Thinking like a prairie:

Soil carbon to perennial conservation

by

Mia M. Keady

A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Environment and Resources)

at the

UNIVERSITY OF WISCONSIN – MADISON

2025

Date of final oral exam: 08/14/2025

The dissertation is approved by the following members of the Final Oral Committee:

Thea Whitman, Associate Professor, Soil and Environmental Sciences

Randall D. Jackson, Professor, Plant and Agroecosystem Sciences

Christopher J. Kucharik, Professor, Plant and Agroecosystem Sciences

Adena R. Rissman, Professor, Forest and Wildlife Ecology

Abstract

The potential for soils to draw down atmospheric CO_2 has fueled interest in natural climate solutions (NCS) to offset anthropogenic climate change. The restoration of plant communities and improved land management are touted for their ability to deliver ecosystem services, including climate stabilization. However, assessing intended outcomes, or the potential for soils to serve as carbon sinks, is hindered by the spatial variability of soil carbon, decadal timescales necessary to detect change, and necessity of assessing soil carbon across the entire soil depth. I examined soil carbon dynamics of remnant and restored prairies in southern Wisconsin across time and depth and assessed conservation investment in dominant agroecosystems across the state. In chapter 1, I assessed differences in soil carbon stocks by above- and below-ground inputs in a long-term detrital input and removal treatment (DIRT) experiment within the world's oldest restored prairie – the UW-Madison Arboretum's Curtis Prairie. I found similar SOC stocks with either form of litter inputs when given enough time and assessed across soil depth. Exclusion of all litter depleted SOC, even persistent forms. In chapter 2, I assessed if Wisconsin prairies were increasing in soil carbon concentration and if stocks varied between remnant and restored prairies. I surveyed 21 prairies (9 restorations and 12 remnants) with previously reported baseline carbon data from the period 1999 to 2006. Restorations were 20 to 87 years old at the time of my sampling (2023). Across all sites, I found no significant change in SOC concentration from baseline in remnant and restored prairies over ~20 years, suggesting prairies maintained SOC but have not been net carbon sinks for climate stabilization within the time-period sampled. Importantly, some prairies increased their SOC over this period, some lost, and some were unchanged, but these patterns were not strongly related to edaphic factors I explored. When assessing the Curtis Prairie over time, soil carbon

concentration significantly increased in the DIRT control treatment over 66 years, with most change happening in the first 15 years, suggesting increases in SOC concentration soon after restoration with slow or variable change in later years. Restored prairies had lower SOC stocks than remnants across the surface 50-cm, reflecting loss of SOC from cultivation prior to restoration, the restoration process itself, and the long time-period necessary to potentially recover SOC stocks. In chapter 3, I looked beyond the small fraction of Wisconsin grasslands with remnant and restored prairie vegetation, to assess what types of agroecosystems we invest conservation dollars in if local and federal conservationists help shape the flow of federal conservation dollars. I assessed average conservation spending from the USDA NRCS Environmental Quality Incentives Program (EQIP) from fiscal year 2014 through 2024 at the county level in Wisconsin. I classified 128 conservation practices into annual cropland, confinement livestock, and perennial practices by expert elicitation and based on the co-occurrence of practices on conservation contracts. I found most of the conservation spending supports annual and confined livestock agroecosystems, with perennial agriculture receiving ~20% of conservation funds. Conservation spending overall was positively correlated with local and federal conservation staff abundance, suggesting conservation staff are important to helping direct federal conservation dollars. All categories of conservation practices were connected to either or both federal or local conservationists. The type of agroecosystem supported by conservation investment may depend on place-specific narratives, relationships, personalities, and priorities. Collectively, my work suggests that litter inputs are necessary to maintain SOC in restored prairies, and restoring and maintaining prairies alone will not mitigate climate change. That said, maintaining SOC is better than losing it and we know perennial grasslands (like prairies) provide many other ecosystem services such as flood reduction, clean water, and

biodiversity. Transforming landscapes of Wisconsin's prairie-savanna region to perennial agroecosystems that provide these many services has been undervalued by federal conservation dollars. Increasing local conservation staff may be key to re-directing federal conservation support to genuinely improving the sustainability of Wisconsin agriculture.

Acknowledgements

To my dear family, thank you. Thank you for supporting Ryan and me moving about the country pursuing degrees. To Ryan, your even demeanor, dedication, and sacrifices to make this endeavor possible are so appreciated. Your feedback and suggestions over the years have enriched the process and outcome. To Willa, you have given your dad and me a love and purpose we will forever cherish. To my dear friends I've made along the way – thank you for your continued support, conversations, love, food, hugs and bike rides.

To my co-advisors, Drs. Randall Jackson and Thea Whiman, thank you for the opportunity to study under your guidance and partake in your labs' spirited work and discussion. I'll forever be grateful for my time at UW and for the friendships and connections I've made. I appreciate your support and encouragement along the way – and to have a place and colleagues to discuss ideas and collaborate across disciplines. To Dr. Chris Kucharik, thank you for kindly providing access to baseline soil carbon data in WI prairies and for collecting and curating this data in the first place! I'm honored you allowed me to build upon the data and surveys you began early in your career. Dr. Adena Rissman, thank you for welcoming me into your lab and supporting my work and evolution as I learned how to work and study in a new field, with new literature and lingo.

To my many lab-mates – you have made this a fun journey – and I appreciate your time, friendship, collaborations, ideas, challenges and pumpkin patch forays. Thank you to past and present lab managers Kelsey Kruger, Erin Pierce and Emily Kaufman. The opportunity to mentor research assistants was one of the highlights of my time and work at UW. Thank you, Louie Thares, Lydia White, Finn Luaders, Olivia Ramsey, Kallysa Taylor and Randi Selvey for the contributions you made to these projects and for the many soils you sieved and fractionated.

A special thank you to Professor Francis Hole for initiating the DIRT experiment at the UW-Madison Arboretum. And, to his once graduate students, Gerald Nielsen and Daniel van Rooyen, for documenting the DIRT experiments in their theses. This data is invaluable and was key to assessing long-term changes in SOC. Thank you to other 'DIRT-network' scientists, Derek Pierson, Kate Lajtha, Kim Townsend, Myrna Simpson, and Knute Nadelhoffer, for your advice and guidance in studying the DIRT experiment. I've been shaped by conversations and collaborations over the last four years with the following colleagues: Yu Lu, Michael Happ, Margaret Krome, Becky Schewe, Christina Anderson, Meagan Duberstein, Brad Herrick, Tim Berry, Adam von Haden, Gregg Sanford, Harry Read, Laura Paine, John Strauser, Sarah Lloyd, Laura Paine and Darcy Kind.

My graduate work was largely funded by the University fellowship and the UW-Madison Arboretum. I am grateful for the opportunity to study and work at UW-Madison, and especially to conduct work in the long-term DIRT experiment in the treasured Curtis prairie. It's been an honor to be a UW-Madison Arboretum Leopold fellow and Research fellow. Thank you to the arboretum staff, researchers, and directors that supported this work. Thank you to Michael Fields Agricultural Institute, and the guidance of Margarett Krome, for supporting my third chapter.

Lastly, thank you to the many organizations and individuals that allowed me to collect soil samples in WI remnant and restored prairies: UW Madison Arboretum, Brad Herrick of Lake Shore Preserve, Andy Gossens of International Crane Foundation, Nate Fayram of WI DNR, Emma Raasch and Graham Steinhauer of Goose Pond Sanctuary, Mark and Sue Martin, David Musolf and Rodger Packard of Faville Grove Sanctuary, Seth McGee of Lake Shore Nature Preserve, Groundswell Conservancy, Southern Wisconsin Bird Alliance, Dane County Parks, Gregg Sanford and Mike Bertram of UW Arlington Research Station, Troy Farm, Madison Area Community Land Trust, and The Nature Conservancy.

Table of Contents

Introduction

I biked into Madison, WI on a rainy morning Tuesday June 29th, 2021, as part of my cross-country bike tour prior to starting my PhD that September. I signed my lease and rode down the Cap-City Trail, which would become familiar over the next four years. Coming off the COVID-19 pandemic and unrest in the Nation's Capitol – a cross-country bike trip was solace in a chaotic world. My two bike comrades and I were greeted by strangers, offered yards to sleep in, and often a home-cooked meal or spotted a fast $20 for breakfast during a rainstorm. We had a front row seat to much of America's Heartland – with ample time to ponder the miles and miles of cropland. I carried Leopold's *A Sand County Almanac* in my pannier and did my best to answer my comrades' questions about why some haybales are covered, others not, some stacked, some square, most round. My upbringing in Lincoln, NE brought me only a sliver of ag knowledge. Much of the once prairie now in tidy rows of feed corn, I somehow dodged the summer job of detasseling corn. My husband's passion for food, home-grown veggies, and history padded my food-system repertoire. My own studies in biology brought me an appreciation for the once sprawling prairie ecosystem. I liked to imagine the rows and rows of wheat in North Dakota as prairie grasses blowing in the wind as we pedaled by - especially with the large, white pelicans amassed on ponds. I reflected on an earlier summer as a plant identification intern in western Nebraska where I experienced the unturned prairie of the Sand Hills – sand deposited as glacial melt from the Rockies 8,000 years ago, stabilized by grasses and forbs, and much of it now grazed by beef cattle. I hoped to apply my recent work studying microbiomes of animals to the soils of the prairie ecosystem. Could the ecosystem I loved, the soils beneath it, and the microbes within it help us with this climate disaster we've created?

We rolled into a drought in Montana that summer. It was hot, and often shadeless. Sometimes we used the shadow of an old grain elevator to try and eat our lunch out of the direct heat of the beating sun. The USDA allowed an emergency haying of CRP acres during a two-week stretch at the end of July. We saw fires start in fields due to the hot machinery and dry biomass. As we made our way across, we soon encountered smog from wildfires in the Pacific Northwest. Our planned route on Highway 20 through Washington was closed. We met people evacuating the area we were heading. Us and the volunteer fire fighters were indulging in a cold treat at the local Dairy Queen on a dry, hot day. After one day of pedaling in poor air quality, we resigned our aspirations of making it to Anacortes, WA and boarded a train to Glacier National Park. We did one more glorious ride up *Going to the Sun Road* and made our return arrangements. The close of my 3,000-mile cross-country bike trip, cut short by anthropogenic climate induced wildfires, brought the next adventure - navigating if our soils can save us from our own CO_2 emissions and the social and political dimensions of how we get our landscape to look a little more like the prairie it once was.

Beginning in the mid-1800s, prairies across the Midwest and Great Plains regions were uprooted, tilled, and converted to row-crop agroecosystems. One-third to one-half of soil organic carbon (SOC) was lost through this process and the losses continue today. The exceptions are roughly 1 to 6% of remnant (untilled) prairie in the eastern Tallgrass Prairie. Larger swaths of prairie remain in areas such as the Konza Prairie in the Flint Hills of Kansas and the Sandhills of Nebraska; both spared because farming was so difficult on steep slopes with shallow and/or sandy soils. Prairies provide essential habitat, biodiversity, water filtration, and soil stabilization, yet are now discontinuous ecosystems, largely isolated and tucked into hillsides on small preserves or private land.

The 1930s and 1940s brought economic hardship, soil loss across the Midwest, federal and local solutions to degradation, and the first seeds of restoration science. Amidst the Great Depression, valuable topsoil that had formed over thousands of years was blown away in just a few years as drought and extractive farming practices brought the Dust Bowl. In response, the US government established the Soil Conservation Service, later renamed to the Natural Resource Conservation Service. States also formed county conservation districts to invest in local solutions alongside federal support. The New Deal formed the Civilian Conservation Corps (CCC) that housed and paid young men across the country to support natural resource and structural development of public lands. The University of Wisconsin-Madison Arboretum acquired land in 1932, and under the direction of the research director Aldo Leopold, began the process of re-establishing "original Wisconsin" ecosystems within the bounds of the Arboretum (*History | UW Arboretum*, 2025). CCC members established plant communities throughout the Arboretum and notably contributed the founding pieces of what developed into the field of restoration ecology. The establishment of the Curtis Prairie in 1936 is now recognized as the first-ever Tallgrass Prairie restoration. The efforts during this time-period to recognize, protect, and restore ecosystems set the seed for how we address conservation needs today and contribute to the evolving debate of how, for whom, and for what we manage agroecosystems.

The UW-Madison Arboretum is home to other advances in ecosystem management, as well as long-term research trials. Central Wisconsin is within a 'tension zone' that climatically provides conditions supporting savanna and woodland systems if prairies are not periodically disturbed by burning and/or grazing. Encroachment of woody species into prairies has been well documented (Brock, 2014; Curtis, 1959; Leach & Givnish, 1996). The UW-Arboretum was an early experimenter with prescribed burning to maintain the disturbance-dependent prairie ecosystem. As part of the quest to establish "original Wisconsin", initiatives to understand Wisconsin plant communities were matched with studying soil development under different ecosystems. Professor Francis Hole established the Detrital Input and Removal Treatment (DIRT) in 1956 to study soil in response to litter manipulations. The UW-Arboretum is home to the only grassland DIRT experiment, with woodland systems replicated elsewhere in North America. This one-of-a-kind trail in the world's oldest restored grassland provides a unique opportunity to study litter effects on SOC in the context of restoration.

The process and goals of ecosystem restoration are typically site specific, but as anthropogenic CO_2 emissions continue to threaten our future climate, ecosystem restoration has become a potential climate mitigation strategy. Terrestrial soil, plant biomass, and the ocean have served as carbon sinks over the millennia; and continue to offset about half of anthropogenic emissions today (*Climate Change*, 2025). The prospect of managing ecosystems in a manner to draw down and store carbon to offset anthropogenic emissions, have been promoted (Fargione et al., 2018; Griscom et al., 2017) and critiqued (Schlesinger, 2022) as viable mechanisms for climate mitigation. Soils store the greatest stock of terrestrial carbon, and prairies certainly have previously accumulated and stored massive amounts of soil organic carbon (SOC) (Yang et al., 2019), but can they be the carbon sink we want and need them to be now and into the future (Bradford et al., 2019)? In this dissertation I explored the following questions 1) Does SOC vary by above- and below-ground litter inputs?, 2) Are restored and remnant Wisconsin prairies building SOC in recent decades?, and 3) What types of agroecosystems do we invest federal conservation dollars in and what shapes the distribution these resources?

References

Bradford, M. A., Carey, C. J., Atwood, L., Bossio, D., Fenichel, E. P., Gennet, S., Fargione, J., Fisher, J. R. B., Fuller, E., Kane, D. A., Lehmann, J., Oldfield, E. E., Ordway, E. M., Rudek, J., Sanderman, J., & Wood, S. A. (2019). Soil carbon science for policy and practice. *Nature Sustainability*, *2*(12), 1070–1072. https://doi.org/10.1038/s41893-019-0431-y

Brock, T. (2014). Legacy Effects in Prairie Restoration: A 73-Year Spatial History. *The Prairie Naturalist*. https://digitalcommons.unl.edu/tpn/14

Climate change: Atmospheric carbon dioxide | NOAA Climate.gov. (2025, May 21). https://www.climate.gov/news-features/understanding-climate/climate-change-atmospheric-carbon-dioxide

Curtis, J. T. (1959). *The Vegetation of Wisconsin: An Ordination of Plant Communities*. University of Wisconsin Press. https://muse.jhu.edu/pub/19/monograph/book/16741

Fargione, J. E., Bassett, S., Boucher, T., Bridgham, S. D., Conant, R. T., Cook-Patton, S. C., Ellis, P. W., Falcucci, A., Fourqurean, J. W., Gopalakrishna, T., Gu, H., Henderson, B., Hurteau, M. D., Kroeger, K. D., Kroeger, T., Lark, T. J., Leavitt, S. M., Lomax, G., McDonald, R. I., … Griscom, B. W. (2018). Natural climate solutions for the United States. *Science Advances*, *4*(11), eaat1869. https://doi.org/10.1126/sciadv.aat1869

Griscom, B. W., Adams, J., Ellis, P. W., Houghton, R. A., Lomax, G., Miteva, D. A., Schlesinger, W. H., Shoch, D., Siikamäki, J. V., Smith, P., Woodbury, P., Zganjar, C., Blackman, A., Campari, J., Conant, R. T., Delgado, C., Elias, P., Gopalakrishna, T., Hamsik, M. R., … Fargione, J. (2017). Natural climate solutions. *Proceedings of the National Academy of Sciences*, *114*(44), 11645–11650. https://doi.org/10.1073/pnas.1710465114

History | UW Arboretum. (2025). https://arboretum.wisc.edu/about-us/history/

Leach, M. K., & Givnish, T. J. (1996). Ecological Determinants of Species Loss in Remnant Prairies. *Science*, *273*(5281), 1555–1558.

Schlesinger, W. H. (2022). Biogeochemical constraints on climate change mitigation through regenerative farming. *Biogeochemistry*. https://doi.org/10.1007/s10533-022-00942-8

Yang, Y., Tilman, D., Furey, G., & Lehman, C. (2019). Soil carbon sequestration accelerated by restoration of grassland biodiversity. *Nature Communications*, *10*(1), 718. https://doi.org/10.1038/s41467-019-08636-w

Chapter 1. Above- and belowground contributions to SOC in world's oldest restored tallgrass prairie

Mia M. Keady, Randall D. Jackson, Thea Whitman

Core ideas

- Similar soil organic carbon concentration accumulation over time with above- and below-ground litter exclusion
- Soil organic carbon stocks similar when above- or below-ground litter excluded
- Bare soils had lower soil organic carbon stocks across depths
- Bare soils had depleted mineral-associated and particulate-associated organic carbon pools
- Absence of below-ground inputs reduces bacterial and fungal abundance and shifts lipid composition

Abstract

Prairies store large amounts of soil carbon via their deep roots and turnover of above-ground biomass. We assessed the relative importance of above- and below-ground litter on soil organic carbon (SOC) accumulation and stocks in the world's oldest restored tallgrass prairie. We found similar SOC stocks and increases in SOC concentration by above- and below-ground litter inputs after 66-years of manipulation. Litter exclusion reduced SOC by ~ 80%, with declines even among soil fractions considered most persistent. Root exclusions resulted in decreased microbial lipid abundance, but with above-ground inputs, maintained SOC stocks similar to control suggesting a possible shift in carbon accumulation pathways. Our results indicate the

importance of above- and below-ground inputs to SOC stocks, and the detriment of bare soils to SOC stocks and soil functions.

Introduction

Soil contains the greatest stock of terrestrial carbon globally, yet the relative importance of factors shaping soil organic carbon (SOC) accumulation and storage remains uncertain. The capacity for soil to be an atmospheric carbon sink has spurred initiatives to mitigate anthropogenic carbon emissions by promoting land management practices that accrue and stabilize SOC. Carbon is stored primarily in soil organic matter (SOM), the living, dead, and decomposing soil fraction. Mechanisms of carbon persistence (or long-term residence) are less well understood with multiple emerging conceptual pathways (Cotrufo et al., 2013; Liang et al., 2017; Rocci et al., 2023). Understanding mechanisms of SOC persistence in tandem with environmental controls (Schmidt et al., 2011) is key to implementing land-management strategies that increase SOC accumulation that contributes to climate mitigation and adaptation.

Conceptual frameworks of SOC persistence, or SOC mean-residence time, have shifted to include biological processes in addition to chemical recalcitrance (Rocci et al., 2023). For instance, chemical composition of plants, microbes, and biological components (proteins, saccharides, glucosamine) are not the sole predictors of SOC mean residence times (Schmidt et al., 2011). Instead, carbon bound to mineral surfaces or protected within aggregates are thought to drive SOC persistence (Liang et al., 2017). Recent work suggests SOC can be conceptualized as two fractions, particulate organic carbon (POC) and mineral-associated organic carbon (MAOC), that differ in their structure, formation, and persistence (Lavallee et al., 2020). POC is largely derived from partially decomposed plant tissue, while MAOC is primarily made up of small, processed organic matter fragments, microbial products, and dead microbial tissues (necromass) that are chemically bonded to mineral particles such as clay and silt (Cotrufo et al., 2015; Lavallee et al., 2020). The chemical bonds make carbon in MAOC harder to access by microbes and thus more persistent. Further, POC and MAOC can both be physically protected from microbial degradation by occlusion within soil aggregates. The persistence of these SOC pools largely depends on whether microbes can access carbon for catabolism and anabolism (Liang et al., 2017; Zhu et al., 2020).

Soil microbes are an essential part of the carbon cycle, both degrading organic matter and respiring carbon as CO_2, as well as contributing to SOM via their carbon-rich cellular tissues (necromass) and extracellular enzymes. Yet, understanding soil microbial community processes as they pertain to the mechanisms that underpinning SOC persistence has proved difficult and led to contrasting frameworks and hypotheses. For instance, the Microbial Efficiency-Matrix Stabilization (MEMS) framework suggests that chemically labile carbon sources (such as those from root exudates or high quality litter) lead to more persistent carbon (such as MAOC), because microbes can easily and efficiently process the carbon, which ends up as mineral-associated necromass (Cotrufo et al., 2013), while others have suggested the addition of labile inputs spur microbial activity, increase respiration, and may ultimately ‘mine’ or ‘prime’ existing carbon and contribute to net SOC loss (Lajtha, Bowden, et al., 2014; Sulzman et al., 2005). Microbial anabolism, which involves uptake, synthesis, and incorporation of carbon into microbial biomass and thus necromass, is thought to create the most stable forms of carbon, referred to as the *entombing* effect (Liang et al., 2017). Anabolism is countered by catabolism and the balance between these two processes over time underpin a stable SOC pool as described in the Microbial Carbon Pump (MCP) framework (Liang et al., 2017). Thus, microbial processing of litter is hypothesized to regulate a stable soil carbon pool (Liang et al., 2017) and is dependent on the ‘efficacy’ or persistence of microbial products (Zhu et al., 2020). Segregating litter (above- vs. below-ground) in long-term field experiments allows us to characterize shifts in microbial composition, quantify MAOC and POC fractions, and track SOC accumulation over time. In doing so, we can empirically test conceptual models of carbon accumulation and persistence in response to litter and microbial perturbations.

The Detrital-Input and Removal Treatment (DIRT), established in 1956 by Dr. Francis Hole in prairie and woodland ecosystems at the University of Wisconsin-Madison's Arboretum (Nielsen & Hole, 1963) manipulates the presence and absence of above- and below-ground inputs (Figure 1, Table 1). The DIRT experimental design has been replicated in woodland and dryland sites in the U.S., Hungary, and Germany (Lajtha et al., 2018). Notably, the UW-Madison Arboretum is the only experiment that includes restored tallgrass prairie. Curtis Prairie is the world's oldest restored prairie with initial plantings by the Civilian Conservation Corps in 1936. Previous work in the Curtis Prairie DIRT experiment reported similar surface-soil carbon stocks between above- and below-ground inputs after 50 yr (Lajtha, Townsend, et al., 2014). Treatments with no litter inputs showed 69 to 71% declines in SOC stocks over time when compared to the undisturbed control plots (Lajtha, Townsend, et al., 2014). MAOC and POC pools also did not vary between above- and below-ground inputs after 50 yr (Lajtha, Townsend, et al., 2014).

We incorporated historical data to assess SOC concentrations over time in surface soils (0 to 10 cm) beginning in 1956 through our most recent sampling in 2022. We assessed SOC stocks among treatments after 66 years, with specific attention to whether above- and below-ground inputs resulted in different SOC stocks across soil depths and whether they affected microbial community composition. We asked 1) How have above- and below-ground inputs influenced SOC concentrations over time? 2) How do total SOC stocks and SOC fractions vary with the presence and absence of above- and below-ground inputs after 66 years? and 3) Is microbial community composition and biomass associated with SOC stocks, and does it vary with the presence and absence of above- and below-ground litter inputs?

Hypotheses

Given the experiment is located within a restored prairie, we expected SOC concentrations to gradually increase over time in treatments receiving some form of litter inputs, with undisturbed treatments accumulating the most SOC followed by treatments with belowground inputs and then those with aboveground inputs. Belowground inputs are hypothesized to have a stronger effect than aboveground litter on SOC given substantial root systems with corresponding rhizosphere and decomposer communities adapted to root inputs in prairie ecosystems.

After 66 years of treatments, we expected DIRT treatments with both above- and below-ground litter inputs would have greater SOC stocks, followed by treatments with belowground inputs and then aboveground inputs. We expected treatments with roots present to have greater proportions of MAOC than aboveground inputs, aligning with the MEMS and MCP frameworks in that root exudates and root turnover provide labile carbon sources, allowing for greater microbial processing and entombing that results in persistent SOC fractions (Cotrufo et al., 2013). We did not expect to observe priming effects (net SOC loss) from roots in response to inputs, given the long duration of the treatments. We predicted microbial biomass and composition to vary according to treatment, with the exclusion of belowground inputs conferring a distinct microbial composition and smaller microbial biomass. The reduction in microbial biomass in root exclusion treatments will correspond with a smaller fraction of MAOC following the entombing effect and MCP framework.

Methods

Site description & experimental design

The UW-Arboretum in Madison, Wisconsin and is home to the Curtis Prairie, renowned as the world's oldest prairie restoration with plantings beginning in the 1930s. The Curtis Prairie is a tallgrass prairie including species such as big bluestem (*Andropogon gerardii*), Indiangrass (*Sorghastrum nutans*), goldenrod (*Solidago sp.*) (Kucharik et al., 2006; Lajtha, Townsend, et al., 2014). The mean annual temperature is 8.4 C and it receives 102 cm annually (*NOAA NCEI U.S. Climate Normals Quick Access*, 2025). The Detrital Input and Removal Treatment (DIRT) experiment is a repeated block design with three replications that each have five treatments: **1)** <u>Control</u> (undisturbed, no burning or mowing), **2)** <u>Burned</u> (spring burned every 2 to 3 years), **3)** <u>Harvested</u> (aboveground biomass mowed and removed annually in late fall), **4)** <u>Mulched</u> (aboveground vegetation removed every 2 weeks by hand to minimize root growth; litter from the harvested treatment added to surface every fall) and **5)** <u>Bare</u> (aboveground vegetation removed every 2 weeks by hand to minimize root growth) (Figure 1, Table 1). Treatment maintenance documentation is limited prior to 2018 (See Appendix for maintenance records and current maintenance protocol).

Treatments are replicated three times in the Curtis prairie (n = 3; Curtis I 43.037435, -89.425931, Curtis II 43.037448, -89.426200, and Curtis III 43.037156, -89.435084). The Curtis Prairie plots were planted in 1940 (Site III), 1950 (Site II), and 1956 (Site I) on land that was previously plowed and cultivated beginning in the 1840s and had also been used as horse pasture (Lajtha, Townsend, et al., 2014; Nielsen & Hole, 1963). All sites were plowed at time of restoration seeding. Prior to land conversion, the Alfisol soil profile suggested a forested ecosystem, but may have supported an oak savanna with prairie vegetation between trees

(Cottam, 1949; Nielsen & Hole, 1963). Arboretum records refer to Curtis III (1940) as the “high prairie” representing a drier habitat than Curtis II (1950) and Curtis I (1956), which are denoted as “low prairies”. All DIRT sites in the Curtis prairie are classified as Grays silt loam.

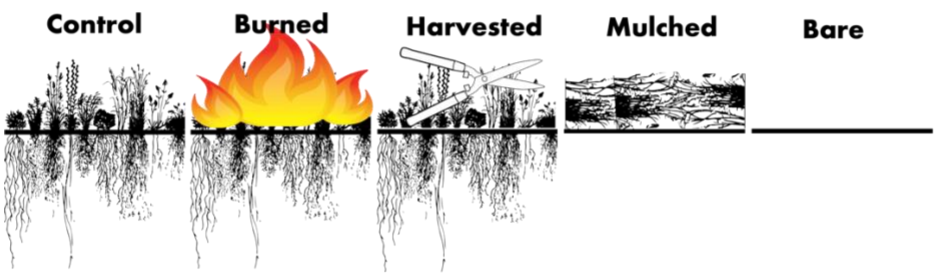


Figure 1. Five treatments of the prairie Detrital Input and Removal Treatments (DIRT) experiment at the UW-Madison Arboretum.

Table 1. Detrital Input and Removal Treatments (DIRT) in restored tallgrass Curtis prairie.

Treatment	Litter inputs		Description
	Aboveground	*Belowground*	
Control	+	+	No burning or harvesting
Burned	+	+	Aboveground vegetation spring-burned every 2 to 3 yr
Harvested	0	+	Aboveground vegetation mowed and removed every fall
Mulched	+	0	Aboveground vegetation removed every 2 weeks by hand to minimize root growth; litter from Harvested treatment added to surface every fall
Bare	0	0	Aboveground vegetation removed every 2 weeks by hand to minimize root growth

Sample collection & processing

One 1-m deep core was collected October 2022 per treatment per site with a JMC Sub-Soil Probe Plus (3-cm diameter), prior to Harvesting and Mulching amendments. Cores were divided in the field into four depths (0 to 10, 10 to 25, 25 to 50, and 50 to 100 cm). If compaction from sampling was >10%, adjustments were made in the field by adjusting depth increments based on depth of hole and depth of soil in liner. In addition to the deep core, at each treatment, four surface cores (0 to 25 cm in depth) were collected and divided into 0 to 10 cm and 10 to 25 cm depths. Surface cores were composited per treatment after sieving to 4 mm to allow for enough soil mass for all planned analyses. Penetrometer readings were taken in each plot three times at the soil surface and averaged to estimate compaction.

Immediately after soil collection and subsampling by depth, soils were kept on ice and transported to the lab where they were kept at 4 °C fridge until sieved. Mass of fresh, field-moist soil samples was recorded. All soils were sieved to 4 mm. A 10-g subsample was dried at 60°C until stable to calculate field moisture for each sample and depth. Mass of roots and gravel were collected and dried for each sample to assess bulk density. The unwashed masses of roots were used as a rough estimate of root abundance. Samples were subdivided with a portion frozen at -20°C and then freeze-dried for PLFA analyses, a portion dried at 55°C for total carbon and nitrogen analysis, and the remaining air dried and stored in WhirlPak bags.

Soil carbon and nitrogen

All soil samples were analyzed for total carbon and nitrogen concentrations with a Thermo Flash EA 1112 (CE Elantech). Concentrations were converted to SOC stocks using equivalent soil mass (von Haden et al., 2020), with Control treatments per site and depth used as references for comparative soil mass. This assesses changes in SOC in relation to the mass of soil per unit area when soils are undisturbed. See Appendix 1 for comparison between fixed-depth and ESM carbon concentrations and stocks. All samples were assessed for inorganic carbon using a hydrochloric acid effervescence test (Nelson & Sommers, 1996). Any sample that reacted was sent to the Kansas State University Soil Testing Laboratory where samples were treated with 1 mL of 1N phosphoric acid in 1-mL increments until no visible reaction and analyzed for total carbon using a LECO TruSpec CN.

MAOC and POC determination

Organic carbon fractions (MAOC and POC) were assessed using size fractionation following a protocol adapted from (Bradford et al., 2008). Briefly, 10 g of air-dried soil were dispersed with 30 mL 0.5% sodium hexametaphosphate for 18 hr. Samples were wet sieved using a 53-µm sieve on Fritsch Analysette 3 vibratory sieve shaker. Material passing through the sieve was considered to contain the MAOC, while material >53 µm was considered to contain the POC. Fractions were dried at 55°C until reaching a stable mass. Material was ground to a fine powder and analyzed for carbon using a Thermo Flash EA1112. A mass-based proportion of MAOC and POC was calculated for each sample and applied to the equivalent soil mass SOC stocks to calculate MAOC and POC stocks.

Historical data

Past soil carbon and total nitrogen concentrations were leveraged from Nielsen and Hole (1963), van Rooyan et al. (1973), Lajtha et al.(2014), and data shared by Dr. Knute Nadelhoffer. We estimated soil carbon concentrations at the initiation of DIRT from nitrogen stocks published in Nielsen and Hole (1963). Soil carbon values in 1959 and 1971 were reported by van Rooyen

(1973) for all three replicates. Estimations and conversions are described below. Lajtha et al. (2014) and K. Nadelhoffer reported soil carbon for only a subset of replicates sampled in 1997 and 2006, which were not included in statistical tests of SOC change over time.

Estimating soil carbon and nitrogen change

Nielsen and Hole (1963) reported nitrogen stocks for all treatments and sties at establishment of DIRT which were calculated from a modified Kjeldahl method (M. L. Jackson, 1958). To compare total nitrogen concentrations from trial establishment to 2022, Kjeldahl concentrations were adjusted with the following linear equation, Total N = 1.048[KN] - 0.010, following Craft et al. (1991). Depth increments from Nielsen and Hole (1963) were adjusted to match the sampling depths of 2022 (0 to 10, 10 to 25, 25 to 50, and 50 to 100 cm) by fitting splines akin to process used in equivalent soil mass (von Haden et al., 2020). We then averaged carbon to nitrogen ratios from control treatments reported in 1997, 2006 and 2022 to estimate the soil carbon concentration at establishment of the trial (estimated CN as 12.46), since carbon values were not reported at this time. This approach assumes that C:N ratios did not change over the course of the trial, but there are not clear trends in C:N within estimates over time. Total C reported for 1959 and 1971 by van Rooyen et al. (1973) were determined using the Walkley and Black method (Walkley & Black, 1934). We multiplied van Rooyen et al.'s total C values by 1.3 to compare to recent carbon concentration (Shamrikova et al., 2022). Bulk density was not reported for past data, so stocks were not calculated or compared over time.

Assaying microbial composition

The living microbial and fungal communities were assayed with phospholipid-fatty-acid (PLFA) analysis in two soil depths: 0 to 10 and 10 to 25 cm. PLFAs were analyzed on an Agilent 6890GC following a protocol adapted from Allison et al. (2005). Briefly, after sieving and removing roots, a sub-sample was freeze-dried, and lipids were extracted with a homogeneous mixture of chloroform, methanol and phosphate buffer (ph 7.4) in a ratio of 1:2:0.8. The mixture was separated into polar and non-polar fractions with water and chloroform. Lipids were extracted from the non-polar fraction and separated by lipid class via silicic acid column chromatography. PLFAs were methylated into fatty acid methyl esters (FAMEs) with an alkaline solution (KOH) and separated and identified using gas chromatography mass spectrometry. PLFA nomenclature is structured as A:BωC with 'A' indicating the number of carbon atoms in the chain, 'B' the number of double bonds and 'C' as position of double bonds from methyl end of the molecule. Suffixes include 'c' for *cis* and 't' for *trans* geometries and prefixes include 'i' for iso, 'a' for anteiso and 'me' for midchain methyl branching (Allison et al., 2005). PLFA justification and assignment can be seen in Appendix 1.

Statistical analyses

We tested changes in soil carbon concentration over time with a linear mixed effect model using packages l*me4*, *lmerTest*,, and *emmeans* for pairwise comparisons (Bates et al., 2015; Kuznetsova et al., 2017; Lenth, 2021). The model structure included treatment and study year as an interaction and site as the random effect. We also tested for differences between treatments within specific years using a similar model, but with study year as a factor. We assessed 2022 soil carbon stocks for each depth increment and MAOC and POC stocks (all calculated using equivalent soil mass) by treatment with site as a covariate using one-way

ANOVA and Tukey's HSD for pairwise comparisons. All data cleaning, statistical tests and visualizations were conducted in R (v4.0.0) (R Core Team, 2020) using packages *ggplot2, dplyr*, *openxlsx, gridExtra, ggpattern, tidyr* and *broom* (Auguie, 2017; FC et al., 2024; Robinson et al., 2022; Schauberger & Walker, 2020; Wickham, 2016; Wickham et al., 2022; Wickham & Girlich, 2022) .

Fungal and bacterial PLFAs absolute abundances were compared between sites and treatments at 0 to 10 and 10 to 25 cm using one-way ANOVA models. We compared microbial composition between sites, treatments, and depth by calculating the relative abundance of lipids within each sample using Bray-Curtis Dissimilarity and used a PERMANOVA model in vegan package (v2.5-6), function *adonis()*. Pairwise comparisons were tested using function pairwise.adonis() in package pairwiseAdonis v0.0.1 (Pedro Martinez Arbizu 2017). We tested dispersion between groups using function *betadisper()* and pairwise differences using function *permutest()* from vegan package.

Results

Soil organic carbon concentration over time

SOC concentrations increased over time in all treatments ($p = 0.002$), except Bare whose slope was significantly different ($p < 0.001$), and negative indicating SOC concentration decreasing over the last 66 years (Figure 3). Slopes for Control, Burned, Harvested and Mulched were not significantly different from each other indicating SOC concentration increased over time (Table S2). But, SOC concentration was affected by treatments when assessed within specific years (Table 2).

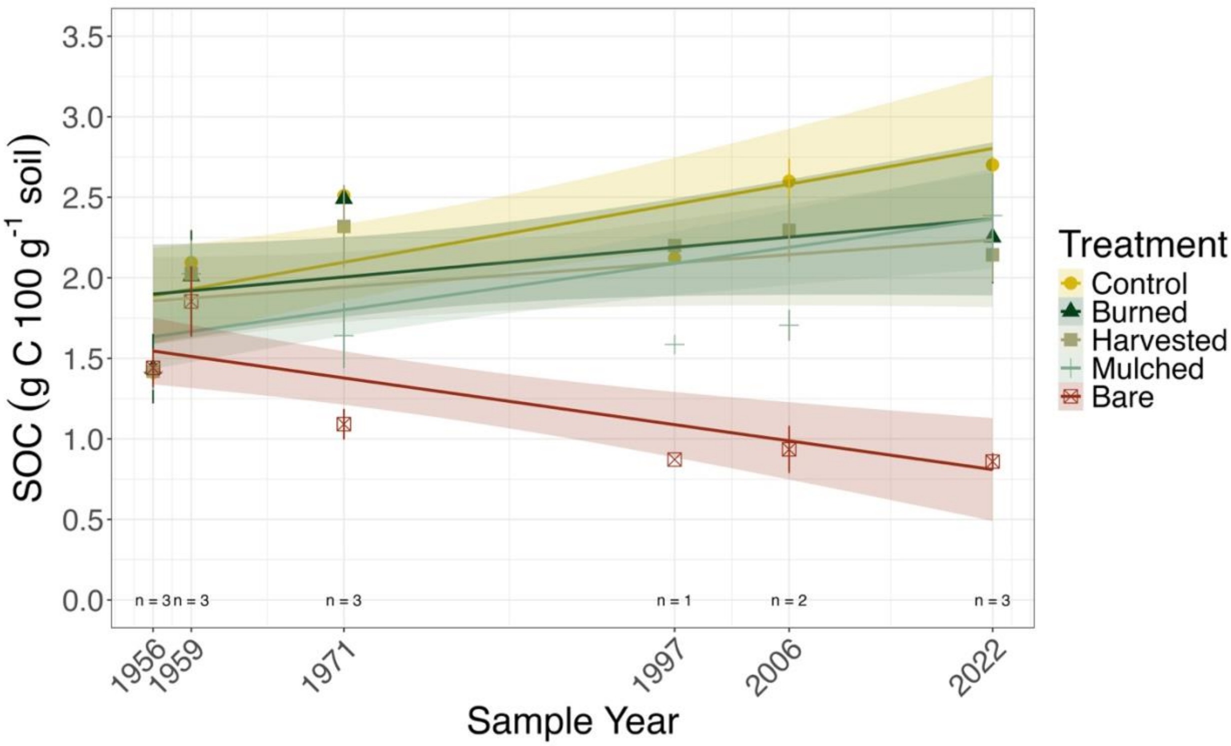


Figure 3. SOC concentrations in surface 10 cm of Detrital Input and Removal Trial at UW-Madison Arboretum over time. Years without data for all replicates (1997 and 2006) are plotted for visual reference but not included in linear fit.

Table 2. Pairwise comparisons of SOC concentrations between treatment levels by study year. P values < 0.05 are bolded.

Treatment	Sample Year	Estimate	SE	df	t Ratio	p value
Bare - Burned	1956	0.004	0.192	34.92	0.022	1.000
Bare - Control		0.028	0.192	34.92	0.146	1.000
Bare - Harvested		0.021	0.192	34.92	0.107	1.000
Bare - Mulched		0.025	0.192	34.92	0.129	1.000
Burned - Control		0.024	0.192	34.92	0.124	1.000
Burned - Harvested		0.016	0.192	34.92	0.086	1.000
Burned - Mulched		0.021	0.192	34.92	0.108	1.000
Control - Harvested		-0.007	0.192	34.92	-0.038	1.000
Control - Mulched		-0.003	0.192	34.92	-0.016	1.000
Harvested - Mulched		0.004	0.192	34.92	0.022	1.000

Bare - Burned	1959	-0.157	0.192	34.92	-0.815	0.92
Bare - Control		-0.241	0.192	34.92	-1.251	0.72
Bare - Harvested		-0.173	0.192	34.92	-0.898	0.9
Bare - Mulched		-0.171	0.192	34.92	-0.891	0. 9
Burned - Control		-0.084	0.192	34.92	-0.435	1.0
Burned - Harvested		-0.016	0.192	34.92	-0.083	1.0
Burned - Mulched		-0.014	0.192	34.92	-0.075	1.0
Control - Harvested		0.068	0.192	34.92	0.353	1.0
Control - Mulched		0.069	0.192	34.92	0.360	1.0
Harvested - Mulched		0.001	0.192	34.92	0.007	1.0
Bare - Burned	1971	-1.400	0.192	34.92	-7.275	**< 0.001**
Bare - Control		-1.418	0.192	34.92	-7.373	**< 0.001**
Bare - Harvested		-1.228	0.192	34.92	-6.381	**< 0.001**
Bare - Mulched		-0.550	0.192	34.92	-2.858	0.05
Burned - Control		-0.019	0.192	34.92	-0.098	1.0
Burned - Harvested		0.172	0.192	34.92	0.894	0.9
Burned - Mulched		0.850	0.192	34.92	4.417	**0.001**
Control - Harvested		0.191	0.192	34.92	0.992	0.86
Control - Mulched		0.869	0.192	34.92	4.515	**0.001**
Harvested - Mulched		0.678	0.192	34.92	3.523	**0.01**
Bare - Burned	2022	-1.392	0.192	34.92	-7.238	**< 0.001**
Bare - Control		-1.842	0.192	34.92	-9.577	**< 0.001**
Bare - Harvested		-1.284	0.192	34.92	-6.675	**< 0.001**
Bare - Mulched		-1.527	0.192	34.92	-7.938	**< 0.001**
Burned - Control		-0.450	0.192	34.92	-2.339	0.16
Burned - Harvested		0.108	0.192	34.92	0.563	0.98
Burned - Mulched		-0.135	0.192	34.92	-0.701	0.96
Control - Harvested		0.558	0.192	34.92	2.902	**0.05**
Control - Mulched		0.315	0.192	34.92	1.639	0.48
Harvested - Mulched		-0.243	0.192	34.92	-1.264	0.72

Litter exclusion reduced SOC stocks

Excluding above- and below-ground biomass inputs for 66 years (i.e., the Bare treatment) resulted in significantly less SOC to 50 cm than all other treatments (Figure 1). Few statistical differences were observed among the other treatments, but Burned had significantly higher SOC stocks than Mulched and Bare treatments at the 10 to 25-cm interval (Figure 1). When assessed cumulatively (0 to 100 cm), Bare SOC stocks were significantly less than other treatments (Figure S1). Among the three blocks, Curtis II (prairie restored in 1950) had higher SOC stocks than Curtis III at 10 to 25 cm ($p = 0.003$) and 25 to 50 cm ($p = 0.02$) and Curtis I at 10 to 25 cm ($p = 0.002$).

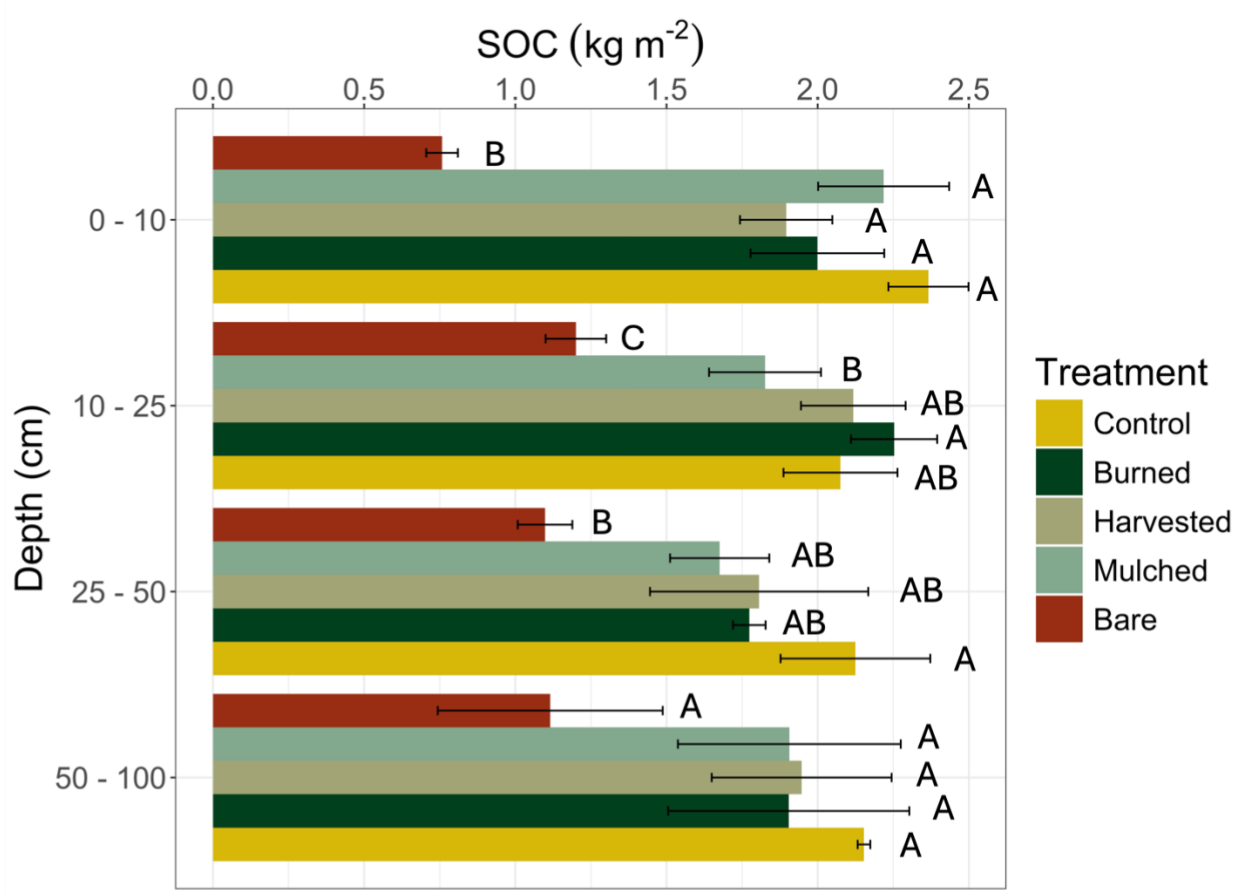


Figure 1. Mean (± SE, n = 3) SOC by treatment and depth in Detrital Input and Removal Trial at UW-Madison Arboretum.

Soil physical properties

Bare soils were observed to be more compacted at the time of sampling requiring 3.6x the force needed to penetrate surface soil compared to Control (158.3 psi and 44.3psi at 0 cm, respectively; Figure S2). Bulk densities also reflected compaction in Bare soils in the 0 to 10-

and 10 to 25-cm intervals, but less so at >25 cm (Figure S3). Mean bulk densities in surface soil (0 to 10 cm; n = 3) were lowest in the Control (0.81 g cm^{-3}), followed by Harvested (0.89 g cm^{-3}), Burned (0.93 g cm^{-3}), Mulched (1.05 g cm^{-3}), and Bare (1.23 g cm^{-3}). Roots were nearly absent in Bare treatments across all depths (Figure S4), confirming treatment manipulation had intended effects on root abundance.

Litter exclusion reduced MAOC and POC

Bare treatments diminished MAOC ($p < 0.001$) and POC ($p < 0.001$) fractions compared to Control, Burned, Harvested, and Mulched treatments, which were not significantly different from each other (Figure 2).

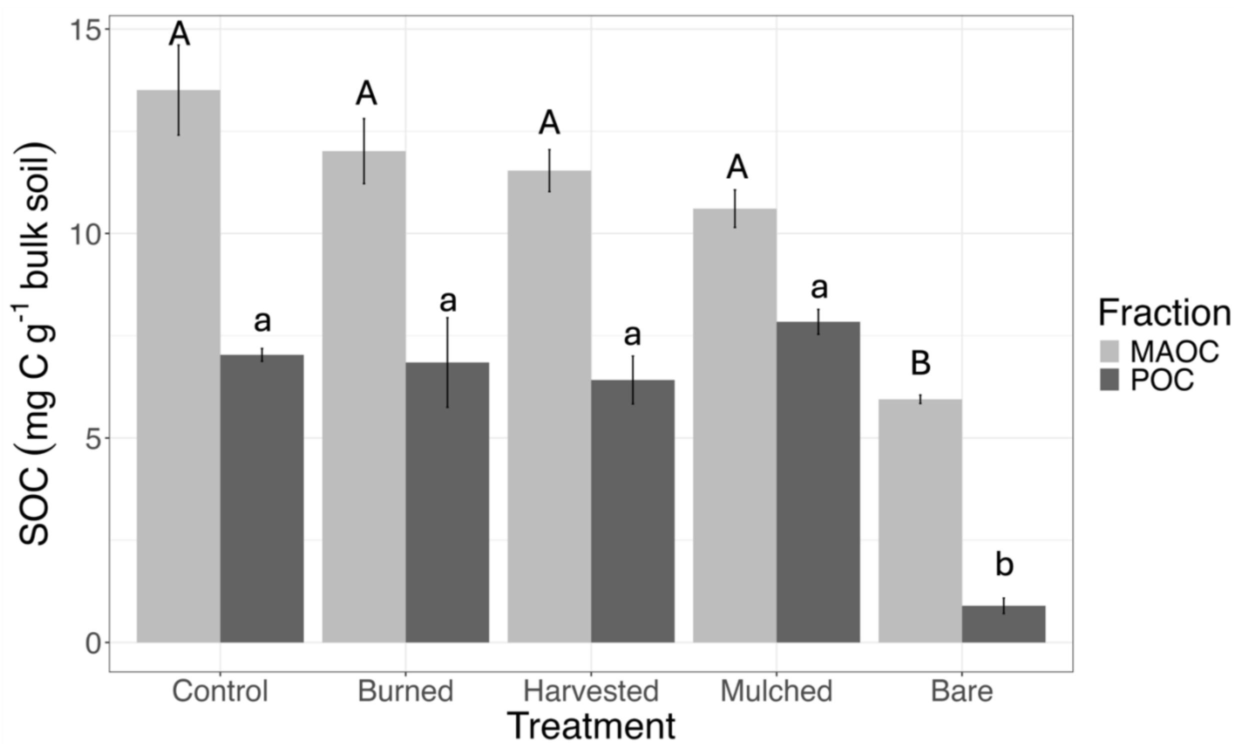


Figure 2. Mean (± SE, n = 3) mineral associated organic carbon (MAOC) and particulate organic carbon (POC) fractions by treatment in the surface 10 cm in Detrital Input and Removal Trial at

UW-Madison Arboretum. Letters indicate significant pairwise comparisons with capitalization specific to fractions.

Root exclusion reduced bacterial and fungal lipid abundance and shifted lipid composition

Bacterial and fungal lipid abundance varied by treatment in surface soils (Figure 4), with no significant differences across site (Figure 4, Table 2). Bare treatments harbored significantly fewer bacterial and fungal lipids than Control, Burned and Harvested the surface 0 to 10- and 10 to 25-cm increments (Table S3). Bacterial and fungal lipid abundance declined in Mulched treatments compared to Control, Burned and Harvested when assessed below 10 cm (Table S3). Declines in fungal and bacterial abundance in Bare and Mulched were across guild assignment (Figure S5).

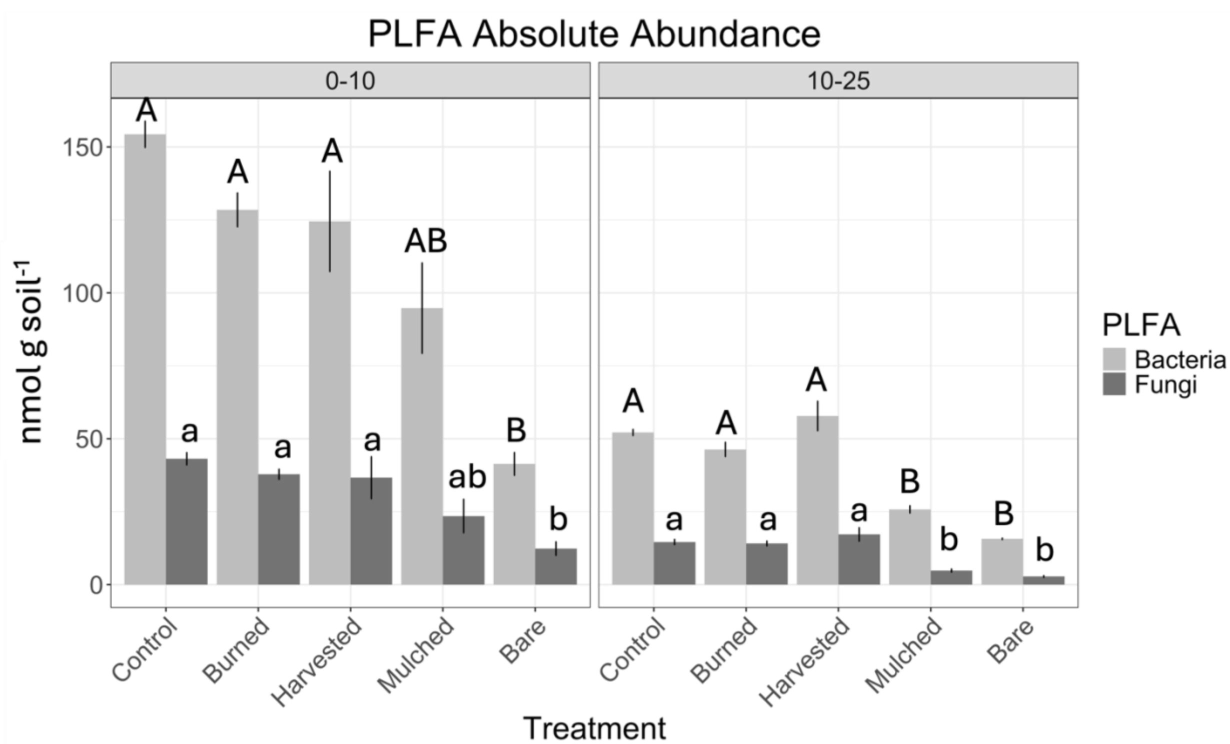


Figure 4. Mean (± SE, n = 3) absolute abundance of extracted lipids (nmol/g soil) by treatment and depth in Detrital Input and Removal Trial at UW-Madison Arboretum. Letters indicate significant pairwise comparisons with capitalization specific to PLFA group.

Table 2. Fungal and bacterial lipid abundance by treatment and site using ANOVA. P values < 0.05 are bolded.

PLFA	Depth (cm)	term	df	SS	Meansq	F statistic	P value
Bacteria	0-10	Site	2	40.26	20.13	0.045	1.0
	0-10	Treatment	4	22337.87	5584.47	12.473	**0.002**
	0-10	Residuals	8	3581.7	447.71		
	10-25	Site	2	38.14	19.07	0.919	0.44
	10-25	Treatment	4	3887.8	971.95	46.856	**< 0.001**
	10-25	Residuals	8	165.9	20.74		
Fungi	0-10	Site	2	2.92	1.46	0.020	0.98
	0-10	Treatment	4	1887.21	471.8	6.526	**0.01**
	0-10	Residuals	8	578.34	72.29		
	10-25	Site	2	5.94	2.97	0.658	0.544
	10-25	Treatment	4	499.08	124.77	27.633	**< 0.001**
	10-25	Residuals	8	36.12	4.52		

We found a differences in microbial composition among treatments ($p = 0.001$, $R^2 = 33.29\%$) and depth ($p = 0.001$ $R^2 = 36.57\%$) when comparing lipid profiles (Figure 5). Within treatments, Bare and Mulched had significantly different lipid composition than Harvested, Burned and Control (Table 3). We found significant differences in dispersion among treatments ($p = 0.04$), but not depth ($p = 0.93$). Bare samples had higher dispersion than Control and Burned ($p = 0.05$).

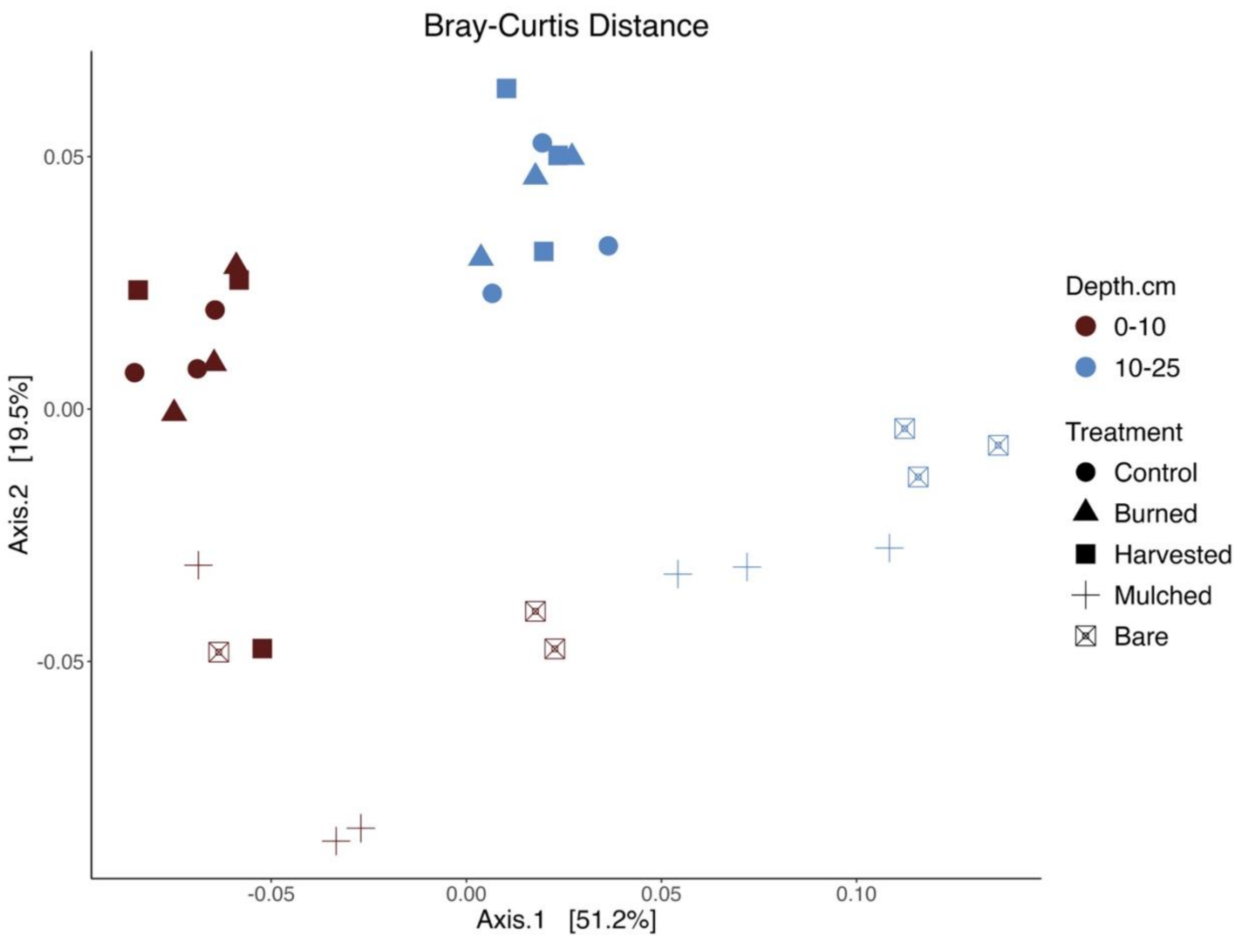


Figure 5. Bray-Curtis distances for microbial lipids extracted by depth and treatment in Detrital Input and Removal Trial at UW-Madison Arboretum.

Table 3. Pairwise comparisons of lipid composition by treatments

Pairs	df	SS	F stat	R^2	P value
Bare vs Mulched	1	0.020	2.17	0.18	0.09
Bare vs Harvested	1	0.033	3.91	0.28	0.03
Bare vs Control	1	0.037	4.56	0.31	0.02
Bare vs Burned	1	0.033	4.19	0.3	0.02
Mulched vs Harvested	1	0.023	3.89	0.28	0.02
Mulched vs Control	1	0.023	4.03	0.29	0.03
Mulched vs Burned	1	0.027	4.99	0.33	0.01
Harvested vs Control	1	0.001	0.13	0.01	0.96

Harvested vs Burned	1	0.001	0.29	0.03	0.82
Control vs Burned	1	0.002	0.55	0.05	0.66

Discussion

SOC concentration over time

Similar rates of SOC concentration increase over time in treatments receiving any litter inputs – above ground, below ground, or both – align with our expectations of incremental gains, given the trial is within a restored prairie. Agricultural disturbance decreases SOC (Dietz et al. 2024), which results in a high theoretical capacity for carbon accumulation when converted back to prairie (von Haden & Dornbush, 2017). Others have reported SOC quickly increasing in the first few years after restoration due to biomass turnover of annual and biannual early successional species while perennials are establishing (Hernández et al., 2013; R. D. Jackson & Stier, 2023; Kucharik, 2007). Our unique long-term dataset followed this trend, with increases in SOC across treatments in the first three years, followed by diverging SOC values in above- vs. below-ground inputs by year 15, and more gradual changes in subsequent sampling, with a gap of 26 years between 1971 and 1997 sampling. The DIRT experiment is replicated in different sections of the Curtis Prairie, with different restoration establishment years (1940, 1950, and 1956). Thus, the SOC increases in the first 15 years of the trial for Control, Burned and Harvested corresponded to different ages of restoration (Restoration age 30, 20, and 15 in 1971), but still fall within what many consider ‘young’ restorations.

SOC concentrations in our recent sampling of Control prairie after nearly 66 to 82 years since establishment remain relatively low (2.5 to 2.8%) compared to other, younger restored prairies in the region (1.8 to 5.1% SOC, 27 to 36 years since establishment, von Haden and Dornbush 2017) but match previous reports from the Curtis prairie (SOC 2.7% in 2001, Kucharik et al. 2006). Increasing SOC concentrations in the Control, Burned, Harvested and Mulched DIRT plots from 1956 to 2022 provide evidence of continued carbon concentration accrual in the Curtis Prairie. Other efforts to assess the carbon balance of the Curtis Prairie have found variability in annual soil respiration and were unable to determine if the prairie served as a carbon sink or source (average NEP ranged from -1.4 to 1.9 and -2.3 to 1.3 Mg C ha^{-1} yr^{-1} for remnant and restored prairies, respectively) (Kucharik et al. 2006). Other prairie restorations in the region have been deemed carbon sources (von Haden and Dornbush 2017), or have reported SOC gains in surface soils, but losses or no change when assessed cumulatively across the entire solum (Dietz et al., 2024).

The unique historical data from 3 years after the initiation of DIRT (1959) offers a glimpse at the initial effects of treatments on SOC. Bare (no litter inputs) and Mulched (root exclusions) initially increase SOC at year-3, followed by observed declines after 15 years. This may be due to root turnover contributions in first few years of removing all litter inputs in Bare treatments and root inputs in Mulched treatments soon after establishment. Bare treatments appear to maintain a low level of SOC, despite theoretical lack of any litter inputs. While in these plots, there is potential for plants to establish in-between regular maintenance or formation of moss at the surface that is difficult to remove without disturbing soil, it perhaps more likely reflects a pool of persistent carbon that remains well protected in aggregates or on mineral surfaces. Other DIRT experiments have noted root encroachment from adjacent areas (Bowden et al., 2024), but we observed little to no roots during soil sieving at these sites (Figure S3), so we do not think this is a dominant mechanism for SOC maintenance.

Mulched (root exclusion) treatments have similar SOC stocks as treatments with root inputs in our recent sampling and have a similar rate of SOC gain overtime. Yet, Mulched treatments have significantly less SOC at earlier sampling time points. The nuance of above- and below-ground litter effects on SOC over time within sampling years may be explained by a few possible mechanisms. First, the physical interface for aboveground litter is the surface soil, which likely slows soil carbon from being transported into deeper soils compared to root litter, which is distributed throughout the soil profile in close proximity to soil minerals. Additionally, any SOC gains in the Mulched treatment in the surface 1 to 2 cm likely are diluted when homogenizing the surface 10 cm of soil. Second, the quantity of carbon entering from aboveground litter likely has increased over time as thatch has accumulated on the surface. The annual harvesting and transfer of aboveground biomass without regular burning would result in more carbon entering the system that would eventually outweigh and likely exceed the absence of carbon inputs from the lack of roots. Aboveground litter turnover is estimated to be 40 to 60% per year in grasslands (Dubeux & Sollenberger, 2020), so about half of each annual deposit of aboveground litter would be available to enter the soil, except for the fraction of that litter converted to carbon dioxide by decomposing biota. The remaining plant litter would enter the soil the following season, again minus the respired fraction. Third, the similar rise in SOC between Control, Harvested and Mulched treatments could indicate that root contributions have no stronger effect on soil carbon than aboveground litter. This would contrast with the MEMS framework, assuming root exudates and fine root turnover provide higher quality inputs than aboveground litter, which are readily incorporated into microbial biomass and ultimately stabilized on mineral surfaces.

Comparisons of SOC between treatments at single time points, could lead to varying conclusions. Ideally, SOC stocks would be compared between these treatments over time using equivalent soil mass (von Haden et al., 2020), however this is difficult due to long term differences in measurements. Stocks were reported as similar between Harvested and Mulched after 50 years of treatment (Lajtha et al. 2014), though comparisons were made without accounting for potential changes in bulk density (Lajtha et al. 2014). Bulk density changed over time in response to treatments, which influences the mass of soil sampled in the same depth increment across years. Specifically, the Mulched treatment had higher bulk density than Undisturbed and Harvested in our recent sampling. The lack of roots tends to compact soil over time, while the addition of litter expands the soil volume resulting sampling that retrieves less of the deepest layer of soil (von Haden et al. 2020), disparities that increase or decrease the amount of soil carbon within a depth interval. These sampling artefacts may have contributed to higher SOC concentration observed in Mulched here. Lastly, the microbial and decomposer communities may have adjusted to applied litter input types and adapted to process and accumulate soil carbon from aboveground litter similar to belowground (Strickland et al., 2009). Shifts observed in the Mulched treatment's lipid community composition could support this hypothesis.

SOC stocks, fractions, and microbial mechanisms

Soil carbon stocks did not vary by above- and below-ground inputs compared to control at all depths after 66 years of litter additions and removal. MAOC and POC fractions also did not vary by inputs compared to control in surface soils indicating that the source of litter (above- or below-ground) has little to no effect on carbon stocks across depth or between fractions in surface soil. We see some indication that the location of litter deposition (at surface or below) may affect the immediate/surrounding soil carbon stock, but not significantly. Presumably the quality (not measured in this study) of litter may also have little effect on soil carbon stocks and persistence when assuming below ground litter is higher quality (root exudates and fine root turnover) than aboveground. These results contrast with our expectation that single sources of litter (above- or below-ground) would have lower carbon stocks than undisturbed treatments receiving both. This could suggest that the quantity of carbon inputs is similar across these treatments despite exclusions of one form or the other. It could also suggest that initial differences in litter chemical composition between above, below, or both diminish as decomposition occurs (Wickings et al., 2012), resulting in similar accumulation of carbon. This second point supports a litter quality convergence hypothesis (Wickings et al., 2012) and contrasts with the MEMs framework, which is predicated on the quality of litter contributing to microbial substrate use efficiency and formation of persistent carbon.

The MCP theory would have predicted that microbial biomass would match persistent carbon fractions (i.e., MAOC), but this is not what we observed. Root exclusion (Mulched) treatments had lower microbial biomass than undisturbed Control in the surface 25 cm, but similar SOC stocks and MAOC fractions (only reported for 0 to 10 cm). It is possible that if we evaluated MAOC fractions in the next depth increment, we would see more distinct differences by treatment, as is the case with microbial biomass at 10 to 25 cm. Nonetheless, despite a smaller microbial community at the surface, a similar amount of carbon is accumulated as in treatments with larger microbial biomass, which may indicate a different set of decomposers and corresponding carbon transformation pathways based on management. Specifically, Mulched treatments may have distinct pathways of transferring carbon into stable pools in response to the thatch buildup, which could harbor distinct organisms burrowed in thatch, their excrement and associated movement. As for the microbes, Liang et al. (2017) described an ex-vivo pathway of carbon transformation via extracellular enzymes compared to in-situ microbial uptake and incorporation. Perhaps the greater proportion of aboveground biomass stimulated more extracellular enzyme activity despite a smaller microbial community. Shifts in microbial community are often driven by substrate use adaptation (Brant et al., 2006; Castañeda-Gómez et al., 2023).

Conclusions

After 66 years of grassland DIRT treatments, we observed increasing SOC concentrations with above- and below-ground litter inputs. Soils with some above- and below-ground inputs resulted in similar SOC stocks across depths, but soils with litter exclusion had lower SOC stocks and lower MAOC stocks indicating loss of older, more stabilized SOC. Absence of belowground inputs reduced bacterial and fungal abundance and shifted lipid composition, but the microbial composition change and biomass reduction did not undermine SOC stocks of Mulched treatments, suggesting a possible adaptation in substrate use from exclusive aboveground inputs and/or alternative pathways of SOC accumulation irrespective of microbial community size. SOC stocks and accumulation are dependent on above- and/or below-ground inputs, but litter exclusion leads to drastic declines in soil carbon and function.

Acknowledgments

Gratitude to the UW-Madison Arboretum's Research Fellowship for funding this work and maintaining the DIRT experiment. To Dr. Francis Hole for initiating the trial and his student's, Gerald Nielsen and Johnathan van Rooyen, whose theses made long-term assessments possible. The researchers and staff at the Arboretum, especially Brad Herrick, Danielle Tanzer, Madeline Smith, Mike Hansen, Petrina Giese, and Josh Goldman. To other DIRT researchers who helped provide guidance along the way including Kate Lajtha, Kate Townsend, Knute Nadelholfer, Derek Peterson, and Myran Simpson. Thank you to my peers in both the Whitman and Jackson labs. Lydia White and Louis Thares for assisting with POC/MAOC fractionations. To Tim Berry, Kelsey Kruger, Erin Pierce, and Harry Read for field and lab work assistance. We thank Nicholas Keuler from the Statistical Consulting group at the University of Wisconsin – Madison for assistance in the analysis of results. Funding support from UW-Madison Arboretum Leopold Fellowship and University Fellowship. Partial support from the Sustainable Agriculture Systems Coordinated Agricultural Program grant no. 2019-68012-29852 from the USDA National Institute of Food and Agriculture and USDA Hatch project (WIS03057).

Supplemental Information

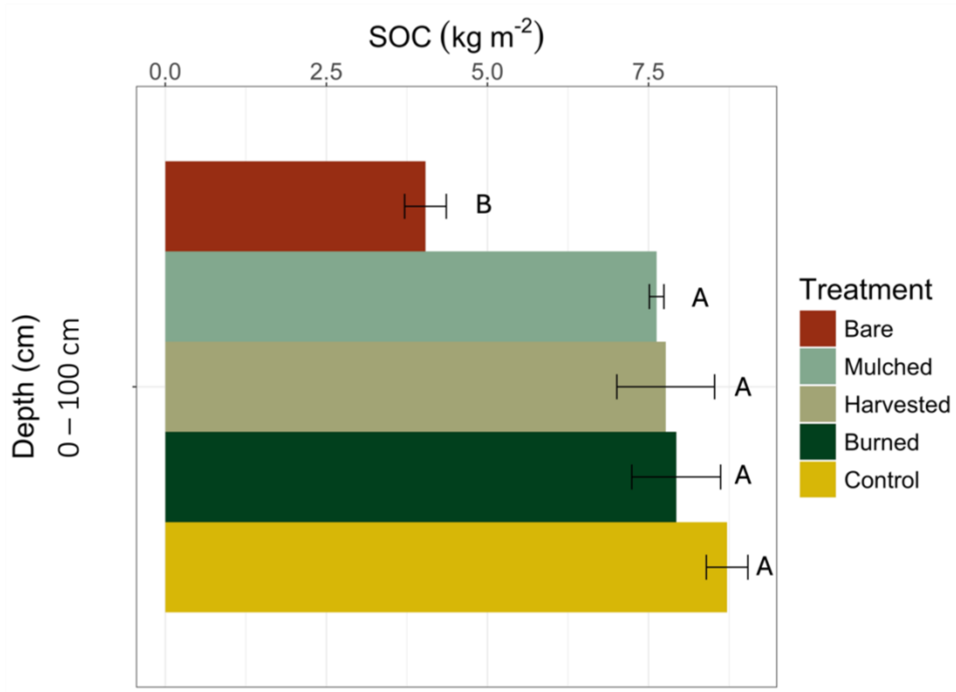


Figure S1. Cumulative SOC stocks by treatment from October 2022 Detrital Input and Removal Trial at UW-Madison Arboretum (n = 3 repeated blocks).

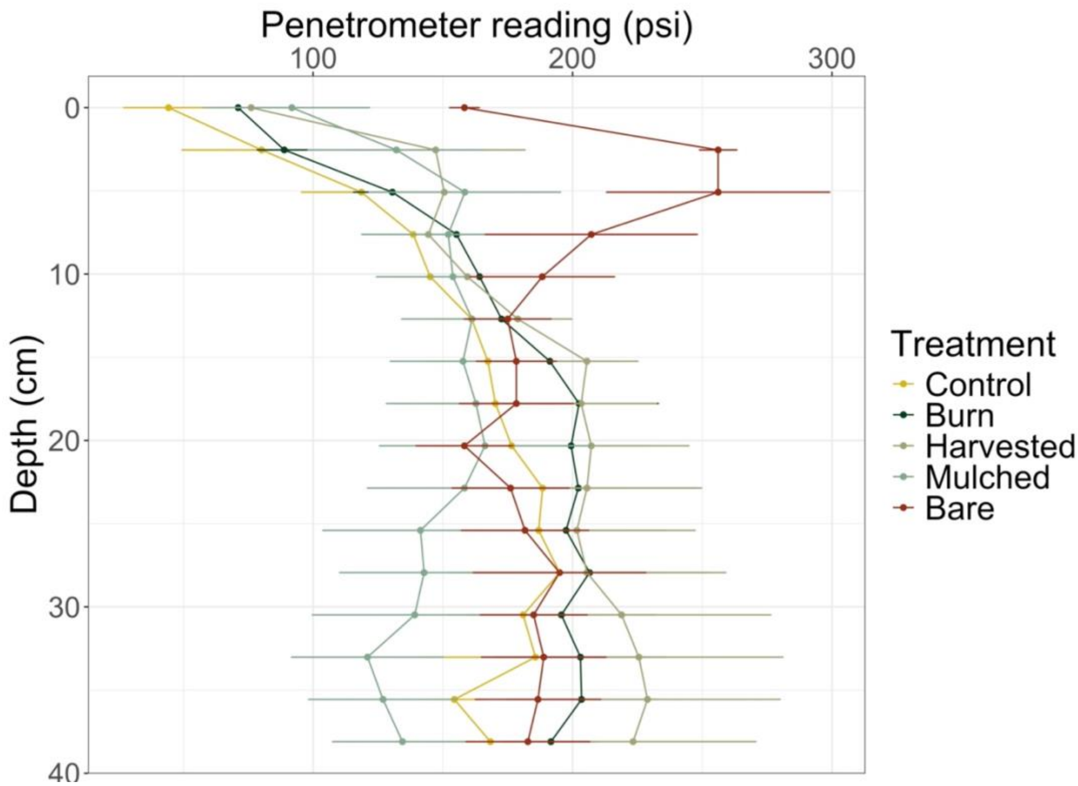


Figure S2. Mean penetrometer readings (psi) with standard error bars by treatment across soil depth collected in October 2022 in Detrital Input and Removal Trial at UW-Madison Arboretum (n = 3 readings per treatment at 3 repeated blocks).

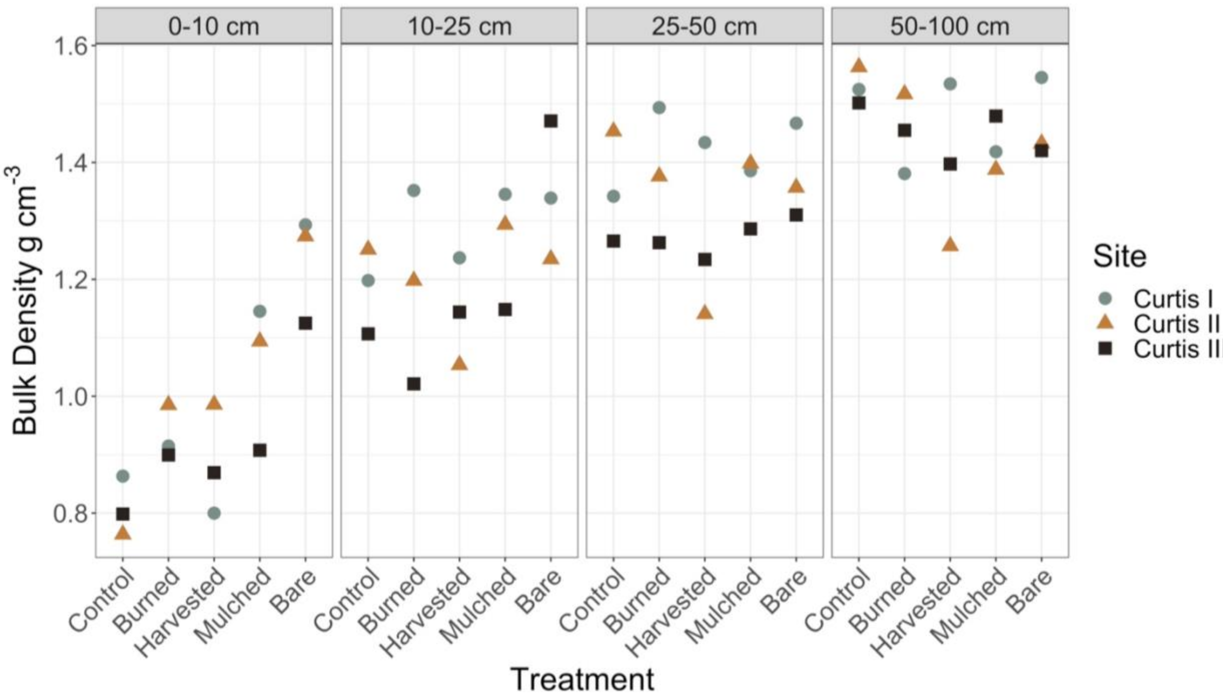


Figure S3. Bulk density (g soil/cm^3) by treatment and depth measured from October 2022 Detrital Input and Removal Trial at UW-Madison Arboretum for each site (3 repeated blocks).

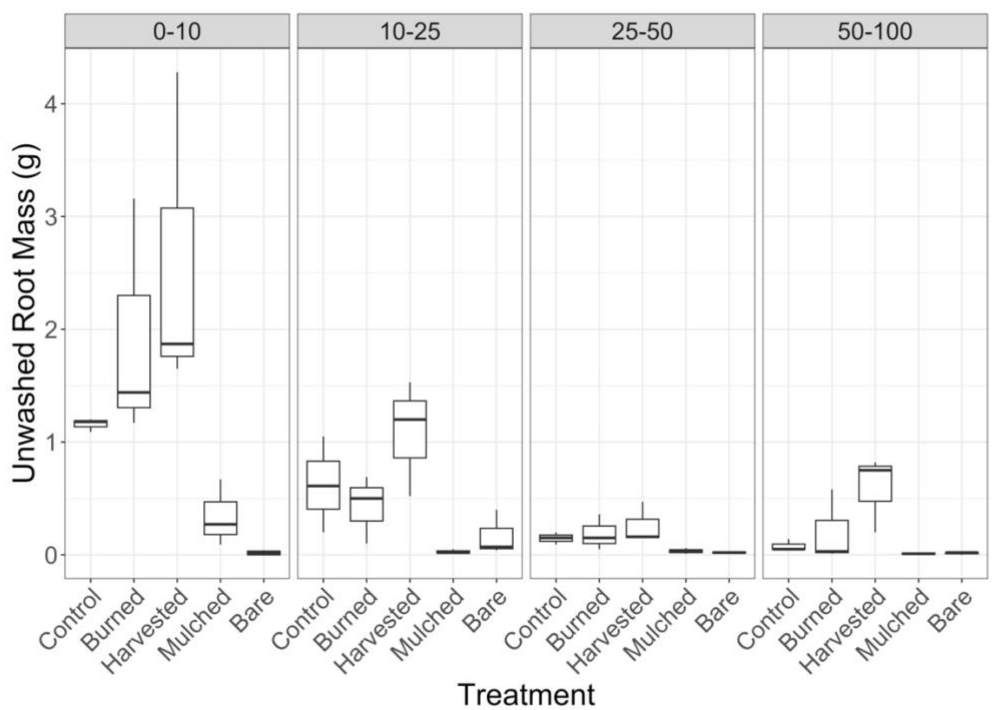


Figure S4. Unwashed root mass (g) by treatment and depth from October 2022 Detrital Input and Removal Trial at UW-Madison Arboretum (n = 3 repeated blocks).

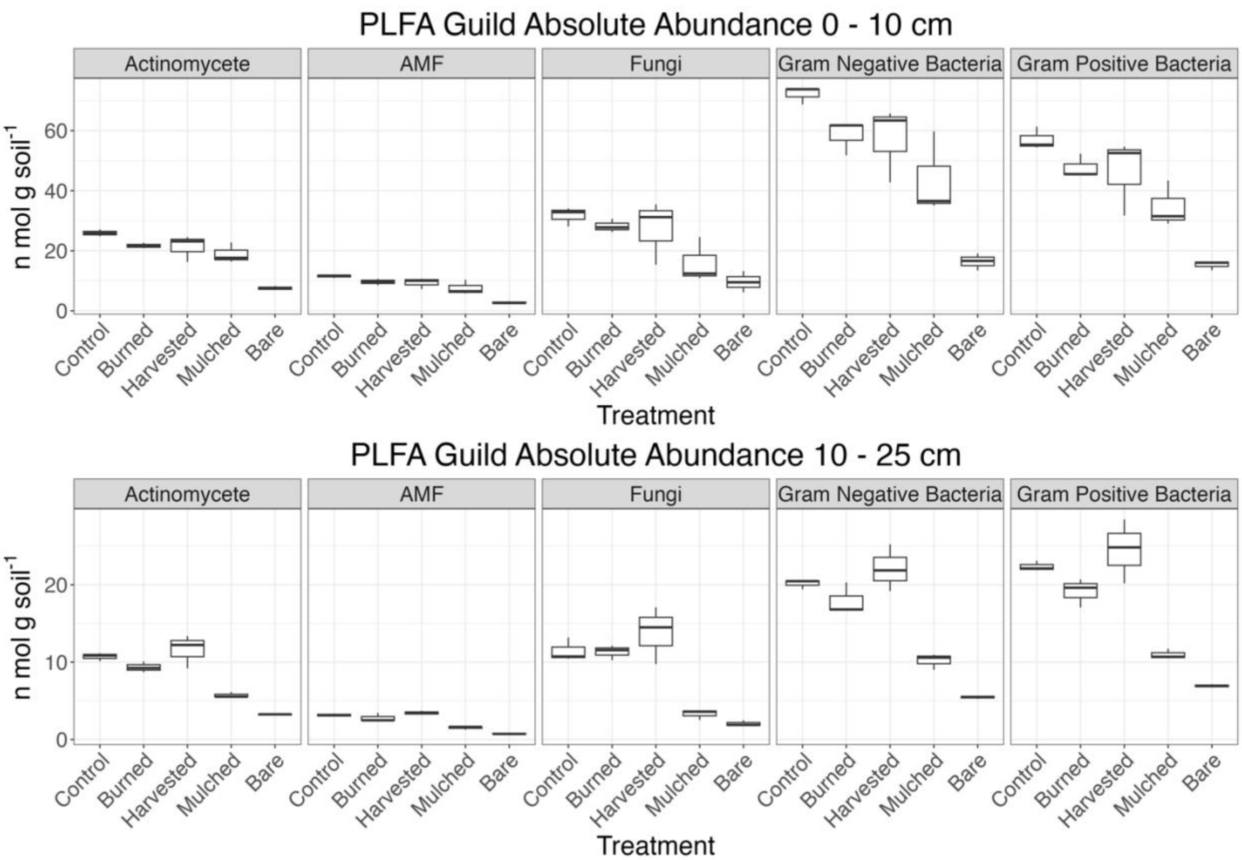


Figure S5. PLFA Guild Absolute Abundance (nmol/g soil) from October 2022 Detrital Input and Removal Trial at UW-Madison Arboretum (n = 3 repeated blocks).

Table S1. Coefficients for SOC concentration over time (numerical). Control treatment is set as baseline intercept, and reported estimates are offsets from control intercept and slope. P values < 0.05 are bolded.

		Estimate	**Std. Error**	**df**	**t value**	**Pr(>|t|)**
Intercept	(Intercept) *Control*	1.889	0.175	10.415	10.819	< 0.001
	TreatmentBare	-0.343	0.200	48	-1.713	0.093
	TreatmentBurned	0.010	0.200	48	0.050	0.961

	TreatmentHarvested	-0.032	0.200	48	-0.160	0.873
	TreatmentMulched	-0.255	0.200	48	-1.270	0.210
Slope	Study.Year *Control*	0.014	0.004	48	3.311	**0.002**
	TreatmentBare:Study.Year	-0.025	0.006	48	-4.225	**< 0.001**
	TreatmentBurned:Study.Year	-0.007	0.006	48	-1.144	0.258
	TreatmentHarvested:Study.Year	-0.008	0.006	48	-1.370	0.177
	TreatmentMulched:Study.Year	-0.003	0.006	48	-0.466	0.643

Table S2. Pairwise comparisons of SOC change over time (numerical) by treatment

Contrast	Estimate	SE	df	t value	p value
Bare - Burned	-0.018	0.006	48	-3.081	**0.027**
Bare - Control	-0.025	0.006	48	-4.225	**0.001**
Bare - Harvested	-0.017	0.006	48	-2.855	**0.048**
Bare - Mulched	-0.022	0.006	48	-3.759	**0.004**
Burned - Control	-0.007	0.006	48	-1.144	0.782
Burned - Harvested	0.001	0.006	48	0.226	0.999
Burned - Mulched	-0.004	0.006	48	-0.678	0.960
Control - Harvested	0.008	0.006	48	1.370	0.649
Control - Mulched	0.003	0.006	48	0.466	0.990
Harvested - Mulched	-0.005	0.006	48	-0.904	0.894

Table S3. Pairwise comparisons of fungal and bacterial lipid abundance

PLFA	Depth.cm	term	contrast	null.value	estimate	conf.low	conf.high	adj.p.value
Bacteria	0-10	Treatment	Burned-Control	0	-25.895	-85.581	33.790	0.590
	0-10	Treatment	Harvested-Control	0	-29.837	-89.523	29.848	0.470
	0-10	Treatment	Mulched-Control	0	-59.557	-119.242	0.129	0.051
	0-10	Treatment	Bare-Control	0	-112.943	-172.629	-53.257	**0.001**
	0-10	Treatment	Harvested-Burned	0	-3.942	-63.628	55.744	0.999
	0-10	Treatment	Mulched-Burned	0	-33.662	-93.347	26.024	0.367
	0-10	Treatment	Bare-Burned	0	-87.048	-146.733	-27.362	**0.006**
	0-10	Treatment	Mulched-Harvested	0	-29.720	-89.405	29.966	0.473
	0-10	Treatment	Bare-Harvested	0	-83.106	-142.791	-23.420	**0.009**
	0-10	Treatment	Bare-Mulched	0	-53.386	-113.072	6.300	0.082

	10-25	Treatment	Burned-Control	0	-5.804	-18.651	7.044	0.557
	10-25	Treatment	Harvested-Control	0	5.643	-7.204	18.490	0.580
	10-25	Treatment	Mulched-Control	0	-26.361	-39.208	-13.514	**0.001**
	10-25	Treatment	Bare-Control	0	-36.450	-49.297	-23.603	**<0.001**
	10-25	Treatment	Harvested-Burned	0	11.446	-1.401	24.294	0.084
	10-25	Treatment	Mulched-Burned	0	-20.557	-33.404	-7.710	**0.004**
	10-25	Treatment	Bare-Burned	0	-30.646	-43.494	-17.799	**<0.001**
	10-25	Treatment	Mulched-Harvested	0	-32.004	-44.851	-19.156	**<0.001**
	10-25	Treatment	Bare-Harvested	0	-42.093	-54.940	-29.246	**<0.001**
	10-25	Treatment	Bare-Mulched	0	-10.089	-22.936	2.758	0.138
Fungi	0-10	Treatment	Burned-Control	0	-5.304	-29.288	18.679	0.934
	0-10	Treatment	Harvested-Control	0	-6.496	-30.480	17.488	0.875
	0-10	Treatment	Mulched-Control	0	-19.630	-43.614	4.354	0.118
	0-10	Treatment	Bare-Control	0	-30.780	-54.764	-6.797	**0.014**
	0-10	Treatment	Harvested-Burned	0	-1.192	-25.175	22.792	1.000
	0-10	Treatment	Mulched-Burned	0	-14.326	-38.310	9.658	0.320
	0-10	Treatment	Bare-Burned	0	-25.476	-49.460	-1.492	**0.037**
	0-10	Treatment	Mulched-Harvested	0	-13.134	-37.118	10.850	0.391
	0-10	Treatment	Bare-Harvested	0	-24.284	-48.268	-0.301	**0.047**
	0-10	Treatment	Bare-Mulched	0	-11.150	-35.134	12.834	0.532
	10-25	Treatment	Burned-Control	0	-0.511	-6.505	5.483	0.998
	10-25	Treatment	Harvested-Control	0	2.602	-3.391	8.596	0.589
	10-25	Treatment	Mulched-Control	0	-9.802	-15.796	-3.808	**0.003**
	10-25	Treatment	Bare-Control	0	-11.809	-17.803	-5.815	**0.001**
	10-25	Treatment	Harvested-Burned	0	3.114	-2.880	9.107	0.437
	10-25	Treatment	Mulched-Burned	0	-9.291	-15.285	-3.297	**0.004**
	10-25	Treatment	Bare-Burned	0	-11.298	-17.292	-5.304	**0.001**
	10-25	Treatment	Mulched-Harvested	0	-12.405	-18.398	-6.411	**0.001**
	10-25	Treatment	Bare-Harvested	0	-14.411	-20.405	-8.417	**<0.001**
	10-25	Treatment	Bare-Mulched	0	-2.007	-8.001	3.987	0.774

References

Allison, V. J., Miller, R. M., Jastrow, J. D., Matamala, R., & Zak, D. R. (2005). Changes in Soil Microbial Community Structure in a Tallgrass Prairie Chronosequence. *Soil Science Society of America Journal*, *69*(5), 1412–1421. https://doi.org/10.2136/sssaj2004.0252

Auguie, B. (2017). *gridExtra: Miscellaneous Functions for "Grid" Graphics*. https://CRAN.R-project.org/package=gridExtra

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), Article 1. https://doi.org/10.18637/jss.v067.i01

Bowden, R. D., Simpson, M. J., Saucedo, N. P., Brozell, K., DiGiacomo, J., & Lajtha, K. (2024). Litter and root sources of soil organic matter in a temperate forest: Thirty years in the DIRT. *Soil Science Society of America Journal*, *88*(3), 593–601. https://doi.org/10.1002/saj2.20634

Bradford, M. A., Fierer, N., & Reynolds, J. F. (2008). Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. *Functional Ecology*, *22*(6), 964–974. https://doi.org/10.1111/j.1365-2435.2008.01404.x

Brant, J. B., Myrold, D. D., & Sulzman, E. W. (2006). Root controls on soil microbial community structure in forest soils. *Oecologia*, *148*(4), 650–659. https://doi.org/10.1007/s00442-006-0402-7

Castañeda-Gómez, L., Lajtha, K., Bowden, R., Mohammed Jauhar, F. N., Jia, J., Feng, X., & Simpson, M. J. (2023). Soil organic matter molecular composition with long-term detrital alterations is controlled by site-specific forest properties. *Global Change Biology*, *29*(1), 243–259. https://doi.org/10.1111/gcb.16456

Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H., & Parton, W. J. (2015). Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience*, *8*(10), 776–779. https://doi.org/10.1038/ngeo2520

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*(4), 988–995. https://doi.org/10.1111/gcb.12113

Cottam, G. (1949). The Phytosociology of an Oak Woods in Southwestern Wisconsin. *Ecology*, *30*(3), 271–287. https://doi.org/10.2307/1932609

Craft, C. B., Seneca, E. D., & Broome, S. W. (1991). Loss on Ignition and Kjeldahl Digestion for Estimating Organic Carbon and Total Nitrogen in Estuarine Marsh Soils: Calibration with Dry Combustion. *Estuaries*, *14*(2), 175. https://doi.org/10.2307/1351691

Dietz, C. L., Jackson, R. D., Ruark, M. D., & Sanford, G. R. (2024). Soil carbon maintained by perennial grasslands over 30 years but lost in field crop systems in a temperate Mollisol. *Communications Earth & Environment*, *5*(1), 1–8. https://doi.org/10.1038/s43247-024-01500-w

Dubeux, J. C. B., & Sollenberger, L. E. (2020). Chapter 4—Nutrient cycling in grazed pastures. In M. Rouquette & G. E. Aiken (Eds.), *Management Strategies for Sustainable Cattle Production in Southern Pastures* (pp. 59–75). Academic Press. https://doi.org/10.1016/B978-0-12-814474-9.00004-9

FC, M., Davis, T. L., & ggplot2 authors. (2024). *ggpattern: "ggplot2" Pattern Geoms*. https://CRAN.R-project.org/package=ggpattern

Hernández, D., Esch, E., Alster, C., McKone, M., & Camill, P. (2013). Rapid Accumulation of Soil Carbon and Nitrogen in a Prairie Restoration Chronosequence. *Soil Science Society of America Journal*, *77*, 2029. https://doi.org/10.2136/sssaj2012.0403

Jackson, M. L. (1958). *Soil chemical analysis*. Prentice-Hall. https://search.library.wisc.edu/catalog/999542273302121

Jackson, R. D., & Stier, J. (2023). Soil C Change Over 14 Years in Grasslands Sown into a Highly Disturbed Soil. *XXV International Grassland Congress (IGC 2023)*, 170–173. https://doi.org/10.52202/071171-0038

Kucharik, C. J. (2007). Impact of Prairie Age and Soil Order on Carbon and Nitrogen Sequestration. *Soil Science Society of America Journal*, *71*(2), 430–441. https://doi.org/10.2136/sssaj2006.0074

Kucharik, C. J., Fayram, N. J., & Cahill, K. N. (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. https://doi.org/10.1111/j.1365-2486.2005.01053.x

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(1), Article 1. https://doi.org/10.18637/jss.v082.i13

Lajtha, K., Bowden, R. D., Crow, S., Fekete, I., Kotroczó, Z., Plante, A., Simpson, M. J., & Nadelhoffer, K. J. (2018). The detrital input and removal treatment (DIRT) network:

Insights into soil carbon stabilization. *Science of The Total Environment*, *640–641*, 1112–1120. https://doi.org/10.1016/j.scitotenv.2018.05.388

Lajtha, K., Bowden, R. D., & Nadelhoffer, K. (2014). Litter and Root Manipulations Provide Insights into Soil Organic Matter Dynamics and Stability. *Soil Science Society of America Journal*, *78*(S1), S261–S269. https://doi.org/10.2136/sssaj2013.08.0370nafsc

Lajtha, K., Townsend, K., Kramer, M., Swanston, C., Bowden, R., & Nadelhoffer, K. (2014). Changes to particulate versus mineral-associated soil carbon after 50 years of litter manipulation in forest and prairie experimental ecosystems. *Biogeochemistry*, *168*, 1–20. https://doi.org/10.1007/s10533-014-9970

Lavallee, J. M., Soong, J. L., & Cotrufo, M. F. (2020). Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global Change Biology*, *26*(1), 261–273. https://doi.org/10.1111/gcb.14859

Lenth, R. V. (2021). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. https://CRAN.R-project.org/package=emmeans

Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, *2*(8), 1–6. https://doi.org/10.1038/nmicrobiol.2017.105

Nelson, D. W., & Sommers, L. E. (1996). Total Carbon, Organic Carbon, and Organic Matter. In *Methods of Soil Analysis* (pp. 961–1010). John Wiley & Sons, Ltd. https://doi.org/10.2136/sssabookser5.3.c34

Nielsen, G. A., & Hole, F. D. (1963). A STUDY OF THE NATURAL PROCESSES OF INCORPORATION OF ORGANIC MATTER INTO SOIL IN THE UNIVERSITY OF

WISCONSIN ARBORETUM. *Wisconsin Acadmey of Sciences, Arts and Letters*, *52*, 213–227.

NOAA NCEI U.S. Climate Normals Quick Access. (2025). https://www.ncei.noaa.gov/access/us-climate-normals/#dataset=normals-monthly&timeframe=30&station=USC00470273

R Core Team. (2020). *R: A language and environment for statistical computing* (Version 4.0.0) [Computer software]. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Robinson, D., Hayes, A., & Couch, S. (2022). *broom: Convert Statistical Objects into Tidy Tibbles*. https://CRAN.R-project.org/package=broom

Rocci, K., Cotrufo, M. F., Ernakovitch, J., Foster, E., Frey, S., Georgiou, K., Grandy, S., Malhotra, A., Reich, P., Schlerman, E., & Wieder, W. R. (2023). *Bridging 20 years of soil organic matter frameworks: Empirical support, model representation, and next steps* [Preprint]. Preprints. https://doi.org/10.22541/essoar.170293436.63506237/v1

Schauberger, P., & Walker, A. (2020). *openxlsx: Read, Write and Edit xlsx Files*. https://CRAN.R-project.org/package=openxlsx

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*(7367), 49–56. https://doi.org/10.1038/nature10386

Shamrikova, E. V., Vanchikova, E. V., Kondratenok, B. M., Lapteva, E. M., & Kostrova, S. N. (2022). Problems and Limitations of the Dichromatometric Method for Measuring Soil Organic Matter Content: A Review. *Eurasian Soil Science*, *55*(7), 861–867. https://doi.org/10.1134/S1064229322070092

Strickland, M. S., Lauber, C., Fierer, N., & Bradford, M. A. (2009). Testing the Functional Significance of Microbial Community Composition. *Ecology*, *90*(2), 441–451.

Sulzman, E. W., Brant, J. B., Bowden, R. D., & Lajtha, K. (2005). Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO2 efflux in an old growth coniferous forest. *Biogeochemistry*, *73*(1), 231–256. https://doi.org/10.1007/s10533-004-7314-6

van Rooyen, D., Boumans, L., & Hole, F. (1973). *Organic carbon and nitrogen status in two Hapludalfs under prairie and deciduous forest as related to moisture regime, some morphological features, and response to manipulation of cover* [1973.]. https://search.library.wisc.edu/catalog/999873179102121

von Haden, A. C., & Dornbush, M. E. (2017). Ecosystem carbon pools, fluxes, and balances within mature tallgrass prairie restorations. *Restoration Ecology*, *25*(4), 549–558. https://doi.org/10.1111/rec.12461

von Haden, A. C., Yang, W. H., & DeLucia, E. H. (2020). Soils' dirty little secret: Depth-based comparisons can be inadequate for quantifying changes in soil organic carbon and other mineral soil properties. *Global Change Biology*, *26*(7), 3759–3770. https://doi.org/10.1111/gcb.15124

Walkley, A., & Black, I. A. (1934). An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Science*, *37*, 29–38. https://doi.org/10.1097/00010694-193401000-00003

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wickham, H., François, R., Henry, L., & Müller, K. (2022). *dplyr: A Grammar of Data Manipulation*. https://CRAN.R-project.org/package=dplyr

Wickham, H., & Girlich, M. (2022). *tidyr: Tidy Messy Data*. https://CRAN.R-project.org/package=tidyr

Wickings, K., Grandy, A. S., Reed, S. C., & Cleveland, C. C. (2012). The origin of litter chemical complexity during decomposition. *Ecology Letters*, *15*(10), 1180–1188. https://doi.org/10.1111/j.1461-0248.2012.01837.x

Zhu, X., Jackson, R. D., DeLucia, E. H., Tiedje, J. M., & Liang, C. (2020). The soil microbial carbon pump: From conceptual insights to empirical assessments. *Global Change Biology*, *26*(11), 6032–6039. https://doi.org/10.1111/gcb.15319

Appendix 1

1. ESM and fixed depth comparisons for SOC stocks and concentrations
    - Figures comparing Equivalent Soil Mass (ESM) and raw SOC data from 2022 DIRT grassland soil carbon assessment by Mia Keady.
2. PLFA indicators
    - Spreadsheet indicating assignment of phospholipid fatty acids to guilds and literature supporting assignment.
3. Wingra and Noe woods DIRT Arboretum project summary
    - Document written by Mia Keady to report results from Leopold graduate fellowship to UW-Madison Arboretum.
4. Location, boundaries and maintenance of *Double Litter* treatment at DIRT site in Wingra Woods, UW-Madison Arboretum
    - Document created by Mia Keady to outline concerns in Wingra woods DIRT maintenance and boundaries of double litter treatment.
5. UW-Madison Arboretum DIRT maintenance document 2018
    - UW-Arboretum's current DIRT maintenance protocol.
6. 1984 Hole Maintenance Protocol
    - Pdf provided by UW-Arboretum.
7. 2008to2009_HolePlotMaintenanceRecords
    - Word doc provided by UW-Arboretum.
8. 2018to2022_HolePlotMaintenanceRecords
    - Xlsx provided by UW-Arboretum.
9. Grassland DIRT burn treatment email correspondence
    - Email correspondence between Mia Keady and UW-Arboretum data coordinator Danielle Tanzer describing re-initiation of burning grassland DIRT treatments.
10. 1950s_Research Map
    - Pdf of hand drawn map from 1950s UW-Arboretum Curtis prairie. Gives context for location and prior use of area where grassland DIRT sites were established.
11. Curtis Legacy Map
    - Pdf of Curtis Legacy map created in 2013 by Molly Fifield Murray based on historical documents.

1. **<u>ESM and fixed depth comparisons for SOC stocks and concentrations</u>**

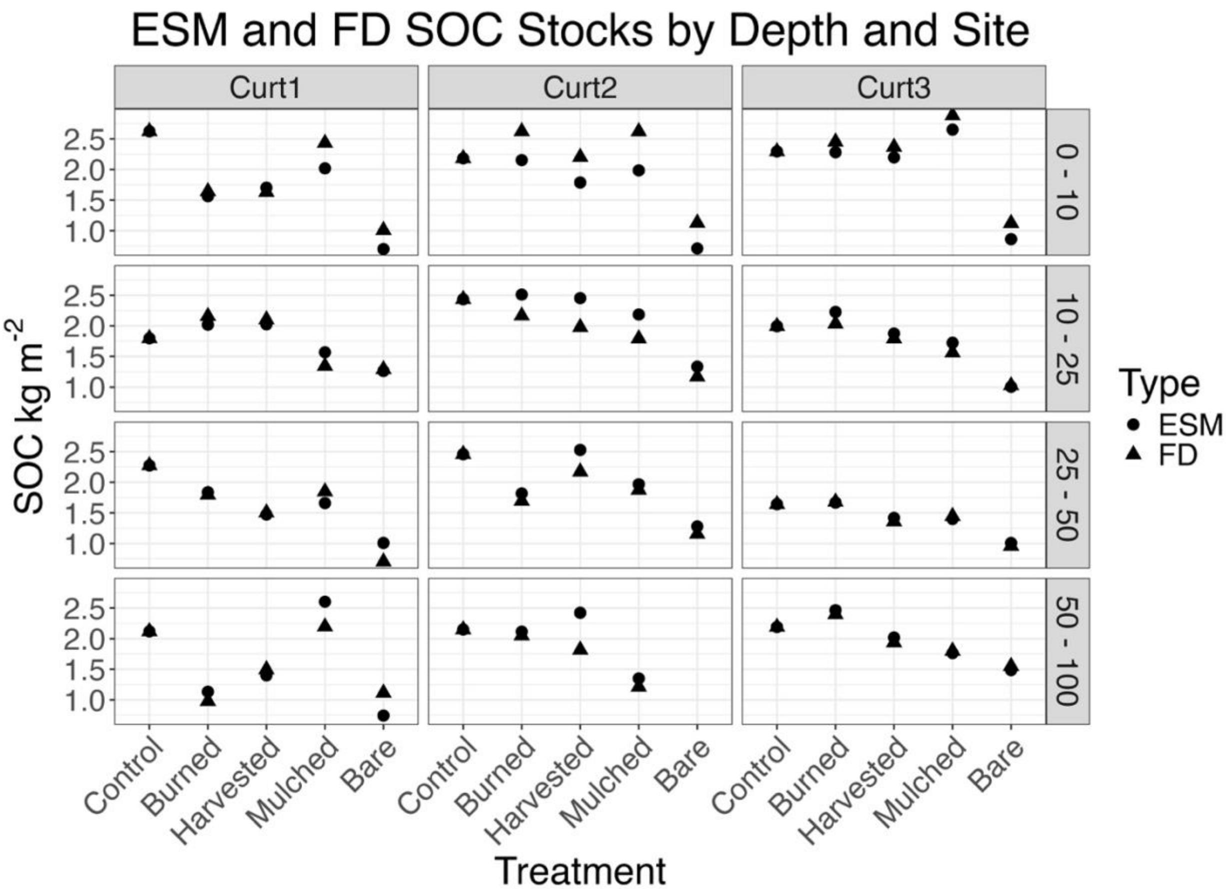


Figure A1. ESM and fixed depth (FD) SOC stock comparison by site and depth.

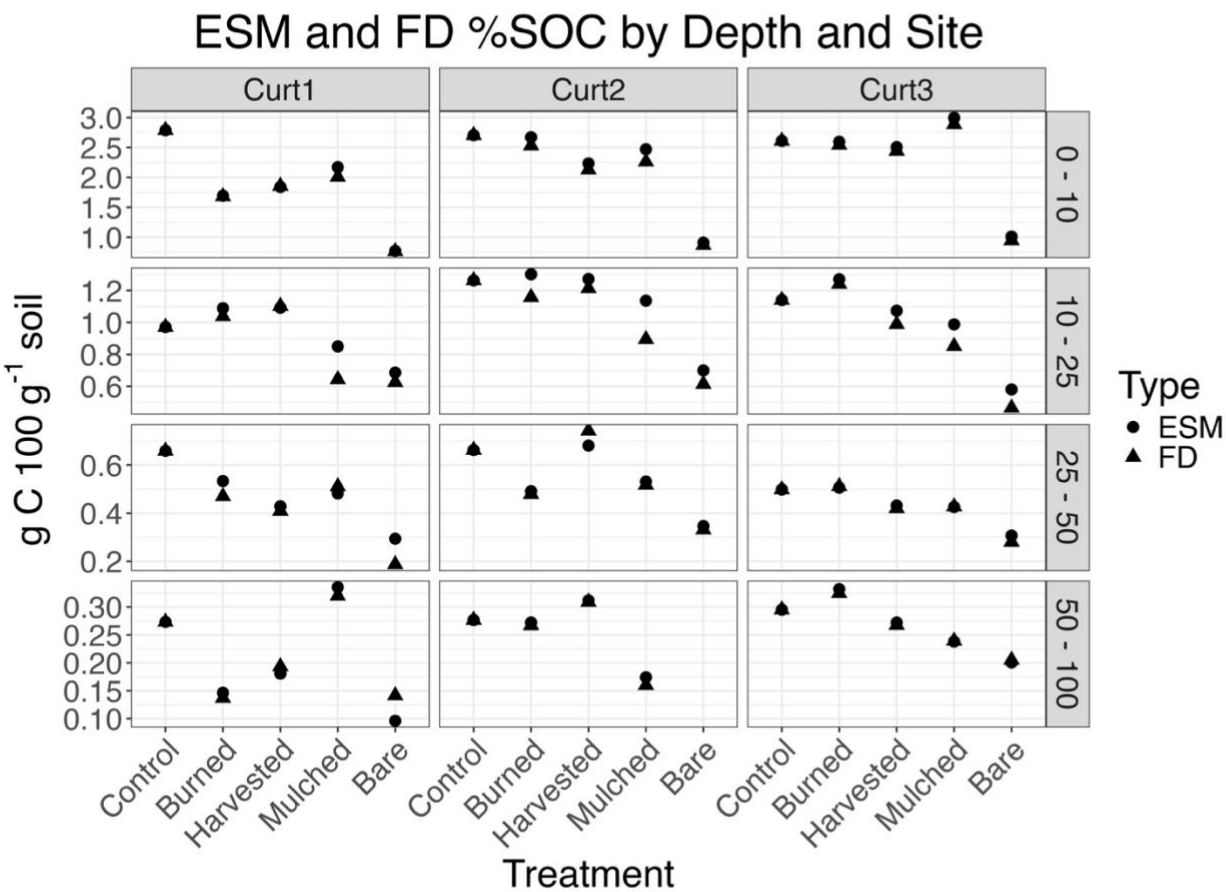


Figure A2. ESM and fixed depth (FD) SOC concentration comparison by site and depth.

2. **PLFA indicators; See excel file attached.**

3. **Wingra and Noe woods Detrital Input and Removal Trial Project Summary**

We observed surprising soil organic carbon concentrations in double litter treatments in 2022 sampling compared to past data (Figures 1 and 2). Compared to past data, we see a decline in SOC concentrations in double litter treatments. Double-litter SOC concentrations are now near or below the control. This would indicate a loss of soil organic carbon in double litter sites while maintaining soil carbon in control and leafless treatments. One explanation could be the introduction of jumping worms or other soil fauna into woodland systems altering soil nutrient cycling. However, only Wingra woods has documented evidence of jumping worms.

We are unable to analyze this data statistically given Noe and Wingra woods are not replicates of each other and are not replicated elsewhere. However, continued long-term monitoring can provide important data to understanding nutrient cycling across input types in different ecosystems.

Given these results were unexpected, we re-sampled the double-litter and control sites in May 2024 to confirm if the observed trends are accurate. **We determined that we should not be confident in Wingra's *double litter* location or that its treatment has been consistently applied over the years since its establishment.** Please see appendix 2 with site history.

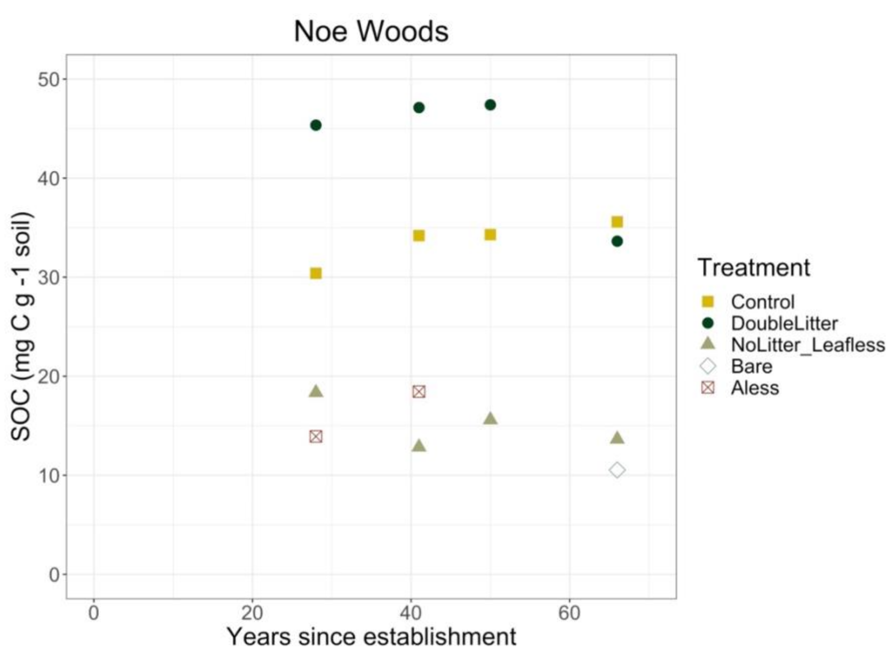


Figure 1. Noe woods soil organic carbon concentrations over time. Past data from Lajtha et al. 2014 and data shared from Knute Nadelhoffer. Past data varies in reporting data for bare and A-less treatments.

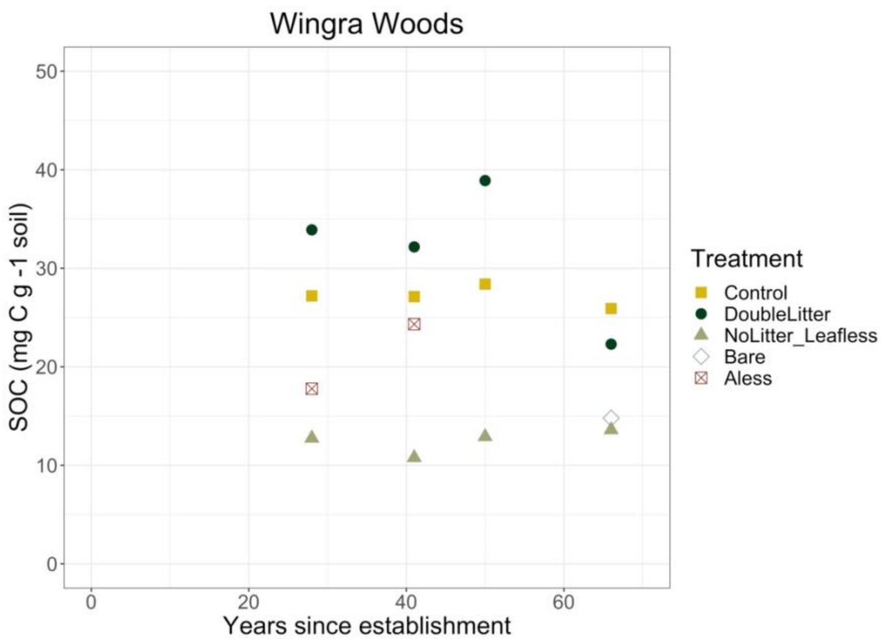


Figure 2. Wingra woods soil organic carbon concentrations over time. Past data from Lajtha et al. 2014 and data shared from Knute Nadelhoffer. Past data varies in reporting data for bare and A-less treatments.

4. <u>Location, boundaries and maintenance of *Double Litter* treatment at DIRT site in Wingra Woods, UW-Madison Arboretum</u>

Location, boundaries, and maintenance of *Double Litter* treatment at DIRT site in Wingra Woods, UW-Madison Arboretum

Mia Keady

November 2024

Introduction

After sampling the Wingra Woods DIRT plots in 2022, the soil carbon concentration values in the *double litter* treatment were lower than expected based on past data. Subsequent conversations, unearthing original documents, and inspecting the plots in person indicated that we should not be confident in the plot's location or that its treatment has been consistently applied over the years since its establishment. The following documents are compiled to give an overview of what we currently know about the plots for this site and their history.

1.) Gerald Nielsen MS thesis 1960 "Figure 3"

Although not necessarily precisely to scale, Nielsen's 1960 thesis map (Fig. 1) does not match current boundaries outlined in the Arboretum's "Hole Plot Maintenance Protocols (Retyped 2018).docx" (Fig. 2).

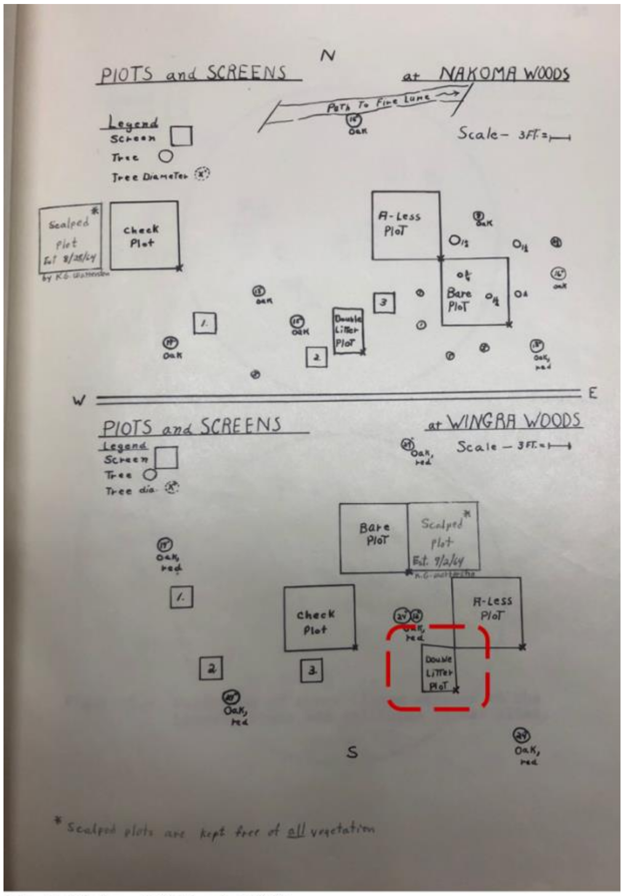
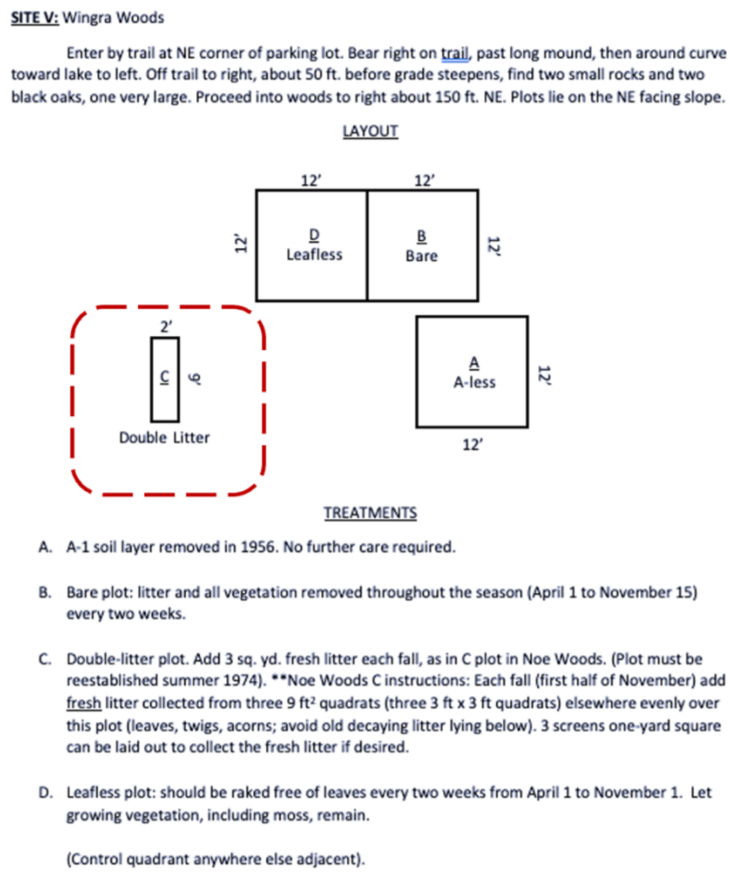


Fig. 1. Nielsen MS thesis 1960



SITE V: Wingra Woods

Enter by trail at NE corner of parking lot. Bear right on trail, past long mound, then around curve toward lake to left. Off trail to right, about 50 ft. before grade steepens, find two small rocks and two black oaks, one very large. Proceed into woods to right about 150 ft. NE. Plots lie on the NE facing slope.

LAYOUT

TREATMENTS

A. A-1 soil layer removed in 1956. No further care required.

B. Bare plot: litter and all vegetation removed throughout the season (April 1 to November 15) every two weeks.

C. Double-litter plot. Add 3 sq. yd. fresh litter each fall, as in C plot in Noe Woods. (Plot must be reestablished summer 1974). **Noe Woods C instructions: Each fall (first half of November) add fresh litter collected from three 9 ft² quadrats (three 3 ft x 3 ft quadrats) elsewhere evenly over this plot (leaves, twigs, acorns; avoid old decaying litter lying below). 3 screens one-yard square can be laid out to collect the fresh litter if desired.

D. Leafless plot: should be raked free of leaves every two weeks from April 1 to November 1. Let growing vegetation, including moss, remain.

(Control quadrant anywhere else adjacent).

Fig. 2. "Hole Plot Maintenance Protocols (Retyped 2018).docx"

2.) Van Rooyan's 1973 thesis (p 49, Table I-A-1)

Van Rooyan's 1973 thesis (p 49, Table I-A-1) notes at the bottom of the table: "**Discontinued because of a blow-down of diseased oak trees"

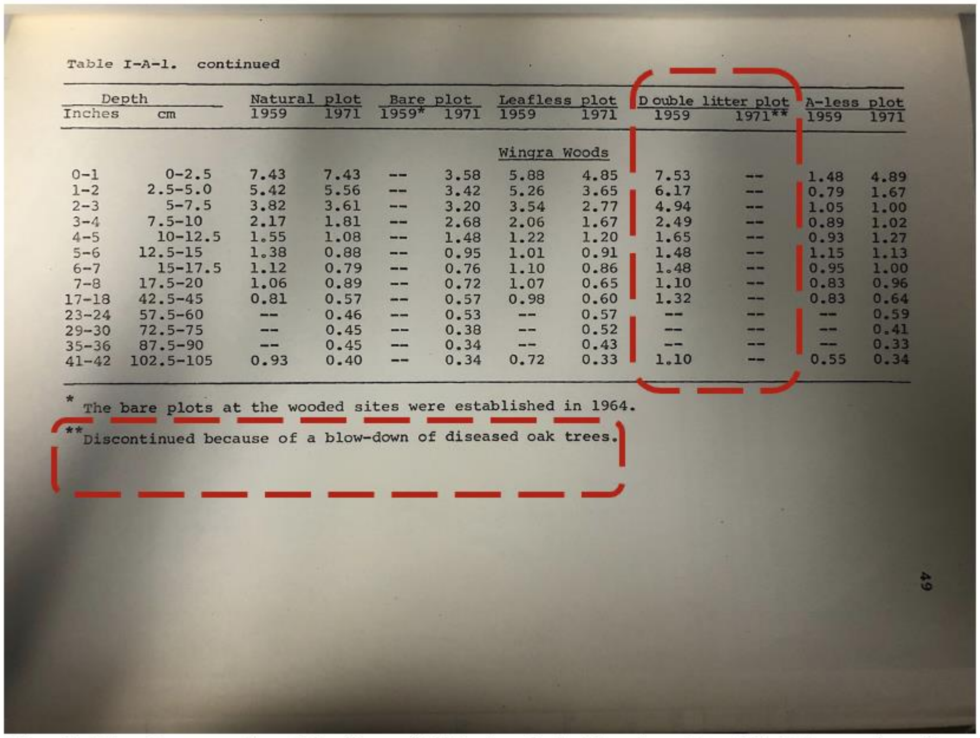


Table I-A-1. continued

Depth		Natural plot		Bare plot		Leafless plot		Double litter plot		A-less plot	
Inches	cm	1959	1971	1959*	1971	1959	1971	1959	1971**	1959	1971
						Wingra Woods					
0-1	0-2.5	7.43	7.43	--	3.58	5.88	4.85	7.53	--	1.48	4.89
1-2	2.5-5.0	5.42	5.56	--	3.42	5.26	3.65	6.17	--	0.79	1.67
2-3	5-7.5	3.82	3.61	--	3.20	3.54	2.77	4.94	--	1.05	1.00
3-4	7.5-10	2.17	1.81	--	2.68	2.06	1.67	2.49	--	0.89	1.02
4-5	10-12.5	1.55	1.08	--	1.48	1.22	1.20	1.65	--	0.93	1.27
5-6	12.5-15	1.38	0.88	--	0.95	1.01	0.91	1.48	--	1.15	1.13
6-7	15-17.5	1.12	0.79	--	0.76	1.10	0.86	1.48	--	0.95	1.00
7-8	17.5-20	1.06	0.89	--	0.72	1.07	0.65	1.10	--	0.83	0.96
17-18	42.5-45	0.81	0.57	--	0.57	0.98	0.60	1.32	--	0.83	0.64
23-24	57.5-60	--	0.46	--	0.53	--	0.57	--	--	--	0.59
29-30	72.5-75	--	0.45	--	0.38	--	0.52	--	--	--	0.41
35-36	87.5-90	--	0.45	--	0.34	--	0.43	--	--	--	0.33
41-42	102.5-105	0.93	0.40	--	0.34	0.72	0.33	1.10	--	0.55	0.34

* The bare plots at the wooded sites were established in 1964.

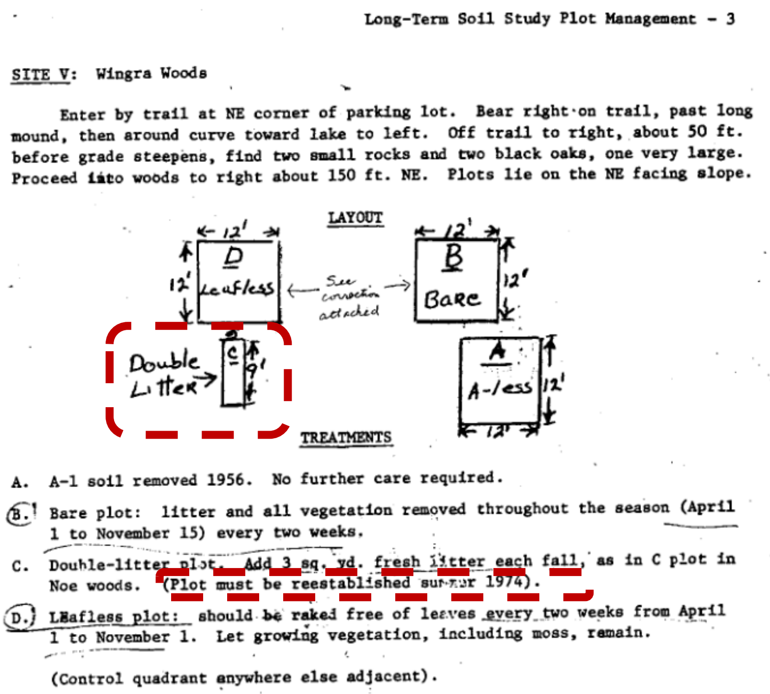
**Discontinued because of a blow-down of diseased oak trees.

49

Fig. 3. Van Rooyan's table from 1973 thesis indicating Double Litter plot discontinued because of tree fall.

3.) 1984 Hole Maintenance Protocol

"1984_HoleMaintenanceProtocol.pdf" (assumed to be the UW-Arboretum's maintenance protocol with comments by Francis Hole) notes double litter plot "reestablished summer 1974". Location of double litter plot in 1984 document appears similar to current location, suggesting re-establishment in 1974 matches current location in Wingra Woods.


Long-Term Soil Study Plot Management - 3

SITE V: Wingra Woods

Enter by trail at NE corner of parking lot. Bear right on trail, past long mound, then around curve toward lake to left. Off trail to right, about 50 ft. before grade steepens, find two small rocks and two black oaks, one very large. Proceed into woods to right about 150 ft. NE. Plots lie on the NE facing slope.

LAYOUT

TREATMENTS

A. A-1 soil removed 1956. No further care required.

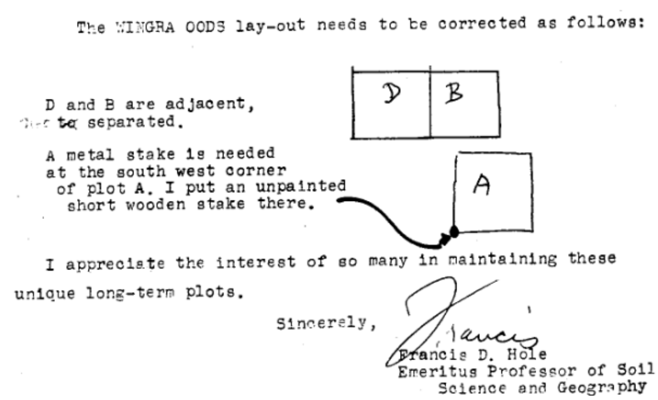
B. Bare plot: litter and all vegetation removed throughout the season (April 1 to November 15) every two weeks.

C. Double-litter plot. Add 3 sq. yd. fresh litter each fall, as in C plot in Noe woods. (Plot must be reestablished summer 1974).

D. Leafless plot: should be raked free of leaves every two weeks from April 1 to November 1. Let growing vegetation, including moss, remain.

(Control quadrant anywhere else adjacent).

Figure 4. UW-Arboretum's "1984_HoleMaintenaceProtocol" with handwritten notes/corrections by Francis Hole.


The WINGRA OODS lay-out needs to be corrected as follows:

D and B are adjacent, not separated.

A metal stake is needed at the south west corner of plot A. I put an unpainted short wooden stake there.

I appreciate the interest of so many in maintaining these unique long-term plots.

Sincerely,

Francis

Francis D. Hole
Emeritus Professor of Soil Science and Geography

Figure 5. Correction to layout presented in UW-Arboretum's "1984_HoleMaintenanceProtocol" (Fig 4).

4.) Knute Nadelhoffer 1997 data file

a. Arb 1997 C master.xlsx

i. Sheet = Field Sampling info

ii. "The plots were not well-marked at the two forest sites. We were able to identify the plot boundaries for Noe Woods but were not sure about the double-litter plot boundaries in the Wingra Woods."

5.) Personal communication with Kim Townsend

a. Indicated boundaries of double litter treatment in Wingra woods were unclear in 2006 sampling. Sampling method involved sampling in and around presumed treatment and used values that aligned with expectations.

6.) Kim Townsend MS thesis "The Legacy of Dr. Francis Hole: Examining Soil Organic Carbon after 50 years of Detrital Manipulation"; Dec 16, 2013

a. Page 59 Appendix: "Francis Hole Plot Site and Treatment Information from an undated typed document on file at the University of Wisconsin Arboretum"

b. Page 60 "There is an additional note that the Double litter plot must be reestablished the summer of 1974".

7.) UW-Arboretum's maintenance records

The UW-Arboretum has maintenance records from 2008-2009 and 2018-2024.

a. "2008to2009_HolePlotMaintenanceRecords.doc"

i. Josh Brown notes in 2008 (Figure 6), "Wingra plots D and B were treated; A and C were not located". Treatment "A" refers to "A-less" and "C" is "double litter".

ii. Erik Olson notes in spring 2009 they were unable to find A-less boundaries (Figure 7).

b. "2018to2022_HolePlotMaintenanceRecords.xlsx"

i. 7/3/18, Site V (Wingra Woods), Plot C (double litter), Notes: "Installed missing rebar at NE, NW, and SW corners (unknown if this was the previous plot location since only one corner remained and it was not labeled)"

2008 Francis Hole Treatment plots

Josh Brown

May 19-23? - (I only have written down the week) – Went with Brad to see Hole plots and check their condition

May 30, 31 – Measured Hole plots' dimensions and began treatments using trowel, rake, and hoe.

June 16 – raked/hoed/cut treatment plots

June 24, 25 – found and measured the East Curtis plots and started treatment, in addition to other plots (before I was only treating Noe and Wingra Woods, and West Curtis Prairie.

July 1 – Treated East Curtis plots so that they would coincide with treatment of three other plots (I treated East Curtis a week early, so I could do all four plots at once, but East Curtis needed extra cutting because it was started later). Started using a brushcutter to treat plots instead of hand tools.

July 12, 13 – Treated all plots with brushcutter.

July 28 – Treated all plots

August 9,10? (not sure of date but sometime the second week of August) – Treated all plots

*Wingra plots D and B were treated; A and C were not located.

Figure 6. UW-Arboretum's "2008to2009_HolePlotMaintenaceRecords" unable to locate treatments A (A-less) and C (double litter).

Notes written by Erik Olson (Spring 2009):

Notes by Olson compiled by Sally Gallagher 6-26-09)

4/8/09 **Site I:** Plots B & C hoed and raked mostly bare of vegetation. C vegetation left and spread evenly. B vegetation removed off site. Very difficult to not disturb top 4" of soil especially in Plot B. Plot B had a lot of moss and short vegetation. Soil was moist and had a lot of worms. Completed on 4/8/09 (1 hour of straight work). Attempted weeding on 5/18/09 : not realistic.

4/10/09 **Site II:** Same as above (see 4/8/09). Although plot B was not as moist and had fewer worms were observed. Completed on 4/10/09 (1 hour straight work). Attempted weeding on 5/18/09: not realistic.

4/13/09 **Site III:** Completed on 4/13/09 by Erik Olson and Brad Herrick. Site was mainly covered in moss and was very easy to scrape off the vegetation with a hoe or upturned rake. Brad suggested that from now on focus on not disturbing the soil vs. removing all the vegetation. So from now on I will remove (for all the bare sites) as much vegetation as possible while disturbing as little soil as possible. Scraping method works best. Note: Plot D or harvested site had roughly 10 year old shrubs within it. Also, area near plots has a high amount of woody vegetation than surrounding area. Not being burned probably is allowing them to establish.

Site IV: Plots B & D were completed on 4/13/09 by Erik Olson. A rake works great for both plots – some hand pulling also. Garlic mustard moving in on edge of bare plot. When raking leafless plot it is difficult to avoid removing any vegetation so some is accidentally removed, but very little. (Total work ~20 mins).

4/15/09 **Site V:** Plots B & D were completed on 4/15/09 by Erik Olson and Brad Herrick. Not much vegetation on either plot but many leaves on the surface. We reflagged (blue flags) the corners of plots B & D using a measuring tape and a compass. Tried to find and mark corners of A ("A-less") plot but could not find them. Try again later. Brad was going to place rebar at corners. (Total raking time ~ 25 mins)

4/24/09 **Site I:** Raked – not as good (upside down rake) Plot B

Site II: Raked – not as good (upside down rake) Plot B

Figure 7. UW-Arboretum's "2008to2009_HolePlotMaintenaceRecords" notes not able to find A-less treatment.

Summary

The Wingra Woods DIRT plots double litter treatment was first documented as discontinued in 1971. The double litter treatment was re-established in the summer of 1974. The location of this re-established plot appears to be in a different location from the original plot. The re-establishment location appears similar to the current plot location. However, sampling in 1997, 2006, and maintenance records from 2008 tried to locate double litter plots but were uncertain of its location. Similarly, as recently as 2018, maintenance records indicate reestablishing missing rebar and uncertainty in plot location. Our understanding is that the new double litter plot was likely re-established to its current location in 1974, yet with irregular maintenance which has led to uncertainty of its boundaries over time.

Recommendations

1. Continue maintenance of the woodland DIRT treatments in the current boundary configuration with clear notation in their management file indicating the Wingra woods double litter treatment does not have the same length of management as the other treatments. Current double litter treatment location likely re-established in 1974.
    a. Continuation of all treatments in their current boundaries, with updated maps made to scale, corresponding GPS coordinates, and detailed maintenance records will allow future researchers to make informed sampling decisions.
    b. Identify boundaries of the original control plot, which could be used in both Wingra and Noe woods research going forward (see Nielsen thesis "check plot").
2. Locate the original boundaries of the double litter treatment in Wingra woods based on the map for Nielsen's thesis in an effort to document the history of the site. However, I do not recommend re-establishing a double litter treatment in the original boundaries.
    a. Given the Nielsen thesis is a hand drawn map, it is not possible to ensure the precise location has been identified. Further, the date of management cessation was first *documented* in 1971, but we don't know for certain when management ended.

5. **<u>UW-Madison Arboretum DIRT maintenance document 2018</u>**

Keady Thesis Ch1 Appendix 1.5

Mia Keady added the year sites (Curtis I, II, and III) were restored after referencing Francis Hole's 1984 map (pictured on page 2).

<u>Long-Tem Soil Study Plots</u>

Begun by Dr. Francis Hole in 1956

MAINTENANCE OF LONG-TERM SOIL STUDY PLOTS

(Five sites initiated in 1956 by Dr. Francis Hole - - locations, identifications, and procedures.)

All corners are marked with steel posts having yellow-painted tops. Plastic tensiometer tubes for probing soil moisture content are set into some of these plots. These should be left in place. They are set <1m deep.

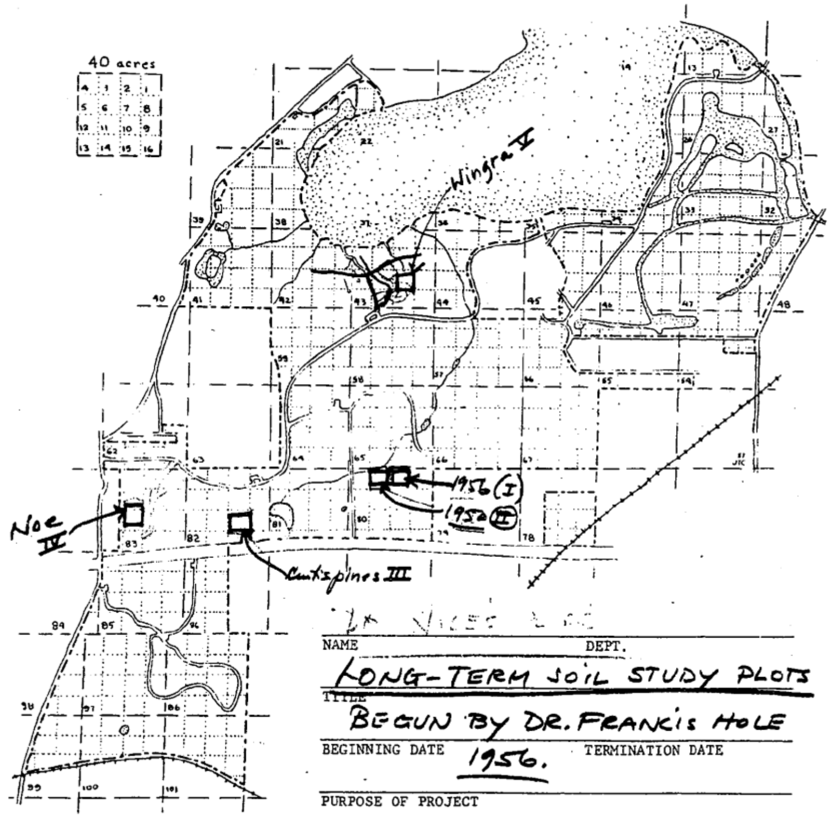
<u>WORK PROGRAM</u>

<u>April 1 to November 1:</u> every 2 weeks

1. Hoe and weed B (bare) plots in all 5 sites.
2. Hoe and weed C (mulched) plots in I, II, and III. Leaving mulch as intact as possible.
3. Rake D (leafless) plots in Site IV (Noe) and Site V (Wingra).

<u>Early November (or late October)</u>

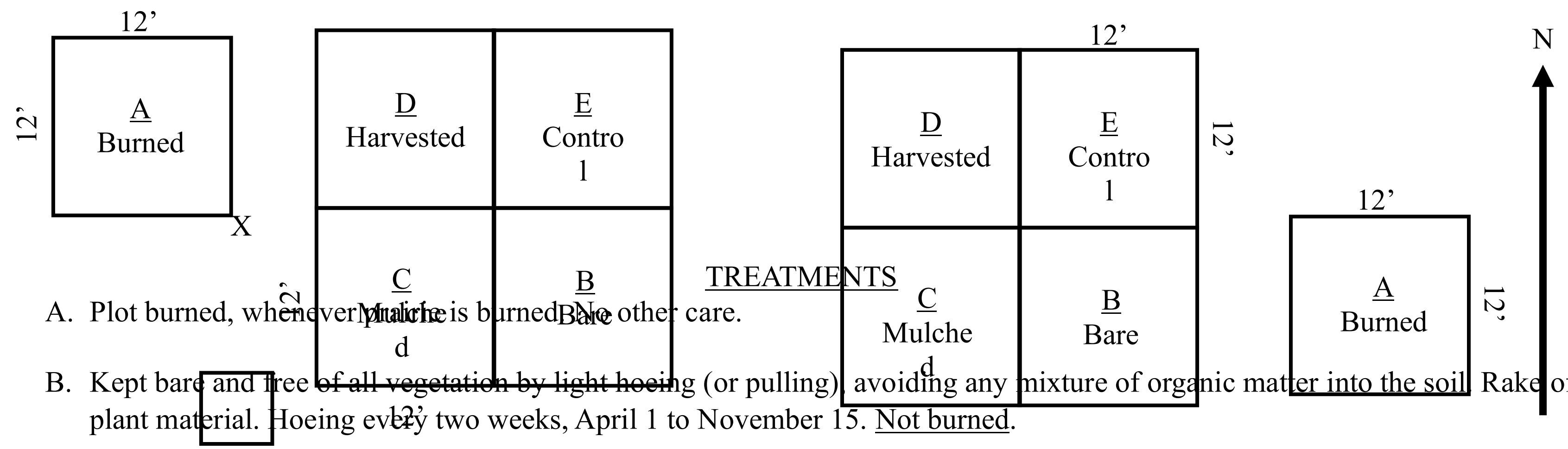
1. Harvest D (harvested) plots in Sites I, II, and III.
2. Rake up harvest and transfer harvest from plots D (harvested) to C (mulched) plots in I, II, and III.
3. Gather <u>new</u> litter from 3 sq. yards of woods and add evenly to C (double-litter) plots in Noe and Wingra.


40 acres
Wingra V
1956 (I)
1950 II
Noe IV
Curtis pines III
NAME
DEPT.
LONG-TERM SOIL STUDY PLOTS
TITLE
BEGUN BY DR. FRANCIS HOLE
BEGINNING DATE
1956.
TERMINATION DATE
PURPOSE OF PROJECT

SITE II (1950 restored): Just west of Site I in eastern end of Curtis Prairie

SITE I (1956 restored): Eastern end, Curtis Prairie *see original document for drawing of location in relation to E. Curtis Firelane

LAYOUT



TREATMENTS

A. Plot burned, whenever prairie is burned. No other care.

B. Kept bare and free of all vegetation by light hoeing (or pulling), avoiding any mixture of organic matter into the soil. Rake off plant material. Hoeing every two weeks, April 1 to November 15. Not burned.

C. Mulched plot: all living plants removed by same light hoeing as B, but left in mulch. Harvested plant material from D spread over this plot evenly every fall (late October, before frost). Not burned.

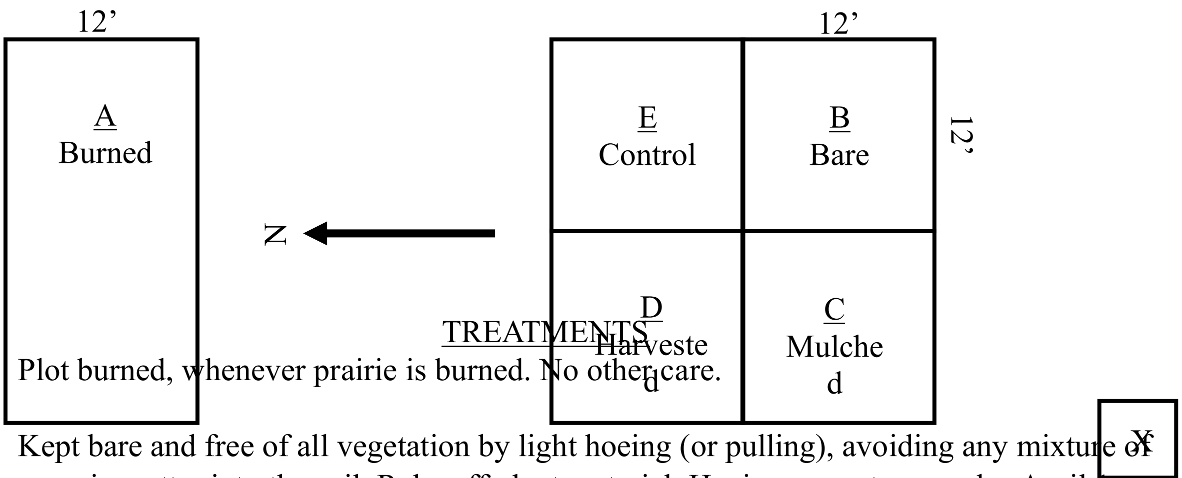
D. Entire plot mowed in later October, harvest raked up and transferred evenly to plot C. No other care. Not burned.

E. Control plot. No treatment EXCEPT that not more than one seeded in-tree should be permitted at a time. The older of any two should be removed. Not burned.

X. Permanent soil profile pit. About 3-1/2 ft. deep, covered.

SITE III (1940 restored): Curtis-Pines Plot, west of desilting pond *see original document for drawing of location in relation to Pines

LAYOUT



TREATMENTS

A. Plot burned, whenever prairie is burned. No other care.

B. Kept bare and free of all vegetation by light hoeing (or pulling), avoiding any mixture of organic matter into the soil. Rake off plant material. Hoeing every two weeks, April 1 to November 15. Not burned.

C. Mulched plot: all living plants removed by same light hoeing as B, but left in mulch. Harvested plant material from D spread over this plot evenly every fall (late October, before frost). Not burned.

D. Entire plot mowed in later October, harvest raked up and transferred evenly to plot C. No other care. Not burned.

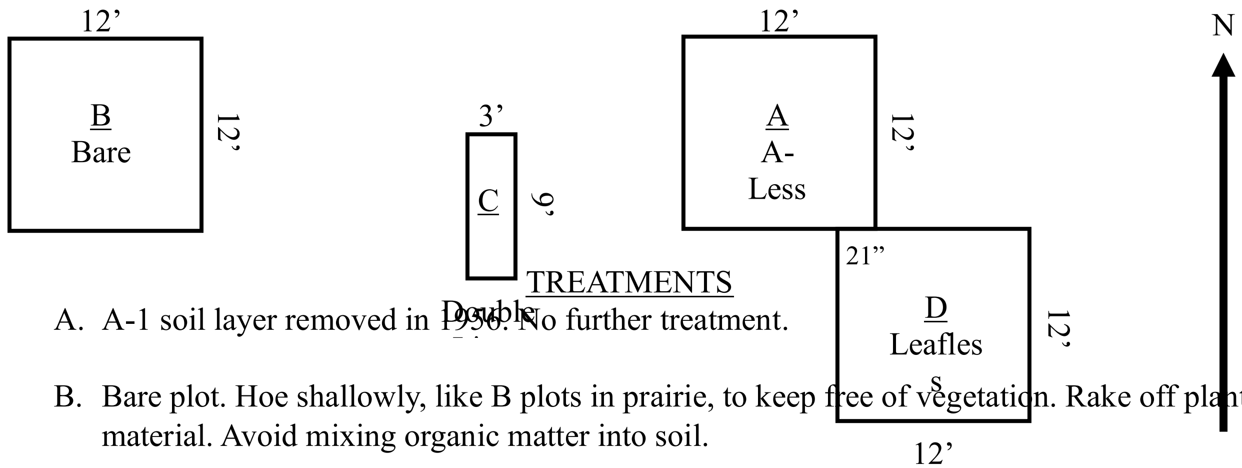
E. Control plot. No treatment EXCEPT that not more than one seeded in-tree should be permitted at a time. The older of any two should be removed. Not burned.

X. Permanent soil profile pit. About 3-1/2 ft. deep, covered.

SITE IV: Noe Woods

Entering from McCaffrey Drive on firelane, proceed straight ahead, then continue on firelane bending around toward right in direction of beltline. Stop before return loop starts, where turn-around arm exists to right of firelane. Enter woods to right (toward McCaffrey Drive), proceeding approximately SW. Plot stakes same as for prairie.

LAYOUT



TREATMENTS

A. A-1 soil layer removed in 1956. No further treatment.

B. Bare plot. Hoe shallowly, like B plots in prairie, to keep free of vegetation. Rake off plant material. Avoid mixing organic matter into soil.

C. Double-litter plot. Each fall (first half of November) add fresh litter collected from three 9 ft^2 quadrats (three 3 ft x 3 ft quadrats) elsewhere evenly over this plot (leaves, twigs, acorns; avoid old decaying litter lying below). 3 screens one-yard square can be laid out to collect the fresh litter if desired.

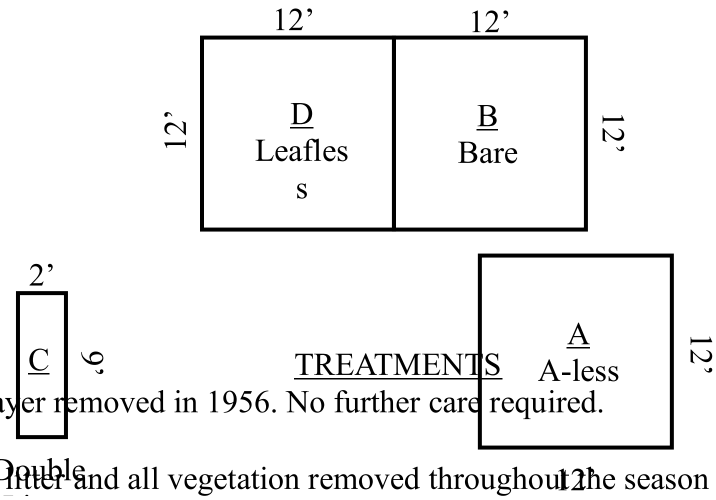
D. Leafless plot. Should be raked free of leaves every two weeks from April 1 to November 15.

(Control Plot: any quadrat elsewhere nearby.)

SITE V: Wingra Woods

Enter by trail at NE corner of parking lot. Bear right on trail, past long mound, then around curve toward lake to left. Off trail to right, about 50 ft. before grade steepens, find two small rocks and two black oaks, one very large. Proceed into woods to right about 150 ft. NE. Plots lie on the NE facing slope.

LAYOUT



TREATMENTS

A. A-1 soil layer removed in 1956. No further care required.

B. Bare plot: litter and all vegetation removed throughout the season (April 1 to November 15) every two weeks.

C. Double-litter plot. Add 3 sq. yd. fresh litter each fall, as in C plot in Noe Woods. (Plot must be reestablished summer 1974). **Noe Woods C instructions: Each fall (first half of November) add fresh litter collected from three 9 ft^2 quadrats (three 3 ft x 3 ft quadrats) elsewhere evenly over this plot (leaves, twigs, acorns; avoid old decaying litter lying below). 3 screens one-yard square can be laid out to collect the fresh litter if desired.

D. Leafless plot: should be raked free of leaves every two weeks from April 1 to November 1. Let growing vegetation, including moss, remain.

(Control quadrant anywhere else adjacent).

6. <u>1984 Hole Maintenance Protocol; See Appendix 1 attachment.</u>

7. **2008to2009 HolePlotMaintenanceRecords; See Appendix 1 attachment.**

2008 Francis Hole Treatment plots
Josh Brown

May 19-23? - (I only have written down the week) – Went with Brad to see Hole plots and check their condition

May 30, 31 – Measured Hole plots' dimensions and began treatments using trowel, rake, and hoe.

June 16 – raked/hoed/cut treatment plots

June 24, 25 – found and measured the East Curtis plots and started treatment, in addition to other plots (before I was only treating Noe and Wingra Woods, and West Curtis Prairie.

July 1 – Treated East Curtis plots so that they would coincide with treatment of three other plots (I treated East Curtis a week early, so I could do all four plots at once, but East Curtis needed extra cutting because it was started later). Started using a brushcutter to treat plots instead of hand tools.

July 12, 13 – Treated all plots with brushcutter.

July 28 – Treated all plots

August 9,10? (not sure of date but sometime the second week of August) – Treated all plots

*Wingra plots D and B were treated; A and C were not located.

Fancis Hole Long-Term Soil Study Plots
1956-Present

Management Notes

Notes written by Erik Olson (Spring 2009):
(Notes by Olson compiled by Sally Gallagher 6-26-09)

4/8/09 **Site I**: Plots B & C hoed and raked mostly bare of vegetation. C vegetation left and spread evenly, B vegetation removed off site. Very difficult to not disturb top 4" of soil especially in Plot B. Plot B had a lot of moss and short vegetation. Soil was moist and had a lot of worms. Completed on 4/8/09 (1 hour of straight work). Attempted weeding on 5/18/09 : not realistic.

4/10/09 **Site II**: Same as above (see 4/8/09). Although plot B was not as moist and had fewer worms were observed. Completed on 4/10/09 (1 hour straight work). Attempted weeding on 5/18/09: not realistic.

4/13/09 **Site III:** Completed on 4/13/09 by Erik Olson and Brad Herrick. Site was mainly covered in moss and was very easy to scrape off the vegetation with a hoe or upturned rake. Brad suggested that from now on focus on not disturbing the soil vs. removing all the vegetation. So from now on I will remove (for all the bare sites) as much vegetation as possible while disturbing as little soil as possible. Scraping method works best. Note: Plot D or harvested site had roughly 10 year old shrubs within it. Also, area near plots has a high amount of woody vegetation than surrounding area. Not being burned probably is allowing them to establish.

Site IV: Plots B & D were completed on 4/13/09 by Erik Olson. A rake works great for both plots – some hand pulling also. Garlic mustard moving in on edge of bare plot. When raking leafless plot it is difficult to avoid removing any vegetation so some is accidentally removed, but very little. (Total work ~20 mins).

4/15/09 **Site V**: Plots B & D were completed on 4/15/09 by Erik Olson and Brad Herrick. Not much vegetation on either plot but many leaves on the surface. We reflagged (blue flags) the corners of plots B & D using a measuring tape and a compass. Tried to find and mark corners of A ("A-less") plot but could not find them. Try again later. Brad was going to place rebar at corners. (Total raking time ~ 25 mins)

4/24/09 **Site I**: Raked – not as good (upside down rake) Plot B

Site II: Raked – not as good (upside down rake) Plot B

5/1/09 **Site: I**: Raked – not as good (upside down rake) Plot C
'
Site II: Raked – not as good (upside down rake) Plot C

Site III: Raked – upside down rake

5/18/09 **Site III:** Weeded by hand (bare plot only). Very quick (15-20 mins)

Site IV: Weeded by hand. Very very quick (5 mins)

5/19/09 **Site I**: Hoed all of Plot B and half of Plot C – very laborious

5/20/09 **Site I**: Finished other half of Plot C

Site II: All of bare Plot B

Summer & Fall 2009 Francis Hole Plot Management
Sally Gallagher

6-29-09

Brad and I toured all sites and decided that all sites should be managed this week (6/29-7/3) since it has been more than 2 weeks since last management at all Hole sites.

Site I – I harvested plots B and C. Soil was very dry/hard/cracked so plant roots did not come up through the soil easily which made snapping them off at the base easier. Many of the plants were much older than 2 weeks and were therefore difficult to get up without the use of hand clippers. Mostly hand picked and used clippers. Used hoe on plot B at the end to clean up but plants were too hard/old to use hoe from the beginning. Use of hoe in plot C was almost impossible due to microtopography. (2-3 hours)

Site II – I harvested plot B. Soil was very dry/hard/cracked so plant roots did not come up through the soil easily which made snapping them off at the base easier. Many of the plants were much older than 2 weeks and were therefore difficult to get up without the use of hand clippers (some grasses and sedges already had lignified shoot bases). Mostly hand picked and used clippers. Used hoe on plot B at the end to clean up but plants were too hard/old to use hoe from the beginning. (1 hour)

6-30-09

Site II – I harvested plot C. Soil was very dry and hard so plants did not come up easily. Many of the plants were much older than 2 weeks and were therefore difficult to get up without the use of hand clippers (some grasses and sedges already had lignified shoot bases). Only used hand clippers and hand pulling. (1-2 hours)

7-6-09

Site III – I harvested plots B anc C. Soil was hard and dry so plant roots did not come up through the soil easily which made snapping them off at the base easier. Most of the plants were old and both plots had very high percent cover. Woody shrubs were well established as were grasses and many nice prairie plants (e.g. indigo, spiderwort). Had to use hand clippers for older plants; hand picked the rest. Used hoe on both plots to scrape up the many small basal leaves (pussytoes?). (3 hours)

Site IV – I harvested plots B and D. Plot B did not have a lot of large plants, but still had a few older seedlings. I used the hoe to get many of the small seedlings up. I forgot to bring the rake, so I tried to hand pick up a few of the larger leaves (mostly oak litter) but a few are remaining. Plot D was very tall and tangled with growth. I found it would be best to not disturb the vegetation by attempting to remove any leaf litter, so I did not technically manage this site. I did look for large branches or large pieces of litter and saw none. (30-40 mins)

Site V – I harvested plots B and D. Neither plot had much new vegetation, so clearing plot B was no problem. I did forget to bring the rake so I picked up as much of the fallen leaf litter as possible, but some still remains. The far NW blue flag of plot B was lying loose so I estimated its general corner and re-staked it (note to self: take measuring tape out next time and find more accurate point for NW corner). I hand removed what litter I could from plot D and left the few small seedlings alone. (20 mins)

7-14-09

Site I – I harvested plots B and C. Soil was quite dry and flaky so roots did not come out of the soil easily. I hand pulled the larger plants in plot B and then hoed the rest to scrape the smaller plants up. I hand pulled all of the plants in plot C since the soil is not even and therefore, hoeing would cause more damage to the topsoil. (45 mins)

Site II – I harvested plots B and C. Soil was quite dry and flaky so roots did not come out of the soil easily. I hand pulled the larger plants in plot B and then hoed the rest to scrape the smaller plants up. I hand pulled all of the plants in plot C since the soil is not even and therefore, hoeing would cause more damage to the topsoil. (45 mins)

7-20-09

Site IV – I harvested plots B and D. Plot B had only very young seedlings. I used the rake turned upside down to scrape away any small seedlings then turned the rake the normal way to rake away the few leaves and branches that had fallen/blown onto the plots. Plot D was very tall and tangled with growth. I found it would be best to not disturb the vegetation by attempting to remove any leaf litter, so I did not technically manage this site. I did look for large branches or large pieces of litter and saw none. (20 mins)

Site V – I harvested plots B and D. Neither plot had much new vegetation, so clearing plot B was no problem. I used the rake turned upside down to scrape away any small seedlings then turned the rake the normal way to rake away the few leaves and branches that had fallen/blown onto the plots. The seedlings in plot D were more numerous and larger than 2 weeks ago. (20 mins)

7-21-09

Site III – I harvested plot B. Most new vegetation was basal-leaved pussy toes. Mostly used hoe to scrape new vegetation off of surface. Came up very easily. Started to rain before I could start plot C.

7-23-09

Site III – I harvested plot C. Not much new vegetation. Had to pick up and move old vegetation from site before hand picking new vegetation. Re-spread old, dead vegetation before leaving.

7-28-09

Site I – I harvested plots B and C. Bare plot had very little new vegetation. What was there came up very easily with the use of light hoeing. Plot C had a bit more vegetation, mostly grasses and rushes that had re-sprout from the last harvesting. All of plot C was hand pulled. Harvested material was then spread as evenly as possible over the top.

Site II – I harvested plots B and C. Bare plot had very little new vegetation. What was there came up very easily with the use of light hoeing. Plot C had a much more vegetation, mostly grasses and rushes that had re-sprout from the last harvesting. All of plot C was hand pulled. Harvested material was then spread as evenly as possible over the top.

8-4-09

Site V - I harvested plots B and D. Neither plot had much new vegetation, so clearing plot B was no problem. I used the rake turned upside down to scrape away any small seedlings then turned the rake the normal way to rake away the few leaves and branches that had fallen/blown onto the plots. (20 mins)

8-5-09

Site III – I harvested plots B and C. The majority of new growth in both plots was basal-leaved pussy toes. I mostly used hoe to scrape new vegetation off of the surface of plot B. Pussy toes came up very easily. I cleared plot C by pushing away dead vegetation then hand picking all living plants. After clearing the whole plot, I re-spread dead vegetation evenly across plot. Soil was very dry. (1.5 hours)

8-6-09

Site IV – I harvested plots B and D. Plot B had only a few very young seedlings. I used the rake turned upside down to scrape away any small seedlings then turned the rake the normal way to rake away the few leaves and branches that had fallen/blown onto the plots. Plot D was very tall and tangled with growth. I found it would be best to not disturb the vegetation by attempting to remove any leaf litter, so I did not technically manage this site. (20 mins)

8-12-09

Site I – I harvested plots B and C. Bare plot had a few new seedlings, mostly grasses and succulent low-laying plants. I used a hoe to scrape up these seedlings. The soil was relatively wet and so made scraping a bit more difficult. Plot C had a few seedlings, mostly grasses and low-laying succulents. All seedlings in this plot were hand-pulled after first pushing aside other dead growth. A small section of Plot C was not finished due to the severe heat of the day (I was feeling heat stroked so left this section undone).

Site II – I harvested plots B and C. Bare plot had a few new seedlings, mostly grasses and succulent low-laying plants. I used a hoe to scrape up these seedlings. The soil was relatively wet and so made scraping a bit more difficult. Plot C had a few seedlings, mostly grasses and low-laying succulents. All seedlings in this plot were hand-pulled after first pushing aside other dead growth.

8-18-09

Site V – I harvested plots B and D. Plot B had no new vegetation, so I only cleared this site of debris. For both plots, I used the rake turned upside down to scrape away the few leaves and branches that had fallen/blown onto the plots.

Site I – I finished plot C that was not completed on 8-12-09 above.

8-25-09

Site III – I harvested plots B and C. The majority of new growth in both plots was basal-leaved pussy toes. I mostly used hoe to scrape new vegetation off of the surface of plot B. Pussy toes came up very easily. I cleared plot C by pushing away dead vegetation then hand picking all living plants. After clearing the whole plot, I re-spread dead vegetation evenly across plot. Soil was wet from dew and recent rains. (1.5 hours)

Site IV – I harvested plots B and D. Plot B had only a few very young seedlings. I used the hoe to scrape away any small seedlings and the few leaves and branches that had fallen/blown onto the plots. Plot D was very tall and tangled with growth. I found it would be best to not disturb the vegetation by attempting to remove any leaf litter, so I did not technically manage this site. (20 mins)

8-28-09

Site I – I harvested plots B and C. Bare plot had a few new seedlings, mostly grasses and succulent low-laying plants. The soil was relatively wet and so made scraping almost impossible; I ended up hand pulling as many plants as possible but the small grass tufts did not come up easily. Plot C had a few seedlings, mostly grasses and low-laying succulents. All seedlings in this plot were hand-pulled after first pushing aside other dead growth.

Site II – I harvested plots B and C. Bare plot had a few new seedlings, mostly grasses and succulent low-laying plants. The soil was relatively wet and so made scraping almost impossible; I ended up hand pulling as many plants as possible but the small grass tufts did not come up easily. Plot C had a few seedlings, mostly grasses and low-laying succulents. All seedlings in this plot were hand-pulled after first pushing aside other dead growth.

9-18-09

Site I – I harvested plots B and C. Plot B had very few new seedlings, mostly small grass clumps. The soil was very dry which made scraping the soil easy with the hoe. Plot C had very few new seedlings as well. Soil was very dry.

Site II – I harvested plots B and C. Plot B had very few new seedlings, mostly small grass clumps. The soil was very dry which made scraping the soil easy with the hoe. Plot C had very few new seedlings as well. Soil was very dry.

Site III – I harvested plots B and C. Plot B had many small basal rosette plants. The soil was very dry and therefore it was easy to scrape the seedlings up using a hoe without disturbing much soil. Plot C had many larger/older basal rosette plants. I started to pull the plants up by hand but that was determined to be taking too long and not being effective, so I switched to hoeing this area. There was some soil disturbance (top 1/2”). Most seedlings were removed. Seedlings and old, dead vegetation was spread evenly across the top.

9-30-09

Site IV – I harvested plot B. Plot B had no new seedlings. There was a good amount of fallen leaf litter and a few small branches on the plot. Plot D was much too overgrown to try to remove what little litter there was. Any attempt to remove litter would be destructive to the understory growth there. A tree had fallen over part of plot D and should be removed once removal will not bee too destructive to the plants.

Site V – I harvested plots B and D. Plot B had no new seedlings. Both plots had quite a bit of fallen leaf litter and acorns as well as a few small branches. I used the rake to scrape off leaves, sticks, and as many acorns as possible. The edges of the plot had been encroached with leaf litter; had to redefine edges

11-4-09

Site I – Brad and I harvested plot D (harvested plot). Using a brush-saw, we cut the plants down as close to soil level as possible. We hand raked the felled prairie grasses and spread them evenly on top of plot C (mulched plot).

Site II – Brad and I harvested plot D (harvested plot). Using a brush-saw, we cut the plants down as close to soil level as possible (hard to get too close due to prairie dropseed tussocks). We hand raked the felled prairie grasses and spread them evenly on top of plot C (mulched plot).

Site III – Brad and I harvested plot D (harvested plot). Using a brush-saw, we cut the plants down as close to soil level as possible. We hand raked the felled shrubs and prairie grasses and spread them evenly on top of plot C (mulched plot). This site was more dogwoods and other shrubs than prairie species.

Site IV – I collected 3 square meters worth of extra litter and spread evenly on top of the double litter plot. Leaves were collected from the south side of the double litter plot.

Site V – I cleared plots B and D of leaves using a rake. I looked for the double litter plot but was only able to find the center marker; no corner markers were located. After chatting with Brad, who couldn't find them earlier in the year, we decided to forgo treating this double litter plot until the following year. This plot should be re-established in the spring of 2010.

8. **<u>2018to2022_HolePlotMaintenanceRecords; See Appendix 1 attachment.</u>**

9. **<u>Grassland DIRT burn treatment email correspondence</u>**

9.) Grassland DIRT burn treatment email correspondence

Email correspondence suggests no documentation of burning 'burned' treatment prior to 2018.

Email correspondence from Danille Tanzer, UW Arboretum Data & GIS Coordinator 12/9/2024:

"For the DIRT plot burn treatments, we're pretty sure they were burned again starting in 2018. So the west plot was burned in 2021 and 2024. And the east plots were burned in 2018 and 2021. The east plots weren't burned in 2022, but the northern part of East Curtis was."

10. **1950s_Research Map; See Appendix 1 attachment.**
11. **Curtis Legacy Map; See Appendix 1 attachment.**

Chapter 2. No general SOC change over two decades in remnant and restored prairies of southern Wisconsin

Mia M. Keady, Christopher Kucharik, Thea Whitman, Randall D. Jackson

Abstract

Anthropogenic climate change requires solutions for drawdown of atmospheric carbon. Managing soils as carbon sinks via grassland restoration and management are touted as one way to do this, but whether prairies in temperature regions are typically accumulating or storing carbon is uncertain. We estimated soil organic carbon (SOC) at 12 remnant and 9 restored tallgrass prairie sites in southern Wisconsin that had been sampled about twenty years previously to assess their potential for change over decadal periods. No general pattern in SOC concentration change emerged from these sites, with several sites gaining, several losing, and several not changing. Remnant prairie SOC stocks remained greater than those of restored prairie. Edaphic characteristics were related to SOC stocks – in particular, stocks were higher in wet, poorly drained soils, with higher silt and clay, classified as Mollisols. Mineral-associated organic carbon (MAOC) concentration increased linearly with SOC concentration, indicating no evidence for SOC saturation in these prairie soils. While grasslands were carbon sinks in past millennia, our results indicate they are not generally increasing in recent decades in southern Wisconsin, indicating low capacity for them to serve as a reliable mechanism of atmospheric carbon drawdown for climate stabilization. Nonetheless, restoring and maintaining intact grasslands continues to be our best option for slowing the contribution of greenhouse gases from these soils to the atmosphere.

Introduction

Efforts to mitigate and adapt to climate change include re-envisioning land management to bolster ecosystem services (Almaraz et al., 2023). Pre-colonial landscapes in the upper Midwest were dominated by tallgrass prairies and oak savannas, boasting dense root systems, diverse plant communities, wildlife habitat, and thousands of years of soil development (Curtis, 1959). During and after conversion to cropped land, repeated tillage of rich Mollisols depleted soil carbon 25 to 50% (Sanderman et al., 2017). Soil carbon was lost through turning the soil and exposing long-protected organic matter to decomposition by microbial communities. Very few remnant (untilled) prairies remain, often on landscapes too troublesome to till (steep slopes, rocky soil, and wet lowlands). However, these prairies serve as our best physical references for soil carbon accumulation and sequestration potential (Brye & Kucharik, 2003; Cahill et al., 2009). At the same time, restored (planted) prairie restorations offer a possible avenue for soil organic carbon (SOC) accumulation after decades of soil depletion (Kucharik et al., 2006).

SOC slowly accumulates in soil via litter decomposition, root turnover and exudation, and microbial production and turnover as necromass. Soil carbon exits the soil environment primarily through microbial and root respiration (carbon lost as CO_2) and dissolved organic carbon through leaching and runoff. Kucharik et al. (2006) estimated above- and below-ground net primary production (NPP) at both remnant and restored parts of Curtis Prairie at the University of Wisconsin-Madison Arboretum to calculate net ecosystem production (NEP) as an estimate of C balance, but variability in soil respiration made it difficult to determine if these prairies served as carbon sinks or sources with respect to the atmosphere (average NEP varied from -1.4 to 1.9 and -2.3 to 1.3 Mg C ha^{-1} yr^{-1} for remnant and restored prairies, respectively). Spatial variability in the remnant plots was double that of the restored plots, likely reflecting soil homogenization effects from past agricultural management (i.e., frequent tillage and uniform crops) and/or the restoration process and subsequent management (i.e., soil preparation and disturbance uniformity). Twenty other remnant and restored prairies were sampled in the early 2000s by Kucharik et al. (2006), establishing baseline SOC and nitrogen stocks for prairies across southern Wisconsin. Nearly 20 years later, re-sampling these remnant and restored prairies allows us to assess whether prairies are accumulating, losing, or maintaining SOC in recent decades.

Edaphic properties such as soil texture, moisture, and organic matter have long been recognized as important factors governing soil carbon accumulation. Low organic matter and water-saturated soils have been identified as priorities for restoration as they have the greatest potential to quickly accumulate SOC (von Haden & Dornbush, 2017). Water-saturated soils limit aerobic microbial activity and thus can drastically slow decomposition, whereas soils with low organic matter have less microbial activity (microbial biomass and enzymes) which may support rapid SOC accumulation until carbon and nitrogen substrates are more available and microbial communities are more active (Bach & Hofmockel, 2015). Younger prairie restorations are thought to accumulate SOC relatively quickly in the short term, but with slower rates of increase with age (Hernández et al., 2013; Kucharik, 2007). However, work in southern Wisconsin, where soils had been homogenized by heavy equipment in preparation for suburban development, showed SOC losses for several years post-restoration with some plots still exhibiting SOC losses after 14 years (Jackson & Stier, 2023).

Soil texture (proportion of sand, silt, and clay sized particles) underlies interactions between mineral surfaces and organic matter, contributing to site-by-site variation. Clay particles are negatively charged, allowing for electrostatic binding with positively charged ions in the soil solution. Exchangeable calcium forms electrostatic bridges between clay surfaces and organic matter and is one of the strongest predictors of SOC in restored prairies, followed by clay content (O'Brien et al., 2015). The sorption of organic matter to mineral surfaces results in what is referred to as mineral-associated organic matter and is thought to be a persistent form of SOC or mineral-associated organic carbon (MAOC). SOC can be conceptualized and physically fractionated into two pools – MAOC that has relatively slower turnover rates (i.e., centurial to millennial turnover, hence considered a more 'stable' pool) and particulate organic carbon (POC) that is mostly comprised of plant tissues decaying at faster rates (i.e., annual to decadal turnover) (Cotrufo et al., 2019; Prairie et al., 2023). That said, the 'stability' and 'persistence' of these pools continues to be debated (Georgiou et al., 2025). Central to this debate is the particular emphasis on whether MAOC saturates as a function of the quantity and quality of finite mineral surfaces (Begill et al., 2023; Cotrufo et al., 2019). While it may seem self-evident that there is a finite amount of sorptive mineral surface in a given soil, organic matter can sorb to extant organic matter, indicating that MAOC accumulation may not be limited by fine silt and clay fractions. Additional nuance in this debate is the question of whether saturation is a constraint on MAOC accumulation from a practical land management perspective or more of a theoretical maximum unlikely to be relevant at temporal scales useful to human decision-making.

Prairie management plays an important role in SOC accumulation. Prairie ecosystems developed in response not only to environmental factors, but also to human management by periodic fires (Roos et al., 2018) and grazing by herbivores of all sizes. Management strategies attempt to re-introduce these disturbances through prescribed burning and managed livestock grazing, but their reported effects on SOC are mixed. Recent work shows contrasting effects of fire on SOC accumulation at the Konza prairie, one of the largest untilled tallgrass prairies in North America. Connell et al. (2020) found SOC concentrations increased with limited burning

(20-yr intervals), whereas Slette et al. (2021) found higher SOC concentration in annually burned prairies than unburned or those with 2- to 4-year frequency. Slette et al. (2021) also found higher soil CO_2 emissions in recently burned prairies, with the historic fire regime influencing the magnitude of this response. Interestingly, Connell et al. (2020) used a longitudinal sampling design and assessed SOC to 25-cm soil depth, whereas Slette et al. (2021) sampled the surface 10-cm and used a space-for-time comparison (i.e., compared across treatments, but not over time). Connell et al. (2020) also showed grazing reduced SOC by 10% compared to ungrazed sites, but grazing management (frequency, intensity, spatial distribution, and species) can shape the C balance of an ecosystem, making simple 'grazed vs. ungrazed' comparisons questionable (Oates & Jackson, 2014).

Few studies assess SOC change over time, while accounting for spatial variation, edaphic characteristics, and management (but see Dietz et al. (2024) and Cordova et al. (2025)). Given the intense focus on drawing down atmospheric C with soils, and the limited remaining remnant prairies in the upper Midwest, understanding the ability of restored and remnant prairie to gain and stabilize SOC is important. We assessed whether and how much remnant and restored prairies accumulated SOC in southern Wisconsin over the past two decades. We then explored whether edaphic properties conditioned SOC accumulation and assessed evidence for MAOC saturation in remnant and restored prairies.

Methods

Soil sampling and characterization

Soil samples were collected from a total of 21 prairies (12 remnant and 9 restored) in south-central Wisconsin (Table 1, Figure 1). Sites were selected based on 1999 and 2006 surveys

(Kucharik & Brye, 2013) allowing for repeated measurements to estimate SOC change. Three 1 m soil cores were collected at each prairie in September and October 2023 with a JMC Sub-Soil Probe Plus (3 cm diameter). Baseline GPS waypoints served as the central sampling location with a 20 m transect running north-south, unless geographic barriers required an east-west transect (i.e., south-facing slope, large trees). One core was collected every 10 m on transect and divided into four depths: 0 to 10, 10 to 25, 25 to 50, and 50 to 100 cm. If compaction from sampling was > 10%, adjustments to depth increments were made in the field by assuming uniform compaction across the core.

Sample masses were collected, and soils were sieved to 2 mm, removing roots and gravel. A 10-g subsample was dried at 60°C until stable to calculate field moisture for each sample and depth. Mass of roots and gravel were collected and dried for each sample to assess bulk density. One independent bulk density core was collected per prairie (5 cm diameter, 20 cm depth) to replicate bulk density sampling at baseline collection (Kucharik & Brye, 2013). Sites with shallow soils resulted in incomplete sampling depth and were excluded from statistical analysis and visualizations.

Table 1. Characteristics of sampled remnant and restored prairies.

Type	Name	Established	Organization	Latitude / Longitude	Soil order	Soil texture	Soil moisture regime	Drainage class
Restored (n = 9)	Curtis Prairie	1936	UW-Madison Arboretum	43.0396, -89.4292	Mollisol	Silt Clay Loam	Aquic	Poorly drained
	Greene Prairie	1946	UW-Madison Arboretum	43.027, -89.4381	Mollisol	Silt Loam	Aquic	Poorly drained
	Faville Restoration	1962	UW-Madison Arboretum	43.14693, -88.88156	Mollisol	Silt Clay Loam	Aquic	Very poorly drained
	Curtis Prairie	1985	UW-Madison Arboretum	43.0403, -89.4293	Mollisol	Silt Clay Loam	Aquic	Poorly drained
	Donald County. Park	1996	Dane County Parks	42.9558, -89.6813	Alfisol	Silt Loam	Udic	Well drained
	International Crane Foundation	1996	International Crane Foundation	43.54708, -89.74936	Entisol	Loamy Sand	Udic	Excessively drained
	Goose Pond	1997	Badgerland Bird Alliance	43.3163, -89.3663	Mollisol	Silt Loam	Udic	Well drained
	UW-Biocore	1997	Lakeshore Nature Preserve	43.0895, -89.4286	Alfisol	Loam	Udic	Well drained
	Troy Garden	2003	Madison Area Community Land Trust	43.137, -89.3908	Alfisol	Silt Loam	Udic	Well drained
Remnant (n = 12)	Arlington	NA	UW-Madison Research Station	43.29077, -89.35986	Mollisol	Silt Loam	Udic	Well drained
	Black Earth Rettenmund Remnant	NA	The Prairie Enthusiasts; Designated DNR State Natural Area	43.13955, -89.77209	Alfisol	Sandy Loam	Udic	Well drained
	Curtis Prairie	NA	UW-Madison Arboretum	43.0399, -89.4285	Mollisol	Silt Clay Loam	Aquic	Poorly drained
	Faville Prairie	NA	UW-Madison Arboretum	43.148, -88.87799	Mollisol	Silt Clay Loam	Aquic	Very poorly drained
	International Crane Foundation	NA	International Crane Foundation	43.5496, -89.7502	Entisol	Loamy Sand	Udic	Excessively drained
	Koltes Prairie	NA	Groundswell Conservancy	43.17806, -89.40054	Mollisol	Loam	Udic	Well drained
	Martin Low Prairie	NA	Mark and Sue Martin	43.18181, -89.08055	Alfisol	Silt Loam	Aquic	Somewhat poorly drained

Snapper Prairie	NA	Badgerland Bird Alliance; Designated DNR State Natural Area	43.162, -88.8893	Mollisol	Silt Clay Loam	Aquic	Very poorly drained
Thousand Rock	NA	The Nature Conservancy	42.98289, -89.84108	Mollisol	Silt Loam	Aquic	Poorly drained
Walking Iron C	NA	Dane County Parks	43.1884, -89.8176	Entisol	Sand	Udic	Excessively drained
Westport Drumlin	NA	DNR State Natural Area	43.1837, -89.3919	Alfisol	Silt Loam	Udic	Well drained
Young prairie remnant	NA	DNR State Natural Area	42.8401, -88.6336	Mollisol	Silt Loam	Aquic	Poorly drained

*Soil order, texture, moisture regime and drainage class based on data shared by Dr. Kucharik and reflect data on Soil Web Survey using site coordinates.

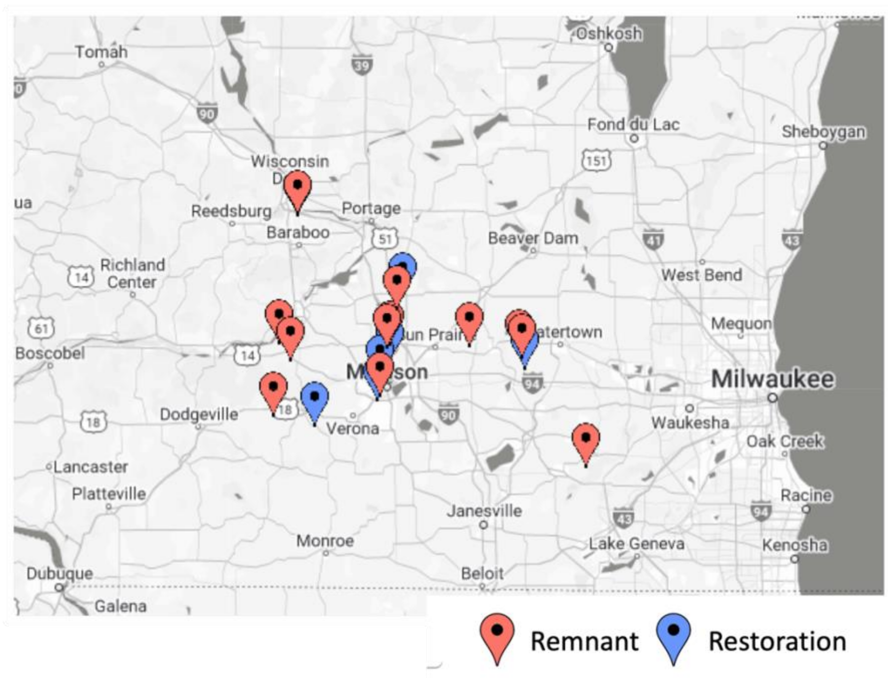


Figure 1. Location of sampled remnant and restored prairies in southern Wisconsin.

Soil texture and soil carbon fractionation (MAOC and POC) were assessed using 10 g of dried and sieved soil composited from three cores for each depth. MAOC and POC were assessed using size fractionation (MAOC < 53 µm and POC 53µm to 2000 µm, protocol adapted from Bradford et al. 2008). The POC fraction mass was used to calculate proportion sand, while a subsample of MAOC fraction was collected in a 50-mL centrifuge tube, shaken vigorously, and set at room temperature for 2 to 4 h to allow sedimentation to separate silt and clay particles (protocol adapted from Kettler et al. (2001)). Suspended clay particles were decanted into a weigh-tin, while the remaining fraction was used to estimate silt mass. Silt and clay fractions were dried to constant weight at 55°C. Soil moisture and drainage classifications were assigned using sampling coordinates on the Web Soil Survey (Soil Survey Staff, 2025). Age of prairie restoration was calculated from restoration establishment date provided by site managers.

Soil samples were analyzed for total carbon and total nitrogen using a Thermo Flash EA1112 (CE Elantech). Soil samples with < 0.2 g carbon 100 g^{-1} soil were below instrument detection levels and removed from analysis. All samples were tested for inorganic carbon using a hydrochloric acid effervescence test (Nelson & Sommers, 1996). Any sample that reacted was sent to the Kansas State University Soil Testing Laboratory where samples were treated with 1 mL of 1-N phosphoric acid in 1-mL increments until no visible reaction and analyzed for total carbon using a LECO TruSpec CN. Soil fractions of samples identified to have inorganic carbon in bulk soil were acidified before analysis for total carbon. MAOC and POC fractions were analyzed for total carbon with a LECO TruSpec CN at Kansas State University Soil Testing Laboratory.

Data analysis

Change in SOC concentration and bulk density between baseline and recent sampling were assessed with linear mixed effect models with sample year and type (remnant vs. restored) as an interaction and site as a random effect. Package lme4, function *lmer* was used for mixed-effect models (Bates et al., 2015; Kuznetsova et al., 2017). We used multi-factor models to test SOC concentration change and 2023 SOC stock (0-50 cm) relationships with edaphic properties and prairie type as an interaction. Linear models were used for numerical variables using function *lm* and *aov* for categorical and ordinal variables. TukeyHSD pairwise comparisons were assessed for significant factors. The effect of age on SOC rates of change and SOC stocks was tested using single-factor linear models for restored prairies. To assess potential MAOC saturation, we assessed the linear regression fit between MAOC (%) and SOC (%). All data analysis and visualizations were performed in R v4.0.0 (R Core Team, 2020). Packages included dplyr, ggplot2, openxlsx, gridExtra, and stringr (Auguie, 2017; Schauberger & Walker, 2020; Wickham, 2016, 2019; Wickham et al., 2022). Drainage class was assessed as an ordinal variable with “somewhat poorly drained” dropped from analyses because only one observation occurred in this class.

Results

SOC concentrations and bulk density

Across all sites, SOC concentrations in the surface 25 cm did not change over time in a consistent way ($p = 0.844$). With roughly equal numbers of each prairie type gaining, losing, and maintaining SOC, no significant interaction was observed for SOC concentration change and prairie type ($p = 0.671$) (Figure 2A & Figure S1). Age of restoration was not related to SOC concentration change ($p = 0.666$) (Figure 2B). We observed a ~36% decline in bulk density between recent and baseline sampling (1999 to 2006) in the surface 10 cm ($p < 0.001$), with no significant difference in rates by prairie type ($p = 0.561$) (Figure 2C).

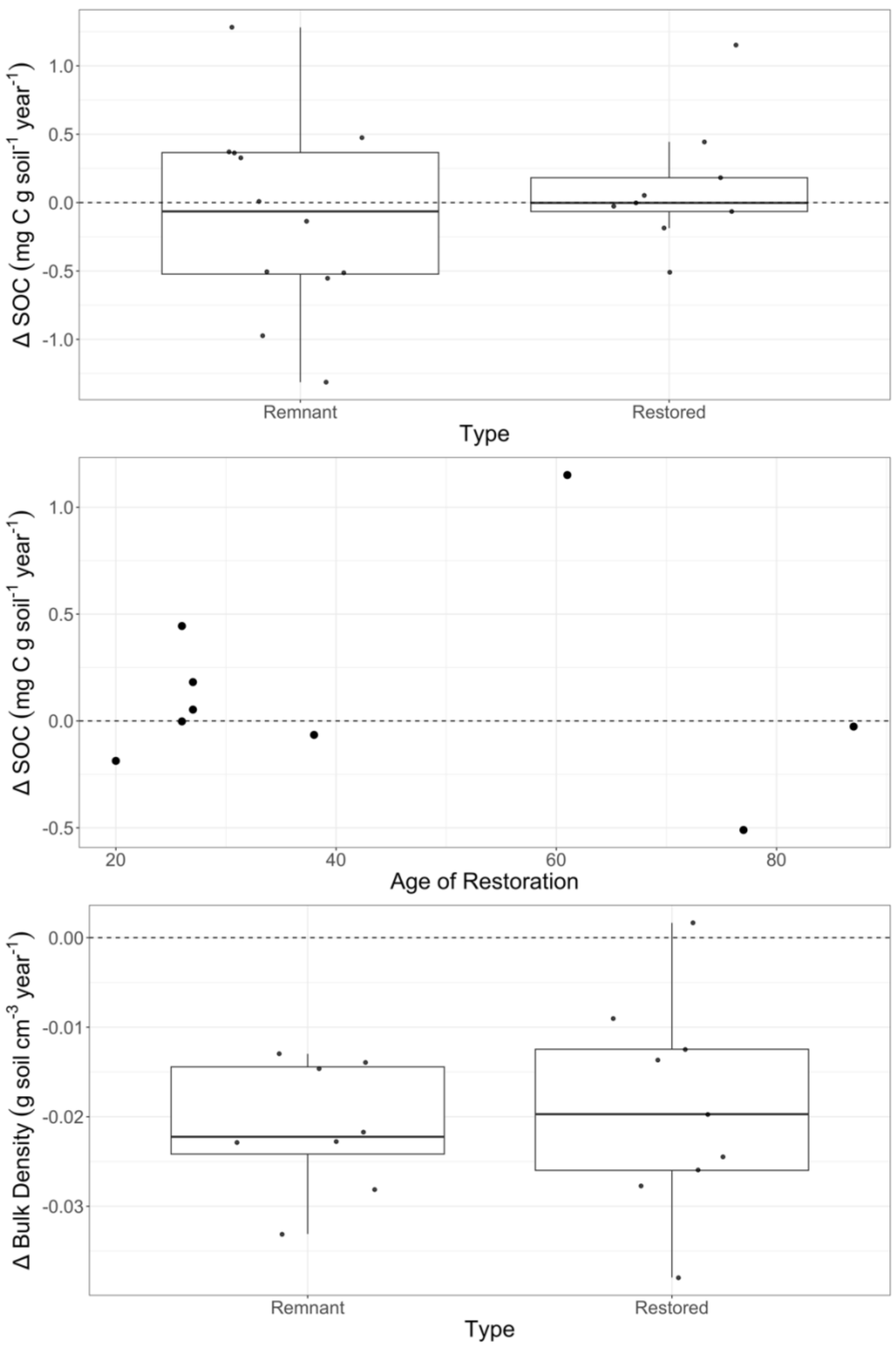


Figure 2. Rate of SOC change in surface 25 cm by A) prairie type and B) restoration age, and C) rate of change for bulk density.

Few relationships were observed between SOC concentration, site, and soil characteristics (Table 1) but SOC concentrations increased in Mollisols (p = 0.016), decreased in Alfisols (p = 0.027) and were unchanged in Entisols (p = 0.319) (Figure 3).

Table 1. Rate of soil carbon concentration change by prairie type, age, and edaphic properties in surface 25 cm analyzed with linear mixed-effect models.

Model		df	F stat	p value
Prairie type	Prairie type	1	0.60	0.45
	Residuals	19		
Texture	Perc.Silt.Clay	1	0.83	0.37
	Prairie type	1	0.35	0.56
	Perc.Silt.Clay:Prairie type	1	0.01	0.91
	Residuals	17		
Soil order	Soil.Order	2	2.77	0.1
	Prairie type	1	0.96	0.34
	Soil.Order: Prairie type	2	1.15	0.34
	Residuals	15		
Soil moisture	Soil.Moisture.Regime	1	0.08	0.78
	Prairie type	1	0.56	0.46
	Soil.Moisture.Regime: Prairie type	1	0.02	0.9
	Residuals	17		
Drainage class	Drainage.Class	3	1.35	0.3
	Prairie type	1	0.3	0.59
	Drainage.Class: Prairie type	3	0.59	0.63
	Residuals	12		

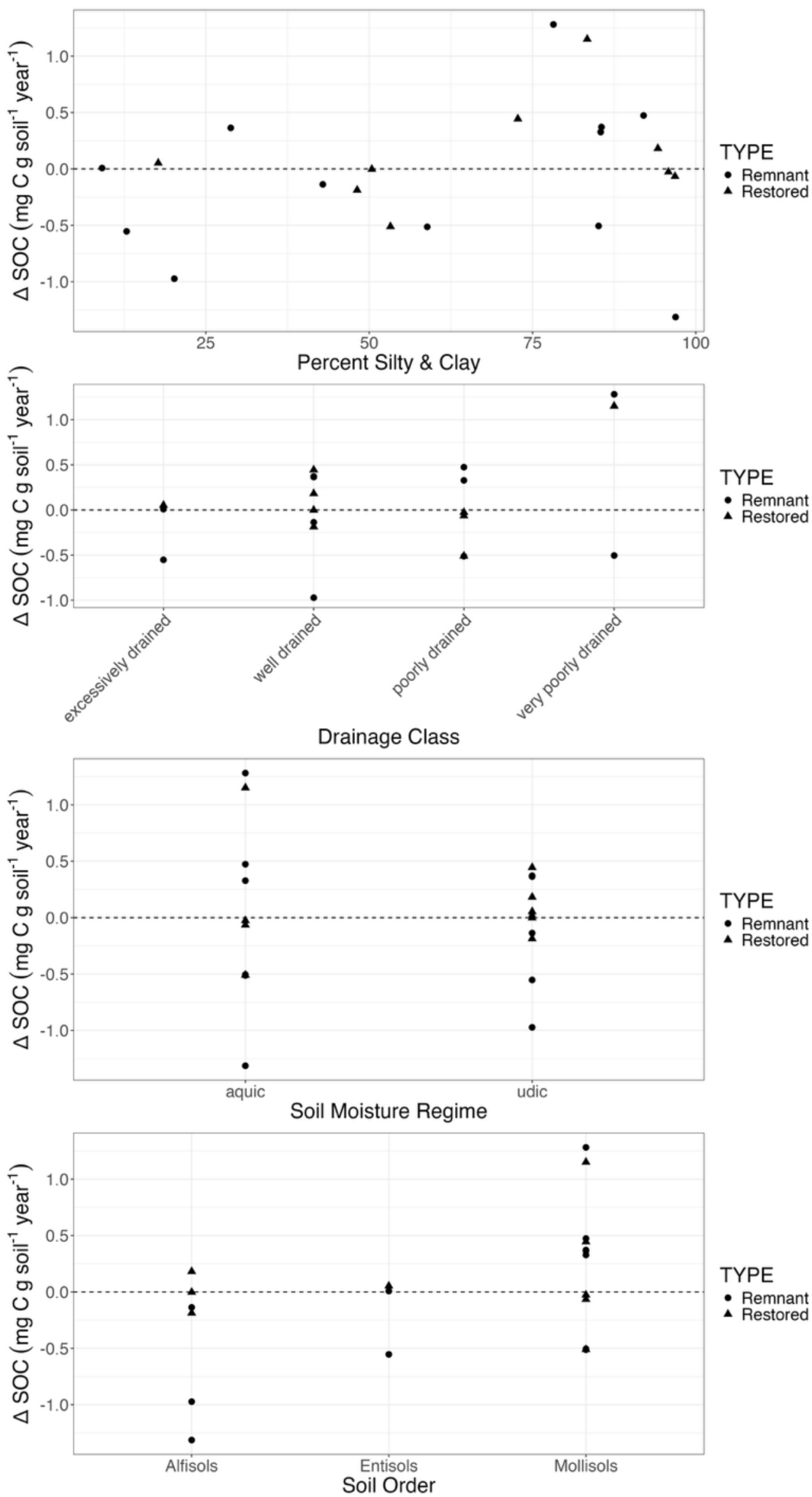


Figure 3. Rate of SOC change (0 to 25 cm) and edaphic properties

Remnant prairies had greater SOC stocks than restored prairies ($p = 0.04$), but SOC stocks did not correspond with age of restorations ($p = 0.127$) (Figure 5). Edaphic variables were strongly related to SOC stocks across prairie type and varied according to soil moisture regime ($p = 0.005$), drainage class ($p < 0.001$), soil order ($p = 0.009$) and percent silt and clay ($p = 0.022$) (Figure 6). SOC stocks were highest among aquic, very poorly drained, Mollisols with greater percent silt and clay. Site variation in carbon concentration by depth can be seen in Figure S2.

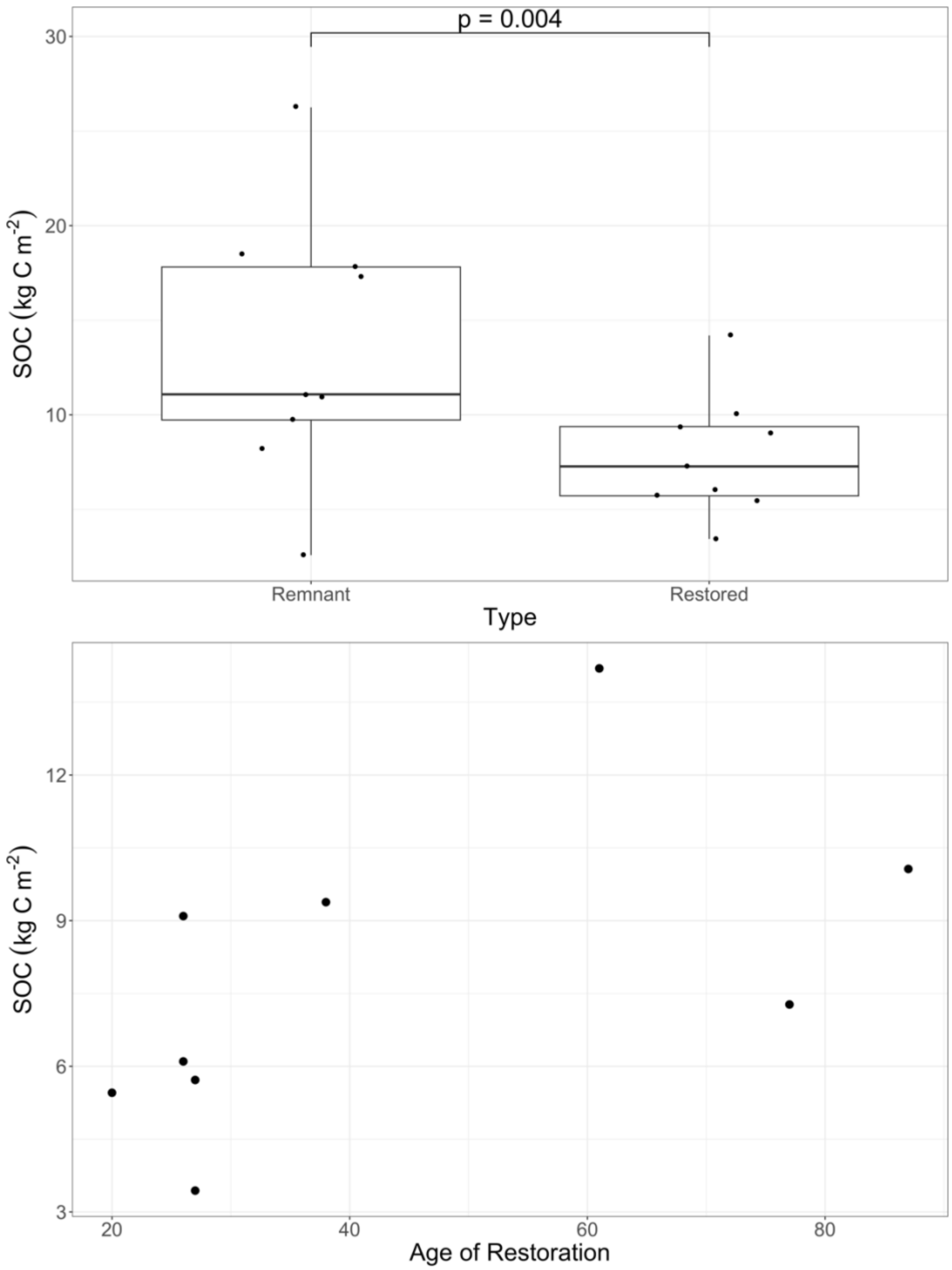


Figure 5. SOC stocks (0 to 50 cm) by A) type and B) age.

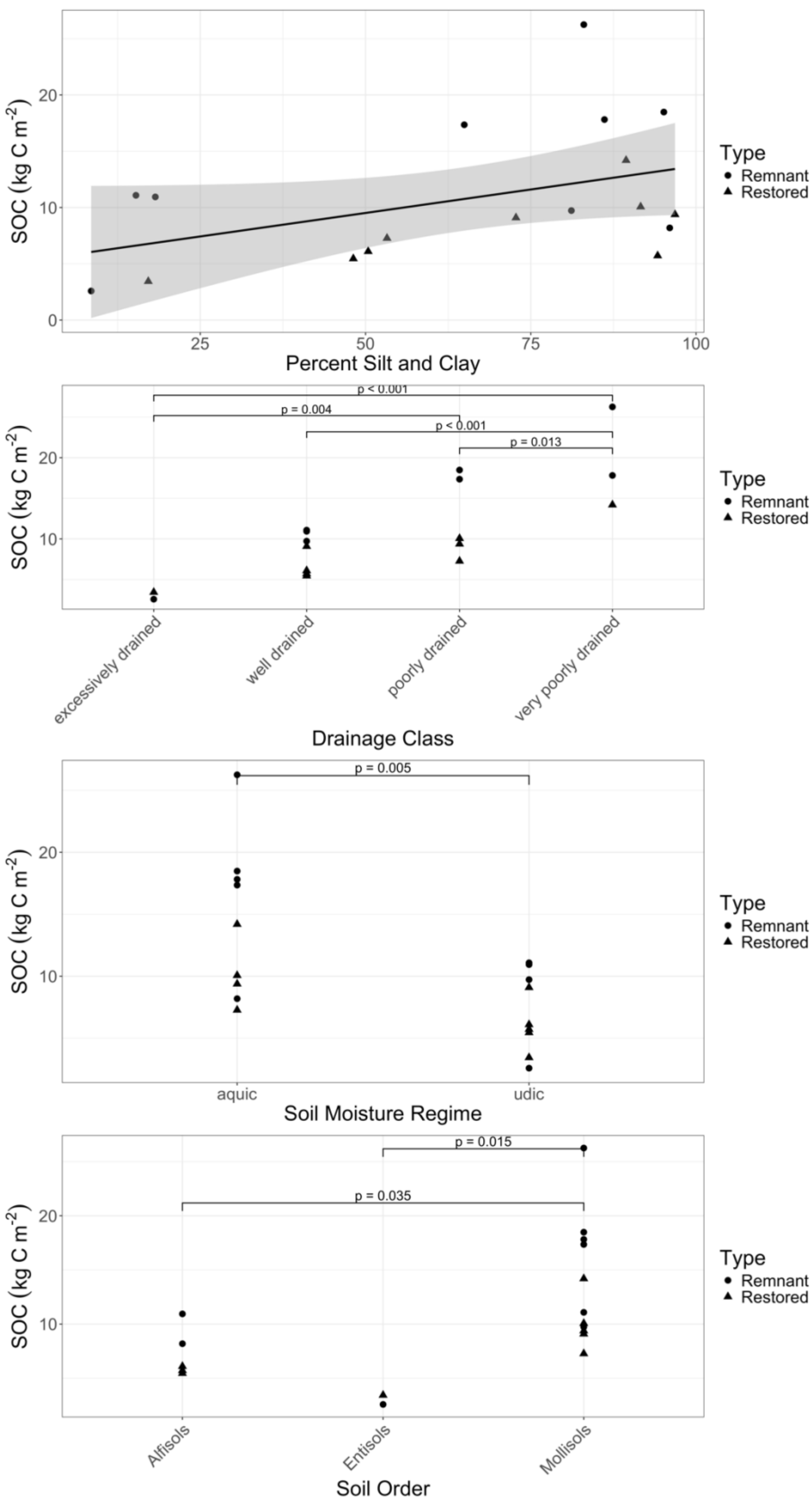


Figure 6. SOC stocks (0 to 50 cm) and edaphic properties.

The relationships between MAOC concentration and SOC concentration were strongly linear and positive providing no evidence that MAOC levels were saturating (Figure 7), with no apparent differences in these relationships in remnant and restored prairies.

Table 3. MAOC concentration by SOC concentration and type analyzed with linear models ($p < 0.05$ bolded).

Depth	Term	Parameter estimate	SE	F statistic	P value
0-10	(Intercept)	-0.029	0.277	-0.1	0.92
	gSOC_100gBulk	0.672	0.042	15.91	**< 0.001**
	TypeRestored	0.486	0.377	1.29	0.22
	gSOC_100gBulk:TypeRestored	-0.088	0.074	-1.19	0.25
10-25	(Intercept)	-0.383	0.312	-1.23	0.24
	gSOC_100gBulk	0.851	0.075	11.42	**< 0.001**
	TypeRestored	0.409	0.411	1.0	0.33
	gSOC_100gBulk:TypeRestored	0.028	0.127	0.22	0.83
25-50	(Intercept)	0.221	0.367	0.6	0.56
	gSOC_100gBulk	0.629	0.152	4.15	**0.001**
	TypeRestored	-0.222	0.463	-0.48	0.64
	gSOC_100gBulk:TypeRestored	0.294	0.283	1.04	0.32

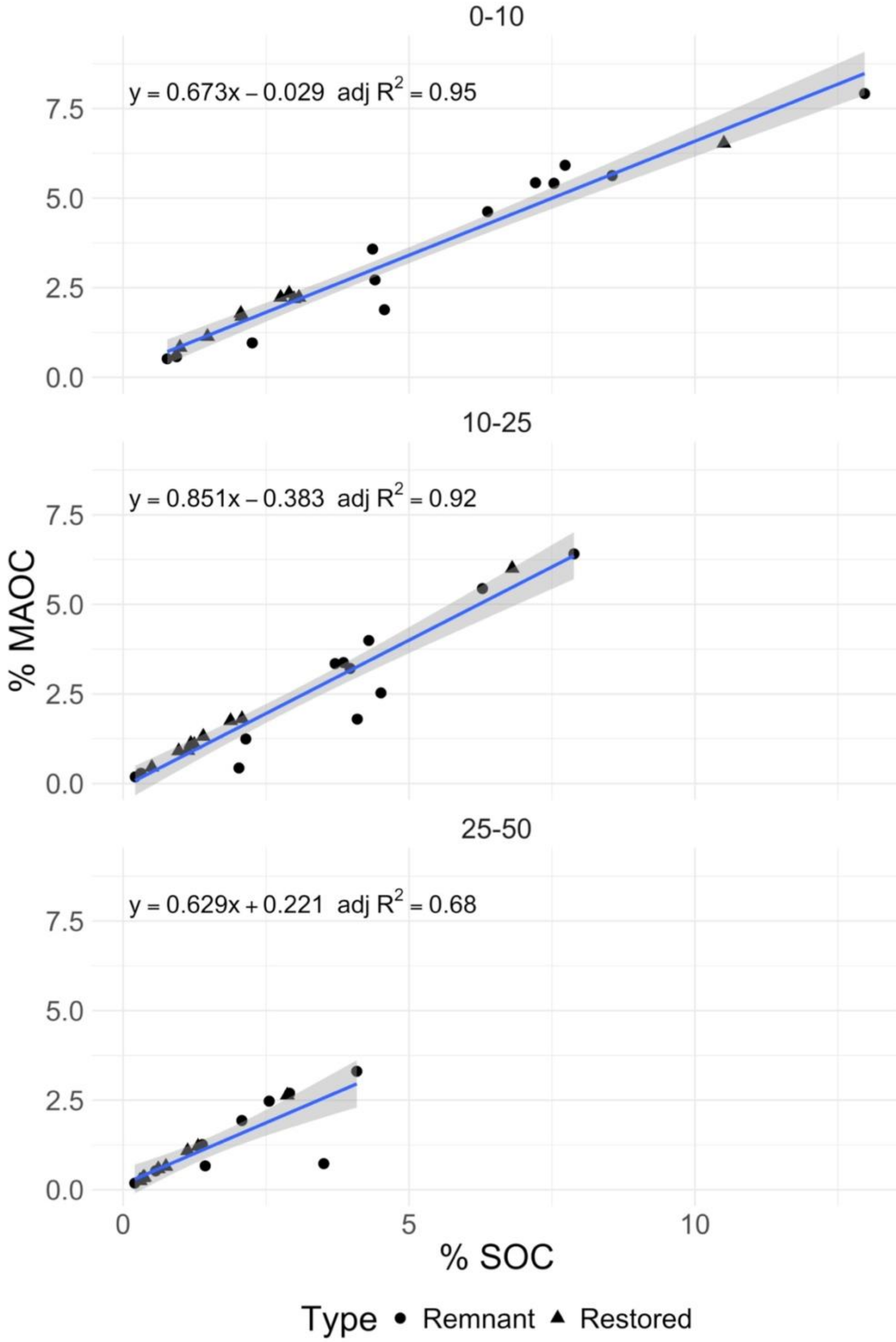


Figure 7. Scatterplots of MAOC and SOC concentrations per bulk soil in remnant and restored prairies by depth increment.

Discussion

No consistent change in prairie SOC

Remnant and restored prairies across southern Wisconsin did not consistently increase or decrease in soil carbon, consistent with past studies in the region showing no significant soil carbon gains in prairies over decadal periods (Brye & Kucharik, 2003; Kucharik, 2007), but contrasting with others that have reported soil carbon accumulation in surface soils (Libbey & Hernández, 2021; O'Brien et al., 2010). Interestingly, Dietz et al. (2024) showed gains in surface soils for restored prairies on Plano silt loam Mollisols, but overall losses because of SOC loss at depth 20 years after establishment.

In restorations, the rate of SOC change was not associated with the age of restoration, counter to our expectations. Other studies have suggested older prairies have a slower rate of accumulation than young prairies (von Haden & Dornbush, 2017). The age of restored prairies in our dataset were all over 20 years old (range 20 – 87), which is older than most studied restorations (Kucharik, 2007; Libbey & Hernández, 2021). This could suggest SOC accumulation rates in restorations older than 20 years have declined to a point of no detectable change and/or require much longer timescales to capture. This may imply past cultivation has limited restoration capacity to reach pre-tilled SOC levels, which others have referred to as the 'legacy of cultivation' (Rosenzweig et al., 2016). If this is the case, restorations in our study are still well below remnant stocks. Modeled predictions suggest restorations require 350 to 450 years to reach remnant soil carbon stocks (Matamala et al., 2008; Rosenzweig et al., 2016). These modeled predictions suggest different rates of recovery for above- and belowground components (i.e., aboveground litter recovering within the first two decades, belowground root biomass requiring 5 decades, and soil carbon stocks requiring 3 to 4 centuries) (Matamala et al., 2008). Perhaps even with our sampling of 'older' restorations, we are still capturing a small period in the restoration process, which may not occur in a linear fashion. In the context of the modelled predictions, the restorations sampled in our study may be in the stage of root biomass recovery, which may facilitate longer-term SOC gains on the order of centuries.

Rates of SOC change varied by site with some accruing, some maintaining and other losing, indicating site effects may shape positive and negative rates of change in a manner that we did not capture. It is broadly understood that site effects such as soil type, moisture, and drainage class influence soil carbon stocks (as seen our study), but rates of change are more multifarious. This is likely due to three inter-related challenges. First, edaphic properties are broadly connected to one another (i.e., sandy soils are more well-drained) and are site-specific. This requires large sample sizes to distinguish and untangle trends across edaphic properties and is still limited by collinearity in statistical regressions. Second, management and land use history also vary across sites and are challenging to quantify and tease apart from edaphic properties (hence requiring even greater sampling sizes). Common approaches include quantifying years since last burn, burn frequency, or categorization of management/intervention intensity (passive vs. active management) (McFarlane et al., 2023). Quantifying land management is more feasible in research trials dedicated to restoration (of which there are relatively few), often designed as chrono-sequences, whereas many restorations occur on private lands or by private organizations which vary in their restoration goals from plant composition to carbon sequestration. Gathering and quantifying establishment techniques and long-term management from private individuals or organizations is challenging and more akin to 'on farm' research. Efforts to make restoration a more predictive science (Brudvig, 2017; McFarlane et al., 2023) move the needle in the right direction, but are still limited by site variation, record keeping, sampling variation, and broad classifications. Passive management signifies absent management, while 'active' management encompasses a wide range of management strategies – from establishment techniques, seeding rates, seed diversity, burn frequency, invasive species management/frequency/techniques. Lastly, the ability to accurately measure changes in soil carbon over time is dependent on sampling methodology and complicated by changes in soil physical properties over time. Comparing soil carbon stocks over time through repeated measures is the gold standard, but requires similar sampling protocols for field collection and soil processing. These are often difficult to keep consistent across studies and sites over time (20+ years). Further, bulk density measurements are essential to calculating soil carbon stocks, yet the process of prairie restoration changes soil physical properties (greater root biomass and increased pore space) and thus, consistently reduces bulk density (Libbey & Hernández, 2021). This expansion of soil results in capturing more surface soil as restoration ages when using a fixed-depth sampling approach. This change in bulk density requires soil carbon stock comparisons be made across the same mass of soil (von Haden et al., 2020). Typically, the baseline bulk density would be used as the reference soil mass, yet when bulk density declines over time, this applies an extrapolation (von Haden et al., 2020). Calculating SOC stocks using the lower, more recent bulk density, ensures any reported change in soil carbon is not due to changes in volume of soil sampled, but doesn't reflect how physical changes to soil contribute to SOC stocks over time. We opted to compare soil carbon concentrations, given differences in bulk density measurements between current and baseline sampling, yet this limits our conclusions to changes in SOC concentration rather than absolute stocks (Don et al., 2023). In comparing SOC concentration, this approach would be expected to skew to more likely reporting an increase in soil carbon change, given that soil carbon concentration typically declines with depth, yet we don't see a consistent pattern of SOC increase. Thus, despite the limitations of this approach, it bolsters our confidence in the observation that the prairies did not consistently accumulate organic matter.

Remnant SOC stocks greater than restored

Remnants had greater SOC stocks than restored prairies to 50 cm. This may be due to the long period of time for soil development (~11,000 years since Laurentide ice sheet) and/or due to the lack of disturbance releasing SOC to the atmosphere via heterotrophic respiration. The large-scale land use change over the last two centuries released ~25-50% (Sanderman et al., 2017) of SOC and nearly eliminated prairie ecosystems with estimates of ~ 1% of untilled prairie remaining. Our results suggest though restoring prairies has numerous environmental benefits (habitat, biodiversity, erosion prevention, water quality protection), SOC stocks are significantly less than remnants. The decline in bulk density compared to ~20 years ago in both remnants and restored prairies suggests robust root and organic matter formation, but may be offset by respiration resulting in no general increase in SOC concentration over time. Protecting prairie soils from further conversion is necessary (Lark, 2020), given restorations may take centuries to recover, which is yet to be seen. SOC accumulation cannot be assumed across land use histories, edaphic properties, and management.

SOC stocks related to edaphic properties

We found Mollisols generally had greater SOC stocks and positive rates of SOC concentration change compared to Alfisol remnants. This difference by soil type may reflect cumulative edaphic and site variables or be a feature of coarse classification with relatively small sample size. Prairies are thought to be atop Mollisol soils, with the mollic epipedon formed in response to long-term organic matter additions via roots, whereas Alfisols are formed under broad leaved forests (Bockheim & Hartemink, 2017). Prairies in Wisconsin exist along a “tension zone”, in which climate, fire and grazing have been responsible for keeping woody species at bay (Cochrane & Iltis, 2000). It is well-documented for forests to succeed prairies in this region without intervention (Brock, 2014; Curtis, 1959), and could explain how prairies may have Alfisol soil classifications, and with land-use history, that may contribute to lower SOC stocks. Remnants in our study are either dry, short-grass prairies on south-facing slopes, or wet-mesic prairies in flood plains, which are both ecosystems that can support tree encroachment. Dry, south-facing prairies on rocky outcrops may not fall within the Mollisol classification of >18 cm organic epipedon due to shallow depth to bedrock and limits on net primary productivity. Yet, not all our dry-mesic prairies were classified as Alfisols (Table 1). It is more likely that the classification used according to NRCS’s Soil Web Survey is too coarse to accurately represent our sampling points. Others have reported lower carbon accumulation rates in Alfisol restorations than Mollisols (Kucharik, 2007), though differences were only in the surface 5 cm.

Aquic, poorly drained soils with higher proportions of silt and clay had higher soil carbon stocks in remnant and restored prairies. This aligns with our expectations given wet, poorly drained soils harbor anoxic conditions, slowing decomposition. Aquic soils (soils experience periods of saturation and anoxic conditions) have previously been documented with lower SOC than udic soils in southern WI (Kucharik & Brye, 2013). Prairie restorations on seasonally saturated soils have been documented to sequester twice as much SOC than mesic prairies (O’Brien et al., 2010). Wet, poorly drained prairies in our study did have higher SOC stocks, yet drainage or soil moisture were not significant predictors of SOC change. High proportions of silt and clay also had greater SOC stocks. Clay particles bind organic matter via electrostatic binding and contribute to soil aggregation protecting organic matter from decomposition (McLauchlan 2006). Clay has been associated with SOC stocks elsewhere (O'Brien et al., 2015), yet not necessarily to SOC rates of accumulation (McLauchlan, 2006). Soil mineralogy underlies the concept of mineral-associated organic matter, with clay-loams expected to have a greater capacity for SOC storage.

No evidence for MAOC saturation

We found a linear relationship between MAOC and total SOC, suggesting the mineral fractions of the soil carbon pools have not reached a maximum observed capacity across remanent and restored prairies and depth in our study. This aligns with other studies across grasslands (Begill et al., 2023), suggesting potential for continued carbon accumulation. Globally, agricultural lands and deep soils are estimated to have MAOC below saturation (Georgiou et al., 2022). We similarly show that soils at 50 cm maintain a linear MAOC/SOC relationship with the potential for MAOC increases as SOC increases. Yet, we did not observe SOC concentration increases over time, suggesting that even though we do not observe a maximum MAOC capacity, there may be biological or management variables resulting in an 'effective maximum' for both SOC and MAOC. A theoretical maximum for soil carbon saturation has been a topic of debate given rising atmospheric CO_2 and propositions for natural climate solutions, including for soil carbon sequestration. However, soil texture (clay+silt), climatic and management variables, and land use history can contribute to observable and effective maxima for particular regions and sites (Georgiou et al., 2025).

Conclusion

We found no consistent change in SOC concentration over two decades in southern Wisconsin prairies. Remnant prairies harbored greater SOC stocks than restorations, highlighting the detrimental effects of land conversion and long-time scales of restoration. Differences in stocks were most pronounced at depth indicating restorations may be recovering at the surface faster than at depth. Wet prairies with poor drainage and finer soil texture had greatest SOC stocks, and thus should be targets for restoration. We found no evidence for MAOC saturating as SOC increases suggesting persistent forms of SOC can continue to accumulate even among untilled prairies. Grasslands have provided ecosystem services for thousands of years, and extant remnants should be protected - given low capacity to predictively drawdown atmospheric carbon.

Acknowledgments

Gratitude to the UW-Arboretum's research fellowship that supported this work. I thank Dr. Chris Kucharik and his team for cataloging and sharing their soil carbon data and prairie locations to be used in this study. Thank you to my peers in both the Whitman and Jackson labs for lab and field work assistance. Thank you to Louis Thares for assisting with POC/MAOC fractionations and soil texture analysis. To Kelsey Kruger, Erin Pierce, and Harry Read for field and lab work assistance. We thank and acknowledge all of the organizations that allowed soil collection for this work:

Brad Herrick, UW Madison Arboretum/ Lake Shore Nature Preserve

Andy Gossens – International Crane Foundation

David Musolf and Rodger Packard – Faville Grove resident managers

Nate Fayram, DNR

TNC WI chapter

Groundswell conservancy

Goose Pond Sanctuary – Emma Raasch and Graham Steinhauer

Southern Wisconsin Bird Alliance,

Seth McGee – Lake Shore Nature Preserve

Dane County Parks

Gregg Sanford & Mike Bertram – UW Arlington Research Station

Troy Farm/Madison Area Community Land Trust

Funding support from UW-Madison Arboretum Research Fellowship and University Fellowship. Partial support from Sustainable Agriculture Systems Coordinated Agricultural Program grant no. 2019-68012-29852 from the USDA National Institute of Food and

Agriculture. We thank Nicholas Keuler from the Statistical Consulting group at the University of Wisconsin – Madison for assistance in the analysis of results

Supplemental Information

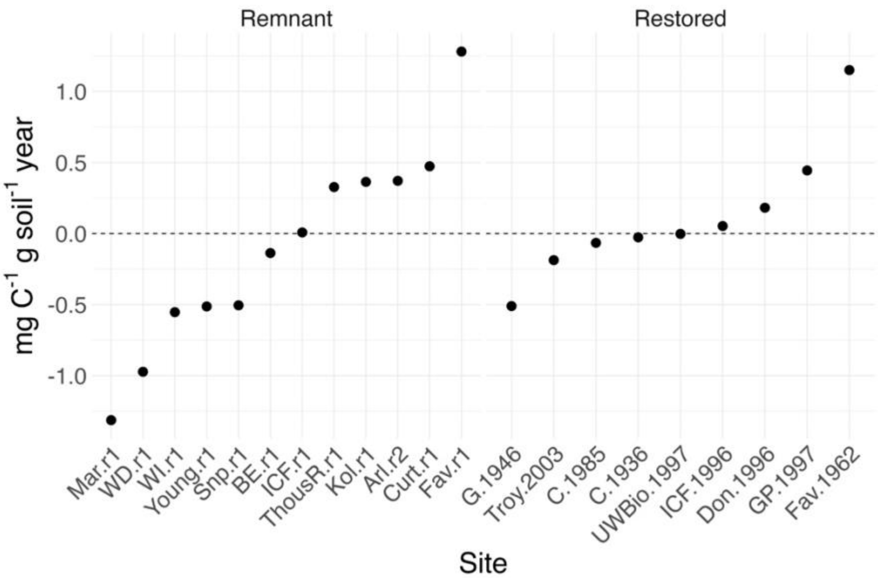


Figure S1. Rate of SOC change (0 to 25 cm) by site.

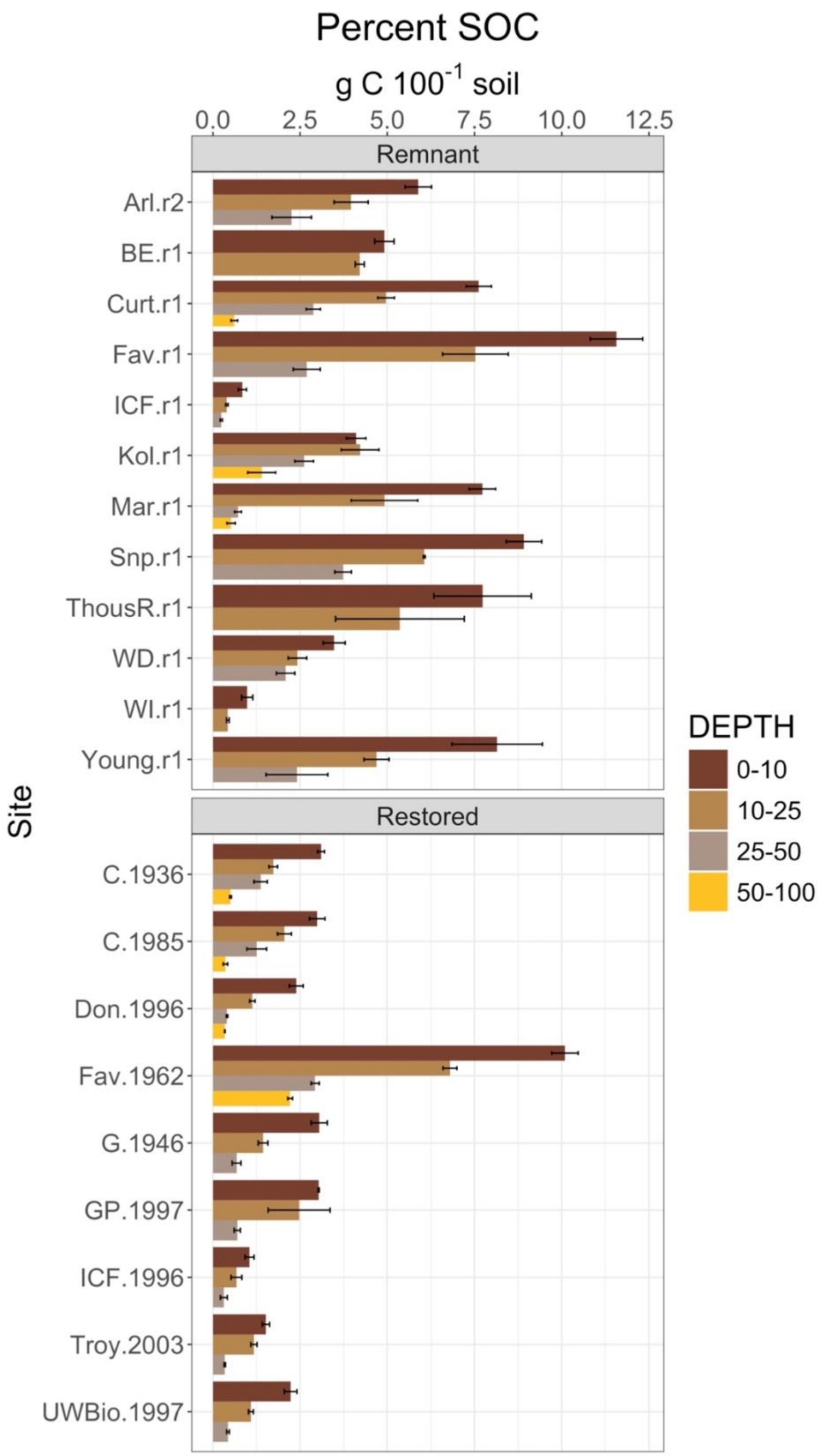


Figure S2. SOC concentration by site and depth

References

Almaraz, M., Simmonds, M., Boudinot, F. G., Di Vittorio, A. V., Bingham, N., Khalsa, S. D. S., Ostoja, S., Scow, K., Jones, A., Holzer, I., Manaigo, E., Geoghegan, E., Goertzen, H., & Silver, W. L. (2023). Soil carbon sequestration in global working lands as a gateway for negative emission technologies. *Global Change Biology*, *29*(21), 5988–5998. https://doi.org/10.1111/gcb.16884

Auguie, B. (2017). *gridExtra: Miscellaneous Functions for "Grid" Graphics*. https://CRAN.R-project.org/package=gridExtra

Bach, E. M., & Hofmockel, K. S. (2015). Coupled Carbon and Nitrogen Inputs Increase Microbial Biomass and Activity in Prairie Bioenergy Systems. *Ecosystems*, *18*(3), 417–427.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), Article 1. https://doi.org/10.18637/jss.v067.i01

Begill, N., Don, A., & Poeplau, C. (2023). No detectable upper limit of mineral-associated organic carbon in temperate agricultural soils. *Global Change Biology*, *n/a*(n/a). https://doi.org/10.1111/gcb.16804

Bockheim, J. G., & Hartemink, A. E. (2017). *The Soils of Wisconsin*. Springer International Publishing. https://doi.org/10.1007/978-3-319-52144-2

Brock, T. (2014). Legacy Effects in Prairie Restoration: A 73-Year Spatial History. *The Prairie Naturalist*. https://digitalcommons.unl.edu/tpn/14

Brudvig, L. A. (2017). Toward prediction in the restoration of biodiversity. *Journal of Applied Ecology*, *54*(4), 1013–1017. https://doi.org/10.1111/1365-2664.12940

Brye, K. R., & Kucharik, C. J. (2003). Carbon and Nitrogen Sequestration in Two Prairie Topochronosequences on Contrasting Soils in Southern Wisconsin. *The American Midland Naturalist*, *149*(1), 90–103.

Cahill, K. N., Kucharik, C. J., & Foley, J. A. (2009). Prairie restoration and carbon sequestration: Difficulties quantifying C sources and sinks using a biometric approach. *Ecological Applications*, *19*(8), 2185–2201. https://doi.org/10.1890/08-0069.1

Cochrane, T. S., & Iltis, H. H. (2000). *Atlas of the Wisconsin prairie and savanna flora*.

Connell, R. K., Nippert, J. B., & Blair, J. M. (2020). Three Decades of Divergent Land Use and Plant Community Change Alters Soil C and N Content in Tallgrass Prairie. *Journal of Geophysical Research: Biogeosciences*, *125*(8), e2020JG005723. https://doi.org/10.1029/2020JG005723

Córdova, S. C., Kravchenko, A. N., Miesel, J. R., & Robertson, G. P. (2025). Soil carbon change in intensive agriculture after 25 years of conservation management. *Geoderma*, *453*, 117133. https://doi.org/10.1016/j.geoderma.2024.117133

Cotrufo, M. F., Ranalli, M. G., Haddix, M. L., Six, J., & Lugato, E. (2019). Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience*, *12*(12), 989–994. https://doi.org/10.1038/s41561-019-0484-6

Curtis, J. T. (1959). *The Vegetation of Wisconsin: An Ordination of Plant Communities*. University of Wisconsin Press. https://muse.jhu.edu/pub/19/monograph/book/16741

Dietz, C. L., Jackson, R. D., Ruark, M. D., & Sanford, G. R. (2024). Soil carbon maintained by perennial grasslands over 30 years but lost in field crop systems in a temperate Mollisol. *Communications Earth & Environment*, *5*(1), 1–8. https://doi.org/10.1038/s43247-024-01500-w

Don, A., Seidel, F., Leifeld, J., Kätterer, T., Martin, M., Pellerin, S., Emde, D., Seitz, D., & Chenu, C. (2023). Carbon sequestration in soils and climate change mitigation—Definitions and pitfalls. *Global Change Biology*, *n/a*(n/a), e16983. https://doi.org/10.1111/gcb.16983

Georgiou, K., Angers, D., Champiny, R. E., Cotrufo, M. F., Craig, M. E., Doetterl, S., Grandy, A. S., Lavallee, J. M., Lin, Y., Lugato, E., Poeplau, C., Rocci, K. S., Schweizer, S. A., Six, J., & Wieder, W. R. (2025). Soil Carbon Saturation: What Do We Really Know? *Global Change Biology*, *31*(5), e70197. https://doi.org/10.1111/gcb.70197

Georgiou, K., Jackson, R. B., Vindušková, O., Abramoff, R. Z., Ahlström, A., Feng, W., Harden, J. W., Pellegrini, A. F. A., Polley, H. W., Soong, J. L., Riley, W. J., & Torn, M. S. (2022). Global stocks and capacity of mineral-associated soil organic carbon. *Nature Communications*, *13*(1), 3797. https://doi.org/10.1038/s41467-022-31540-9

Hernández, D., Esch, E., Alster, C., McKone, M., & Camill, P. (2013). Rapid Accumulation of Soil Carbon and Nitrogen in a Prairie Restoration Chronosequence. *Soil Science Society of America Journal*, *77*, 2029. https://doi.org/10.2136/sssaj2012.0403

Jackson, R. D., & Stier, J. (2023). Soil C Change Over 14 Years in Grasslands Sown into a Highly Disturbed Soil. *XXV International Grassland Congress (IGC 2023)*, 170–173. https://doi.org/10.52202/071171-0038

Kettler, T. A., Doran, J. W., & Gilbert, T. L. (2001). Simplified Method for Soil Particle-Size Determination to Accompany Soil-Quality Analyses. *SOIL SCI. SOC. AM. J.*, *65*.

Kucharik, C. J. (2007). Impact of Prairie Age and Soil Order on Carbon and Nitrogen Sequestration. *Soil Science Society of America Journal*, *71*(2), 430–441. https://doi.org/10.2136/sssaj2006.0074

Kucharik, C. J., & Brye, K. R. (2013). Soil Moisture Regime and Land Use History Drive Regional Differences in Soil Carbon and Nitrogen Storage Across Southern Wisconsin. *Soil Science*, *178*(9), 486–495. https://doi.org/10.1097/SS.0000000000000015

Kucharik, C. J., Fayram, N. J., & Cahill, K. N. (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. https://doi.org/10.1111/j.1365-2486.2005.01053.x

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Lark, T. J. (2020). Protecting our prairies: Research and policy actions for conserving America's grasslands. *Land Use Policy*, *97*, 104727. https://doi.org/10.1016/j.landusepol.2020.104727

Libbey, K., & Hernández, D. L. (2021). Depth Profile of Soil Carbon and Nitrogen Accumulation over Two Decades in a Prairie Restoration Experiment. *Ecosystems*, *24*(6), 1348–1360. https://doi.org/10.1007/s10021-020-00588-3

Matamala, R., Jastrow, J. D., Miller, R. M., & Garten, C. T. (2008). Temporal Changes in C and N Stocks of Restored Prairie: Implications for C Sequestration Strategies. *Ecological Applications*, *18*(6), 1470–1488. https://doi.org/10.1890/07-1609.1

McFarlane, S. L., Kochanski, J. M., Gratton, C., & Damschen, E. I. (2023). Intervention intensity predicts the quality and duration of prairie restoration outcomes. *Restoration Ecology*, *31*(8), e13993. https://doi.org/10.1111/rec.13993

McLauchlan, K. K. (2006). Effects of soil texture on soil carbon and nitrogen dynamics after cessation of agriculture. *Geoderma*, *136*(1), 289–299. https://doi.org/10.1016/j.geoderma.2006.03.053

Nelson, D. W., & Sommers, L. E. (1996). Total Carbon, Organic Carbon, and Organic Matter. In *Methods of Soil Analysis* (pp. 961–1010). John Wiley & Sons, Ltd. https://doi.org/10.2136/sssabookser5.3.c34

Oates, L. G., & Jackson, R. D. (2014). Livestock Management Strategy Affects Net Ecosystem Carbon Balance of Subhumid Pasture. *Rangeland Ecology & Management*, *67*(1), 19–29. https://doi.org/10.2111/REM-D-12-00151.1

O'Brien, S. L., Jastrow, J. D., Grimley, D. A., & Gonzalez-Meler, M. A. (2010). Moisture and vegetation controls on decadal-scale accrual of soil organic carbon and total nitrogen in restored grasslands. *Global Change Biology*, *16*(9), 2573–2588. https://doi.org/10.1111/j.1365-2486.2009.02114.x

O'Brien, S. L., Jastrow, J. D., Grimley, D. A., & Gonzalez-Meler, M. A. (2015). Edaphic controls on soil organic carbon stocks in restored grasslands. *Geoderma*, *251–252*, 117–123. https://doi.org/10.1016/j.geoderma.2015.03.023

Prairie, A. M., King, A. E., & Cotrufo, M. F. (2023). Restoring particulate and mineral-associated organic carbon through regenerative agriculture. *Proceedings of the National Academy of Sciences*, *120*(21), e2217481120. https://doi.org/10.1073/pnas.2217481120

R Core Team. (2020). *R: A language and environment for statistical computing* (Version 4.0.0) [Computer software]. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Roos, C. I., Zedeño, M. N., Hollenback, K. L., & Erlick, M. M. H. (2018). Indigenous impacts on North American Great Plains fire regimes of the past millennium. *Proceedings of the National Academy of Sciences*, *115*(32), 8143–8148. https://doi.org/10.1073/pnas.1805259115

Rosenzweig, S. T., Carson, M. A., Baer, S. G., & Blair, J. M. (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. https://doi.org/10.1016/j.apsoil.2016.01.001

Sanderman, J., Hengl, T., & Fiske, G. J. (2017). Soil carbon debt of 12,000 years of human land use. *Proceedings of the National Academy of Sciences*, *114*(36), 9575–9580. https://doi.org/10.1073/pnas.1706103114

Schauberger, P., & Walker, A. (2020). *openxlsx: Read, Write and Edit xlsx Files*. https://CRAN.R-project.org/package=openxlsx

Slette, I. J., Liebert, A., & Knapp, A. K. (2021). Fire history as a key determinant of grassland soil CO2 flux. *Plant and Soil*, *460*(1), 579–592. https://doi.org/10.1007/s11104-020-04781-0

Soil Survey Staff. (2025). *Web Soil Survey*. Web Soil Survey. https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx

von Haden, A. C., & Dornbush, M. E. (2017). Ecosystem carbon pools, fluxes, and balances within mature tallgrass prairie restorations. *Restoration Ecology*, *25*(4), 549–558. https://doi.org/10.1111/rec.12461

von Haden, A. C., Yang, W. H., & DeLucia, E. H. (2020). Soils' dirty little secret: Depth-based comparisons can be inadequate for quantifying changes in soil organic carbon and other mineral soil properties. *Global Change Biology*, *26*(7), 3759–3770. https://doi.org/10.1111/gcb.15124

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wickham, H. (2019). *stringr: Simple, Consistent Wrappers for Common String Operations*. https://CRAN.R-project.org/package=stringr

Wickham, H., François, R., Henry, L., & Müller, K. (2022). *dplyr: A Grammar of Data Manipulation*. https://CRAN.R-project.org/package=dplyr

Chapter 3. Conservation Staff Key to Federal Conservation Investments in Perennial, Annual Crop, and Confinement Livestock Operations

Mia Keady, Yu Lu, Michael Happ, Margaret Krome, Becky Schewe, Randall D. Jackson, Adena R. Rissman

Keywords: incentives, cost-share, conservation, expenditures, staff capacity

Abstract

Conservation incentives are meant to improve the environmental and social outcomes of agriculture, but factors shaping how these incentives are distributed across agroecosystems is unclear. Drawing on U.S. Farm Bill - Environmental Quality Incentives Program (EQIP) data in Wisconsin, USA, we categorized conservation practices based on expert elicitation and the co-occurrence of practices on contracts. We then regressed local and NRCS staff abundance with county level expenditures. Averaged across fiscal years 2014 through 2024, expenditures were \$9.28M yr^{-1} on annual cropland practices such as cover crops and nutrient management, \$8.69M yr^{-1} for confinement livestock infrastructure, and \$5.85M yr^{-1} to support perennial grazing, forestry, habitat, and agroforestry, and \$3.6M yr^{-1} for multi-system practices across these categories. Most conservation funding flowed to annual cropping systems and confinement livestock operations to support conservation interventions that reduce, but do not eliminate, resource degradation. About ~20% of conservation funding supported perennial systems like managed-grazing, forestry, habitat and agroforestry which have well-demonstrated positive ecological outcomes. We found that more local and federal conservation staff correlated to more

federal conservation expenditures overall. All categories of conservation practices (perennial, annual, confinement) were connected to either or both federal or local conservationists. More investment in conservation staff who support agroecological transformation, rather than interventions into systems with steep environmental and societal costs, is critical to address the urgent need for agroecosystems that provide for people today and in future generations.

Introduction

Growing and distributing food, fiber, and fuel in a manner that cares for both people and the land is one of the grand challenges of our time (Anderson et al., 2021; Ewert et al., 2023; FAO, 2018). Conservation cost-share programs coupled with technical assistance are one pathway to incrementally and voluntarily address soil erosion, biodiversity and habitat loss, and water quality concerns. Yet conservation adoption rates are low (Guo et al., 2023) and conservation practices have a range of efficacies dependent on environmental and management factors (Cates et al., 2018; Ladoni et al., 2016; Liebig et al., 2022). Heterogeneity across the landscape precludes one-size-fits-all solutions to the diverse, operation specific resource concerns (Blanco-Canqui, 2022; Plastina et al., 2020). When conservation practices are implemented, a very small proportion of conservation dollars support top-tier practices or practices that contribute to ecosystem services, rather than simply slowing degradation (Basche et al., 2020; Cates et al., 2018). Meanwhile, physical structures and facilities receive the largest share (~51%) of cost-share funding across the U.S. (Basche et al., 2020). Understanding drivers of conservation investment in transformative agroecosystems that contribute to ecosystem stability are needed if we are to meet demands for food, fiber, and fuel while maintaining environmental integrity for future generations.

Sustainable agricultural and natural resources management enhances biodiversity, reduces greenhouse gas emissions, improves water quality, maintains soil carbon, and re-invigorates rural spaces (Horrigan et al., 2002; Jackson, 2024; Lal, 2008; Reynolds et al., 2021). Perennial agroecosystems, or systems that support multi-year herbaceous or tree/shrub crops, meet many of the desired outcomes associated with sustainable agriculture (Jackson, 2022, 2024; Reynolds et al., 2021; Rui et al., 2022). Managed grazing, forestry, and agroforestry are perennial agriculture or timber production systems that reduce soil erosion, improve water quality, provide wildlife and pollinator habitat, and sequester or maintain soil carbon, but only a fraction of farmland is devoted to perennial practices in the United States. Pathways for perennial agriculture are hindered by incremental changes to US agricultural policy, limited market development, and gaps in conservation cost-share support (A. R. Rissman, Fochesatto, et al., 2023; Scott et al., 2022). Well-managed perennial grassland and forestry are critical conservation tools that produce food, fiber, and bioenergy and improve soil health and water quality (Franzluebbers et al., 2012; Spratt et al., 2021; Winsten, 2024), but they lack the subsidy and insurance support provided to row crop agriculture (A. R. Rissman, Fochesatto, et al., 2023).

Understanding incentives propelling transitions to sustainable agriculture, forestry, and habitat can inform and direct approaches that optimize broad-scale adoption of best management practices on farms and forests, ensuring desired sustainability outcomes. While studies of individual manager behavior and social psychological drivers of adoption are common (Prokopy et al., 2019), analyses of policy, institutions, administrations, and narratives have seen a resurgence of interest for understanding conservation adoption and transformations to land management (A. R. Rissman, Kazer, et al., 2023; Strauser & Stewart, 2024). Cost-share incentives in the United States are distributed federally through the U.S. Farm Bill to local landowners via an application process within the United States Department of Agriculture (USDA) Natural Resource Conservation Service (NRCS). Cost-share dollars disproportionally support high dollar industrial agricultural systems rather than perennial transformation, missing opportunities to support systems addressing environmental and climate concerns (Happ, 2024). Adoption of conservation practices supported by cost-share incentives has been studied as a generalized concept, or with emphasis on one or few practices (Adhikari et al., 2023; Kalcic et al., 2015; Surdoval et al., 2024). Understanding drivers of cost-share incentives for perennial systems has implications for reaching sustainability goals, reducing soil erosion and improving water quality, yet are currently indistinguishable from drivers of cover crop adoption.

Access to and relationships with conservation staff may advance sustainability and land stewardship goals through the coupling of cost-share incentives with technical assistance (Moynihan & Landuyt, 2009). In the United States, technical assistance to facilitate flow of financial assistance to farms comes from the USDA NRCS, university extension, county conservationists, private consultants, and non-governmental organizations (NGOs). Together, these advisors play a significant role in how agroecosystems are managed, the ecosystem services provided by these enterprises and their land base, and our capacity to mitigate and adapt to environmental degradation and climate change.

Federalism, or shared influence between local and federal governments, has been an intentional design feature of conservation agriculture to promote local farmer voices and priorities in land use policy (Gilbert, 2015). Local conservation districts or county departments have important roles that shape conservation planning and implementation (Wardropper & Rissman, 2019). Likewise, federal NRCS staff contribute to conservation planning, with greater farmer to professional interaction increasing conservation practice adoption (Morris & Arbuckle, 2021).

Drawing on county and contract-level EQIP data for 11 years in Wisconsin, we asked:

1) How were federal conservation dollars distributed across perennial, annual, and confinement livestock systems?
2) Were federal EQIP expenditures associated with county conservation and federal NRCS conservation staff abundance?

Methods

Conservation program and agencies

Voluntary conservation cost-share programs in the US are supported by the Farm Bill proposed by Congressional agricultural committees and passed by Congress approximately every five years, historically. Funding is allocated to the USDA and distributed via NRCS, an agency first established in 1935 as the Soil Conservation Service in response to Dust Bowl-era soil loss from a combination of long-term weather patterns and extractive agriculture. NRCS (renamed in 1994) strives to "deliver conservation solutions so agricultural producers can protect natural resources and feed a growing world" (NRCS, 2025). The Environmental Quality Incentive Program (EQIP) is NRCS's predominant cost-share program to address natural resource concerns via financial and technical assistance for landowners and producers. Landowners apply for EQIP contracts by working directly with NRCS approved conservation technical assistants (CTAs). CTAs include NRCS staff, county conservation staff (termed conservation district staff in other states), and non-profit or private firm consultants. County conservation districts were created locally by states during the same time-period as the establishment of the Soil

Conservation Service (now NRCS) to promote a local approach to addressing resource concerns (Walker & Parks, 1946). Conservation districts in Wisconsin are organized as members of local Land and Water Conservation Departments (LWCD). CTAs work one-on-one with landowners to develop a conservation plan that includes a site visit and personalized suggestions to address conservation concerns and goals. EQIP applications are competitive and ranked based on state priority resource concerns (*Center for Rural Affairs*, 2022). Payment rates for cost-share practices vary by state and by practice and are reviewed and adjusted each year to reflect current costs.

Study area

Wisconsin is dominated by forests in the north and row crops and pasture in the south. Central and southern WI was primarily converted to agriculture from deciduous forest, oak savanna and prairie between 1850 and 1939 (Curtis, 1959; Curtis & McIntosh, 1951; Huang et al., 2019). Wisconsin has diverse topography that lends itself to multiple land uses. Agricultural systems include large dairies concentrated to the east, potato production in the Central Sands and annual corn and soybean row cropping across the southern and central regions. The southwest Driftless Region is characterized by steep slopes and was unglaciated in the last glacial maximum. The state's mean annual temp is 43.8F (6.56C) and 864.87 mm MAP between 1991-2020 (Wisconsin State Climatology Office, 2025).

Data acquisition

We collaborated with state NRCS and Wisconsin Land and Water (the statewide association of county conservation departments, known as conservation districts in other states) to access county level conservation spending and county conservation staff. We accessed publicly available datasets to quantify land cover and farm demographics. Data cleaning and formatting was conducted in R version 4.0.0 (R Core Team, 2020). All spatial datasets were projected to NAD 1983 HARN WI ™ using the *project* tool in ArcGIS Pro. Statistical tests were conducted in Stata (v18.5). Figures were created with R version 4.0.0 (R Core Team, 2020) and Flourish (*Flourish*, 2025).

Conservation expenditures

EQIP expenditure data for fiscal years 2014 thought 2024 were shared by Wisconsin NRCS. We averaged expenditures by practice for each county for fiscal years 2014 through 2024. We classified conservation practices as perennial, annual, or confinement livestock (Figure 1). Classification was informed by NRCS practice standards and practice co-occurrence based on a dataset of de-identified contracts. Practices commonly used across the categories were classified as multi-system. Practices that did not fall neatly within these categories were classified as 'other'.

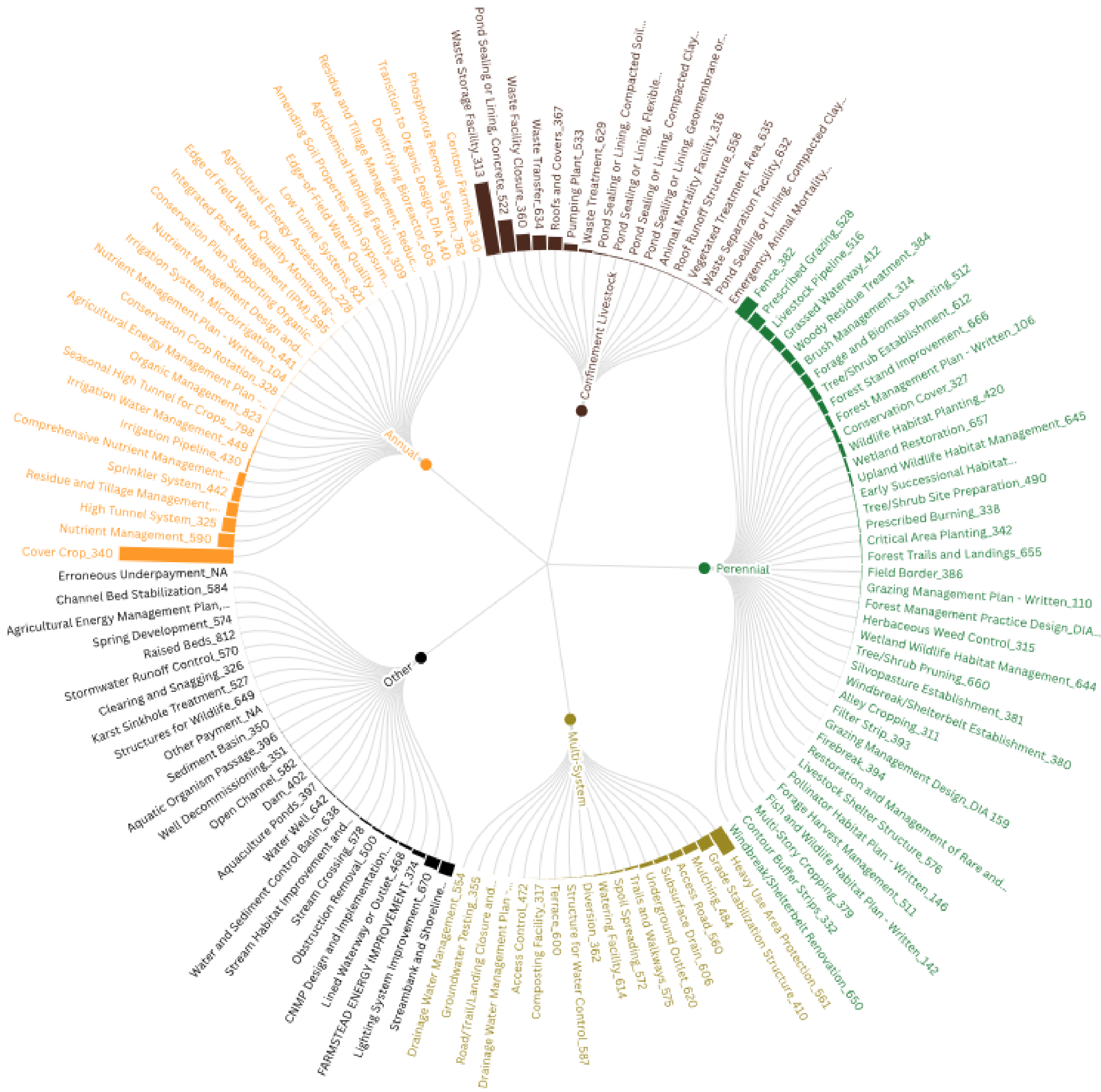


Figure 1. Classification of EQIP practices into five categories (annual row crop, confinement livestock, perennial, multi-system, and other). Practices are ordered within each category by high to low spending. Bars on the inner circle represent average annual dollars spent by practice FY2014 through FY2024.

Conservation staff

NRCS staffing data from 2015 to 2023 were obtained via FOIA request 2023-NRCS-02523-F. We removed national headquarter positions (~5.73% of Wisconsin positions over this period) from the dataset. State and area NRCS staff were removed per personal communication with NRCS to ensure dataset reflects field staff. A quarter of WI counties (~26%) do not have field offices, but are served by adjacent counties. County NRCS offices that serve multiple counties were counted as a fraction of the counties served (i.e., staff shared across 4 counties were assigned a value of 0.25). The dataset linked staff to the city in which their home office was located. We used the US Census Bureau Place 2024 TIGER/Line Shapefile to merge Wisconsin city coordinates with associated NRCS staffing data. We calculated the average NRCS staff (2015 through 2023) within a county using *Spatial Join*, join operation 'join one to many', match option 'completely contains' and summarized total staff numbers by county using *Summary Statistics* in ArcGIS Pro.

Wisconsin county conservation staffing data were accessed via Wisconsin Land and Water's member directory as of 29 May 2025. At the recommendation of Wisconsin Land and Water staff, we removed positions specific to aquatic invasive species, zoning and sanitation, erosion control/stormwater specialists, property managers/analysts, and recycling specialists. Positions shared across counties were counted as a fraction of the counties served (i.e., staff shared across 4 counties were assigned a value of 0.25 FTE). While county staff were provided as of May 2025, those local staff numbers were seen as generally consistent with their levels in prior years (personal communication August 2025) in contrast with NRCS staff numbers which dropped substantially during 2025 (Schewe, 2025).

Land cover

Land cover maps for 2023 from the National Land Cover Database at 30-m resolution were downloaded (Dewitz, 2023). We used the *Tabulate* tool in ArcGIS Pro to calculate the area covered by each land classification. We excluded open water from the state and calculated proportions of terrestrial land cover. We grouped the following classifications: *Forest* (sum deciduous, evergreen, mixed), *Grassland* (sum of grassland herbaceous and pasture hay), *Wetlands* (sum woody wetlands and emergent herbaceous wetlands).

Agricultural census

The average size of farm (acres) per county and number of milk cows per farm per county from the 2022 Agricultural Census were included in empirical models. Model results for the number of milk cows are reported in thousands to improve interpretability of coefficients.

Empirical models

We assessed drivers of conservation expenditures with six county-level ordinary least squares linear regression models for overall average annual expenditures and each category of conservation practices. Response variables reflect county average expenditures from FY2014 through FY2024. NRCS staff and county staff were analyzed in independent models (reported as models a and b) due to substantial differences in coefficients when assessed together. Co-variates included average farm size in acres (2022) and the proportion of land cover most associated with practice categories (2023). All model residuals were assessed for normality. Expenditures were natural log transformed. Relationships between county conservation staff and expenditures were assessed for robustness by removing four counties (St. Croix, Sauk, Dane, and Outagamie) with high staff abundance and reported in supplemental material.

Table 2. Linear regression models of conservation expenditures.

Response variable (average $ per county FY14 throughFY24)	Predictor variables
Overall expenditures	Model 1. NRCS staff + County staff + Avg farm size (ac) + Proportion Cultivated Cropland
Perennial expenditures	Model 2. NRCS staff + County staff + Avg farm size + Proportion (Grassland + Forest + Shrubland)
Grazing expenditures	Model 3. NRCS staff + County staff + Avg farm size + Proportion Grassland
Forestry expenditures	Model 4. NRCS staff + County staff + Avg farm size + Proportion Forestland
Annual row crop expenditures	Model 5. NRCS staff + County staff + Avg farm size + Proportion Cultivated Cropland
Confinement livestock expenditures	Model 6. NRCS staff + County staff + Avg farm size + Proportion Cultivated Cropland + No. Milk Cows

Results

Total conservation expenditures

Annual row crop practices received the largest share of conservation dollars (\$9.28M yr^{-1}) followed closely by confined livestock practices (\$8.69M yr^{-1}) (Table 1). Perennial practices received an average \$5.85M yr^{-1}, while \$3.6M yr^{-1} supported practices commonly applied across systems, and \$2.31M yr^{-1} supported practices that did not fit within our categorization. Perennial, annual, and confinement livestock conservation dollars had different patterns of distribution across the state (Figure 2). Similarly, certain counties implemented grazing and forestry practices more than others (Figure 3). Conservation spending increased over time, with annual row crops and confinement livestock consistently receiving a greater share of conservation dollars (Figure 4).

Table 1. EQIP practices categorized by system type. Average per year spending by category for WI FY 2014 through- FY 2024.

Category	Mean conservation expenditure per year (FY 2014-2024)	Top 5 Practices	Top 5 Avg Expenditure per year	Category Rationale
Annual	$9.28 M (30.98% of avg spending)	Cover Crop_340	$5,966,481	Practice applies or supports annual cropping system
		Nutrient Management_590	$843,605	
		High Tunnel System_325	$693,609	
		Residue and Tillage Management, No-Till_329	$587,387	
		Sprinkler System_442	$443,438	
Confinement Livestock	$8.69 M (29.25% of avg spending)	Waste Storage Facility_313	$3,797,574	Practice applies or supports confinement livestock system
		Pond Sealing or Lining, Concrete_522	$1,697,173	
		Waste Facility Closure_360	$885,555	
		Waste Transfer_634	$778,173	
		Roofs and Covers_367	$700,068	
Perennial	$5.85 M (19.68% of avg spending)	Fence_382	$1,074,435	Practice applies or supports maintenance of perennial land use
		Prescribed Grazing_528	$676,005	
		Livestock Pipeline_516	$478,800	
		Grassed Waterway_412	$459,651	
		Woody Residue Treatment_384	$433,468	
Multi-System	$3.60 M (12.10% of avg	Heavy Use Area Protection_561	$1,384,762	Practices applied across

	spending)	Grade Stabilization Structure_410	$648,369	agricultural and land use systems
		Mulching_484	$391,622	
		Access Road_560	$358,291	
		Subsurface Drain_606	$242,239	
Other	$2.31 M			
Total	$29.73 M			

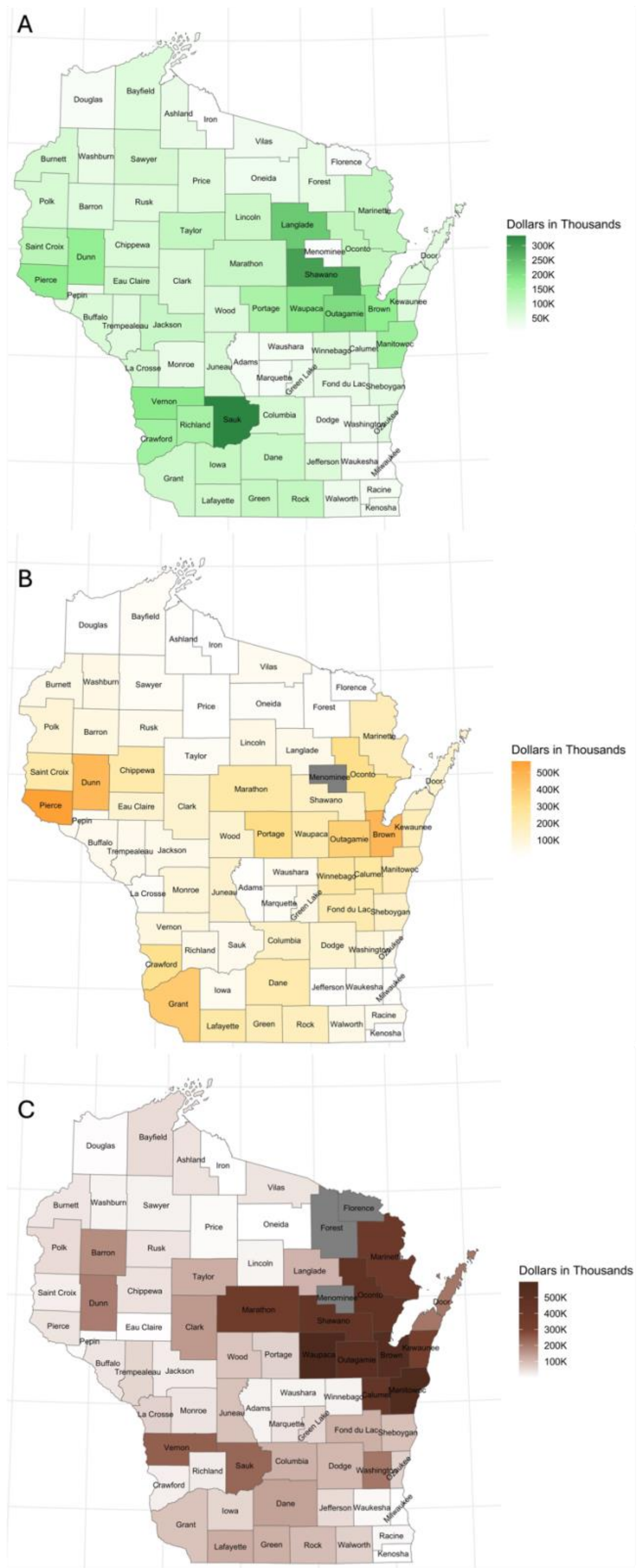


Figure 2. Distribution of average annual EQIP expenditures for a) perennial, b) annual and c) confinement livestock

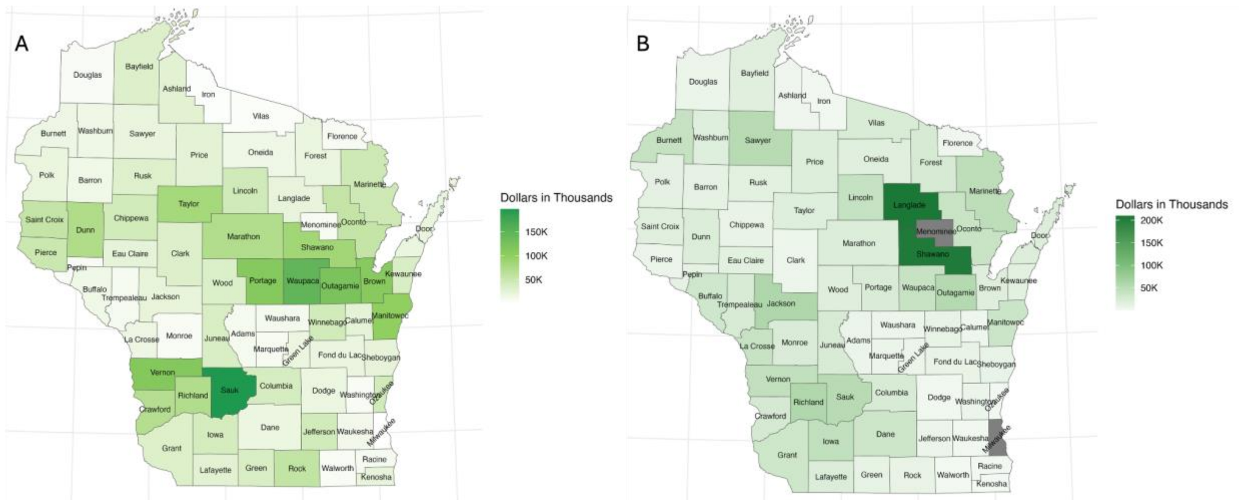


Figure 3. Distribution of average annual expenditures for a) grazing and b) forestry practices.

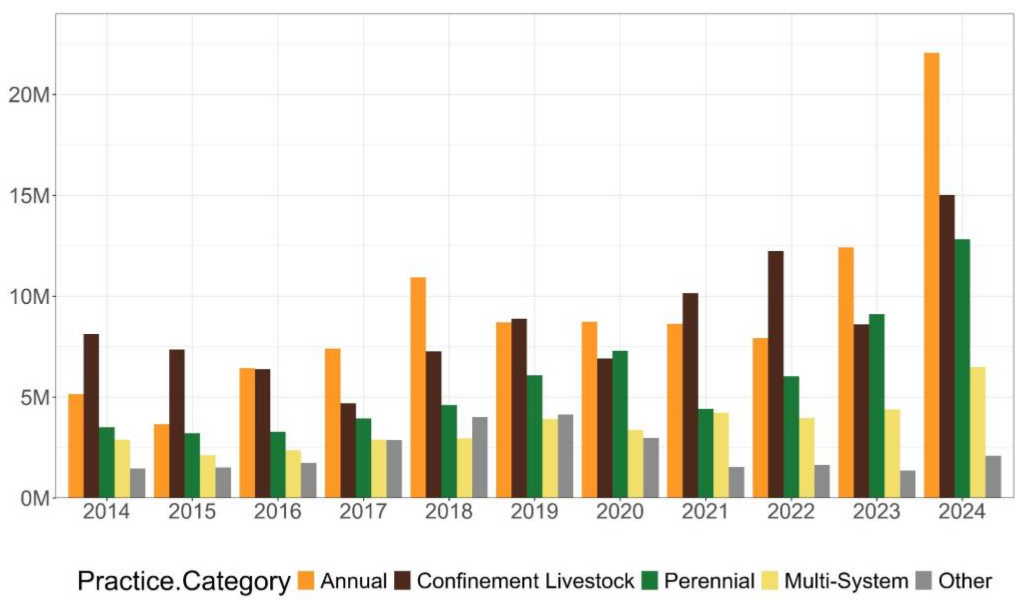


Figure 4. Bar chart of EQIP conservation spending by year and category.

Conservation practice contract co-occurrence

There were 12,893 EQIP contracts from FY 2014 through 2024, with an average of 1,172 contracts per year. Top practices based on frequency included cover crops, forest management plans, mulching, critical area planting and fence (Figure 5). Practices commonly used together include prescribed grazing with forage and biomass planting, fence, livestock pipeline, and watering facility. Heavy use protection area is used ~50% of time with biomass planting, fence, livestock pipeline, prescribed grazing, watering facility, and 25-50% with waste storage facilities, pumping plants, and waste transfer (Figure 6). See full co-occurrence matrix in Table S1.

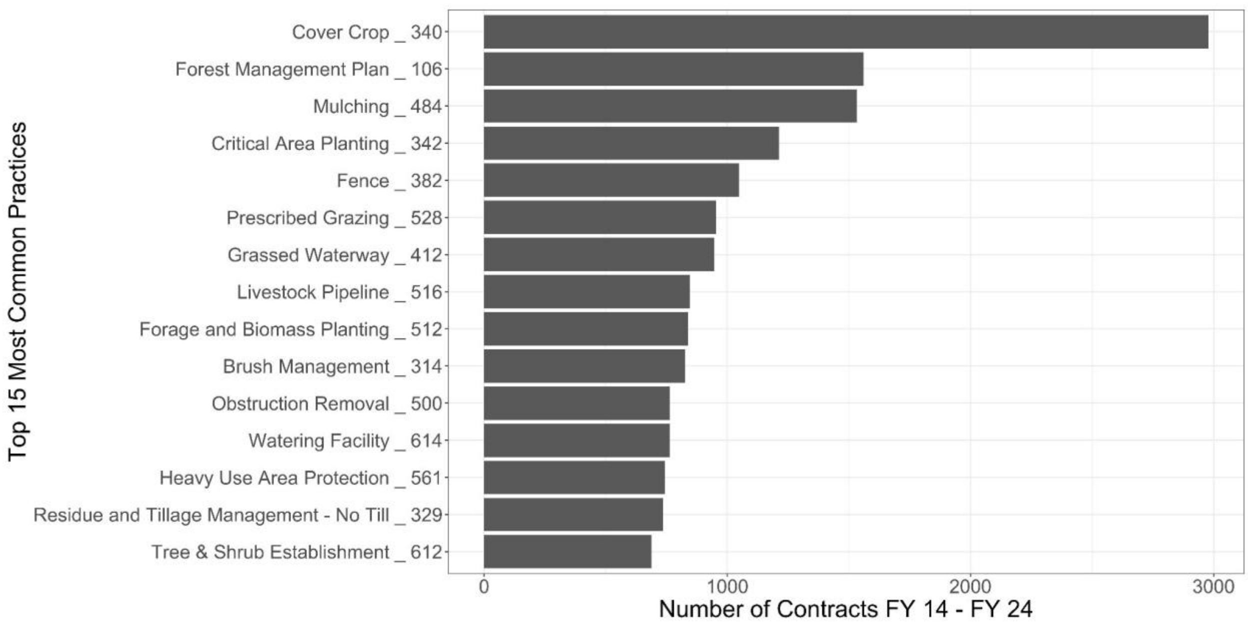
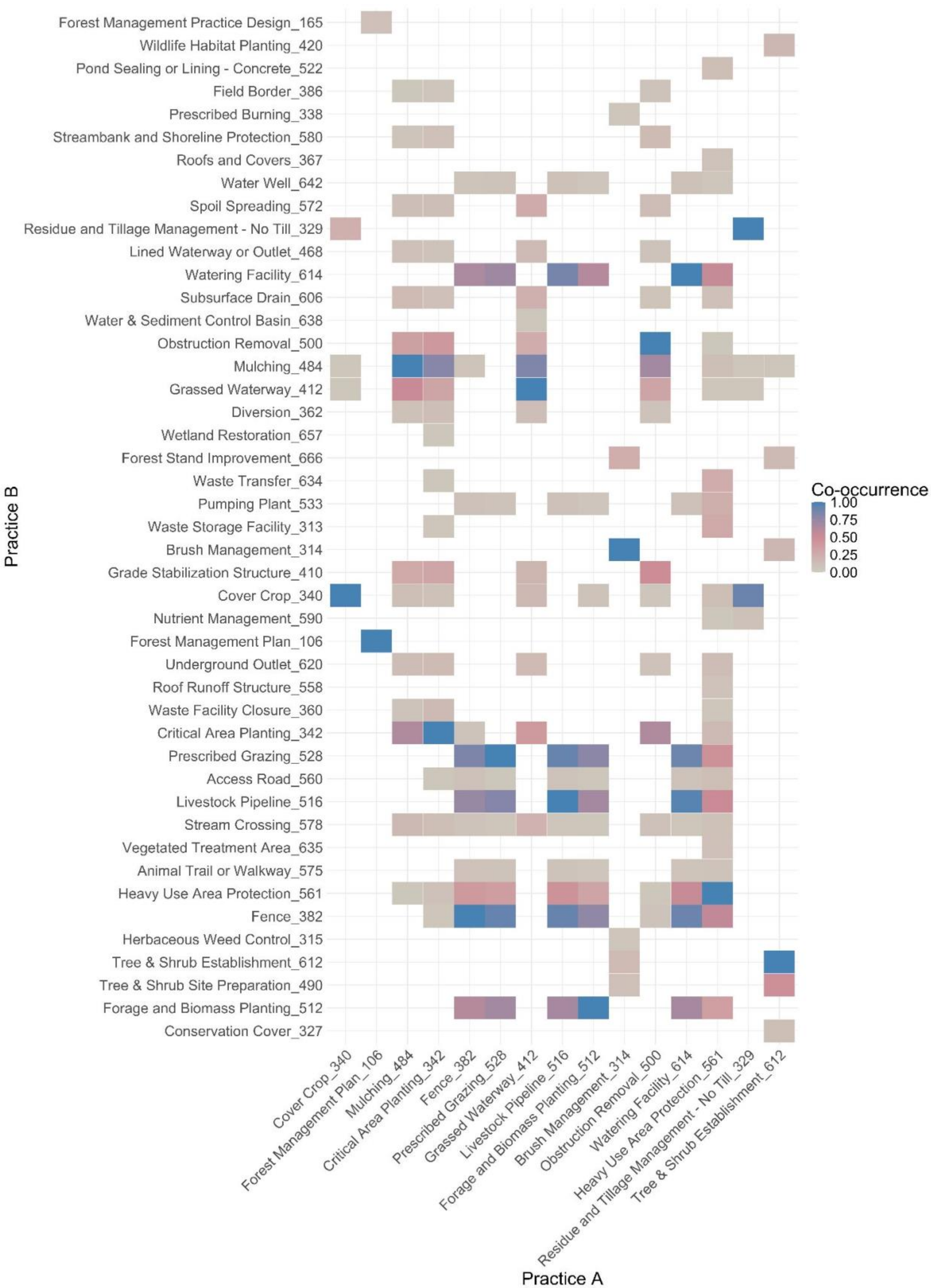


Figure 5. Top 15 practices based on frequency in contracts from FY 14 - 24.



Forest Management Practice Design_165
Wildlife Habitat Planting_420
Pond Sealing or Lining - Concrete_522
Field Border_386
Prescribed Burning_338
Streambank and Shoreline Protection_580
Roofs and Covers_367
Water Well_642
Spoil Spreading_572
Residue and Tillage Management - No Till_329
Lined Waterway or Outlet_468
Watering Facility_614
Subsurface Drain_606
Water & Sediment Control Basin_638
Obstruction Removal_500
Mulching_484
Grassed Waterway_412
Diversion_362
Wetland Restoration_657
Forest Stand Improvement_666
Waste Transfer_634
Pumping Plant_533
Waste Storage Facility_313
Brush Management_314
Grade Stabilization Structure_410
Cover Crop_340
Nutrient Management_590
Forest Management Plan_106
Underground Outlet_620
Roof Runoff Structure_558
Waste Facility Closure_360
Critical Area Planting_342
Prescribed Grazing_528
Access Road_560
Livestock Pipeline_516
Stream Crossing_578
Vegetated Treatment Area_635
Animal Trail or Walkway_575
Heavy Use Area Protection_561
Fence_382
Herbaceous Weed Control_315
Tree & Shrub Establishment_612
Tree & Shrub Site Preparation_490
Forage and Biomass Planting_512
Conservation Cover_327
Practice B
Cover Crop_340
Forest Management Plan_106
Mulching_484
Critical Area Planting_342
Fence_382
Prescribed Grazing_528
Grassed Waterway_412
Livestock Pipeline_516
Forage and Biomass Planting_512
Brush Management_314
Obstruction Removal_500
Watering Facility_614
Heavy Use Area Protection_561
Residue and Tillage Management - No Till_329
Tree & Shrub Establishment_612
Practice A
Co-occurrence
1.00
0.75
0.50
0.25
0.00

Figure 6. Heat map showing top 15 practices by frequency in contracts as percent co-occurrence of practice A with practice B relative to total use of practice A. Co-occurrence represents how often practices are used together on EQIP contracts from FY 14 - 24. Filtered co-occurrence to > 5% used with top 15 practices.

Conservation staff

Wisconsin had an average of 246.6 NRCS employees from 2015 through 2023, and Wisconsin county conservation staff were 391 as of May 2025. NRCS and county conservation staff abundance were not highly correlated. NRCS staff numbers had a smaller distribution (0.5 to 5 staff per county), while county staff numbers ranged from 1 to 10 staff per county with four counties having > 10 staff (Figure 7).

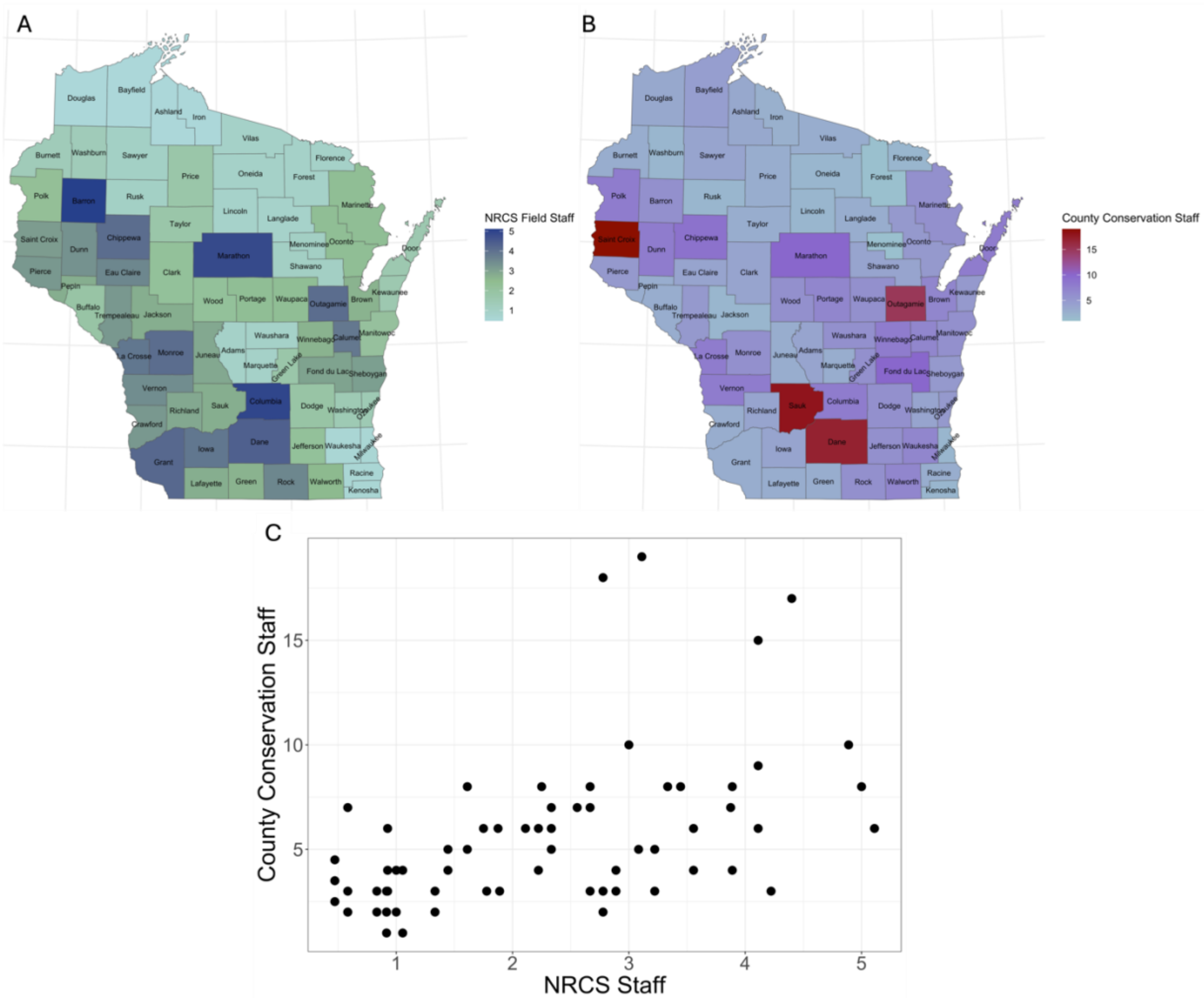


Figure 7. A) Distribution of NRCS staff abundance by county, B) county conservationist abundance by county, and C) scatterplot of county conservation staff abundance by NRCS staff abundance.

Staff abundance and conservation expenditures

County conservation staff and NRCS field staff abundance were positively correlated with federal EQIP spending overall and for all categories of spending except for confinement livestock (no relationship between NRCS field staff and confinement) (Figure 8 & 9, Table 3). Positive relationships between county staff and forestry expenditure were not robust when removing counties with high staff (Table S2). Forestry expenditures and NRCS staff don't appear to show a significant relationship, yet the model controls for forestry cover and supports a significant correlation. Farm size did not predict conservation spending, with the exception of forestry practices where more dollars flowed to bigger farm properties. More cultivated cropland meant more conservation spending overall and more annual-row crop conservation support. Proportion of forested land corresponded with forestry practices, while proportion of grassland was not significantly related to grazing expenditures. Lastly, the number of milk cows per county (milk cows ranged from 0 - 66,631 per county) was positively associated with confinement livestock conservation.

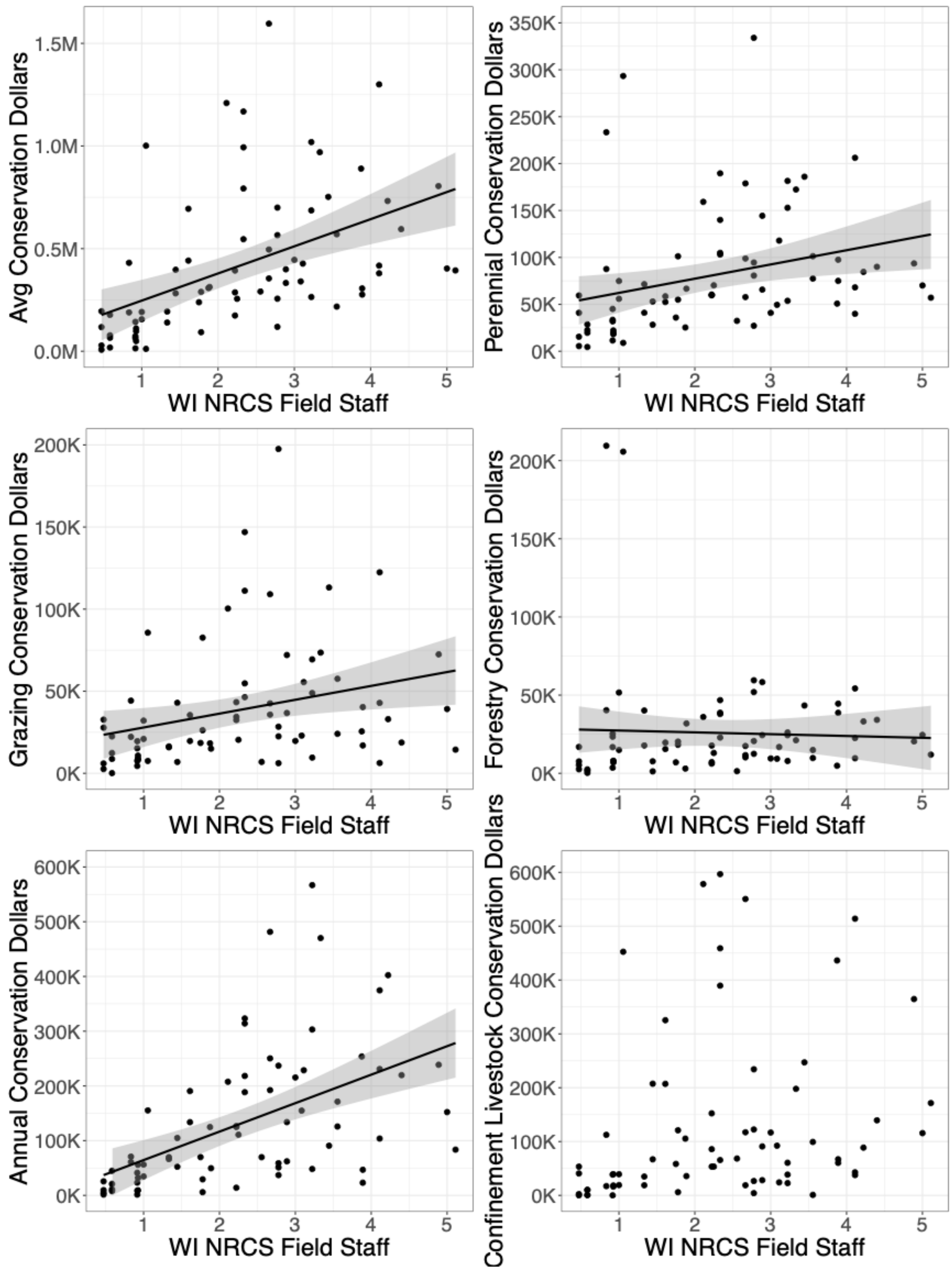


Figure 8. Associations between NRCS field staff and conservation spending categories. Line of fit displayed when significant relationship present.

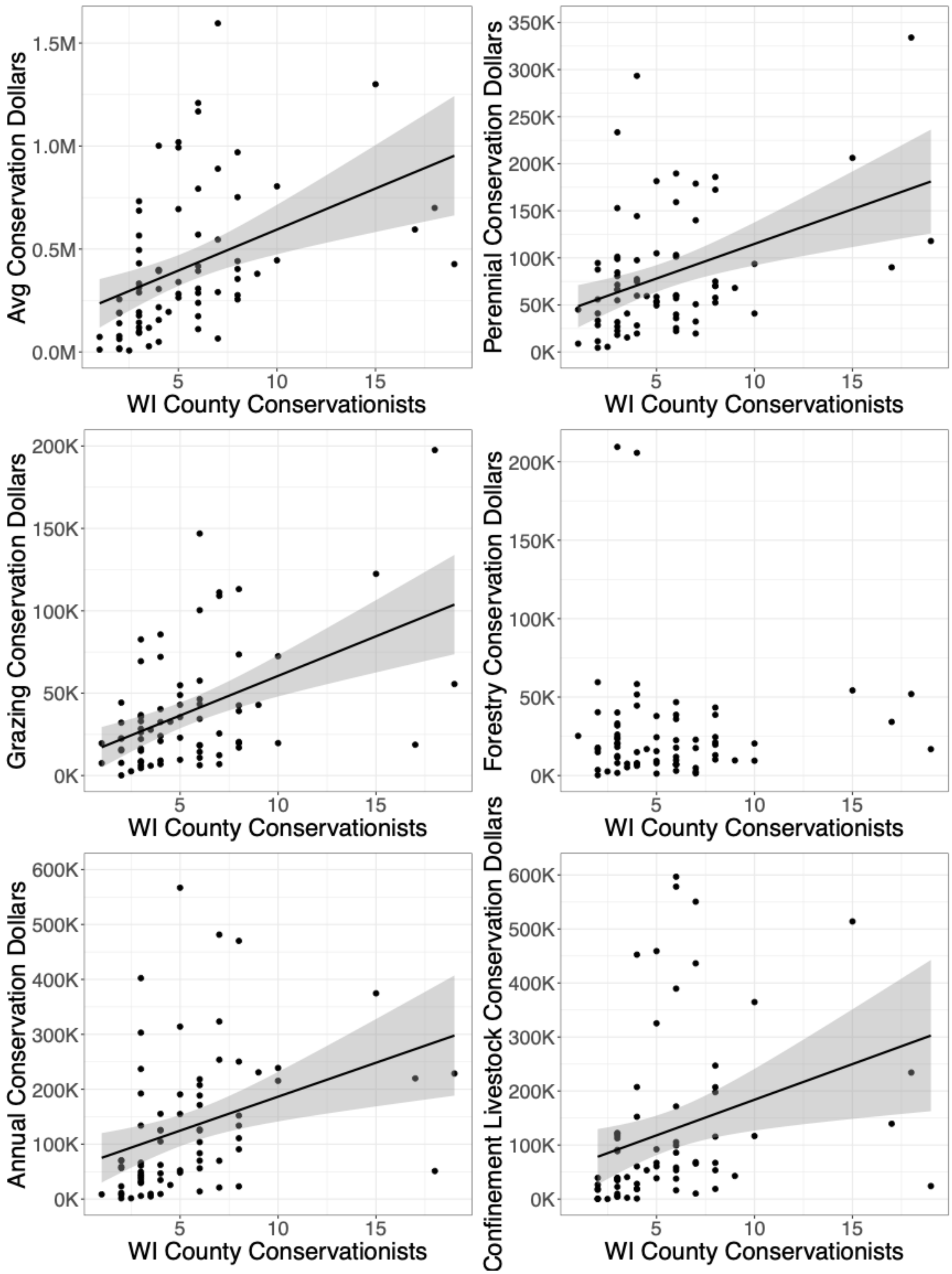


Figure 9. Associations between local county conservationists and conservation spending categories. Line of fit displayed when significant, robust relationship present.

Table 3. Linear regression models (1-6) with coefficients per variable, robust standard errors in parenthesis, and p-value significance indicated by asterisks (*** p<0.01, ** p<0.05, * p<0.1).

	Model											
	1		2		3		4		5		6	
Potential predictors	Overall (ln $)		Perennial (ln $)		Grazing (ln $)		Forestry (ln $)		Annual (ln $)		Confinement (ln $)	
Model version	a	b	a	b	a	b	a	b	a	b	a	b
NRCS Staff	0.35 ***		0.29 ***		0.25 ***		0.35 ***		0.42 ***		0.25	
	(0.08)		(0.07)		(0.08)		(0.11)		(0.10)		(0.17)	
County Land + Water Staff		0.07 ***		0.09 ***		0.10 ***		0.10 ***		0.07 **		0.13 *
		(0.02)		(0.02)		(0.03)		(0.03)		(0.03)		(0.07)
Average size of farm (acres)	0.00	0.00	0.00	0.00	0.00	0.00	0.01 *	0.01 *	0.00	0.00	0.00	0.01
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)	(0.01)
Prop cultivated crops	0.02 ***	0.03 ***							0.03 ***	0.04 ***	0.03	0.03
	(0.01)	(0.01)							(0.01)	(0.01)	(0.02)	(0.02)
Prop perennial (grassland + shrubland + forest)			0.00	0.01								
			(0.00)	(0.00)								

Prop grassland					0.00	0.01						
					(0.02)	(0.02)						
Prop forest							0.02***	0.02***				
							(0.01)	(0.01)				
Milk cows (1000)											0.05***	0.05***
											(0.01)	(0.01)
Constant	9.09***	9.21***	9.91***	9.87***	9.37***	9.01***	6.86***	6.95***	9.06***	9.29***	7.22***	6.72***
	(0.75)	(0.88)	(0.56)	(0.68)	(0.69)	(0.75)	(0.82)	(0.94)	(0.86)	(1.03)	(1.78)	(1.84)
Observations	70	70	70	70	70	70	70	70	70	70	68	68
R-squared	0.48	0.39	0.22	0.16	0.12	0.17	0.30	0.24	0.50	0.40	0.34	0.36

Discussion

Federal conservation dollars primarily flowed to annual row crop and confinement agriculture

Federal conservation dollars in Wisconsin flowed to a few practices within annual row crop and confined livestock agroecosystems, namely cover crops and waste storage facilities. Row cropping (corn and soybeans) largely feeds confinement livestock, maintaining a close tie between these two systems. In addition to receiving the majority of conservation dollars, annual row crops are the primary beneficiaries of commodity subsidies, commodity price floors, and crop insurance (Bland & Kallins, 2025). The investment by the UDSA into perennial land use is relatively small compared to confinement and annual systems, despite perennial agroecosystem potential for addressing pressing environmental concerns (Glover et al., 2010; Jackson, 2022, 2024; Reynolds et al., 2021; Rui et al., 2022; Wepking et al., 2022).

Conservation supported by polycentric governance

Local and federal field conservation staff were positively associated with federal EQIP expenditures across practice types, with exceptions in confinement livestock and forestry systems. The combined contributions of both local conservationists and federal field staff to the distribution of conservation expenditures is an example of polycentric governance, with distinct levels of government working simultaneously to manage common resources (Ostrom 2010). Local and federal staff are part of an intentionally federalist system in which local and state decision makers share influence with federal agencies over priorities and expenditures. Our findings suggest both localized and federal approaches to conservation have been effective in influencing conservation adoption in Wisconsin.

Local and federal conservation staff are shaped by different objectives, policies and resources. Conservation districts are guided by conservation committees that include elected members of county boards. The mission of federal staff is influenced by the federal agency with state priorities and input from State Technical Committees that advise NRCS. Counties with greater local staff abundance and substantial agricultural land, such as Dane, Sauk, and St. Croix counties, have been able to leverage federal funds to meet their priorities. Drivers of county staff abundance such as budgetary support from county property and sales taxes along with state aid, greater political will for conservation, substantial water quality concerns from agriculture, and industrial consolidation that creates high conservation demand should be examined in future research.

Staff abundance – mechanism for change

Greater local and federal staff abundance corresponded to higher conservation investment. The influence of staff abundance on conservation expenditures may manifest from increased capacity, greater conservation staff specialization, access to training and professional development, closer farmer relationships, and administrative support for grant applications. Relationships and access to technical service providers has been suggested to improve conservation adoption (Wauter and Mathijis 2014). Future work assessing place-based priorities, narratives, and networks would advance our understanding of how staff abundance advances conservation spending toward different kinds of land use change.

Staff support for agricultural transformation

Perennial and grazing-specific perennial practices can transform agroecosystems into systems that both provide food and maintain ecosystem integrity. Local and federal staff advanced perennial and grazing-specific practices in Wisconsin, suggesting conservation staff can be key to advancing agroecosystem transformation to systems with high environmental and societal value. Our results suggest both row-cropping and perennial practices are connected to staff abundance. Others have found county conservationists reinforce the status-quo of industrial agriculture rather than advocating for high value soil and water conservation practices (Comito et al., 2013). County conservation staff water quality concern and use of long-term weather projections were correlated with greater adaptations to extreme weather events, though number of office staff was only marginally significantly associated with greater adaptation actions (Wardropper and Rissman 202). Conservation priorities are tied to staff expertise and regional identities and narratives about conservation agriculture (Strauser et al., 2022). The potential for county staff to direct their time and efforts towards transformative conservation practices with high conservation/biodiversity value should be recognized as an important mechanism for land use change and further studied.

Confinement livestock investment tied to milk cows

The number of milk cows in a county corresponds to confinement livestock conservation practices as expected, but the average size of farm per county was not a significant factor. The connection between milk cows and confinement conservation suggests concentrated animal feeding operations (CAFOs) influence how conservation dollars are spent in the north-east portion of the state. Though large concentrations of animals and their waste lead to extensive environmental concerns, specifically related to water quality (Miralha et al., 2022; Raff & Meyer, 2022) and soil contamination (Liu et al., 2015), the use of limited conservation dollars to remedy a high polluting industry (Levandowski, 2020) has been an issue of significant debate (Happ, 2022). Confinement-livestock conservation practices are among the most expensive to implement. This results in fewer operations receiving high dollar contracts rather than many landowners receiving modest cost-share support. Prior to the 2002 Farm Bill, EQIP did not support construction of large waste facilities related to confinement agriculture. Lobbying by dairy, meat and egg industries opened EQIP funds to support confinement ag practices and dedicated 60% of EQIP to livestock operations (Imhoff & Badaracco, 2019). Wisconsin state regulations allow agencies to require farm changes to meet water quality standards, but only if most to all of the upgrades are paid by the government through conservation cost-share. Cost-share serves an important role in bridging the disconnect between water quality regulations and the operation of farm practices (Rissman et al., 2024). We did not see a connection between confinement livestock conservation expenditures and farm size at the county scale. This relationship would be better tested at the farm rather than the county scale. The lack of relationship between federal staff and confinement livestock conservation may reflect a reliance on private consultants by large dairy farms seeking specialized information, rather than from federal NRCS staff (Eanes et al., 2017, 2019; Frisvold & Deva, 2012).

Grazing practices were not targeted to counties with more grassland

Land cover was a significant predictor for forestry and annual row crops, but did not correspond to grazing expenditures. Prescribed grazing in the state likely occurs on a small subset of what is broadly classified as ‘grassland’. The implementation of managed grazing is usually a part of a land cover transition from row crops while some farms transition existing pasture to rotational grazing. Mayerfeld et al. (2023) report 20% of farms with pasture practice rotational grazing, with the majority of livestock farms not using rotational grazing. The transition to managed grazing has the potential to restore ecosystem function (Wepking et al., 2022), but application of managed grazing was not driven by the existing grassland cover in the county.

Conclusions

Provisioning food, fuel and fiber while protecting ecosystem services requires agency investments in conservation solutions beyond cover crops and waste storage facilities. Perennial agroecosystems address many perpetual environmental concerns perpetrated by annual and confinement agroecosystems. Yet, perennial agroecosystems receive less conservation investment than both annual row crop and confinement livestock systems. The future of conservation should strive for agroecological transformations with high conservation value if we want to steward the land for future generations. Our work suggests local conservationists and federal field staff are important keys for conservation and transformative land use. Future research should examine mechanisms behind staff contributions to different types of agroecosystems. Further, assessing the effects of the rapid rise of NRCS staff and conservation cost share in 2024 under the Inflation Reduction Act and Bipartisan Infrastructure Law (USDA Staff, 2024) and decline in 2025 under direction from the Department of Government Efficiency (Mercier, 2025) is merited. Local conservationists, funded from county, state, and district sources, may help stabilize the effect of dramatic federal changes to staff (Andersson & Ostrom, 2008).

Supplemental Information

Table S1. Co-occurrence Matrix of EQIP practices that co-occur on contracts from FY 2014 through 2024.

- See excel file in Ch 3 appendix.
    - EQIP practices are listed in *column A* and as *column names*. Cell values represent the proportion of contracts where *column A* practice is used with column header practice relative to total use of *column A* practice.

Table S2. Linear regressions when removing high local county conservationist (counties > 10 staff removed) to test robustness of relationships.

	Model					
Potential predictors	(1) Overall (ln $)	(2) Perennial (ln $)	(3) Grazing (ln $)	(4) Forestry (ln $)	(5) Annual (ln $)	(6) Confinement (ln $)
County Land + Water Staff	0.12***	0.10***	0.12***	0.10	0.15**	0.34**
	(0.05)	(0.04)	(0.04)	(0.07)	(0.06)	(0.14)
Average farm size (acres)	0.00	0.00	0.00	0.01*	0.00	0.01
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)
Prop cultivated crops	0.02***				0.03***	0.02
	(0.01)				(0.01)	(0.02)
Prop perennial (grassland + shrubland + forest)		0.01				
		(0.00)				
Prop grassland			0.02			
			(0.02)			
Prop forest				0.03***		
				(0.01)		
Milkcows1000						0.04***
						(0.01)
Constant	9.05***	9.82***	8.90***	6.96***	9.05***	5.88***
	(0.90)	(0.72)	(0.78)	(1.05)	(1.09)	(2.08)

Observations	66	66	66	66	66	64
R-squared	0.38	0.09	0.12	0.23	0.40	0.39

Robust standard errors in parentheses
*** p<0.01, ** p<0.05, * p<0.1

Acknowledgements

Thank you to Margaret Krome and Michael Fields Agricultural Institute for funding this work.

Thank you to Christina Anderson and Meagan Duberstein for conservation staff consultation.

References

Adhikari, R. K., Wang, T., Jin, H., Ulrich-Schad, J. D., Sieverding, H. L., & Clay, D. (2023). Farmer perceived challenges toward conservation practice usage in the margins of the Corn Belt, USA. *Renewable Agriculture and Food Systems*, *38*, e14. https://doi.org/10.1017/S1742170523000042

Anderson, C. R., Bruil, J., Chappell, M. J., Kiss, C., & Pimbert, M. P. (2021). Conceptualizing Processes of Agroecological Transformations: From Scaling to Transition to Transformation. In *Agroecology Now!* (pp. 29–46). Palgrave Macmillan, Cham. https://doi.org/10.1007/978-3-030-61315-0_3

Andersson, K. P., & Ostrom, E. (2008). Analyzing decentralized resource regimes from a polycentric perspective. *Policy Sciences*, *41*(1), 71–93. https://doi.org/10.1007/s11077-007-9055-6

Basche, A., Tully, K., Álvarez-Berríos, N. L., Reyes, J., Lengnick, L., Brown, T., Moore, J. M., Schattman, R. E., Johnson, L. K., & Roesch-McNally, G. (2020). Evaluating the Untapped Potential of U.S. Conservation Investments to Improve Soil and Environmental Health. *Frontiers in Sustainable Food Systems*, *4*. https://www.frontiersin.org/articles/10.3389/fsufs.2020.547876

Blanco-Canqui, H. (2022). Cover crops and carbon sequestration: Lessons from U.S. studies. *Soil Science Society of America Journal*, *86*(3), 501–519. https://doi.org/10.1002/saj2.20378

Bland, M., & Kallins, L. (2025, July 21). *How the Federal Reconciliation Bill Will Affect Farms and Food Policy*. National Conference of State Legislatures. https://www.ncsl.org/state-legislatures-news/details/how-the-federal-reconciliation-bill-will-affect-farms-and-food-policy

Cates, A. M., Sanford, G. R., Good, L. W., & Jackson, R. D. (2018). What do we know about cover crop efficacy in the North Central United States? *Journal of Soil and Water Conservation*, *73*(6), 153A-157A. https://doi.org/10.2489/jswc.73.6.153A

Center for Rural Affairs. (2022, April 18). What to Know about EQIP Application Rankings and Advance Payment Option. https://www.bing.com/videos/riverview/relatedvideo?q=EQIP+ranking&mid=55478E82B4896BB49B4855478E82B4896BB49B48&FORM=VIRE

Comito, J., Wolseth, J., & Morton, L. W. (2013). The State's Role in Water Quality: Soil and Water Conservation District Commissioners and the Agricultural Status Quo. *Human Organization*, *72*(1), 44–54.

Curtis, J. T. (1959). *The Vegetation of Wisconsin: An Ordination of Plant Communities*. University of Wisconsin Press. https://muse.jhu.edu/pub/19/monograph/book/16741

Curtis, J. T., & McIntosh, R. P. (1951). An Upland Forest Continuum in the Prairie-Forest Border Region of Wisconsin. *Ecology*, *32*(3), 476–496. https://doi.org/10.2307/1931725

Dewitz, J. (2023). *National Land Cover Database (NLCD) 2021 Products* [Data Release]. Earth Resources Observation and Science (EROS) Cente. https://doi.org/10.5066/P9JZ7AO3

Eanes, F. R., Singh, A. S., Bulla, B. R., Ranjan, P., Fales, M., Wickerham, B., Doran, P. J., & Prokopy, L. S. (2019). Crop advisers as conservation intermediaries: Perceptions and policy implications for relying on nontraditional partners to increase U.S. farmers' adoption of soil and water conservation practices. *Land Use Policy*, *81*, 360–370. https://doi.org/10.1016/j.landusepol.2018.10.054

Eanes, F. R., Singh, A. S., Bulla, B. R., Ranjan, P., Prokopy, L. S., Fales, M., Wickerham, B., & Doran, P. J. (2017). Midwestern US Farmers Perceive Crop Advisers as Conduits of Information on Agricultural Conservation Practices. *Environmental Management*, *60*(5), 974–988. https://doi.org/10.1007/s00267-017-0927-z

Ewert, F., Baatz, R., & Finger, R. (2023). Agroecology for a Sustainable Agriculture and Food System: From Local Solutions to Large-Scale Adoption. *Annual Review of Resource Economics*, *15*(Volume 15, 2023), 351–381. https://doi.org/10.1146/annurev-resource-102422-090105

FAO. (2018). *The 10 elements of agroecology*. https://openknowledge.fao.org/handle/20.500.14283/i9037en

Flourish. (2025). Flourish. https://flourish.studio/

Franzluebbers, A. J., Paine, L. K., Winsten, J. R., Krome, M., Sanderson, M. A., Ogles, K., & Thompson, D. (2012). Well-managed grazing systems: A forgotten hero of conservation. *Journal of Soil and Water Conservation*, *67*(4), 100A-104A. https://doi.org/10.2489/jswc.67.4.100A

Frisvold, G. B., & Deva, S. (2012). Farm Size, Irrigation Practices, and Conservation Program Participation in the Us Southwest. *Irrigation and Drainage*, *61*(5), 569–582. https://doi.org/10.1002/ird.1676

Gilbert, J. (2015). *Planning Democracy: Agrarian Intellectuals and the Intended New Deal*. Yale University Press. https://www.jstor.org/stable/j.ctt1bh4bp7

Glover, J. D., Culman, S. W., DuPont, S. T., Broussard, W., Young, L., Mangan, M. E., Mai, J. G., Crews, T. E., DeHaan, L. R., Buckley, D. H., Ferris, H., Turner, R. E., Reynolds, H. L., & Wyse, D. L. (2010). Harvested perennial grasslands provide ecological benchmarks for agricultural sustainability. *Agriculture, Ecosystems & Environment*, *137*(1), 3–12. https://doi.org/10.1016/j.agee.2009.11.001

Guo, T., Marquart-Pyatt, S. T., Beethem, K., Denny, R., & Lai, J. (2023). Scaling up agricultural conservation: Predictors of cover crop use across time and space in the US upper Midwest. *Journal of Soil and Water Conservation*, *78*(4), 335–346. https://doi.org/10.2489/jswc.2023.00084

Happ, M. (2022). *Payments for Pollution: How federal conservation programs can better benefit farmers and the environment*. https://www.iatp.org/payments-pollution-how-federal-conservation-programs-can-better-benefit-farmers

Happ, M. (2024). *Costly versus Cost-Effective: How EQIP can be improved to serve more farmers and the climate*. Institute for Agriculture and Trade Policy.

Horrigan, L., Lawrence, R. S., & Walker, P. (2002). How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environmental Health Perspectives*, *110*(5), 445–456. https://doi.org/10.1289/ehp.02110445

Huang, J., Hartemink, A. E., & Zhang, Y. (2019). Climate and Land-Use Change Effects on Soil Carbon Stocks over 150 Years in Wisconsin, USA. *Remote Sensing*, *11*(12), Article 12. https://doi.org/10.3390/rs11121504

Imhoff, D., & Badaracco, C. (2019). The Conservation Era. In *The Farm Bill* (pp. 49–57). Island Press, Washington, DC. https://doi.org/10.5822/978-1-61091-975-3_8

Jackson, R. D. (2022). Grazed perennial grasslands can match current beef production while contributing to climate mitigation and adaptation. *Agricultural & Environmental Letters*, *7*(1), e20059. https://doi.org/10.1002/ael2.20059

Jackson, R. D. (2024). America's Dairy Grassland – Wisconsin milk production that regenerates people and land. *Agroecology and Sustainable Food Systems*, *48*(6), 898–915. https://doi.org/10.1080/21683565.2024.2344027

Kalcic, M. M., Frankenberger, J., Chaubey, I., Prokopy, L., & Bowling, L. (2015). Adaptive Targeting: Engaging Farmers to Improve Targeting and Adoption of Agricultural Conservation Practices. *JAWRA Journal of the American Water Resources Association*, *51*(4), 973–991. https://doi.org/10.1111/1752-1688.12336

Ladoni, M., Basir, A., Robertson, P. G., & Kravchenko, A. N. (2016). Scaling-up: Cover crops differentially influence soil carbon in agricultural fields with diverse topography. *Agriculture, Ecosystems & Environment*, *225*, 93–103. https://doi.org/10.1016/j.agee.2016.03.021

Lal, R. (2008). Soils and sustainable agriculture. A review. *Agronomy for Sustainable Development*, *28*(1), 57–64. https://doi.org/10.1051/agro:2007025

Levandowski, R. (2020). Polluting 'til the Cows Come Home: How Agricultural Exceptionalism Allows CAFOs Free Range for Climate Harm. *Georgetown Environmental Law Review*, *33*, 151.

Liebig, M. A., Acosta-Martinez, V., Archer, D. W., Halvorson, J. J., Hendrickson, J. R., Kronberg, S. L., Samson-Liebig, S. E., & Vetter, J. M. (2022). Conservation practices induce tradeoffs in soil function: Observations from the northern Great Plains. *Soil Science Society of America Journal*, *86*(6), 1413–1430. https://doi.org/10.1002/saj2.20375

Liu, X., Zhang, W., Hu, Y., Hu, E., Xie, X., Wang, L., & Cheng, H. (2015). Arsenic pollution of agricultural soils by concentrated animal feeding operations (CAFOs). *Chemosphere*, *119*, 273–281. https://doi.org/10.1016/j.chemosphere.2014.06.067

Mayerfeld, D., Keeley, K. O., Rickenbach, M., Rissman, A., & Ventura, S. J. (2023). Evolving conceptions of silvopasture among farmers and natural resource professionals in Wisconsin, USA. *Frontiers in Sustainable Food Systems*, *7*. https://www.frontiersin.org/articles/10.3389/fsufs.2023.983376

Mercier, S. (2025, May 8). *Impact of DOGE Cuts on USDA Staff and Programs*. AgWeb. https://www.agweb.com/opinion/impact-doge-cuts-usda-staff-and-programs

Miralha, L., Sidique, S., & Logsdon Muenich, R. (2022). The spatial organization of CAFOs and its relationship to water quality in the United States. *Journal of Hydrology*, *613*, 128301. https://doi.org/10.1016/j.jhydrol.2022.128301

Morris, C., & Arbuckle, J. G. (2021). Conservation plans and soil and water conservation practice use: Evidence from Iowa. *Journal of Soil and Water Conservation*, *76*(5), 457–471. https://doi.org/10.2489/jswc.2021.00166

Moynihan, D. P., & Landuyt, N. (2009). How Do Public Organizations Learn? Bridging Cultural and Structural Perspectives. *Public Administration Review*, *69*(6), 1097–1105. https://doi.org/10.1111/j.1540-6210.2009.02067.x

NRCS. (2025). *About NRCS* |. About NRCS |. https://www.nrcs.usda.gov/about

Plastina, A., Liu, F., Miguez, F., & Carlson, S. (2020). Cover crops use in Midwestern US agriculture: Perceived benefits and net returns. *Renewable Agriculture and Food Systems*, *35*(1), 38–48. https://doi.org/10.1017/S1742170518000194

Prokopy, L. S., Floress, K., Arbuckle, J. G., Church, S. P., Eanes, F. R., Gao, Y., Gramig, B. M., Ranjan, P., & Singh, A. S. (2019). Adoption of agricultural conservation practices in the United States: Evidence from 35 years of quantitative literature. *Journal of Soil and Water Conservation*, *74*(5), 520–534. https://doi.org/10.2489/jswc.74.5.520

R Core Team. (2020). *R: A language and environment for statistical computing* (Version 4.0.0) [Computer software]. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Raff, Z., & Meyer, A. (2022). CAFOs and Surface Water Quality: Evidence from Wisconsin. *American Journal of Agricultural Economics*, *104*(1), 161–189. https://doi.org/10.1111/ajae.12222

Reynolds, J., Bell, M. M., Grace, J., Gratton, C., Jackson, R. D., Keeley, K. O., & Mayerfeld, D. (2021). An agroecological vision of perennial agriculture. *Agroecology and Sustainable Food Systems*, *45*(10), 1470–1479. https://doi.org/10.1080/21683565.2021.1918313

Rissman, A., Booth, E., Olson, M., & Vir, S. (2024). *Bridging a major disconnect: Ideas for farm standards and systems to achieve phosphorus goals in Wisconsin lakes and streams*. https://minds.wisconsin.edu/handle/1793/89619

Rissman, A. R., Fochesatto, A., Lowe, E. B., Lu, Y., Hirsch, R. M., & Jackson, R. D. (2023). Grassland and managed grazing policy review. *Frontiers in Sustainable Food Systems*, *7*. https://www.frontiersin.org/articles/10.3389/fsufs.2023.1010441

Rissman, A. R., Kazer, A., DeMets, C., & Martell, E. (2023). Sustaining land and people over time: Relationships with successor landowners on conservation easements. *People and Nature*, *5*(2), 542–556. https://doi.org/10.1002/pan3.10436

Rui, Y., Jackson, R. D., Cotrufo, M. F., Sanford, G. R., Spiesman, B. J., Deiss, L., Culman, S. W., Liang, C., & Ruark, M. D. (2022). Persistent soil carbon enhanced in Mollisols by well-managed grasslands but not annual grain or dairy forage cropping systems. *Proceedings of the National Academy of Sciences*, *119*(7). https://doi.org/10.1073/pnas.2118931119

Schewe, R. (2025, March 14). USDA Staffing Cuts Hurt Farmers and Rural Communities. *National Sustainable Agriculture Coalition*. https://sustainableagriculture.net/blog/usda-staffing-cuts-hurt-farmers-and-rural-communities/

Scott, E. I., Toensmeier, E., Iutzi, F., Rosenberg, N. A., Lovell, S. T., Jordan, N. R., Peters, T. E., Akwii, E., & Leib, E. B. M. (2022). Policy pathways for perennial agriculture. *FRONTIERS IN SUSTAINABLE FOOD SYSTEMS*, *6*, 983398. https://doi.org/10.3389/fsufs.2022.983398

Spratt, E., Jordan, J., Winsten, J., Huff, P., van Schaik, C., Jewett, J. G., Filbert, M., Luhman, J., Meier, E., & Paine, L. (2021). Accelerating regenerative grazing to tackle farm, environmental, and societal challenges in the upper Midwest. *Journal of Soil and Water Conservation*, *76*(1), 15A-23A. https://doi.org/10.2489/jswc.2021.1209A

Strauser, J., & Stewart, W. P. (2024). Moving beyond production: Community narratives for good farming. *Agriculture and Human Values*. https://doi.org/10.1007/s10460-024-10550-x

Strauser, J., Stewart, W. P., & Leitschuh, B. (2022). Producing Regions: Connecting Place-Making with Farming Practices. *Society & Natural Resources*, *35*(9), 1012–1020. https://doi.org/10.1080/08941920.2022.2101080

Surdoval, A., Jain, M., Blair, E., Wang, H., & Blesh, J. (2024). Financial incentive programs and farm diversification with cover crops: Assessing opportunities and challenges.

Environmental Research Letters, *19*(4), 044063. https://doi.org/10.1088/1748-9326/ad35d8

USDA Staff. (2024, December 4). *Biden-Harris Administration Sets Record Investment in Private Lands Conservation in 2024 Thanks to Inflation Reduction Act | Natural Resources Conservation Service*. https://www.nrcs.usda.gov/programs-initiatives/acep-agricultural-conservation-easement-program/news/biden-harris-0

Walker, H., & Parks, W. R. (1946). Soil Conservation Districts: Local Democracy in a National Program. *The Journal of Politics*, *8*(4), 538–549. https://doi.org/10.2307/2125533

Wardropper, C. B., & Rissman, A. R. (2019). Adaptations to extreme storm events by conservation organizations. *Climatic Change*, *152*(1), 85–101. https://doi.org/10.1007/s10584-018-2342-8

Wepking, C., Mackin, H. C., Raff, Z., Shrestha, D., Orfanou, A., Booth, E. G., Kucharik, C. J., Gratton, C., & Jackson, R. D. (2022). Perennial grassland agriculture restores critical ecosystem functions in the U.S. Upper Midwest. *Frontiers in Sustainable Food Systems*, *6*. https://www.frontiersin.org/articles/10.3389/fsufs.2022.1010280

Winsten, J. R. (2024). Low-overhead dairy grazing: A specific solution to a vexing problem. *Journal of Soil and Water Conservation*, *79*(2), 27A-31A. https://doi.org/10.2489/jswc.2024.0122A

Wisconsin State Climatology Office. (2025). *Statewide Climate Normals*. Wisconsin State Climatology Office. https://climatology.nelson.wisc.edu/wisconsin-historic-climate-data/statewide-climate-normals/

Conclusions

The UW-Madison Arboretum hosts 'Madison Reads Leopold' each year, and for the past two years I've participated in reading Leopold's recount of honoring the last *Silphium* in a roadside cemetery in *Prairie Birthday*. The prairie ecosystem has been changed to such an extent, it's not recognized as prairie by most but instead, a corn field. Sampling remnant prairies in central WI (Chapter 2) felt like a treasure hunt; coming around a curve in the road and spotting the little knoll tucked into a sea of row-cropping. I was surprised to find short-grass prairie species in this region, often sunning themselves on steep south-facing slopes with shallow soils. The remnants lived up to their storied heterogeneity including steep hills with rocky, shallow soils, to very sandy soils, and wet low-lying areas likely submerged seasonally. The rich organic horizons atop gleyed soils underscoring periodic wet, anoxic conditions. Remnant prairies harbor carbon stocks that may take several generations to recover if lost to cultivation. Wet, poorly drained, high silt and clay, Mollisol prairies had greater SOC stocks, but edaphic properties didn't explain variation in rates of SOC concentration change. Both remnant and restored prairies maintained soil carbon concentrations over ~20 years on average, with about the same number of sites increasing as were decreasing, resulting in no general trend. At one restored prairie site, the world's oldest restored ecosystem (Curtis Prairie), I found soil carbon concentrations increased over 66 years, with most change happening in the first 15 years after establishment. Prairies provide many ecosystem functions including water filtration, erosion prevention, and essential habitat – making them key to climate adaptation. But my results make clear that they can't be considered a significant part of Wisconsin's contribution to mitigating anthropogenic climate change. The fact that deep, rich Mollisols developed over thousands of years of tallgrass prairie vegetation shows that prairies were net atmospheric C sinks under past climates, but in the early decades of the 21st Century, SOC sequestration in southern Wisconsin prairies was not the rule, aligning with other findings in southern Wisconsin agroecosystems (Dietz et al., 2024; Kucharik et al., 2006; Oates & Jackson, 2014; von Haden & Dornbush, 2017). That said, agroecosystems with soil disturbance are regularly sources of atmospheric C, and maintaining SOC is better than losing it. We must protect the few remnant prairies in southern Wisconsin (and their large stocks of SOC) from land use change, and nudge our agroecosystems toward perennial grasslands and savannas, while continuing to study the restoration process over decades and centuries. And of course, we must dramatically reduce fossil fuel combustion because no amount of land-use and land-cover change is likely to make soils carbon sinks in ways that significantly offset these atmospheric C sources.

The 21 prairies I resampled should serve as long-term experimental units for continued SOC monitoring. SOC stock comparisons are possible across the entire soil depth (i.e., 50 to 100 cm depending on inorganic C and bedrock) and sampling points are georeferenced. We observed declines in bulk density over 20 years suggesting organic matter likely is forming, but this did not translate to increases in SOC concentrations. Organic matter inputs may be accumulating, but if outputs via oxidation of older organic matter are equivalent, carbon accumulation will not be observed. Future sampling of these sites could provide clarity around changes in soil bulk density over time, with careful attention to replicating methods. The changing climate is a factor that future research must consider, in addition to the potential for directional plant community change. Tracking weather patterns and changes in rainfall and temperature by site could add context to how prairies are responding. Are the wet prairies becoming wetter and more waterlogged? If winters are milder, is there greater annual soil respiration in recent decades? SOC dynamics for these sites could be simulated with ecosystem models to better understand climate effects on SOC. Assessing pyrogenic carbon (PyC) in remnant and restored prairies, in relation to burn history could inform contributions of PyC to total C stocks. Methods to assess PyC are currently most apt in manipulated lab experiments rather than *in situ* quantitative assessment of PyC stocks. Lastly, including and understanding management effects on SOC change will be important for advancing restoration ecology.

I had the privilege and challenge of working within a long-term research trial at the UW-Madison Arboretum DIRT experiment (Chapter 1). It is a rare opportunity to partake in >60 years of an experimental trial. Professor Francis Hole's study of liter inputs on soil formation has withstood the test of time and remains relevant in the 21st Century. The restored prairie was resilient to litter manipulations, with similar stocks at 66 years of manipulation in treatments with either above-ground, below-ground, or both types of litter input – a somewhat surprising result given the treatments with both above- and below-ground litter inputs have likely received more total C inputs than systems with only one or the other. Further, grasslands are adapted to root inputs, and I expected the exclusion of roots would have dramatic effects on SOC. Instead, root exclusion effects were most evident at 10 to 25-cm depth increment, where we saw lower SOC stocks than burned treatments, and significant declines in bacterial and fungal abundance. Yet, when assessed across the entire soil profile, SOC stocks were similar to control. Future work should directly measure both the quantity and quality of carbon flowing into and out of each treatment to clarify if treatments receive similar amounts of carbon. Specifically, do root exclusion (Mulched) treatments receive greater inputs of carbon than other treatments as a result of years of thatch accumulation at the soil surface. Greater carbon in root exclusion inputs could explain similar SOC stocks by input type and how the carbon concentration 'caught up' to control, burned, and harvested after 66 years. The lack of carbon inputs in the Bare treatment has decreased SOC stocks, and we observed declines in both MAOC and POC fractions, indicating even persistent forms of SOC are decreasing when soil is bare. Bare soil is not characteristic of prairies, but is common in annual-row cropping agroecosystems – where soils often are bare for part of the year (post-harvest to planting) - likely reducing SOC.

Going forward our understanding of SOC and treatment effects of the DIRT experiment would be advanced by considering potential microclimate effects imposed by treatments, documenting species composition over time, and understanding shifts in soil fauna in response to litter manipulation. Maintenance of these experimental sites with clear documentation over time is key to ensuring future questions can be addressed with novel tools and frameworks. Historical gaps in DIRT record keeping and concerns about treatment boundaries for woodland DIRT sites are outlined in Chapter 1 Appendix.

The on-going and impending effects of climate change require us to consider mitigation and adaptation strategies. Federal conservation programs (Chapter 3) are a mechanism for gradual change, but largely support harm reduction in industrial agroecosystems, with just 20% of funding in Wisconsin supporting perennial agroecosystems. Local and federal conservation staff correlated to overall conservation spending, indicating the importance of technical service support to farmers. Confinement livestock systems received more federal dollars where local conservation staff were more abundant. Conservation staff numbers were also related to directing federal conservation dollars to perennial agroecosystems. Future work should focus on 'why' staff abundance seems to drive support for perennial conservation practices. More staff could relate to greater capacity for specialization, access to training and professional development, more extensive networks/farmer relationships, and/or administrative support for permitting and contract applications. Future work should also consider drivers of why certain counties have more staff (particularly at the county level) including county and state budgetary support, political will for conservation, and local environmental quality concerns. Conservation programs should take proactive approaches to maximize the environmental benefits of on-farm practices if we are to address environmental degradation and plan for climate mitigation and adaptation. The future of conservation should aspire to work across-farms to meet local and regional goals and prioritize cost-share support for practices with the greatest environmental benefits.

Over the last four years I've traipsed through tallgrass prairies, sifted through theses in the basement of the Soil Science Department, and traversed the interdisciplinary challenge of understanding and framing my work within the larger sociopolitical dynamics of how we manage our agroecosystems. Leopold may be pleased to know *Silphium* persists in the county - despite our largely mono-cropped agroecosystems - there are slivers of perenniality propelling new prairie birthdays across the landscape.

References

Dietz, C. L., Jackson, R. D., Ruark, M. D., & Sanford, G. R. (2024). Soil carbon maintained by perennial grasslands over 30 years but lost in field crop systems in a temperate Mollisol. *Communications Earth & Environment*, *5*(1), 1–8. https://doi.org/10.1038/s43247-024-01500-w

Kucharik, C. J., Fayram, N. J., & Cahill, K. N. (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. https://doi.org/10.1111/j.1365-2486.2005.01053.x

Oates, L. G., & Jackson, R. D. (2014). Livestock Management Strategy Affects Net Ecosystem Carbon Balance of Subhumid Pasture. *Rangeland Ecology & Management*, *67*(1), 19–29. https://doi.org/10.2111/REM-D-12-00151.1

von Haden, A. C., & Dornbush, M. E. (2017). Ecosystem carbon pools, fluxes, and balances within mature tallgrass prairie restorations. *Restoration Ecology*, *25*(4), 549–558. https://doi.org/10.1111/rec.12461