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Enhancing Soil Carbon Sequestration through Grassland Restoration of Former

Row Crop Fields at Irvine Prairie

A Thesis Submitted in Partial Fulfillment

of the Requirements for the Degree of Master of Science

Joshua Joel Mixdorf

University of Northern Iowa

December 2023

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Abstract

The sequestration of atmospheric carbon into the soil is an important mechanism to combat the current global warming trend and the depletion of soil organic matter. Plants capture carbon from the atmosphere and sequester it in the soil, where it is utilized by soil organisms; these processes increase soil organic matter, decrease the amount of carbon in the atmosphere and fuel microbial respiration that drives nutrient production needed by plants. In this study I measured the accumulation of soil organic matter on land that had been in constant row crop rotation for many years and was converted into prairie in increments of 10-15 ha per year starting in 2018. This provided a gradient to see how conversion affects the total soil organic carbon over time. I examined this using shallow (top 15 cm) and deep (0-45 cm) core samples. Deep core samples provide information as to how deep and fast the soil is sequestering the soil organic carbon. I also examined the effect that restoration has on the soil's bulk density. Both the percent and density of soil organic matter increased by over 40% in the first five years after restoration. The top 15 cm accumulated 1,416 kg of soil organic matter per ha per year. With soil between 15 and 45 cm trending similar to the top 15 cm, Irvine Prairie is sequestering 4,248 kg of soil organic matter per ha per year, or approximately 2,464 kg C per ha per year. Finally, I developed a novel, improved technique for collecting deep core samples utilizing a corer tip that was modified and fitted with an auger to reduce surface tension on the corer tube. The auger core tip collected soil samples with minimal compaction while the original corer tip was found to be pressing the core sample into the ground rather than collecting all of the sample when soil moisture was high.

This Study by: Joshua Joel Mixdorf

Entitled: Enhancing Soil Carbon Sequestration Through Grassland Restoration of Former

Row Crop Fields at Irvine Prairie

has been approved as meeting the thesis requirements for the

Degree of Master of Science

Date	Dr. Kenneth Elgersma, Chair, Thesis Committee
Date	Dr. Laura Jackson, Thesis Committee Member
Date	Dr. Mark Myers, Thesis Committee Member
Date	Dr. Stephanie Huffman, Dean, Graduate College

Dedication

This thesis is dedicated to my ancestors. It is hard to target just one member of my family to thank since my family is rooted in agriculture. Both sides of my family come from farming backgrounds, and both raised livestock. As will be seen in this thesis this is a dwindling trend. Without them to be my role models, I would have never had the passion for an ecologically friendly and supportive farm. It is too easy to farm in a manner that takes advantage of the land and yields little rewards back to the ecosystem however it is much more challenging but rewarding to take care of the land and in turn the ecosystem that it supports. My parents and ancestors have instilled these values in me and have therefore impacted me more than they can ever imagine. My farm will always value the ecosystem it supports before all things. I want to thank them with all my heart for this lesson and gift.

Acknowledgements

I would like to thank Dr. Laura Jackson and Dr. Mark Myers for serving on my thesis committee. I would also like to thank all the professors who I worked with at the University of Northern Iowa.

I would like to thank Cathy Irvine for donating the land to the University of Northern Iowa. Without her commitment to nature and the environment this study and many others currently in progress would not be possible.

I am also thankful for my family who have supported me through my academic career. They have been instrumental in helping me accomplish my goals. They have been there to advised me and shared in many of my responsibilities to allow me to accomplish my goals. Not many people can say they have achieved so many goals while still running a farm and a full-time career and a military career. All this is possible due to my family.

I am especially thankful for my advisor and chair of my thesis committee at the University of Northern Iowa, Dr. Kenneth Elgersma, for accepting me as a graduate student, for his guidance in ecology, and his support throughout the entirety of my time at the University of Northern Iowa. My first class with Dr. Elgersma was our graduate level ecology class in which his study on polyculture prairie restoration which incorporated switchgrass was the inspiration for our new pasture mix of nine species. I appreciate all of his guidance and patience in the writing process. I am grateful for his patience and instruction, and I know without his help I could never have accomplished this study. He has been incredibly supportive of my many military absences, farming obligations and working full time. I thank you sincerely.

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Chapter 1: Introduction

Background

Climate change and rising temperatures are considerable issues facing the world today and have a global impact. The largest atmospheric component contributing to global warming and climate change is carbon dioxide (CO₂), whose rising concentrations explain most of the increase in atmospheric carbon (C). Carbon dioxide was responsible for two-thirds of the total heat influence from humans alone in 2021 (IPCC, 2022, Lindsey, 2023). It is estimated that "globally, ground soils hold three times more carbon than the atmosphere, and the role of soil organic matter (SOM) as a regulator of climate has been recognized by scientists for decades" (Bossio et al., 2020). Large areas of grassland in the Midwestern United States have been and continue to be converted to croplands (Laingen & Craig, 2011, Wright & Wimberly, 2013, Lark et al. 2022). Similarly large areas of land across the globe have been turned from forests and native prairie lands into lands used predominantly for crop production, and this has implications for soil carbon. Shete et al. (2015) studied land contracts in Ethiopia and estimated 2.2 million hectares (ha) of land had been transferred to investors from 1993 to 2013. Spatiotemporal satellite images were utilized to estimate the area of developed lands where it was found 6,519.1 of 7,867.2 ha came from forest lands (Shete et al., 2015).

Land conversion to row crops that began early in US history continues today. As the United States expanded and European settlers moved west, the government promoted farming and expansion in the Great Plains through the Homestead Act of 1862, which gave settlers 160 acres. This played a major role in the agricultural development of the Great Plains. Later advancements in farming practices in the second half of the 20th

1

century continued the growth of farming across the Great Plains. Laingen and Craig (2011) found that counties in the Corn Belt west of the 100th meridian line had 48,000 irrigated acres producing 17 million bushels of maize in 1950. By 2007, that increased to 2.6 million acres of land producing 596 million bushels of maize in the same counties. As another example, Laingen and Craig found in 1950 North Dakota produced 9.3 million bushels of maize and by 2006 that increased to 155.4 million bushels per year. Only two years later in 2008, that number nearly doubled again to 285.2 million bushels (Laingen and Craig, 2011).

In the 21st century, federal policies promoting ethanol production led to further conversion from grassland to row crops. Wright and Wimberly found that between 2006 and 2011, the Western Corn Belt had lost 530,000 ha of grassland to row crop land conversion across an area containing just five states: North and South Dakota, Minnesota, Iowa, and Nebraska. This land was predominantly converted for maize and soybean production, driven by demand for biofuels (Wright and Wimberly, 2012). This loss in grassland by agricultural intensification and reallocation of land use has only grown more prevalent in the last decade or so due to the EPA's Renewable Fuel Standard which has fueled the expansion of row crops in the upper Midwest. This land use change has large effects on soil organic carbon (SOC) as will be discussed. The SOC is an important component of soil organic matter (SOM), making up approximately 58% of SOM by mass (Howard & Howard 1990).

Looking at livestock production, shifts from grazing livestock on large areas of grassland to confinement or lot operations has been a predominant trend. This shift in production methods has led to large areas of grassland used for livestock production to be turned into row crops. In cattle operations 128 feedlots, which only accounts for 0.5% of all U.S. feedlots, feed 44.1% of all the U.S. inventory of cattle on feed (USDA report, 2017). Hog production has followed a similar pattern. In the past two decades the number of hog farms across the U.S. declined by more than 70% while hog production rose by more than 30%. While the number of producers has declined drastically, three quarters of these producers had fewer than 100 head of hogs while they only accounted for 0.8% of the U.S. inventory. The majority of hogs are now raised in confinement buildings rather than on a pasture and eat a grain-based diet. I have personally seen the shift from grasslands to cultivated crops. Our cattle farm is one of the very few operations that has more land in pasture and hay production than grain production. In the last 25 years, I have seen more and more farmers tearing out fence lines and cutting down tree lines separating fields in order to gain even five more feet around their fields.

Land cover and land use can vary widely and can thus be divided into categories, such as forests, pastures, secondary forests, plantations, and crops (Guo & Gifford, 2002). Throughout this review, I will consider prairie, native grassland, hay, and pasture as a single category. Transitions from different land cover categories can affect the amount of carbon stored in the soils. Different land use categories have a large effect on the health of the soil and its ability to sequester carbon into the soil. Ihori et al. (1995) found that when cultivating grassland there was an average carbon loss of 26%. Hans Jenny formulated an equation now known as the Jenny Equation to characterize the formation of soil. The equation: S = f(cl, o, r, p, t, ...) describes soil formation (S), as a function of climate (CL), organisms (O) in and on the soil, topography (R), the initial state of material (aka parent material, P), and time (T) (Jenny, 1958). Because land use and land

cover change affects many of these factors and most notably influences vegetation and other organisms in and on the soil, these changes are expected to influence soil formation processes, including SOM formation and subsequent C sequestration.

Carbon Sequestration

It is important to look at how farming techniques affect SOC levels by both releasing it and sequestering it. Modern farming practices cause increasing amounts of SOC to be lost from the soil to the atmosphere due to reduced plant inputs and increased aeration and oxidation of organic matter. Increases in cultivated farmland practices have evolved over the years to increase crop production and weed control. However, the tradeoff is that modern row crops deplete SOC, reduce soil structure, and leave the soil bare through the winter and spring months making the soil vulnerable to erosion and nutrient leaching. Utilizing cover crops as an erosion preventer and green manure (plant material that is not used for production), is a method of protecting the soil. Poeplau and Don found cover crops added SOC to the soil in the same magnitude as other organic matter sources. Within the first 20 years of implementing cover crops, 50% of the SOC sequestration occurs. This is similar in magnitude to changing the land use categories to afforestation of cropland. Cover crops result in a landscape that mimics the perennial cover found in native tallgrass prairie. These factors make large-scale implementation of cover crops a viable strategy to combat Earth's rising temperatures and to reduce nutrient leaching and wind and water erosion (Poeplau & Don, 2015).

Other crop management techniques that help agricultural fields mimic prairie are reduced-till and no-till agriculture. Govednik et al. found no-till operations provided the highest SOC and microbial C (C_{mic}) levels in the first 0-20 cm of the soil level and little

difference in the 30-60 cm compared to conventional methods. They also found that the C_{mic} concentrations correlated to SOC concentrations and that the no till method had higher concentrations of SOC and C_{mic} in the first 0-10 cm (Govednik et al., 2023). No till practices can help to prevent soil erosion and nutrient leaching better than tilling operations. Guo and Gifford (2002) found that converting untilled pasture to tilled crop fields reduced the soil's C concentration by 85% within 30-50 years after conversion. Shete et al. (2015) found the conversion of forest to crops resulted in a SOC loss of 16% to 61%, depending on land and crop usage.

In the past, farmers utilized a large portion of agricultural land as pasture for animals to graze. Pastureland closely mimics native prairie by offering a polyculture plant-base, year-round soil coverage, deep rooted plant systems, and long periods between being plowed. Plant communities in pastures are also typically diverse. Plant diversity can play a major role in soil health. Yang et al. (2019) found that when converting crop fields back to grasslands, the diversity of the grassland affects soil accretion. They found a 178% increase in SOC in a 16 species mix compared to a monoculture restoration over 22 years (Yang et al, 2019). Guo and Gifford found when crop land is converted back to pasture, there was a 19% increase in C concentration in the soil overall, with this percentage varying with depth. The deeper the soil core the less the C concentration they found, and the first 20 cm had the highest concentration at about 29% carbon change decreasing to about 11% at 100 cm. Deeper soil sequestered less C, revealing topsoil is the most active in sequestering C (Gou & Gifford, 2002). It has also been found that when comparing pasture lands to continuous cultivation land the SOC, microbial C (C_{mic}), and C_{mic}/SOC was higher in pasture soils, suggesting the below-ground

C input from the deep-rooted systems of pastureland is much greater than other land categories (Sparling et al., 1992).

Bulk Density

Soil bulk density is an important characteristic of soil that arises during the soil formation process described by Jenny's equation and plays a large role in soil ecosystem function. Bulk density is calculated as the ratio of the dried mass of soil to its total volume. Soil bulk density is affected by many of the factors in Jenny's equation. The soil type (parent material), plant growth (organisms), and climate (e.g., frost heaving) are a few factors that affect bulk density. Bulk density in turn affects the soil's ability to sequester carbon. However bulk density is not always an easy characteristic to measure. Direct methods use core samples where the soil sample is collected by taking a known volume of a sample and then dried to get the mass over volume. One method is the core method which has the most cost advantages; however, its accuracy can vary based on many factors such as operator experience, environment, and depth of sample. One of the key issues with the core method is that the process of coring soil can itself compact the soil, resulting in artificially inflated estimates of bulk density. This is especially true for small-diameter cores because of the core's high surface area to volume ratio, which increases the amount of friction placed on the soil in the core as the corer is pushed into the ground (Campbell, 1994). This problem can be partially mitigated by using a largerdiameter core, but the resulting trade-off is that larger cores require a much greater force to push into the ground, and result in large, bulky samples. As a result, there are tradeoffs in the utility of different coring techniques. This method does not work in all areas, and where areas of high stone volume are present, an alternative to direct measurement is using methods such as excavation methods or other indirect methods such as using gamma radiation (Al-Shammary et al. 2018). The radiation method was found by Al-Shammary et al. (2018) to be the most accurate; however, it is the most expensive method and can still be affected by the sample depth and user experience.

A soil's bulk density may reveal a great deal about the soil and its ability to sequester C. For a given soil type, as the soil's bulk density rises, its ability to hold nutrients and water decreases due to compaction. Varying soil types and land categories have different bulk densities, and changes in land use categories can shift the soil's bulk density and its ability to sequester carbon. When grasslands are converted to maize, bulk density rises by 7-28.5% depending on the region. When grassland is converted to sugarcane, the bulk density increased 46.7%. Lastly, when forest is converted to cotton, bulk density increased 16% (Shete et al., 2015). The increase in Midwest bulk densities from these conversions may be due to the loss of SOC and the use of heavy equipment (Shete et al., 2015). Ruser et al. (2006) reviewed established potato fields and studied the tire-packed inter-rows, unpacked inter-rows, and the cultivated ridge. Samples were taken from depths of 0-5 cm. It was found the ridge contained the highest SOC level along with the lowest bulk density (Ruser et al., 2006). Matamala et al. (2008) compared restored prairies to cultivated fields and found the oldest restored prairie had a lower bulk density than the cultivated fields, yet the youngest restored prairie had a higher bulk density than the cultivated field (Matamala et al., 2008).

The effects of bulk density indicate that it is a variable that can be affected by soil and plant types. The conversion of a native scattered tree plot to pongomia and pigeon pea caused a decrease in bulk density. This decrease may have occurred due to pongomia and pigeon pea improving SOC by fixing atmospheric nitrogen (Shete et al., 2015). It is important to examine the bulk density of samples from different depths and soil types to gain an understanding of its effects on SOC, and to apply the findings to other results (Gifford & Roderick, 2003). This makes accurate measurement of soil bulk density important. Because bulk density affects the calculation of soil organic carbon content and because bulk density can be influenced by a number of factors, there is a need for developing tools and techniques to accurately and quickly determine bulk density in soils under a wide range of conditions.

Experimental Approach

It is important to study the effects that restoring agricultural fields to prairie lands has on C sequestration. I studied soil C sequestration at the Irvine Prairie Restoration project (IPR), which is approximately 32 hectares and has been restored in approximately 10-15 ha. sections annually starting in 2018. The IPR began with the field tilled and crop stubble at a minimum. The Tallgrass Prairie Center plotted areas of the field and planted several seed mixtures (see Table 1) with varying species at different rates across the field. Though restoration is ongoing, the plots in this study were planted between the years 2018-2022. The Tallgrass Prairie Center then established monitoring plots, marking the locations with fiberglass poles and GPS plotting. In this study, I use the IPR area to look at the gradient of time from conversion from cropland to prairie and to see its effect on the carbon sequestration in the restored prairie fields. I expect to see areas that have been in prairie longer to have a decrease in bulk density and an increase in SOC. I randomly selected plot points over a course of six years. In the first four years only shallow core samples were collected. The last two years I had access to a Giddings trailer-mounted hydraulic corer for deep core samples, and I collected both shallow and deep cores. As previously noted, a common challenge in collecting deep soil cores is preserving accurate bulk density of the core (Al-Shammary et al. 2018). Because an immense amount of pressure is required to extract deep cores, the core that is collected is often artificially compacted during the extraction process. To overcome this limitation of the coring technique, I hypothesized that adding an auger to the exterior of the coring tip would reduce the artificial compaction of the extracted core sample. To test this hypothesis, I compared the bulk density of samples taken with a smooth tip corer to samples taken with an augered tip. I hypothesized that the auger addition would reduce the soil compaction with the deep corer due to it breaking the tension of the soil as it is drilled rather than just pushing through the soil. If I was able to reduce the soil compaction in the core sample this would give a more accurate bulk density in the sample.

Chapter 2. Carbon Sequestration in Irvine Prairie Restoration Project Methods

Sample Collection

The Irvine Prairie restoration site is located at 42.22522°N, 92.26483°W. and lies in the eastern portion of Iowa, which has a humid continental climate. The predominant soil type at the site is Dinsdale silty clay loams and is moderately well drained. It is more than 80 inches to the restrictive layer. Soil samples were collected from the Irvine Prairie over the course of four summers (2018-2020 and 2022). Samples were not collected in 2021 due to an extreme drought that left the soil very compact and hard. Samples collected in 2018 were 25 points placed equidistant along 4 transects laid out equidistant from each other across the restored area to create a grid of points. The samples were taken in approximately 30 m intervals. These points were then stored in the Garmin GPS for future reference. Samples from 2019-2022 were collected approximately 1 m SE from the permanent vegetation monitoring points that had previously been established by the University of Northern Iowa's Tallgrass Prairie Center. These points were resampled each year, but were different from the 2018 points, which were not resampled. The samples consisted of cores that were shallow (15 cm) and deep (45 cm). Soil samples collected in 2019, 2020, and 2022 were all shallow; in 2022 I was able to use a hydraulic deep corer. Before the samples were taken, the litter and duff were removed to expose bare soil. The shallow cores taken in 2018 were 1.75 cm ID and 2019, 2020, and 2022 samples were 1.95 cm ID. All shallow core samples were taken to a depth of 15 cm. The deep core samples were 6.35 cm ID and were taken to a depth of 45 cm using the Giddings trailer-mounted hydraulic corer. Each core was divided into 15 cm sections and

placed in freezer bags labeled with the plot, the date, and 0-15, 15-30, 30-45 for the section of the core. All samples were placed in a cooler on ice for storage while in the field. They were then taken to the lab and placed in the freezer until they could be analyzed.

Sample Preparation

The samples were taken out of the freezer and left to thaw completely on the lab bench. Prelabeled tins were weighed empty with their lids and weights recorded. The core samples were weighed while still in the bag to get the whole wet core mass; several empty bags were weighed, and the average bag weight was subtracted from each sample weight to get the net weight of the wet soil in grams. A subsample of each sample was then dried to determine soil moisture content by filling a pre-weighed tin roughly three quarters full of wet soil and reweighed to determine wet subsample mass. The tins were then placed in the drying oven with their lids propped open at 105°C for a minimum of 72 hours. A single tin was then removed from the oven and weighed before being placed back in the oven and allowed to dry for an additional five hours. That same tin was then reweighed to ensure the weight was constant. All samples were then pulled from the oven and placed in a desiccator for an hour to cool. The lids were placed over the samples as they were pulled out of the desiccator and their weight recorded. The pre-recorded tin weight was subtracted to determine dry soil mass and soil moisture content of the subsample. The soil moisture content of each subsample was used to calculate the dry mass of each soil core. The bulk density of each core was calculated by dividing dry soil mass by the volume of the core.

To determine organic matter content in each core, the dried subsamples were screened through a 2 mm sieve to remove any existing rocks and debris. Around 5-10 g of the pass-through sample was weighed and placed into clean, prelabeled, and preweighed crucibles. The crucibles with dry soil were weighed and placed in the muffle furnace at 500°C for 200 minutes. After 200 minutes, the furnace was shut off, but the samples remained there overnight to cool. The crucibles were then placed in the desiccator for a minimum of one hour to further cool and remove any moisture condensate. The weight of each sample was recorded, and the empty crucible weight was subtracted to get the final weight of the sample. The percent of dry soil mass lost during combustion was used to calculate organic matter content of the soil core. For analysis, organic matter content was expressed both as a percentage of dry soil mass (mass: mass %) and on a volumetric basis (g C per cubic centimeter).

Sample Analysis

The data collected was analyzed using R version 4.2.2 (R Core Team, 2022). I examined the data for outliers, and two data points were removed (one shallow 0-15 cm core and one deep 30-45 cm core). Both the outliers were from the 2022 data, and both were extreme outliers, strongly suggesting errors in the sampling process or data processing.

To account for differences in soil compaction caused by the wide (6.35 cm) and narrow (1.95 or 1.75 cm) cores, the bulk density from the wide and narrow cores were compared side-by-side in 2018. Soil cores were collected using both core types in each sampled location. The ratio of bulk density between the two core types was then calculated to compare the wide and narrow core samples. The ratio of bulk density of wide to narrow cores was on average 0.82, so the bulk density of all narrow cores was multiplied by this ratio to adjust for soil compaction caused by the use of narrow cores and to allow for comparison across wide- and narrow-core samples.

In order to determine the change in soil properties over time since restoration to prairie, I analyzed the bulk density, organic matter percent, and organic matter density (g OM per cm³) using a repeated measures mixed effects model to examine linear change over time in the top 15 cm. A repeated measures model was used to account for the fact that measurements were taken at the same locations through time. The number of years since planting was a fixed factor and the sample location was a random factor in the models. Because the 2018 data were quite different compared to other years (perhaps due to collection methodological differences in the field and the gridding point differences), analyses were done two ways: including 2018 and excluding 2018 data.

Because different restored areas were planted using different seed mixtures (Table 1), I also tested for any differences due to seed mix composition by including "seed mix" as a fixed term in the repeated measures analysis. Because seed mix showed no significant effect on results, most likely due to the seed mixes being very similar in species composition, this term was not included in subsequent analyses.

I also examined the vertical distribution of carbon from the deep core samples collected in 2022. Cores were sectioned vertically into 0-15 cm, 15-30 cm, and 30-45 cm sections. Each section was processed separately, and the data were analyzed to characterize the vertical depth profile changes in bulk density, carbon concentration, and carbon content. The samples came from areas that had been restored in various years past. This space-for-time substitution can show if there is a gradient of carbon sequestered vertically over time. This data set is very limited since data were collected in only one year. Understanding the data set is limited; it may not show if there is a significant effect over time but with more sampling this would present more information.

Figure 1

Map of Irvine Prairie with dates each section was restored and geolocated plot points where soils were sampled in 2019-2022.



0 37.5 75 150 225 300 Meters

Results

It was important to first figure out if the seed mixture used affected the SOM since the sampling points used were in different seed mixtures. When looking at whether

the seed mixes affected the SOM there was no significant difference (p = 0.4107). Because there was no significant effect of seed mix and all the seed mixes are very similar to each other in plant diversity and species composition (Table 1), I did not include this factor in subsequent analyses.

Table 1

Seed Mix	species count	Seeds per ft ²	acres
Irvine Mesic West Hilltop	71	39.63	8.68
Irvine West Mesic Midslope	78	41.01	10.04
Irvine Wet-Mesic Toe	77	54.48	3.88
Irvine Wet-Mesic Lowland	70	52.39	2.93
Irvine Central Mesic Midslope	72	40.75	15.00
Central Slopes Spring North Re-Seed	43	12.95	0.95
Irvine Central Wet-Mesic Waterway	81	60.48	3.64
Southeast Mesic Midslope	89	93.32	2.00
Diversity Mix Timelapse	64	38.61	0.13
Irvine East Central Wet-Mesic Waterway	81	70.18	2.82
Irvine Dry-Mesic Midslope	69	48.94	2.02
Irvine East Central Mesic Midslope	79	46.43	4.52
Diverse Grass Strips Reseed	69	39.27	0.25
STRIPS Diverse Grass	69	40.1	11.90
Northeast Mesic Midslope	86	65.96	2.27

Irvine Prairie Seed mixtures

During the Irvine Prairie restoration total organic matter (Figure 2) and SOM (Figure 3) increased over time. The lowest SOM was found in the first year that the field

was converted from row crop to grassland, and SOM increased gradually the longer it was in grassland. The total SOM (Figure 2) in the year of restoration is 4.23% and increased to 5.38% in the first year after restoration, an increase of 27%. The SOM continued to increase over time and peaked at 5.98% (Figure 2). This was a 41% increase in SOM over time, however this was only marginally significant (p = 0.0858) due to high variability. The individual plot locations were sampled repeatedly throughout the study to determine the locations effect on SOM (Figure 3). Since the IPR was not restored all at the same time but over several years, the newly planted areas' SOM were tracked by years since planting and by location. When I exclude data from the 2018 collection, which were unusually different from the data from subsequent years, time since planting did not have a significant effect (Table 3). Sampling point location had a strong effect on SOM (Table 2). This effect remained consistent with or without 2018 data (Table 3), indicating clear and strong spatial variability in SOM across the restoration area, likely associated with topography.

The effect of time since restoration on organic matter content in the soil. The x-axis displays the number of years elapsed since converting from row crops to prairie. Year 0 indicates soils were sampled in the same year that prairie restoration occurred.



The response of soil percent organic matter to the number of years elapsed since grassland restoration. Lines connect values sampled at the same location through time. Colors indicate the year in which particular locations were restored.



Table 2

ANOVA analyses of the effects of sampling location and time since restoration on %OM, Bulk Density, and OM density. Analyses were performed using all available data, including the 2018 data.

		Percent OM		Bulk density g/cm ³		OM density g/cm ³	
Source	Df	F	р	F	р	F	р
Location	1, 100	562.1	<0.0001	13002	<0.0001	626.9	<0.0001
Time	1, 74	3.03	0.0858	4.232	0.0422	13.49	0.0005

Table 3

ANOVA analyses of the effects of sampling location and time since restoration on %OM, Bulk Density, and OM density. Analyses were performed excluding the 2018 data.

		Perce	Percent OM		Bulk density g/cm ³		OM density g/cm ³	
Source	Df	F	р	F	р	F	р	
Location	1, 75	3097	<0.0001	7865	<0.0001	4447	<0.0001	
Time	1, 74	1.1464	0.2878	6.63	0.012	8.52	0.0047	

Soil bulk density increased slightly but consistently over time elapsed since restoration (Figure 4). The first year of seeding, bulk density was 1.086 g/cm³, and increased to 1.092g/cm³ one year after seeding. The bulk density continued to increase to 1.121 g/cm³ the second year after planting. The third year after planting had a slight drop to 1.050 g/ cm³ but increased back up to 1.171 g/cm³ after four years (Figure 4, Figure 5). The bulk density increased from 1.086 to 1.171, an increase of 8% over the five years. This is a statistically significant increase over time (Table 2). If we include the 2018 data, the p-value is 0.042, however when we exclude the 2018 data, we see a rise in

significance (p = 0.012). Bulk density was also significantly affected by sample location (Table 2). The location's effect on the bulk density is seen to be significant with the 2018 data and when excluding it (Table 3).

Figure 4





The response of Bulk Density as grams soil/cubic cm over the number of years elapsed since grassland restoration.



The first year planted had the lowest average organic matter density (0.045 g OM/cm^3) and significantly (p = 0.0005) increased to 0.063 g OM/cm^3 after five years (Figure 6, Figure 7). The buildup of SOM over time was quite steady and consistent (Figure 6) The increase in OM across time was significant, whether the 2018 data were included (p=0.0005) or excluded (p=0.0047) from the analysis (Table 2, Table 3). Organic matter density increased by 40% over the five years. The organic matter density was affected by the location of the samples significantly as well (Table 3, Figure 7).

Deep cores had lower SOM density than shallow cores (Figure 8). The average SOM at 0-15 cm was 0.063 g/cm³. This decreased by 8% to 0.058 g/cm³ at 15-30 cm. The

30-45 cm SOM decreased a further 17% from the 15-30 cm samples and a total of 24% overall to 0.048 g/cm³. The deep core samples were also affected by years since sampling, though the overall effect of time in this smaller subset of data was not significant likely due to a smaller sample size (Table 4). Figure 9 shows that from zero years to four years since planting, the SOM at 0-15 cm deep had a slight increasing trend from 0.060 to 0.063 g/cm³. From 15-30 cm the one year to four years since planting also showed a more substantial increasing trend from 0.052 to 0.064 g/cm³. Despite the depletion of SOM as the depth increases, the 30-45 cm samples from one to four years planted again had a substantial increase over time at these three different soil depths, as indicated by similar slopes in their regression lines (Fig. 9) and a non-significant interaction (p = 0.836) between depth and time (Table 4).

Table 4

ANOVA analysis of depth, time, and depth vs time effects on SOM density on deep core samples as from period of restoration.

		OM density g/cm ³		
Source	Df	F	р	
Depth	2	9.9897	0.0002	
Time	1	0.9622	0.3306	
Depth X Time	2	0.1797	0.8360	
Error	59			

Linear Regression of organic matter density as kg per hectare vs years after restoration.



SOM as kg per hectare over years for each sample average per year. Colors indicate the year in which particular locations were restored. Lines link the values measured at individual locations that were sampled multiple years.



Linear regression of SOM as kg per hectare over three sampling depths for each point sampled with the sample average per depth.



The response of soil percent organic matter to the number of years elapsed since grassland restoration by depth of sampling. By random chance, no deep-core samples were collected in 2022 in the area being restored in 2022, leading to no data for year 0 at depths below 15 cm.



Discussion

Soil Organic Carbon Sequestration

Organic matter density increased significantly over a 5-year period at a rate that is equivalent to 1,416 kg of organic matter per hectare per year in the top 15 cm. While less information was available at lower depths, the data suggest a similar rate of accumulation between 15 and 45 cm. If equivalent, this implies an accumulation rate of 4,248 kg of organic matter per hectare per year in the top 45 cm, where most organic matter is found. Assuming 58% carbon in this organic matter (Howard & Howard 1990), the Irvine Prairie restoration is sequestering 2,464 kg C per hectare per year over its first 5 years. This is a substantial accumulation of organic matter that is improving soil quality and sequestering significant amounts of CO₂ from the atmosphere. Maize cultivation is estimated in the literature to sequester 200-400 kg C per hectare per year (Clay et al. 2012). Soybeans produce fewer bushels per acre and even less fodder than maize. This leaves the ground fallow for longer and creates a higher C emission and much lower C sequestration than maize (Eranki et al., 2019). Restoration from row crops to grassland therefore improves C sequestration and soil quality. Using cover crops has shown to improve the carbon sequestration of row crops but still falls short of permanent grassland or grazing land.

When switching from cultivated row crops to grassland, the 2018 data results were much lower than all subsequent years' data. Because the reason for this difference is unknown and to ensure the results are robust, I compared results with and without the 2018 data. When excluding the 2018 data from the analyses it did not change the overall conclusions for conditions except the soil %OM. In this case, the effect of time since restoration changed from marginally significant with 2018 data included, to no significance when excluding 2018 data. The following year in 2019 the SOM had recovered to 5.38%. The SOM showed an increasing trend over time. One potential explanation for this is that in 2021 starting in April large parts of Iowa suffered drought. The area of IPR had suffered an abnormal dry to extreme drought from which it did not recover from until the middle of May of 2022. I found that the SOM was the lowest the

initial year and continued to increase over time. The first to the third year after being planted there was a rise in SOM and then it started to decrease afterward. Another potential explanation could be that the plants were contributing more energy to the development of the above ground biomass rather than the root system at this time.

Bulk Density

Bulk density has been shown to decrease as SOC increases (Shete et al., 2015); however, I found that bulk density was lowest the first year of restoration and increased after that. It should be noted that this increase is not statistically significant, meaning that the simplest explanation for this is that it is due to random chance associated with random sampling. If this was not a random chance however, one reason for this could be due to residual legacy effects of plowing up of the field for converting from row crop to grassland. During cultivation, bulk density is reduced through tillage, and for a short period of years after tillage ends, the soil is expected to settle, increasing bulk density for a short amount of time (Hill & Cruse 1985). This is expected to be a transient phenomenon and as prairie roots grow into the soil, they will upheave the soil and reduce bulk density over time.

Seed Mix Effect

Previous studies have found that the composition, species richness, and functional group richness of seed mixes can influence the rate of grassland C sequestration (Yan et al., 2022). The seed mixes that the Tallgrass Prairie Center used to plant the IPR did vary in the composition and number of species; however, the diversity of all mixes was high, with the lowest species richness being 43 species. In order to determine if species diversity influences C sequestration, it would be best to compare a wider range of

diversities. Previous research has also shown that the effects of diversity are most apparent at low levels of species richness (e.g., monocultures), with little effect seen once diversity exceeds a few dozen species (Yang et al., 2019). However, since this restoration project was not designed to test these mechanisms but instead targeted for high-diversity grassland restoration, this study was not designed to examine diversity effects. Another potential explanation for the lack of seed mix effects could be since there is not a large variation in plant composition visible at the site, it may take a longer period to see differentiation in the SOC. As the different seed varieties diverge in composition through natural succession, they may create a diversity gradient across the different seed mixes; it will take a longer-term study to understand how successional changes in the plant community affect C sequestration (Walker et al. 2010).

Chapter 3: Comparing Deep Core Compaction Based on Corer Tip Types Methods

Sample Collection

Soil samples were collected from the Irvine Prairie Restoration site in the spring and summer of 2023. All samples in this data set were deep core samples that were 6.35 cm ID and were taken to a depth of 45 cm deep using the Giddings trailer-mounted hydraulic corer (Giddings Machine Co., Windsor, CO). The corer platform was set up at randomized locations at approximately 1 m SE from the permanent vegetation monitoring points that had previously been established by the University of Northern Iowa's Tallgrass Prairie Center. As the standard smooth-bore corer presses into the soil, the tension is cut by the smooth tip as It rotates, however there is still a lot of downward force applied that can compact the soil. To test this hypothesis, a smooth tip was compared to an identical tip that had a quarter inch strip of stainless steel welded around its exterior, with a one-inch rise per revolution. Hereafter I refer to this as the augered tip. The auger strip was welded with one revolution wrapped around the tip (Figure 10). Anticipating cutting the exterior pressure with the auger I hypothesized the augered tip would reduce this pressure and help to reduce the compaction of the sample.



The augered tip is pictured on the left and the smooth tip is pictured on the right.

Two core samples were collected at each location, approximately 50 cm apart. The same corer tube was used for both samples; however, the tip of the corer tube was changed between the smooth tip and the augered tip. The base of the corer on the Giddings trailer was set to swing 50 cm to the left and the right to take samples from adjacent areas without having to move the coring machine or disturb the soil. The corer was set up at the randomized locations and the tip was set to either smooth or augered. At each new location, the order in which the two tips were used was alternated from the last plot's tip; as a result, each tip was used first half of the time. This was done to prevent any effects of the order in which coring took place on the final results.

For each sample, the tube was marked at 45 cm, and before the samples were taken the litter and duff were removed to expose bare soil. The corer was then lowered into place at the soil surface before starting the downward pressure and rotation. A constant downward pressure was applied in order to keep the corer boring into the soil, and the rotation was set to full forward rotation in all samples, regardless of which tip was in use. Once the corer reached the 45 cm mark on the tube, the downward pressure and rotation was stopped. Then a reverse rotation and upward pressure was applied to extract the corer. The corer tube was pulled off the machine and before extracting the core from the tube, the length of the intact core was measured to determine the amount of soil compaction that had occurred. To get the length of the core, the top cap of the coring tube was removed. A meter stick was dropped down to the highest spot on the core sample to measure the distance from the soil surface to the top of the corer (Figure 11). To determine the compaction distance, I measured the distance between the top of the soil inside of the core to the 45-cm mark on the core. This distance was zero when no compaction occurred, and greater than zero when compaction did occur.

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Example demonstrating measurement of compaction in the tube using a meter stick to measure the distance between the surface of the soil inside of the core tube and the surface of the soil outside of the core tube.



Soil was then extracted from the core, divided evenly into three sections (0-15 cm, 15-30 cm, 30-45 cm), then placed in freezer bags and labeled. The bags were stored in a cooler on ice in the field, transported back to the lab, then placed in the refrigerator until they could be analyzed. I collected 9 pairs of cores (18 total) in the spring (April 7) of 2023 and 10 pairs (20 total) samples in the summer (June 26) of 2023.

Sample Preparation

The samples were taken out of the refrigerator and left to warm up inside the bags on the lab bench to prevent excess condensation. Prelabeled tins were weighed empty with their lids and weights recorded. The core samples were weighed while still in freezer bags; several empty bags were weighed, and the average weight was subtracted from each sample weight to get the net weight of the wet soil in grams. A subsample of each core was then dried to determine soil moisture content by filling a pre-weighed tin roughly three quarters full of wet soil and reweighed to determine wet subsample mass. The tins were then placed in the drying oven with their lids propped open at 105°C for a minimum of 72 hours. A single tin was then removed from the oven and weighed before being placed back in the oven and allowed to dry for an additional five hours. That same tin was then reweighed to ensure the weight was constant. All samples were then pulled from the oven and placed in a desiccator for at least an hour to cool. The lids were placed over the samples as they were pulled out of the desiccator and their weight recorded, and the pre-recorded tin weight was subtracted to determine dry soil mass and soil moisture content of the subsample. The soil moisture content of each subsample was used to calculate the dry mass of each soil core. The bulk density of each core was calculated by dividing dry soil mass by the volume of the corer tube (as a constant value).

Statistical analysis

Data were analyzed using R version 4.2.2 (R Core Team 2022). The augered core sample lengths were compared to the smooth core sample lengths using paired t-tests to determine if there was a difference in compaction (compaction distance). Core compaction could occur in one of two ways: one possibility is that the corer compacts the soil inside of the tube as the tube descends into the earth, resulting in a shorter core length. Alternatively, the corer could be pressing the sample down into the earth (below the 45-cm core depth) instead of compacting the sample in the corer tube. In order to determine if the soil was being compacted in the corer tube or just pressed down into the

earth below the tube, the bulk densities of both samples were calculated and compared to one another using paired t-tests. If the core samples were being compacted, one influencing factor may be soil moisture. To determine if the soil moisture affected the compaction, we fit nested ANOVAs, with the two samples nested within each plot to account for pairing of the samples, both with and without soil moisture as a covariate in the model.

Results

Two cores (one pair) from the June sample set had extremely low soil moisture and very high bulk density, suggesting that they did not dry completely in the drying oven, resulting in inaccurate measurements. This sample set was excluded from subsequent analyses, though excluding those samples from the dataset did not significantly change overall average bulk density ($t_{72} = 0.51304$, p = 0.610). When comparing the compaction distance of the augered tip samples to the smooth core samples, the augered tip resulted in significantly less compaction of the sample (Figure 13). Averaged across the two sample collections (spring and summer) the auger tip had a significantly lower compaction distance (paired t; $t_{17} = 2.601$, p = 0.019). The smooth tip had an average compaction distance of 6.9 cm and the augered tip was 3.6 cm. That is 92% more compaction from the smooth tip versus the augered tip. However, the difference in compaction distance was only evident in spring (paired t; $t_8 =$ 3.211, p = 0.012), when compaction was 115% greater for the smooth tip (12.2 cm) than the augered tip (5.7 cm, Figure 12. Compaction did not differ among the tip types in the summer (paired t; $t_8 = 0.222$, p = 0.8299), with mean difference in compaction of only 0.08 cm.

Comparison of compaction distance by tip type by date of collection. April is higher moisture then in June.



Compaction distance in cm over core tip type on the left (A) and compaction by date of collection on the right (B).



In order to determine if compaction was occurring in the corer tube or from the core being pressed into the earth, the bulk density of the samples were compared. Bulk density was calculated as grams soil / cubic cm using dried soil weight. For both the wet soil mass (Fig. 16) and the bulk density based on dry soil mass (Fig. 15), the augered tip core samples had a higher soil mass and bulk density than the smooth tip core samples. Averaged across dates, this difference between tip types was less evident (Figure 14). Average bulk density of the smooth tip cores was 1.12 g/cm³ compared to 1.16 g/cm³ for the augered cores. This 4% increase in bulk density of the augered cores was small but

marginally significant in spring (paired t; $t_8 = 2.033$, p = 0.0765) but not during the summer (paired t; $t_8 = 1.466$, p = 0.1808, Figure 14). There was a significant interaction between date and tip (p = 0.0163, Table 5). When looking at whether the date of collection played a role in the bulk density, the spring collection had a lower bulk density than in the summer (Figure 15). As expected, there was also a large amount of natural variability in bulk density from location to location across the site (p < 0.0001, Table 5).

Figure 14

Comparing bulk density as grams per cubic cm by tip type on the left (A) and bulk density by date of collection on the right (B).





Bulk density expressed as grams per cubic cm by date and tip type.

Table 5

ANOVA analyses of the effects of tip type on compaction, bulk density, and wet core

		Compaction Bulk den		ensity g/cm ³	Wet core mass		
Source	Df	χ^2	р	χ^2	р	χ^2	р
Location	1	64.33	1.1e-15	357.9	< 2.2e-16	433.7	< 2.2e-16
Auger	1	20.02	7.7e-06	7.37	0.0066	11.85	0.0006
Date	1	23.85	1.0e-06	4.37	0.0366	2.77	0.0959
Auger: Date	1	9.77	0.0018	5.77	0.0163	8.60	0.0034

mass. Analyses were performed using the two collections in 2023.

The wet soil mass from the two collection dates is shown in Figure 16. In April, the augered corer picked up significantly (paired t; $t_8 = 2.559$, p = 0.0337) more mass than the smooth tip corer, however this disappeared in June. Thus, both the bulk density and wet soil mass were reduced in the smooth core in spring only.

To understand why the effect of the corer type might differ between the spring and summer dates, I examined soil moisture. Soil moisture was higher in April than in June, although the difference was only marginally significant (p=0.0959 Table 5, Figure 17). Soil moisture did not directly affect the mass of the soil in the corer tube (p = 0.2498). However, there is some evidence that soil moisture influenced the bulk density of the samples. When adding soil moisture to the bulk density model, the effect of soil moisture is significant (p = 0.0061), but the effect of collection date is no longer significant (p=0.1901). This suggests the effect of collection date in the model is actually driven by soil moisture; "date of collection" is acting as a surrogate to the soil moisture.

Table 6

ANOVA analyses of the effects of soil moisture and tip type on core bulk density. Analyses were performed using the two collections in 2023.

Source	Df	χ²	р
Location	1	79.074	< 2.2e-16
Soil moisture	1	7.532	0.0061
Auger	1	12.581	0.0004
Date	1	1.717	0.1901
Auger: Date	1	9.450	0.0021



Comparing wet soil mass by collection date and auger tip type.





Discussion

These results strongly suggest that the augered core tip provides a more accurate assessment of bulk density and a more accurate soil profile by reducing soil compaction. The smooth tip core samples' average compaction depth was over double that of the augered tip. This supports my hypothesis that the augered tip would cut the tension of the exterior wall and allow the corer to penetrate the soil more effectively.

Interestingly, the results also suggest that at least some of the compaction occurring with the smooth tip core is compaction of soil below the core, and not just compaction within the core. Bulk density of the smooth tip samples was less than the auger tip samples (Figure 14A, Figure 15), suggesting the augered tip was collecting a larger volume of soil and the smooth tip was pushing soil down into the earth below the core. Since the samples were collected only 50 cm apart and with the same conditions of coring except the tip, they should have very similar bulk densities. If in fact the augered tip is cutting the exterior pressure and the smooth tip is having higher compaction inside of the core, the bulk density for the smooth tip should have been higher than that of the augered tip. Since the smooth bore tip has a lower bulk density and a higher compaction and when this compaction is higher it has less soil mass than the augered tip; this indicates that the smooth tip is not compacting the sample into the core tube but is compacting the sample into the earth and is not collecting the entire sample. This means the augered tip is picking more of the sample up and is increasing the accuracy of the information being collected.

I observed differences in compaction between sampling dates. However, when soil moisture was included in the model, the effect of date was no longer significant. This suggests that the effect of date was due to differences in soil moisture across dates. This is reasonable due to the soil's tendency to compact more as the moisture increases. When looking at the compaction data from the spring to the summer the smooth tip had the highest compaction in the spring and lowest bulk density. However, the spring was much higher in soil moisture than in the summer.

Chapter 4: Conclusion

Agriculture is a major factor in today's effects on climate and plays a vital role in providing food for the world. As described in Chapter 1, agriculture practices have changed drastically over the last century and even in the last decade. The loss of grasslands, pastures, and forests to row crops has contributed huge amounts of carbon to the atmosphere. Agriculture produces 30% of the annual greenhouse gases and releases 4.6 Gt CO²/yr (Tubiello et al., 2013) Row crops sequester less carbon into the soil compared to many other land uses. Agriculture also takes fuel to run equipment which further contributes carbon to the atmosphere. As we look at ways to capture this atmospheric carbon and redeposit it into the soil where it is beneficial to the soil, we need to find the most effective way to balance food production along with soil and atmospheric health.

The SOC was affected greatly by the switch from cultivated row crop to grassland and was seen to equal 1,416 kg per hectare per year accumulation of SOC. This is essential to help the health of the soil over time and sustains the soil microbial communities which then provide nutrients for the plants. The increase in SOC also plays a major role in helping to sequester the atmospheric carbon rather than contributing to greenhouse gasses. In this study I also found significant amounts of carbon were being sequestered into deeper layers of soil.

A very viable means to help with today's problems of grassland losses and the need to sequester carbon would be to employ a mixture of techniques presented in this study. One example would be planting grassland strips at the headlands of row crop fields, which are usually the most compacted areas of the field and are entrance points to the field. This would reduce nutrient runoff, provide habitat for wildlife, and sequester carbon into the soil. Another avenue would be to use cover crops in the off season to help sequester carbon and to reduce soil runoff, produce green manure, decrease soil compaction, and reduce the need for herbicides. Another important factor to look at is to move to the more traditional farming techniques and have fewer animals raised in big feedlots and move those animals back to a pasture-based land usage to help create grasslands and reduce large manure management confines.

However, studying these land use changes provides challenges for accurate measurements. Having the right tools for the job are essential. When comparing the deep core samples to the shallow core samples I didn't see a big difference between them. One explanation may be from the smooth bore tip pressing the soil into the ground rather than collecting all the sample. This may be leading to comparing shallow soil samples as deep core samples since they are being pressed down and in the deep core samples' place. This can lead to inaccurate data. More accurate tools need to be used to correct and prevent this.

One technique I designed was to create the augered tip corer, which reduces compaction of the sample into the earth. By reducing this compaction, the augered tip reduced the surface tension on the exterior of the corer enough to be able to pick up the sample with more accuracy. This accuracy is important for understanding the bulk density and the deep core SOC. The fact we see that the smooth tip corer is pushing soil into the earth rather than picking it up has important implications for soil carbon sequestration research. When looking at soil samples and trying to gather the data from deep samples in wetter soils this may lead to inaccuracies since what is assumed to be the full core sample will in reality only be a partial core profile. Future studies should consider a method similar to this to get a more accurate result when examining bulk density and depth gradients, especially in moist soil.

This small short-term study found that IPR sequesters 2,464 kg C per hectare per year. To understand long-term consequences of restoration, this study should continue over decadal timescales in order to see the long-term effects of grassland restoration on soil C dynamics. Another aspect to examine is how different soil types affect SOC accumulation and bulk density. The IPR is a great area for further studies for years to come. Looking at the deep corer samples throughout the year and in subsequent years will be important to determine if the deep SOC samples may be skewed and getting full data at the last 30-45 cm of the soil sample.

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