M. M., and C. W. Rice. 2004. Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. Soil Science Society of America Journal 68(3):809-816.

Muneer, M., and J. M. Oades. 1989. The role of Ca-organic interactions in soil aggregate stability. III. Mechanisms and models. Soil Research, 27(2), 411-423.

Neal Smith Wildlife Refuge. 2015. Resource Management. US Fish and Wildlife Service.

https://www.fws.gov/refuge/Neal_Smith/what_we_do/resource_management.html

(accessed 20 Nov. 2017).

Oades, J. M., and A. G. Waters. 1991. Aggregate hierarchy in soils. Soil Research 29(6):815-828.

Obour, A. K., M. M. Mikha, J. D. Holman, and P. W. Stahlman. 2017. Changes in soil surface chemistry after fifty years of tillage and nitrogen fertilization. Geoderma 308:46-53.

O'Brien, S. L., and J. D. Jastrow. 2013. Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands. Soil Biology and Biochemistry 61:1-13.

Palmer, J. A., K. E. Schilling, T. M. Isenhart, R. C. Schultz, and M. D. Tomer. 2014. Streambank erosion rates and loads within a single watershed: Bridging the gap between temporal and spatial scales. Geomorphology 209:66-78.

Plaza, C., D. Courtier-Murias, J. M. Fernández, A. Polo, and A. J. Simpson. 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: a central role for microbes and microbial by-products in C sequestration. Soil Biology and Biochemistry 57:124-134.

Poffenbarger, H. J., J. E. Sawyer, D.W. Barker, D. C. Olk, J. Six, and M. J. Castellano. 2018. Legacy effects of long-term nitrogen fertilizer application on the fate of nitrogen fertilizer inputs in continuous maize. Agriculture, Ecosystems & Environment, 265:544-555.

Regelink, I. C., C. R. Stoof, S. Rousseva, L. Weng, G. J. Lair, P. Kram, N. P. Nikolaidis, M. Kercheva, S. Banwart, and R. N. Comans. 2015. Linkages between aggregate formation, porosity and soil chemical properties. Geoderma 247:24-37.

Rosenzweig, S. T., M. A. Carson, S. G. Baer, and J. M. Blair. 2016. Changes in soil properties, microbial biomass, and fluxes of C and N in soil following post-agricultural grassland restoration. Applied Soil Ecology 100:186-194.

Samson, F., and F. Knopf. 1994. Prairie Conservation in North America. BioScience 44(6):418-421.

Schilling, K. E., and P. Drobney. 2014. Restoration of prairie hydrology at the watershed scale: Two decades of progress at Neal Smith National Wildlife Refuge, Iowa. Land 3(1):206-238.

Schilling, K.E., J. A. Palmer, E. A. Bettis, P. Jacobson, R. C. Schultz, T. M. Isenhart. 2009. Vertical distribution of total carbon, nitrogen and phosphorus in riparian soils of Walnut Creek, southern Iowa. Catena 77:266–273.

Schilling, K. E., and C. A. Thompson. 2000. Walnut Creek Watershed monitoring project, Iowa: Monitoring water quality in response to prairie restoration. Journal of the American Water Resources Association 36(5):1101-1117.

Silva, L. C., T. A. Doane, R. S. Corrêa, V. Valverde, E. I. Pereira, and W. R. Horwath. 2015. Iron-mediated stabilization of soil carbon amplifies the benefits of ecological restoration in degraded lands. Ecological Applications 25(5):1226-1234.

Six, J., S. D. Frey, R. K. Thiet, and K. M. Batten. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70(2):555-569.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research 79(1):7-31.

Six, J., R. T. Conant, E. A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil 241(2):155-176.

Six, J. A., E. T. Elliott, and K. Paustian. 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32(14):2099-2103.

Sleutel, S., S. De Neve, T. Németh, T. Tóth, and G. Hofman. 2006. Effect of manure and fertilizer application on the distribution of organic carbon in different soil fractions in long-term field experiments. European Journal of Agronomy, 25(3):280-288.

Smith, P., M. Bustamante, H. Ahammad, H. Clark, H. Dong, E. A. Elsiddig, H. Haberl, R. Harper, J. House, M. Jafari, O. Masera, C. Mbow, N. H. Ravindranath, C. W. Rice, C. Roble do Abad, A. Romanovskaya, F. Sperling, and F. Tubiello. 2014. Agriculture, Forestry and Other Land Use (AFOLU). In: O. Edenhofer, R. Pichs-Madruga, Y. Sokona. E. Farahani, S. Kadner, and K. Seyboth, (ed.), Climate change 2014: Mitigation of climate change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press, Cambridge, UK. p. 811–922

Stewart, C. E., K. Paustian, R. T. Conant, A. F. Plante, and J. Six. 2008a. Soil carbon saturation: evaluation and corroboration by long-term incubations. Soil Biology and Biochemistry 40(7):1741-1750.

Stewart, C. E., A. F. Plante, K. Paustian, R. T. Conant, and J. Six. 2008b. Soil carbon saturation: linking concept and measurable carbon pools. Soil Science Society of America Journal 72(2):379-392.

Tix, D., J. A. Hebberger, and I. Charvat. 2006. Influence of aboveground biomass removal on nitrogen mineralization in a restored tallgrass prairie. Restoration Ecology 14(4):561-568.

Wade, J., H. Waterhouse, L. M. Roche, and W. R. Horwath. 2018. Structural equation modeling reveals iron (hydr) oxides as a strong mediator of N mineralization in California agricultural soils. Geoderma, 315: 120-129.

Warncke, D., and J.R. Brown. 1998. Potassium and other basic cations. p. 31–33. In: J.L. Brown (ed.) Recommended chemical soil test procedures for the North Central region. North Central Regional Publ. No. 221 (Rev.). Missouri Exp. Stn. Publ. SB 1001. Univ. of Missouri. Columbia.

West, T. O., and J. Six. 2007. Considering the influence of sequestration duration and carbon saturation on estimates of soil carbon capacity. Climatic Change 80(1):25-41.

Wilson, G. W., C. W. Rice, M. C. Rillig, A. Springer, and D. C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecology Letters 12(5):452-461.

Winters, D. 1974. Tenant Farming in Iowa, 1860-1900: A Study of the Terms of Rental

Leases. Agricultural History, 48(1):130-150.

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Table 1S Seeding mixtures for reconstructed prairie sites

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Figure 1S Map of study site. (Adapted from Schilling and Drobney, 2014)
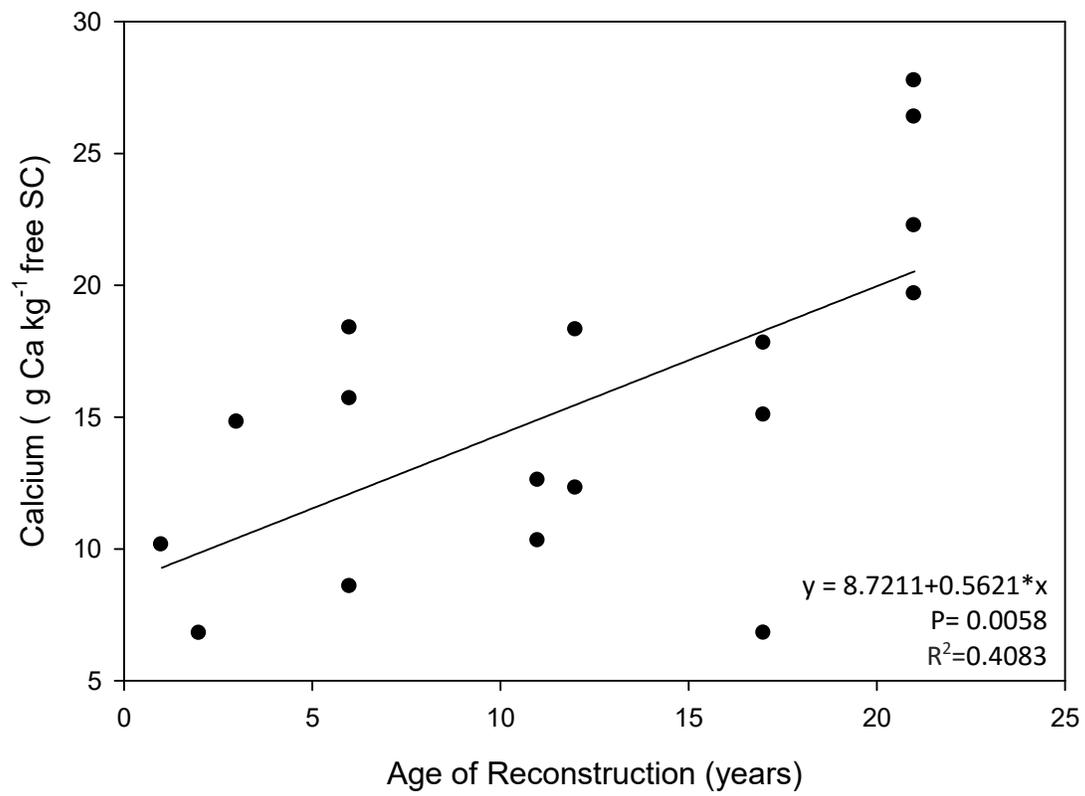
Figure 2S Ammonium acetate extractable calcium located on silt/clay outside of microaggregates (free SC) plotted against the age of the reconstructed grassland.
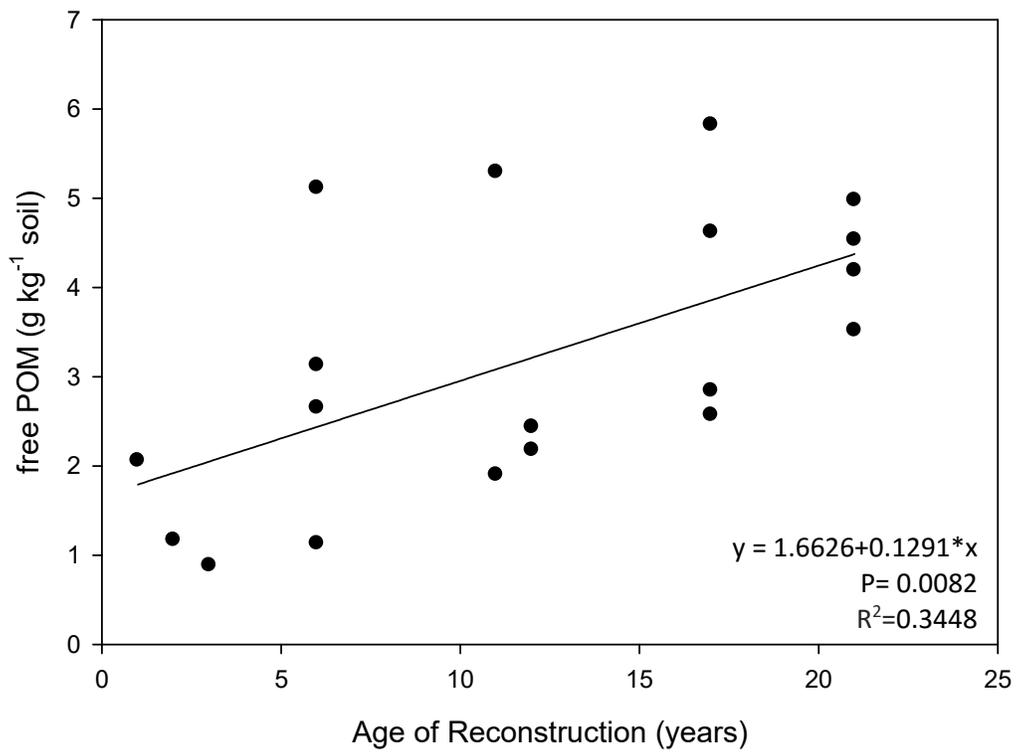
Figure 3S Concentration of particulate organic matter outside of microaggregates 250-53 microns in size (free POM) compared to the age of reconstructed grassland.
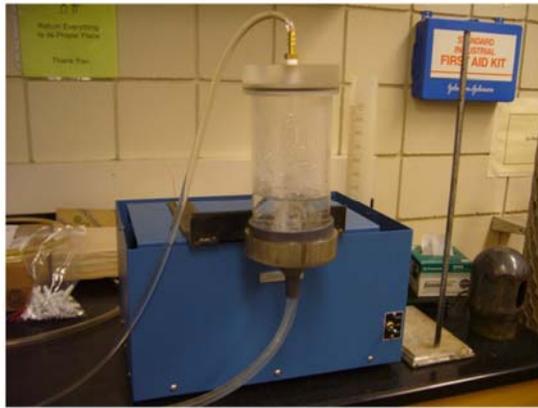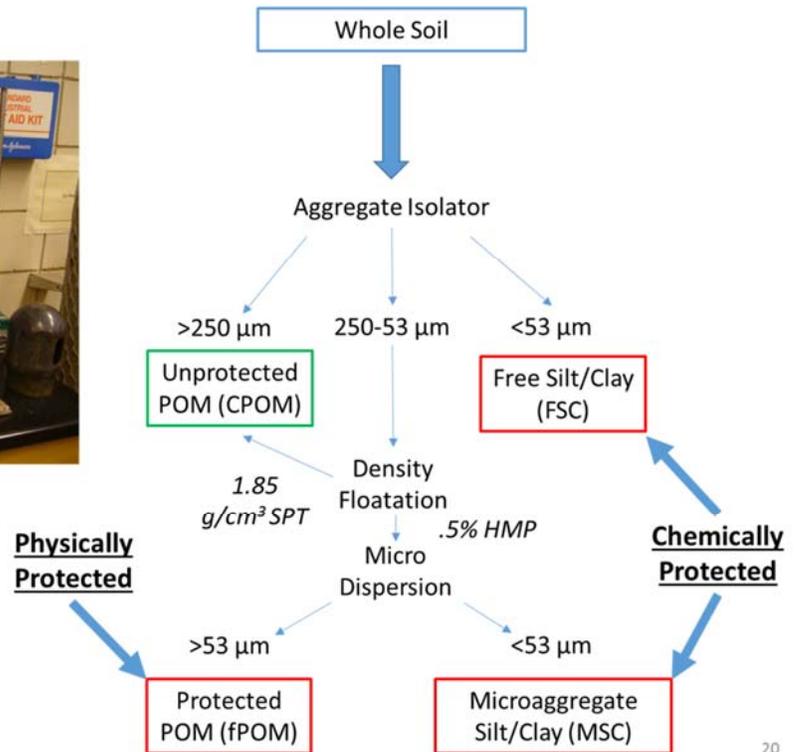
Figure 4S Diagram of the modified fractionation procedure from Six et al. 2004 used in the study as well as an example picture of an aggregate isolator on the upper left. MRT stands for "mean resonance time", with MRT's of 5 years or less defined as "unprotected" pools and 100 years or more as (protected) pools.
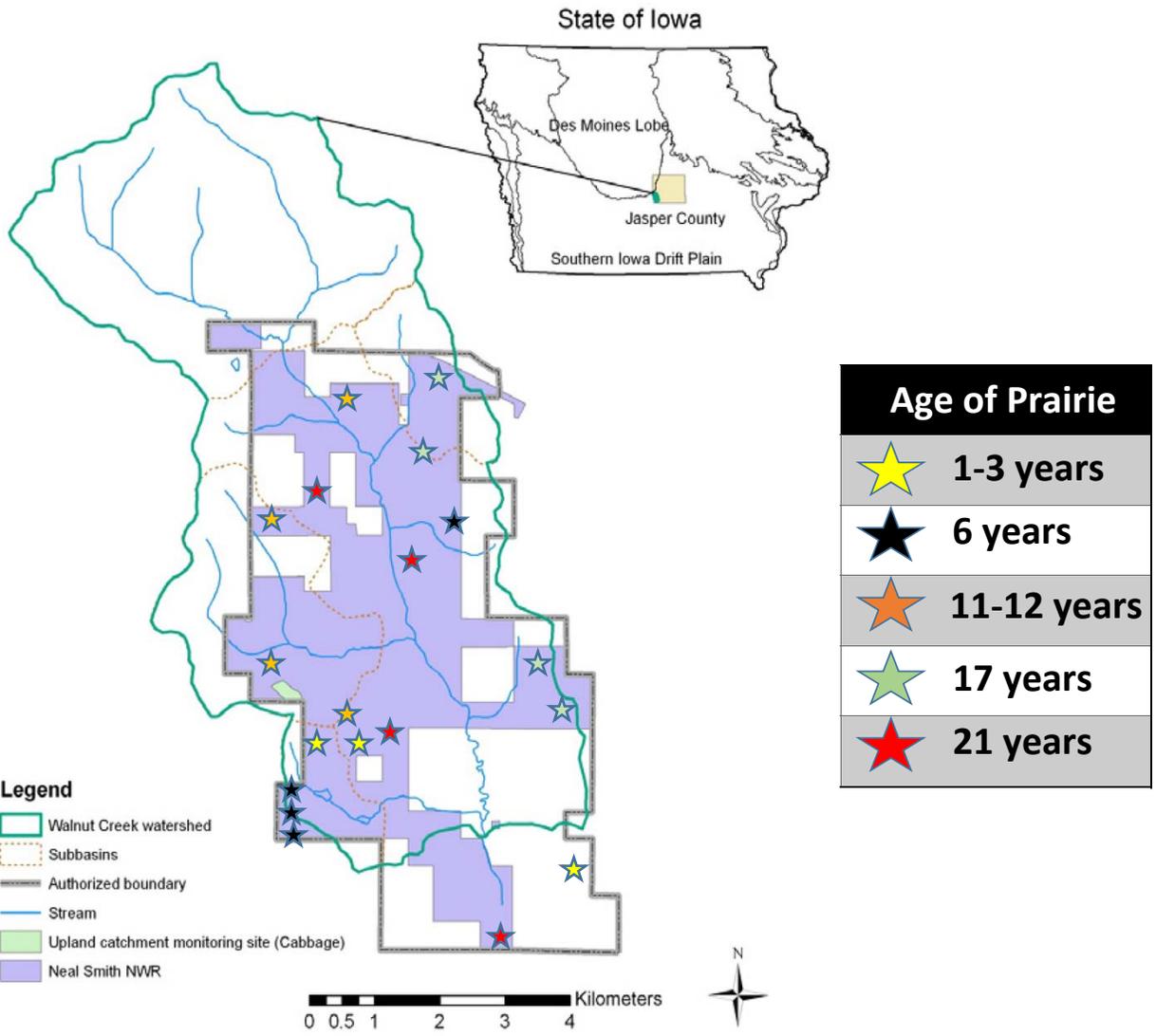
Table 1S   Seeding mixtures for reconstructed prairie sites.

Reconstruction Year	Species
1998	Achillea millefolium lanulosa
1998	Amaranthus sp.
1998	Ambrosia artemisiifolia
1998	Ambrosia trifida
1998	Andropogon gerardii
1998	Aster sp.
1998	Aster pilosus
1998	Bidens sp.
1998	Bidens polylepis
1998	Chamaecrista fasciculata
1998	CHENOPODIUM ALBUM
1998	Cirsium sp.
1998	Dalea purpurea
1998	DAUCUS CAROTA
1998	Elymus canadensis
1998	Eryngium yuccifolium
1998	Eupatorium sp.
1998	Gaura biennis
1998	Helianthus sp.
1998	HYPERICUM PERFORATUM
1998	Lactuca canadensis
1998	LACTUCA SERRIOLA
1998	Lespedeza capitata
1998	Monarda fistulosa
1998	Muhlenbergia spp.
1998	Oenothera biennis centralis
1998	Panicum virgatum
1998	Polygonum pensylvanicum laevigatum
1998	Polygonum sp.
1998	Ratibida pinnata
1998	Rudbeckia hirta
1998	RUMEX CRISPUS
1998	Schizachyrium scoparium
1998	Scirpus heterochaetus
1998	Scirpus sp.
1998	SETARIA FABERII
1998	Silphium perfoliatum
1998	Solidago rigida
1998	Sorghastrum nutans
1998	Sporobolus asper
1998	TRIFOLIUM PRATENSE

1998	Verbena stricta
2003	Achillea millefolium lanulosa
2003	Amaranthus sp.
2003	Ambrosia artemisiifolia
2003	Ambrosia sp
2003	Ambrosia trifida
2003	Amorpha canescens
2003	Andropogon gerardii
2003	Anemone cylindrica
2003	Anemone virginiana
2003	Artemisia ludoviciana
2003	Asclepias amplexicaulis
2003	Asclepias sp.
2003	Asclepias tuberosa
2003	Asclepias verticillata
2003	Aster azureus
2003	Aster ericoides
2003	Aster novae-angliae
2003	Aster sp.
2003	Astragalus canadensis
2003	Baptisia bracteata var glabrescens
2003	Baptisia lactea
2003	Baptisia sp.
2003	Bidens polylepis
2003	Bouteloua curtipendula
2003	Brickellia eupatorioides
2003	Carex sp
2003	Castilleja coccinea
2003	Ceanothus americanus pitcheri
2003	Chamaecrista fasciculata
2003	Chenopodium sp.
2003	Cirsium altissimum
2003	Coreopsis palmata
2003	Coreopsis tripteris
2003	Crotalaria sagittalis
2003	Croton capitatus
2003	Dalea candida
2003	Dalea purpurea
2003	Daucus carota
2003	Delphinium virescens
2003	Desmanthus illinoensis
2003	Desmodium illinoense
2003	Dichanthelium oligosanthes

	scribnerianum
2003	Echinacea pallida
2003	Elymus canadensis
2003	Elymus virginicus
2003	Eriochloa villosa
2003	Eryngium yuccifolium
2003	Eupatorium altissimum
2003	Euphorbia corollata
2003	Euthamia graminifolia
2003	Gaura biennis
2003	Gentiana alba
2003	Gentiana puberulenta
2003	Gentiana x curtissii
2003	Helianthus grosseserratus
2003	Helianthus rigidus
2003	Helianthus sp.
2003	Heliopsis helianthoides
2003	Heuchera richardsonii
2003	Hieracium longipilum
2003	Hypoxis hirsuta
2003	Koeleria macrantha
2003	Lespedeza capitata
2003	Liatris aspera
2003	Liatris cylindracea
2003	Liatris punctata
2003	Liatris pycnostachya
2003	Lithospermum canescens
2003	Lithospermum incisum
2003	Lobelia siphilitica
2003	Mirabilis nyctaginea
2003	Monarda fistulosa
2003	Oenothera biennis centralis
2003	Onosmodium molle hispidissimum
2003	Panicum virgatum
2003	Parthenium integrifolium
2003	Pedicularis canadensis
2003	Penstemon digitalis
2003	Phlox maculata
2003	Phlox pilosa
2003	POA PRATENSIS
2003	Potentilla arguta
2003	Prenanthes racemosa
2003	Psoralidium batesii

2003	Pycnanthemum pilosum
2003	Pycnanthemum virginianum
2003	Ratibida pinnata
2003	Rosa arkansana suffulta
2003	Rosa sp
2003	Rudbeckia hirta
2003	Ruellia humilis
2003	RUMEX CRISPUS
2003	Schizachyrium scoparium
2003	Setaria faberii
2003	SETARIA GLAUCA
2003	Silene stellata
2003	Silphium integrifolium
2003	Silphium laciniatum
2003	Silphium perfoliatum
2003	Solidago hispida
2003	Solidago rigida
2003	Solidago sp.
2003	Solidago speciosa
2003	Sorghastrum nutans
2003	Sporobolus asper
2003	Sporobolus heterolepis
2003	Stipa spartea
2003	Teucrium canadense
2003	Tradescantia ohiensis
2003	Tradescantia sp
2003	Tridens flavus
2003	Verbena hastata
2003	Verbena sp.
2003	Verbena stricta
2003	Vernonia fasciculata
2003	Veronicastrum virginicum
2003	Viola pedatifida
2003	Zizia aurea
2004	Ambrosia sp.
2004	Amorpha canescens
2004	Anaphalis margaritacea
2004	Andropogon gerardii
2004	Anemone canadensis
2004	Anemone cylindrica
2004	Anemone virginiana
2004	Artemisia ludoviciana
2004	Asclepias incarnata

2004	Asclepias sp.
2004	Asclepias tuberosa
2004	Asclepias verticillata
2004	Aster azureus
2004	Aster ericoides
2004	Aster laevis
2004	Aster novae-angliae
2004	Aster pilosus
2004	Aster sp.
2004	Astragalus canadensis
2004	Baptisia bracteata var glabrescens
2004	Baptisia lactea
2004	Bidens polylepis
2004	Bouteloua curtipendula
2004	Brickellia eupatorioides
2004	Bromus inermis
2004	Cacalia atriplicifolia
2004	Cacalia plantaginea
2004	Carex spp.
2004	Ceanothus americanus pitcheri
2004	Ceanothus herbaceus pubescens
2004	Chamaecrista fasciculata
2004	Chelone glabra
2004	Coreopsis palmata
2004	Coreopsis tripteris
2004	Dalea candida
2004	Dalea purpurea
2004	DAUCUS CAROTA
2004	Desmodium canadense
2004	Desmodium illinoense
2004	Echinacea pallida
2004	Elymus canadensis
2004	Elymus virginicus
2004	Eryngium yuccifolium
2004	Eupatorium altissimum
2004	Eupatorium perfoliatum
2004	Eupatorium purpureum
2004	Euphorbia corollata
2004	Euthamia graminifolia
2004	Gaura biennis
2004	Gentiana ×curtisii
2004	Gentiana alba
2004	Gentiana andrewsii

2004	Gentiana puberulenta
2004	Gentiana x curtissii
2004	Gnaphalium obtusifolium
2004	Hackelia virginiana
2004	Helenium autumnale
2004	Helianthus maximiliani
2004	Helianthus rigidus
2004	Helianthus sp.
2004	Heliopsis helianthoides
2004	Heuchera richardsonii
2004	Hypericum pyramidatum
2004	Koeleria macrantha
2004	Lespedeza capitata
2004	Liatris aspera
2004	Liatris cylindracea
2004	Liatris punctata
2004	Liatris pycnostachya
2004	Liatris sp.
2004	Liatris squarrosa
2004	Lilium michiganense
2004	Lilium philadelphicum andinum
2004	Lobelia siphilitica
2004	MEDICAGO SATIVA
2004	Monarda fistulosa
2004	Muhlenbergia sp.
2004	Oenothera biennis centralis
2004	Onosmodium molle
2004	Parthenium integrifolium
2004	Pediomelum argophyllum
2004	Phlox pilosa
2004	Physostegia virginiana
2004	Potentilla arguta
2004	Prenanthes racemosa
2004	Psoralidium batesii
2004	Pycnanthemum pilosum
2004	Pycnanthemum virginianum
2004	Ratibida pinnata
2004	Rosa arkansana suffulta
2004	Rudbeckia hirta
2004	Rudbeckia subtomentosa
2004	Ruellia humilis
2004	RUMEX CRISPUS
2004	Schizachyrium scoparium

2004	Scirpus sp.
2004	Setaria glauca
2004	Silene stellata
2004	Silphium integrifolium
2004	Silphium laciniatum
2004	Silphium perfoliatum
2004	Solidago rigida
2004	Solidago sp.
2004	Solidago speciosa
2004	Sorghastrum nutans
2004	Sporobolus asper
2004	Sporobolus heterolepis
2004	Sporobolus sp.
2004	Stipa spartea
2004	Teucrium canadense
2004	Thalictrum dasycarpum
2004	Tradescantia ohiensis
2004	Tridens flavus
2004	TRIFOLIUM PRATENSE
2004	Verbena hastata
2004	Verbena stricta
2004	Vernonia sp.
2004	Veronicastrum virginicum
2004	Zizia aurea
2009	Ambrosia trifida
2009	Amorpha canescens
2009	Andropogon gerardii
2009	Asclepias incarnata
2009	Asclepias sp.
2009	Asclepias sullivantii
2009	Asclepias tuberosa
2009	Asclepias verticillata
2009	Aster azureus
2009	Aster Ericoides
2009	Aster laevis
2009	Aster novae-angliae
2009	Aster praealtus
2009	Aster sp.
2009	Baptisia bracteata var glabrescens
2009	Baptisia lactea
2009	Baptisia sp.
2009	Bouteloua curtipendula
2009	Brickellia eupatorioides

2009	Cacalia atriplicifolia
2009	Cacalia plantaginea
2009	Carex bicknellii
2009	Carex lupulina
2009	Carex sp
2009	Carex vulpinoidea
2009	Ceanothus americanus pitcheri
2009	Centaurea americana
2009	Chamaecrista fasciculata
2009	Chelone glabra
2009	Cicuta maculata
2009	Coreopsis palmata
2009	Coreopsis sp.
2009	Dalea candida
2009	Dalea purpurea
2009	Dalea sp.
2009	Delphinium virescens
2009	Desmodium canadense
2009	Desmodium sp
2009	Dichanthelium sp
2009	Echinacea pallida
2009	Elymus canadensis
2009	Epilobium coloratum
2009	Eryngium yuccifolium
2009	Eupatorium altissimum
2009	Eupatorium perfoliatum
2009	Eupatorium purpureum
2009	Eupatorium sp.
2009	Euphorbia corollata
2009	Euphorbia sp.
2009	Euthamia graminifolia
2009	Helenium autumnale
2009	Helianthus grosseserratus
2009	Helianthus maximiliani
2009	Helianthus petiolaris
2009	Helianthus rigidus
2009	Helianthus sp.
2009	Heliopsis helianthoides
2009	Heuchera richardsonii
2009	Hieracium sp.
2009	Koeleria macrantha
2009	Lespedeza capitata
2009	Liatris aspera

2009	Liatris ligulistylis
2009	Liatris pycnostachya
2009	Liatris squarrosa
2009	Lobelia siphilitica
2009	Lobelia spicata
2009	Monarda fistulosa
2009	Muhlenbergia sp.
2009	Oenothera biennis centralis
2009	Parthenium integrifolium
2009	Penstemon digitalis
2009	Phlox pilosa
2009	Physostegia virginiana
2009	Polytaenia nuttallii
2009	Potentilla arguta
2009	Prenanthes alba
2009	Prunella vulgaris
2009	Pycnanthemum virginianum
2009	Ratibida pinnata
2009	Rosa sp
2009	Rudbeckia hirta
2009	Rudbeckia laciniata
2009	Rudbeckia subtomentosa
2009	Rudbeckia triloba
2009	Ruellia humilis
2009	Schizachyrium scoparium
2009	Scleria triglomerata
2009	Setaria faberii
2009	Silphium integrifolium
2009	Silphium laciniatum
2009	Silphium perfoliatum
2009	Sisyrinchium campestre
2009	Solidago rigida
2009	Solidago sp.
2009	Solidago speciosa
2009	Sorghastrum nutans
2009	Sporobolus asper
2009	Sporobolus heterolepis
2009	Stipa spartea
2009	Teucrium canadense
2009	Tradescantia ohiensis
2009	Tridens Flavus
2009	Verbena hastata
2009	Vernonia fasciculata

2009	Veronicastrum virginicum
2009	Viola pedatifida
2009	Zizia aurea
2012	Ambrosia trifida
2012	Amorpha canescens
2012	Andropogon gerardii
2012	ARCTIUM LAPPA
2012	Aster sp.
2012	Bouteloua curtipendula
2012	BROMUS JAPONICUS
2012	Euphorbia nutans
2012	Cirsium altissimum
2012	Dalea purpurea
2012	Daucus carota
2012	ECHINOCHLOA CRUS-GALLI
2012	Elymus canadensis
2012	Elymus canadensis
2012	Lactuca serriola
2012	Lespedeza capitata
2012	Monarda fistulosa
2012	Ratibida pinnata
2012	Rudbeckia hirta
2012	SETARIA GLAUCA
2012	Setaria faberii
2012	Sorghastrum nutans
2012	Sporobolus asper
2013	Ambrosia artemisiifolia
2013	Amorpha canescens
2013	Andropogon gerardii
2013	Anemone cylindrica
2013	Asclepias tuberosa
2013	Aster azureus
2013	Aster ericoides
2013	Aster laevis
2013	Aster sp.
2013	Baptisia bracteata var glabrescens
2013	Baptisia lactea
2013	Bouteloua curtipendula
2013	Brickellia eupatorioides
2013	Bromus inermis
2013	Carex brevior
2013	Ceanothus americanus pitcheri
2013	Centaurea sp.

2013	Chamaecrista fasciculata
2013	CHENOPODIUM ALBUM
2013	CICHORIUM INTYBUS
2013	Coreopsis palmata
2013	Dalea candida
2013	Dalea purpurea
2013	Daucus carota
2013	Delphinium virescens
2013	Echinacea pallida
2013	Elymus canadensis
2013	Elymus virginicus
2013	Eryngium yuccifolium
2013	Eupatorium altissimum
2013	Euphorbia corollata
2013	Festuca paradoxa
2013	Gentiana alba
2013	Helenium autumnale
2013	Helianthus grosseserratus
2013	Heliopsis helianthoides
2013	Heuchera richardsonii
2013	Hypericum prolificum
2013	Koeleria macrantha
2013	Lactuca canadensis
2013	Lespedeza capitata
2013	Liatris aspera
2013	Liatris pycnostachya
2013	Liatris sp.
2013	Lobelia spicata
2013	Medicago sativa
2013	Mirabilis sp.
2013	Monarda fistulosa
2013	Muhlenbergia sp.
2013	Panicum virgatum
2013	Parthenium integrifolium
2013	Penstemon digitalis
2013	Phlox pilosa
2013	Potentilla arguta
2013	Pycnanthemum virginianum
2013	Ratibida pinnata
2013	Rosa spp.
2013	Rudbeckia hirta
2013	Rudbeckia subtomentosa
2013	Ruellia humilis

2013	RUMEX CRISPUS
2013	Schizachyrium scoparium
2013	Scirpus sp.
2013	SETARIA FABERIi
2013	SETARIA GLAUCA
2013	Silphium integrifolium
2013	Silphium laciniatum
2013	Silphium perfoliatum
2013	Sisyrinchium campestre
2013	Solidago nemoralis
2013	Solidago rigida
2013	Solidago sp.
2013	Solidago speciosa
2013	Sorghastrum nutans
2013	Spartina pectinata
2013	Sporobolus asper
2013	Sporobolus heterolepis
2013	Stipa spartea
2013	Tradescantia ohiensis
2013	Verbena stricta
2013	Vernonia baldwinii
2013	Vernonia sp.
2013	Veronica arvensis
2013	Viola pedatifida
2013	Zizia aurea
2014	Amorpha canescens
2014	Andropogon gerardii
2014	Anthemis cotula
2014	Asclepias syriaca
2014	Aster azureus
2014	Aster ericoides
2014	Aster laevis
2014	Aster oblongifolius
2014	Aster pilosus
2014	Aster sp.
2014	Baptisia bracteata var glabrescens
2014	Baptisia lactea
2014	Bouteloua curtipendula
2014	Brickellia eupatorioides
2014	Ceanothus americanus pitcheri
2014	Chamaecrista fasciculata
2014	Dalea candida
2014	Dalea purpurea

2014	Delphinium virescens
2014	Echinacea pallida
2014	Elymus canadensis
2014	Elymus virginicus
2014	Eryngium yuccifolium
2014	Eupatorium altissimum
2014	Eupatorium sp.
2014	Gentiana alba
2014	Guizotia abyssinica
2014	Heliopsis helianthoides
2014	Heuchera richardsonii
2014	Juncus tenuis
2014	Koeleria macrantha
2014	Lactuca canadensis
2014	Lespedeza capitata
2014	Liatris aspera
2014	Lobelia spicata
2014	Mirabilis nyctaginea
2014	Monarda fistulosa
2014	Penstemon digitalis
2014	Phlox pilosa
2014	Polytaenia nuttallii
2014	Potentilla arguta
2014	Pycnanthemum virginianum
2014	Ratibida pinnata
2014	Rosa spp.
2014	Rudbeckia hirta
2014	Ruellia humilis
2014	Schizachyrium scoparium
2014	Scirpus sp.
2014	Silene stellata
2014	Silphium integrifolium
2014	Silphium laciniatum
2014	Solidago canadensis
2014	Solidago nemoralis
2014	Solidago rigida
2014	Solidago sp.
2014	Solidago speciosa
2014	Sorghastrum nutans
2014	Sporobolus asper
2014	Sporobolus heterolepis
2014	Tradescantia ohiensis
2014	Tradescantia ohiensis

2014	Verbena stricta
2014	Verbesina alternifolia
2014	Vernonia baldwinii
2014	Vernonia fasciculata
2014	Viola pedatifida