BELOWGROUND DYNAMICS OF PRAIRIE STRIPS IN U.S. MIDWEST CROPPING SYSTEMS

By

Corinn Elizabeth Rutkoski

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Integrative Biology – Doctor of Philosophy
Environmental Science and Policy – Dual Major

2024

ABSTRACT

The expansion of conventional row crop agriculture has altered soil biodiversity and function across the Midwest United States. The tallgrass prairie ecosystem that historically dominated this region is characterized by rich soil food webs that carry out important ecosystem services; therefore, restoring prairies within agricultural landscapes may reverse the harms wrought by intensive agricultural management. Prairie strips are a farm conservation practice in which tallgrass prairie vegetation is established in row crop fields, comprising up to 25% of a field area in linear strips ranging from 30 to 120 feet in width. Prairie strips reintroduce many features of the tallgrass prairie ecosystem to row crop farms, including increased plant, insect, and wildlife diversity, enhanced pollination, and reduced erosion and nutrient runoff. It remains unknown whether prairie strips also reintroduce belowground biota and soil processes characteristic of the tallgrass prairie. In this dissertation, I investigated the belowground changes associated with prairie strip establishment in Michigan and Iowa cropping systems.

In Chapter 1, I measured soil microbial community composition during the first four years of prairie strip establishment within two low-intensity cropping systems in Michigan. I found that prairie strips quickly enriched prairie-associated microbial phyla and functional groups within just a few months after prairie strip planting, that land use history is an important driver of prairie strip microbial community composition, and that prairie strips did not affect microbial communities of surrounding cropland soils, suggesting that prairie taxa do not spill over into surrounding cropland soils early in their establishment. To understand whether prairie strips have a stronger effect on adjacent cropland soil microbial communities later in their succession, I conducted a similar study at a site in Iowa with more mature prairie strips in Chapter 2. I showed that similar to newly planted prairie strips in Michigan, 12 year old prairie

strips altered soil microbial communities beneath the prairie strip, but not in adjacent cropland soils. Prairie strips also increased soil microbial biomass carbon and potential enzyme activities beneath the prairie strip, and increased potential activity of some extracellular enzymes in adjacent cropland soils.

In Chapter 3, I measured early soil carbon indicators and soil organic matter fractions during three years of prairie strip establishment and at multiple distances from prairie strips in two cropping systems. This study showed that prairie strips do not exhibit a linear increase in soil carbon indicators during three years of establishment, but some indicators do show a temporary increase compared to surrounding cropland. Prairie strips increased soil active C relative to adjacent cropland in 2 of the 3 years we measured. Prairie strips did not increase soil active C in surrounding cropland soils, nor did they increase C stocks in organic matter fractions after 3 years. Early increases in prairie strip soil active C suggest that prairie strips will show more pronounced increases in soil C stocks relative to cropland in coming years.

Finally, in Chapter 4, I examined prairie strips' effect on insecticide movement across the landscape and insecticide degradation by soil microbial communities. I measured the neonicotinoid clothianidin in soil, plant tissue, and groundwater. Prairie strips at this site accumulated neonicotinoids in plant tissue, but at concentrations below levels toxic to pollinator insects. This study also showed that prairie strips planted in cropland managed with neonicotinoid-treated seeds do not reduce neonicotinoid runoff into downslope soils. Lastly, I report findings from an experiment that tests degradation of neonicotinoid insecticides by soil microbial communities in prairie strips and surrounding cropland. Altogether, this dissertation shows that prairie strip effects on surrounding cropland is minimal, but beneath the strips, prairie strips quickly restore soil biota and soil C cycling functions of the tallgrass prairie.

In loving memory of Andy Fogiel,
whose generous and creative spirit lives on
in the Kellogg Biological Station community.

ACKNOWLEDGEMENTS

Thank you to my mentor, Sarah Evans, for helping me become a better communicator in research and in relationships, for exemplifying thoughtful and community-minded leadership, and for empowering me to weave my many interests into my work. Thank you to the many past and present members of the Evans Lab who have generously lent advice and hands-on help – Tayler Ulbrich, Brandon Kristy, Lukas Bell-Dereske, Jennifer Jones, Glade Bogar, Ceco Maples, Emily Burgess, Moriah Young, Caitlin Broderick, Zahraa Al Tameemi, Madaris Serrano-Perez, and especially lab manager Holly Vander Stel who makes this lab a wonderful place to work. Thank you to my committee – Lisa Schulte Moore, Phil Robertson, and Nick Haddad – for helping me make my research more meaningful and for always being available to answer my questions. Thank you to my collaborators at Iowa State University who helped me plan and execute my projects - Marshall McDaniel, Laura Burns, Dwayne Schrunk, Jessica Stephenson, Chris Witte, Jacob Studt, Cole Dutter, Maura Hall, and Tim Youngquist. Thank you to the many Kellogg Biological Station staff who provided logistical and administrative support for my projects, including Andy Widner, Brian Balcom, Sarah Reimer, Melanie Hitchcock, Kevin Kahmark, Stacey Vanderwulp, Dave Weed, Bridget Powers, Nate Stonerock, Daniel Gorentz, Stephan Ozminski, and Andy Fogiel. Thank you to my many mentors and friends who helped me connect science to societal needs – especially Kara Haas, Liz Schultheis, Nameer Baker, Kathryn Docherty, Brook Wilke, Misty Klotz, Sarah Roy, and Julie Doll. Thank you to my dear friends for coloring this chapter of my life with adventure and laughter and growth – Kristi Droppers, Alice Dykstra, Lindsey Kemmerling, Stephanie Clark, Trevor Grabill, Cathy McMinn, Tayler Ulbrich, Carson Branch, Morgan Bradetich, Anna Lee Roeder, Stephen Tuscher, Daniela Herrera, Allison La Chapelle, Ally Hutchens, Pam Woodruff, and Ellen Badger Hanson. Thank

you to Karlie Rautenberg and Rachel Smead for a lifetime of support and sisterhood. Thank you to my family – the Braggs and Telfers and Rutkoskis and Cromers and Logans and many others – for cheering me on. Thank you to Robert Logan for making me laugh every single day of the last six years and for helping me see what I am capable of. Thank you to my sister, Caitlin, for showing me just how loyal and loving a person can be. Thank you to my grandfather Brian Bragg for teaching me how to live a life of curiosity, adventure, and kindness. Thank you to my grandmothers, Marilyn Raupp Rutkoski, Nancy Burnstrum Telfer, and Susan Stong Bragg, for exemplifying reverence for nature and lifelong personal growth, for teaching me to allow lightness and silliness into every day, and for empowering me to create. And finally, thank you to my parents, Lisa and David, for a lifetime of sacrifices and support and love that enabled me to achieve this milestone.

TABLE OF CONTENTS

INTRODUCTION

Agricultural expansion and intensification in the Midwest United States has led to an extreme loss of biodiversity and ecosystem services (IPBES 2019; UNEP and IUCN 2021). Belowground biodiversity and functioning in soils have also declined due to intensive agricultural land management (Grandy and Robertson 2006; Poeplau et al. 2011; Sanderman et al. 2017; FAO et al. 2020). The use of large, heavy equipment in agricultural fields can cause both compaction and fragmentation of soil aggregates, and over time, soil that is regularly disturbed exhibits lower water infiltration, greater nitrous oxide emissions, greater chemical runoff, and lower crop yields (Van Veen and Paul 1981; Grandy and Robertson 2006; Syswerda et al. 2011; Robertson et al. 2015; Millar and Robertson 2015; Cusser et al. 2020). Agrochemicals applied as seed treatments or foliar sprays infiltrate into soil and alter soil pH (Brady and Weil 2017), shift belowground community composition (Fierer and Jackson 2005; Jach-Smith and Jackson 2018), and limit soil organic matter formation (Millar and Robertson 2005; Brady and Weil 2017). Compared to diverse perennial systems, annual monocultures also show a reduced capacity for carbon storage, due to lower belowground biomass, litter additions, and diversity of pore space for microbial carbon use (Córdova et al. 2018; Kravchenko et al. 2019; Sprunger et al. 2017; Sprunger et al. 2018). The U.S. Midwest needs land management strategies that deliver crop yields without compromising soil functionality.

The tallgrass prairie ecosystem that historically comprised most of the U.S. Midwest (Li et al. 2023) harbors a diverse soil food web that carries out critical ecosystem services (Van Veen and Paul 1981; Anderson 2006; Helzer 2009; McClain et al. 2021). Reintroducing prairie within agricultural landscapes may therefore reverse losses of soil biota and functionality that occurred during agricultural intensification. The Iowa State University Science-based Trials of

Rowcrops Integrated with Prairie Strips (STRIPS) research team developed a management strategy called prairie strips to implement prairie systems on Midwestern farms. Prairie strips are strips of tallgrass prairie vegetation established in row crop fields, comprising up to 25% of a field area in linear strips ranging from 30 to 120 feet in width (Schulte et al. 2017). The STRIPS team has published over a decade's worth of research demonstrating socioeconomic and ecological benefits of prairie strips, both within the prairie areas and in the cropland surrounding prairie strips. Prairie strips have also now been established at Michigan State University's W.K. Kellogg Biological Station to compare prairie strip effects across Midwest states.

Prairie strips provide suite of ecological benefits on farms without compromising crop yields beyond the area taken out of production (Schulte et al. 2017). As habitat corridors, prairie strips support increased diversity of insects, birds, and other wildlife within the strips and, in some cases, in surrounding cropland (Schulte et al. 2017; Kemmerling et al. 2022; Kemmerling et al. 2023; Borchardt et al. 2023; Stephenson et al. 2024). Prairie strips also support healthier and more productive pollinator populations (Zhang et al. 2023). At the landscape scale, across both prairie and cropland, prairie strips reduce erosion (Schulte et al. 2017; Stephenson et al. 2024), nutrient runoff (Hernandez-Santana et al. 2013, Pérez-Suárez et al. 2014, Zhou et al. 2014; Hladik et al. 2017) and transport of antimicrobial resistance contaminants from manure application (Alt et al. 2023) into downslope and offsite areas. Most research on prairie strips has focused on their aboveground effects, but prairie strips also have the potential to restore the diversity and functional capacity of prairie soil communities.

Belowground effects of prairie strips may be similar those of larger prairie restorations. Prairie restoration enhances soil structure (Baer et al. 2002, Herzberger et al. 2014, Sprunger et al. 2017, Mackelprang et al. 2018, Kravchenko et al. 2019), increases organic matter inputs

(Johnson et al. 2001, Allison et al. 2005, Köhl et al. 2014, Säle et al. 2015, Carson and Zeglin 2018, Zhu et al. 2020), and changes the composition of soil food webs (Bach et al. 2018, Upton et al. 2018, Plassart et al. 2008, Barber et al. 2017, Mackelprang et al. 2018). The unique configuration of prairie strips' within cropland may also cause prairie strip establishment to effect soil biota and function in surrounding cropland. Organisms may disperse from prairie strip soils to cropland soils (Warmink et al. 2011, Coleman and Wall 2015, van Elsas et al. 1991, Choudoir et al. 2018, Langenheder and Lindström 2019, Chaudhary et al. 2020, Kemmerling et al. 2022), or prairie roots may extend into cropland and contribute organic matter (Sprunger et al. 2018; Middleton et al. 2018). On the other hand, cropland management activities could alter abiotic conditions (Weil and Brady 2017, Maher et al. 2010, Stewart et al. 2007) and inhibit soil biota from successfully dispersing from prairie strips (Jeske et al. 2018; Säle et al. 2015; Warmink et al. 2011; Johnsen et al. 2001; Ferrero et al. 2022; Dutter et al. 2024).

Only a handful of studies have measured belowground responses to prairie strips, and most have taken place in mature prairie strips after 10 or more years of establishment. Older prairie strips exhibit enhanced soil carbon pools compared to cropland, including greater dissolved organic carbon (Smith et al. 2014), soil organic carbon (Pérez-Suárez et al. 2014), and microbial biomass carbon (Dutter et al. 2024), but have little effect on soils in adjacent cropland (Dutter et al. 2023; Dutter et al. 2024). One of the few studies in newer prairie strips showed increased water infiltration in prairie strips compared to surrounding cropland after 3 years, further reducing landscape-level soil erosion and agrochemical runoff (Henning et al. 2024). The rate at which belowground changes in prairie strips occur, the degree to which prairie strips affect soils in surrounding cropland, and the ways in which effects differ across cropping systems are all key knowledge gaps in prairie strip research.

My dissertation addresses these gaps by describing how prairie strips affect belowground communities and their functioning in cropping systems across the Midwest. In Chapter 1, I investigate soil microbial communities in newly-established prairie strips and adjacent cropland in two Michigan cropping systems. I measure bacterial and fungal community composition during the first four years of prairie strip establishment and at multiple distances from prairie strips within two low-intensity cropping systems. The study clarifies whether, and how quickly, prairie strip soil communities can be expected to resemble those of larger-scale prairie restorations. The project also evaluates the effect of agricultural land use history in prairie strip communities, and the extent to which prairie strips can introduce bacteria and fungi to surrounding cropland soils early in their establishment.

In Chapter 2, I test whether prairie strips have a stronger effect on adjacent cropland soil microbial communities later in their succession. In row crop fields with prairie strips and without prairie strips in Iowa, I measure soil bacterial and fungal communities, as well as soil carbon and nitrogen, over two years. This chapter shows the extent to which prairie strips can, and cannot, be expected to alter soil microbial communities beneath the strip and in adjacent cropland soils after 12 years of establishment in a no till cropping system. The study also shows how planting prairie strips in a contour configuration may alter surrounding soil nutrient cycling differently under corn and soybean crop rotation phases.

Chapter 3 builds on these findings to determine whether early compositional changes in prairie strip soil microbial communities translate to changes in soil carbon cycling in prairie strips. I measure early soil carbon indicators, as well as carbon in soil organic matter fractions, in prairie strips and surrounding cropland soils in two Michigan cropping systems. This chapter clarifies whether prairie strips build soil carbon stocks within their first three years, and also

shows how soil carbon fractions in both prairie strips and cropland reflect crop rotational phases and management practices. Finally, Chapter 4 describes how prairie strips affect landscape-scale movement of neonicotinoid insecticides, and whether prairie strips accumulate insecticides in their soils and plant tissue. In this study, I measure neonicotinoids in surface soil, deep soil, plant tissue, and groundwater at a commercial farm in central Iowa comprised of two catchments: one with prairie strips and one without prairie strips. The study shows whether prairie strips planted amid insecticide-treated crop seeds can serve as insecticide-free habitat for visiting insects, and whether they can reduce insecticide runoff into downslope and offsite areas.

In summary, this dissertation aims to identify how prairie strips affect belowground biodiversity and function during the first few years of their establishment to address knowledge gaps in the agroecology literature and to help land managers make sound decisions about prairie strip implementation.

LITERATURE CITED

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, 1412–1421. https://doi.org/10.2136/sssaj2004.0252

Alt, L.M., Flater, J.S., Howe, A., Moorman, T.B., Schulte, L.A., Soupir, M.L., 2023. Prairie strips remove swine manure associated antimicrobial resistance genes and bacteria from runoff. Agriculture, Ecosystems & Environment 349, 108469. https://doi.org/10.1016/j.agee.2023.108469

Anderson, R.C., 2006. Evolution and origin of the Central Grassland of North America: climate, fire, and mammalian grazers. Journal of The Torrey Botanical Society 133, 626–647. https://doi.org/10.3159/1095-5674(2006)133[626:eaootc]2.0.co;2

Bach, E.M., Williams, R.J., Hargreaves, S.K., Yang, F., Hofmockel, K.S., 2018. Greatest soil microbial diversity found in micro-habitats. Soil Biology & Biochemistry 118, 217–226. https://doi.org/10.1016/j.soilbio.2017.12.018

Baer, S.G., Kitchen, D.J., Blair, J.M., Rice, C.W., 2002. Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecological Applications 12, 1688–1701. https://doi.org/10.1890/1051-0761(2002)012[1688:ciesaf]2.0.co;2

Barber, N.A., Chantos-Davidson, K.M., Peralta, R.A., Sherwood, J.P., Swingley, W.D., 2017. Soil microbial community composition in tallgrass prairie restorations converge with remnants across a 27-year chronosequence. Environmental Microbiology 19, 3118–3131. https://doi.org/10.1111/1462-2920.13785

Borchardt, K. Kadelka, C., Schulte, L.A., Toth, A.L. 2023. An ecological networks approach reveals restored native vegetation benefits wild bees in agroecosystems. Biological Conservation 287, 110300–110300. https://doi.org/10.1016/j.biocon.2023.110300

Carson, C.M., Zeglin, L.H., 2018. Long-term fire management history affects N-fertilization sensitivity, but not seasonality, of grassland soil microbial communities. Soil Biology & Biochemistry 121, 231–239. https://doi.org/10.1016/j.soilbio.2018.03.023

Chaudhary, B., Nolimal, S., Sosa-Hernández, M.A., Egan, C., and Kastens, J. 2020. Trait-based aerial dispersal of arbuscular mycorrhizal fungi. New Phytologist 228:238-252. https://doi.org/10.1111/nph.16667

Choudoir, M.J., Barberán, A., Menninger, H.L., Dunn, R.R. and Fierer, N., 2018. Variation in range size and dispersal capabilities of microbial taxa. Ecology 99(2), pp.322-334. https://doi.org/10.1002/ecy.2094

Coleman, D.C. and Wall, D.H., Soil Fauna: Occurrence, Biodiversity, and Roles in Ecosystem Function in: Paul, E.A. (Ed) Soil Microbiology, Ecology and Biochemistry (Fourth Edition), Academic Press, 2015, pp. 111-149. https://doi.org/10.1016/B978-0-12-415955-6.00005-0

Córdova, S.C., Olk, D.C., Dietzel, R., Mueller, K.E., Archontouilis, S.V., Michael J. Castellano, Castellano, M.J., 2018. Plant litter quality affects the accumulation rate, composition, and stability of mineral-associated soil organic matter. Soil Biology & Biochemistry 125, 115–124. https://doi.org/10.1016/j.soilbio.2018.07.010

Cusser, S., Bahlai, C.A., Swinton, S.M., Robertson, G.P., Haddad, N.M., 2020. Long-term research avoids spurious and misleading trends in sustainability attributes of no-till. Global Change Biology 26, 3715–3725. https://doi.org/10.1111/gcb.15080

Dutter, C., Damiano, L.A., Niemi, J., Miller, B.A., Schulte L.A., Liebman, M., Helmers, M., Cruse, R., and McDaniel, M.D., 2023. Contour Prairie Strips Affect Surrounding Soil but Only Have Slight Effects on Crop Yield. Field Crops Research 296: 108905. https://doi.org/10.1016/j.fcr.2023.108905.

Dutter, C., Rutkoski, C.E., Evans, S.E., McDaniel, M.D., Contour Prairie Strips Alter Soil Microbial Communities and Functioning Both Below the Prairie Strip and in Adjacent Cropland. Available at SSRN: https://ssrn.com/abstract=4542718 or http://dx.doi.org/10.2139/ssrn.4542718

FAO, ITPS, GSBI, SCBD, and EC. 2020. State of knowledge of soil biodiversity - Status, challenges and potentialities, Report 2020. Rome, FAO.

Ferrero, F., Prencipe, S., Tabacco, E., Ferrocino, I., Borreani, G., Spadaro, D. 2022. Effects of a prothioconazole- and tebuconazole-based fungicide on the yield, silage characteristics, and fungal mycobiota of corn harvested and conserved as whole-crop and high-moisture ear silages. Journal of Applied Microbiology. https://doi.org/10.1093/jambio/lxac033

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103, 626–631. https://doi.org/10.1073/pnas.0507535103

Grandy, A.S., Robertson, G.P., 2007. Land-Use Intensity Effects on Soil Organic Carbon Accumulation Rates and Mechanisms. Ecosystems 10, 59–74. https://doi.org/10.1007/s10021-006-9010-y

Helzer, Chris. The Ecology and Management of Prairies in the Central United States. 1 ed. University of Iowa Press, 2009. Project MUSE, https://doi.org/10.1353/book3566.

Herzberger, A., Duncan, D.S., Jackson, R.D., 2014. Bouncing Back: Plant-Associated Soil Microbes Respond Rapidly to Prairie Establishment. PLOS ONE 9. https://doi.org/10.1371/journal.pone.0115775

Hernandez-Santana, V., Xiaobo Zhou, Zhou, X., Helmers, M.J., Asbjornsen, H., Kolka, R., Tomer, M.D., 2013. Native prairie filter strips reduce runoff from hillslopes under annual row-crop systems in Iowa, USA. Journal of Hydrology 477, 94–103. https://doi.org/10.1016/j.jhydrol.2012.11.013

Henning, E.J., Kolka, R.K., Helmers, M.J. 2024. Infiltration within native prairie vegetative strips embedded in row crop fields across Iowa. Journal of Soil and Water Conservation 79 (1) 43-52; https://doi.org/10.2489/jswc.2024.00146

Hladik M.L., Bradbury S., Schulte L.A., Helmers M., Witte C., Kolpin D.W., Garrett J.D., Harris M. 2017. Neonicotinoid insecticide removal by prairie strips in row-cropped watersheds with historical seed coating use. Agriculture, Ecosystems & Environment 241:160-167. https://doi.org/10.1016/j.agee.2017.03.015

Intergovernmental Platform on Biodiversity and Ecosystem Services (IBES). 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. S. Díaz, J. Settele, E. S. Brondízio, H. T. Ngo, M. Guèze, J. Agard, A. Arneth, P. Balvanera, K. A. Brauman, S. H. M. Butchart, K. M. A. Chan, L. A. Garibaldi, K. Ichii, J. Liu, S. M. Subramanian, G. F. Midgley, P. Miloslavich, Z. Molnár, D. Obura, A. Pfaff, S. Polasky, A. Purvis, J. Razzaque, B. Reyers, R. Roy Chowdhury, Y. J. Shin, I. J. Visseren-Hamakers, K. J. Willis, and C. N. Zayas (eds.). IPBES secretariat, Bonn, Germany. 56 pages. https://doi.org/10.5281/zenodo.3553579

Jach-Smith, L.C., Jackson, R.D., 2018. N addition undermines N supplied by arbuscular mycorrhizal fungi to native perennial grasses. Soil Biology & Biochemistry 116, 148–157. https://doi.org/10.1016/j.soilbio.2017.10.009

Johnsen, K., Jacobsen, C.S., Torsvik, V., Sørensen, J.T., 2001. Pesticide effects on bacterial diversity in agricultural soils – a review. Biology and Fertility of Soils 33, 443–453. https://doi.org/10.1007/s003740100351

Kemmerling, L.R., Rutkoski, C.E., Evans, S.E., Helms, J.A., Cordova-Ortiz, E.S., Smith, J.A., Vázquez Custodio, J.A., Vizza, C., Haddad, N.M. Prairie Strips and Lower Land Use Intensity Increase Biodiversity and Ecosystem Services. Frontiers in Ecology and Evolution. https://doi.org/10.3389/fevo.2022.833170

Kemmerling, L.R., McCarthy, A.C., Brown, C.S., Haddad, N. 2023. Butterfly biodiversity increases with prairie strips and conservation management in row crop agriculture. Insect Conservation and Diversity. https://doi.org/10.1111/icad.12675

Köhl, L., Oehl, F., van der Heijden, M.G.A., 2014. Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. Ecological Applications 24, 1842–1853. https://doi.org/10.1890/13-1821.1

Kravchenko, A., Guber, A.K., Razavi, B.S., Koestel, J., Quigley, M., Robertson, G. P.,

Kuzyakov, Y., 2019. Microbial spatial footprint as a driver of soil carbon stabilization. Nature Communications 10, 3121–3121. https://doi.org/10.1038/s41467-019-11057-4

Langenheder, S., Lindström, E.S., 2019. Factors influencing aquatic and terrestrial bacterial community assembly. Environmental Microbiology Reports 11:306-315. doi:10.1111/1758-2229.12731.

Li, X., Tian, H., Lu, C., Pan, S., 2023. Four-century history of land transformation by humans in the United States (1630–2020): annual and 1\,km grid data for the HIStory of LAND changes (HISLAND-US). Earth System Science Data 15, 1005–1035. https://doi.org/10.5194/essd-15-1005-2023

Mackelprang, R., Grube, A.M., Lamendella, R., da Conceição Jesus, E., Copeland, A., Liang, C., Jackson, R.D., Rice, C.W., Kapucija, S., Parsa, B., Tringe, S.G., Tiedje, J.M., Jansson, J.K., 2018. Microbial Community Structure and Functional Potential in Cultivated and Native Tallgrass Prairie Soils of the Midwestern United States. Frontiers in Microbiology 9, 1775–1775. https://doi.org/10.3389/fmicb.2018.01775

McClain, W.E., Charles M. Ruffner, Ruffner, C.M., Ebinger, J.E., Spyreas, G., 2021. Patterns of Anthropogenic Fire within the Midwestern Tallgrass Prairie 1673–1905: Evidence from Written Accounts. Natural Areas Journal 41, 283–300. https://doi.org/10.3375/20-5

Millar, N. and G. P. Robertson. 2015. Nitrogen transfers and transformations in row-crop ecosystems. Pages 213-251 in S. K. Hamilton, J. E. Doll, and G. P. Robertson, editors. The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability.

Pérez-Suárez, M., Castellano, M.J., Kolka, R.K., Asbjornsen, H., Helmers, M.J., 2014. Nitrogen and carbon dynamics in prairie vegetation strips across topographical gradients in mixed Central Iowa agroecosystems. Agriculture, Ecosystems & Environment 188, 1–11. https://doi.org/10.1016/j.agee.2014.01.023

Plassart, P., Vinceslas, M.A., Gangneux, C., Mercier, A., Barray, S., Laval, K., 2008. Molecular and functional responses of soil microbial communities under grassland restoration. Agriculture, Ecosystems & Environment 127, 286–293. https://doi.org/10.1016/j.agee.2008.04.008

Robertson, G. Philip, Robertson, G. P., Gross, K.L., Hamilton, S.K., Landis, D.A., Schmidt, T.J., Schmidt, T.M., Snapp, S.S., Swinton, S.M., 2015. Farming for ecosystem services: An ecological approach to production agriculture. BioScience 33–53. https://doi.org/10.1093/biosci/biu037

Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., Urs Zihlmann, Zihlmann, U., van der Heijden, M.G.A., Oehl, F., Oehl, F., 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biology & Biochemistry 84, 38–52. https://doi.org/10.1016/j.soilbio.2015.02.005

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

Schulte, L.A., Niemi, J., Helmers, M.J., Liebman, M., Arbuckle, J.G., David E. James, James, D.E., Kolka, R.K., O'Neal, M.E., Tomer, M.D., Tomer, M.D., Tyndall, J.C., Asbjornsen, H., Drobney, P., Neal, J., Van Ryswyk, G., Witte, C., 2017. Prairie strips improve biodiversity and the delivery of multiple ecosystem services from corn–soybean croplands. Proceedings of the National Academy of Sciences of the United States of America 114, 11247–11252. https://doi.org/10.1073/pnas.1620229114

Smith, T.E., Kolka, R.K., Zhou, X., Kolka, R., Tomer, M.D., Cruse, R. Effects of native perennial vegetation buffer strips on dissolved organic carbon in surface runoff from an agricultural landscape. *Biogeochemistry* 120, 121–132 (2014). https://doi.org/10.1007/s10533-014-9985-y

Sprunger, C.D., Oates, L.G., Jackson, R.D., Robertson, G.P., 2017. Plant community composition influences fine root production and biomass allocation in perennial bioenergy cropping systems of the upper Midwest, USA. Biomass & Bioenergy 105, 248–258. https://doi.org/10.1016/j.biombioe.2017.07.007

Sprunger, C.D., Robertson, G.P., 2018. Early accumulation of active fraction soil carbon in newly established cellulosic biofuel systems. Geoderma 318, 42–51. https://doi.org/10.1016/j.geoderma.2017.11.040

Stephenson, J.A., M. Liebman, J. Niemi, R. Cruse, J. Tyndall, C. Witte, D. James, and M. Helmers. 2024. The influence of prairie strips sown in midwestern corn and soybean fields on sediment discharge throughout the year. Journal of Soil and Water Conservation: In press.

Stephenson, M.D., Schulte, L.A., Klaver, R.W. 2024. The relative contributions of habitat area, configuration, and vegetative diversity on snake and lizard presence in agricultural landscapes. Conservation Science and Practice. https://doi.org/10.1111/csp2.13100

Syswerda, S.P., Corbin, A.T., Mokma, D.L., Kravchenko, A., Robertson, G. Philip, Robertson, G. P., 2011. Agricultural Management and Soil Carbon Storage in Surface vs. Deep Layers. Soil Science Society of America Journal 75, 92–101. https://doi.org/10.2136/sssaj2009.0414

United Nations Environment Programme and International Union for Conservation of Nature. 2021. Nature-based solutions for climate change mitigation. Nairobi and Gland. https://www.unep.org/resources/report/nature-based-solutions-climate-change-mitigation

Upton, R.N., Bach, E.M., Hofmockel, K.S., 2018. Belowground response of prairie restoration and resiliency to drought. Agriculture, Ecosystems & Environment 266, 122–132. https://doi.org/10.1016/j.agee.2018.07.021

van Elsas, J.D., Trevors, J.T. and van Overbeek, L. S., 1991. Influence of soil properties on the vertical movement of genetically-marked pseudomonas fluorescens through large soil microcosms. Biology and Fertility of Soils, 10(4), 249-255. https://doi.org/10.1007/BF00337375

Van Veen, J.A., Paul, E.A., 1981. Organic carbon dynamics in grassland soils. Background information and computer simulation. Canadian Journal of Soil Science 61, 185–201. https://doi.org/10.4141/cjss81-024

Warmink, J., Nazir, R., Corten, B. and van Elsas, J.D., 2011. Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. Soil Biology and Biochemistry 43:760-765. http://doi.org/10.1016/j.soilbio.2010.12.009

Weil, R.R., Brady, N.C., 2017. Soil Organic Matter, in: The Nature and Properties of Soil Fifteenth Edition. Pearson Education, Inc., pp. 526–582.

Zhang, G., Murray, C. J., St. Clair, A. L., Cass, R. P., Dolezal, A. G., Schulte, L. A., Toth, A. L., & O'Neal, M. E. (2023). Native vegetation embedded in landscapes dominated by corn and soybean improves honey bee health and productivity. Journal of Applied Ecology, 60, 1032–1043. https://doi.org/10.1111/1365-2664.14397

Zhou X., Helmers M.K., Asbjornsen H., Kolka R., Tomer M.D. and Cruse R.M. 2014. Nutrient removal by prairie filter strips in agricultural landscapes. Journal of Soil and Water Conservation 69(1): 54-64 https://doi.org/10.2489/jswc.69.1.54.

Zhu, L., Dickson, T.L., Zhang, Z., Dere, A., Xu, J., Bragg, T.B., Tapprich, W., Lu, G., 2021. Effects of burning and mowing on the soil microbiome of restored tallgrass prairie. European Journal of Soil Science 72, 385–399. https://doi.org/10.1111/ejss.12980

CHAPTER 1: PRAIRIE STRIPS QUICKLY ALTER SOIL BACTERIAL AND FUNGAL COMMUNITIES IN STRIPS, BUT NOT IN SURROUNDING CROPLAND

ABSTRACT

The conversion of tallgrass prairie to row crop agriculture in the U.S. Midwest has reduced soil biodiversity and function. Prairie strips are zones of perennial vegetation integrated into row crop farm fields that can increase native plant, insect, and wildlife diversity in agricultural landscapes, and may also enrich belowground diversity of soil bacteria and fungi. Within a 35 year old cropping system experiment, we introduced prairie strips in two treatments - one with reduced chemical inputs and one with no chemical inputs – and measured soil microbial communities every year for the first four years of prairie strip establishment, hypothesizing that communities will shift in soils under prairie vegetation, and that subsequent dispersal from prairie communities will alter composition in surrounding cropland. We assessed alpha diversity, beta diversity, and abundance of prairie-associated microbial phyla in prairie strip soils and in cropland soils at multiple distances from prairie strips. We found that as early as two months after planting, prairie strip bacterial and fungal communities diverged from cropland community composition, exhibited higher diversity than in cropland soils, and were enriched in microbial phyla characteristic of remnant tallgrass prairie soils. Prairie strips harbored different communities in the two cropping systems, indicating that land use history shapes communities early in prairie strip establishment. Microbial communities in surrounding croplands were not altered by the presence of prairie strips, neither on average nor by distance. We show that prairie strips restore tallgrass prairie soil biota in multiple cropping systems, but prairie taxa are not likely to spill over into even low-intensity cropland soils due to cropland management practices that appear to inhibit microbial dispersal and establishment.

INTRODUCTION

Tallgrass prairie ecosystems contain highly diverse soil food webs that carry out essential ecosystem functions, including decomposition, nutrient cycling, soil aggregation, and disease suppression (Bach and Hofmockel 2016; Sprunger and Robertson 2018; vanVeen and Paul 1981; Shaw et al. 2016; Bakker et al. 2010). The proliferation of large-scale agriculture in the U.S. has led to an almost complete loss of the tallgrass prairie ecosystem and its perennial cover, rich soil biodiversity, and broad ecological functions (Samson and Knopf 1994; Syswerda et al. 2011; Tsiafouli et al. 2013; Gardi et al. 2013; Robertson et al. 2014; Wagg et al. 2014; Geisen et al. 2019; Banjeree et al. 2019). There is a need for land management strategies that support crop production while restoring habitat for soil biota.

Prairie strips are zones of prairie vegetation planted on row crop farms to support multiple conservation outcomes, and they may also increase belowground biodiversity and functionality in soils (USDA 2020, USDA 2022). In both Iowa and Michigan, converting 10% of a row crop farm to prairie strips has been shown to increase plant, bird, and insect diversity within months to years after planting (Schulte et al. 2017; Kemmerling et al. 2022; Kemmerling et al. 2023). Soon after establishment, prairie strips change soil physiochemical properties, including soil erodibility (Stephenson et al. 2024), infiltration (Hernandez-Santana et al. 2013), and organic carbon and nitrogen pools (Iqbal et al. 2014; Smith et al. 2014). Prairie strips may also shift belowground communities of soil fauna early in their establishment, including soil bacterial and fungal communities that carry out key ecosystem functions (Schmidt et al. 2007; Morris and Blackwood 2007) including plant nutrient acquisition, stress tolerance, pathogen resistance, and community succession (Lekberg and Koide 2014; Poole et al. 2018; Koziol and Bever 2017), as well as soil carbon storage (Zhang et al. 2013; Kallenbach et al. 2016).

Microbial changes in prairie strips may be similar to those that occur in larger prairie restorations (Badger-Hanson and Docherty 2022). Microbial biomass often increases following prairie restoration due to soil physical changes such as enhanced soil aggregation via reduced tillage and greater root biomass (Baer et al. 2002, Herzberger et al. 2014, Sprunger et al. 2017, Mackelprang et al. 2018, Kravchenko et al. 2019), as well as chemical changes, including organic matter inputs from mowing, grazing, rhizodeposition, and prescribed fire (Johnson et al. 2001, Allison et al. 2005, Köhl et al. 2014, Säle et al. 2015, Carson and Zeglin 2018, Zhu et al. 2020). Overall microbial diversity does not respond consistently to prairie restoration, and has been shown to increase (Bach et al. 2018, Upton et al. 2018, Plassart et al. 2008), decrease (Barber et al. 2017, Mackelprang et al. 2018), or show no change (Jangid et al. 2010) relative to agricultural soils. Microbial diversity also can be sensitive to plant successional stage (Barber et al. 2023), and is not necessarily indicative of microbial function (Shade 2017).

Changes in plant community composition and soil edaphic properties during prairie restoration act as environmental filters that shape microbial assemblages (Fierer and Jackson 2006; Lauber et al. 2008; Hargreaves et al. 2015) and cause particular fungal and bacterial groups to increase in abundance (Allison et al. 2005, Herzberger et al. 2014, Duncan et al. 2016, Barber et al. 2017, Mackelprang et al. 2018, Barber et al 2023), and a similar microbial selection process may occur during prairie strip establishment. Compared to cropland soils, restored prairie soils contain a higher proportion of carbon-rich fungal biomass and gram-negative bacteria (Drijber et al. 2000, Allison et al. 2005, Herzberger et al. 2014), as well as higher abundances of particular taxonomic groups (Allison et al. 2005, Herzberger et al. 2014, Duncan et al. 2016, Barber et al. 2017, Mackelprang et al. 2018, Barber et al 2023). While these prairie-associated microbes may be enriched on farms through the establishment of prairie strips, agricultural land use legacies and

edge effects of cropland management could make prairie strip microbial communities unique from other restorations.

Establishing prairie strips may also shift microbial communities in adjacent cropland soils. Microbes may disperse from prairie strips to cropland soils via air, water, fungal hyphae, and microbivores (Warmink et al. 2011, Coleman and Wall 2015, van Elsas et al. 1991, Evans et al. 2017; Choudoir et al. 2018, Langenheder and Lindström 2019, Chaudhary et al. 2020). Biotic spillover has already been observed for higher order taxa in fields with prairie strips. Kemmerling et al. (2022) report overall greater abundance of dung beetles and spiders one year after prairie strip establishment, and abundances that decline with distance from the prairie strip. There is also evidence that soil nematodes disperse outward from prairie strips into neighboring cropland soils; in Michigan croplands, fungivore nematode abundance and nematode community maturity indices decrease with distance from the prairie strip (Tvisha Martin, personal communication). On the other hand, cropland management activities including crop rotation sequence, agrochemical inputs, and soil disturbance could alter cropland abiotic conditions so strongly that any microbes dispersing from prairie strips are unable to colonize and persist. As a result, prairie strip and cropland communities could be filtered into two distinct assemblages (Johnsen et al. 2001, Fierer and Jackson 2006, Jangid et al. 2008, Jones et al. 2022). Indeed, Dutter and Rutkoski et al. (2024) recently measured microbial communities within and adjacent to 12-year-old prairie strips in Iowa and showed that mature prairie strips and adjacent cropland had distinct bacterial and fungal community composition, and that distance from prairie strip (0.3 m to 9 m from the prairie strip edge) was not an important predictor of community composition in cropland soils. This suggests that either dispersal is negligible, or more likely, that filtering due to cropland management overrides establishment of any novel taxa and spillover signature.

Here we leverage a 35 year old land use experiment where prairie strips have been planted in two cropping systems to test the hypothesis that prairie strip soil microbial communities diverge from surrounding cropland communities, exhibit increased abundance of prairie-associated microbial taxa commonly found in prairie restorations (Dutter et al. 2024, Barber et al. 2017), and show distinct community structure in each cropping system due to land use history (Jangid et al. 2011). We also hypothesize that prairie strips will change bacterial and fungal communities of surrounding cropland soils and increase the abundance of prairie-associated microbial phyla via dispersal, as has been observed for higher order taxa (Kemmerling et al. 2022).

METHODS

Site description

This study was conducted at the W.K. Kellogg Biological Station Long Term Ecological Research (KBS LTER) site located in Hickory Corners, Michigan, United States (occupied Anishinaabe land, 85° 24'W, 42° 24' N). The KBS LTER site is comprised of two mixed mesic Typic Hapludalf soil series – Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) – and site topography is nearly level with some small rolling hills (Crum and Collins 1995, Robertson and Hamilton 2015). The average annual temperature January-December is 9.74°C and the average annual precipitation January-December is 1005 mm per year (Robertson and Hamilton 2015).

The KBS LTER Main Cropping System Experiment (MCSE) was established in 1987, and includes a matrix of replicated experimental plots managed under a variety of annual, perennial and unmanaged successional systems. Our study took place in two of the annual cropping system treatments – Reduced Input and Biologically Based (Organic) – each situated in a randomized complete block design with 1 ha (90 x 110 m) plots and each replicated in six blocks (Figure 1.1). Both treatments are planted with a corn-soybean-winter wheat rotation that includes a red clover

cover crop following wheat prior to corn, and a rye cover crop following corn prior to soybean. Reduced Input plots are planted with pesticide-treated winter wheat and corn seed and untreated soybean seed, receive banded herbicide and starter nitrogen fertilizer every year, and receive preventative fungicide (Prosaro, Bayer CropScience, U.S.) during winter wheat years to prevent wheat head scab. Fertilizer, herbicide, and fungicide in the Reduced Input treatment are each applied in 33% of the row crop field area (Robertson and Hamilton 2015). Organic plots are planted with untreated corn, soybean, and wheat seed and receive no chemical inputs, compost, or manure. Both Reduced Input and Organic plots receive post-planting cultivation each year, and Organic plots are rotary-hoed to control weeds (Figure 1.2). To summarize, the cropping systems differ in agrochemical inputs and tillage frequency: Reduced Input plots receive agrochemical inputs (N fertilizer, herbicides, and pesticides) and infrequent rotary hoeing, while Organic plots receive no agrochemical inputs and increased rotary hoeing (Figure 1.2).

In April 2019, a prairie seed mix was sown down the center of each Reduced Input and Organic plot, configured as a 15 m wide strip running parallel to crop rows and comprising five percent of each plot area (Figure 1.1). The seed mix, purchased from Native Connections in Kalamazoo, Michigan, United States, was comprised of four grass species and 18 forb species (listed in Kemmerling et al. 2022). To reduce weeds and promote establishment of seeded species, prairie strips were mowed in June 2019 (after soil sample collection) and July 2020, and underwent prescribed burning in March 2021 and April 2022. Prairie strips in Reduced Input plots received potash and phosphate fertilizer in May 2020 and May 2021 (Figure 1.2). Otherwise, prairie strips received no agrochemical inputs and were managed identically across crop management treatments. Our study took place from June 2019 to June 2022, capturing the better part of four crop years: winter wheat in 2019, corn in 2020, soybean in 2021, and winter wheat in 2022.

Experimental design and sample collection

We compared soil microbial communities among crop management treatments and among distances from the prairie strip using a replicated transect design within each plot (Figure 1.1). Soil samples were collected from three transects perpendicular to each prairie strip with sampling locations at seven distances: -20, -5 ,-1, 0, 1, 5, and 20 m from the prairie strip edge in east and west directions (the station at 0 m was located in the center of the prairie strip), such that 21 individual soil samples were collected from each plot (Figure 1.1). Soil samples were collected in mid to late June in each sampling year. Samples were collected two months post-planting on June 25 2019, approximately one year post-planting on June 18 2020, approximately two years post-planting on June 22 2021, and approximately three years post-planting on June 10 2022 (4 years * 2 treatments * 6 replicates * 7 distances * 3 transects, n=1008 samples total). Each sample was sieved to <2mm within 24 hours of collection and a 5 g subsample was stored at -80°C prior to DNA extraction. We also collected belowground plant biomass for two plant species at all center transect sampling locations on June 10 2022 (Year 3) to assess mycorrhizal colonization: *Andropogon gerardi* (big bluestem) was collected from prairie strip sampling locations and *Triticum aestivum* (winter wheat) was collected from cropland sampling locations. We measured mycorrhizal colonization of big bluestem in prairie strips because big bluestem and winter wheat associate with mycorrhizae at comparable rates (Plenchette et al. 1983; Wilson and Harnett 1998).

Soil microbial community analysis

We characterized microbial communities in all prairie strip and cropland soil samples using a high-throughput amplicon sequencing Illumina MiSeq platform (Illumina, CA, USA). For each soil sample, we extracted genomic DNA using the Qiagen MagAttract KF PowerSoil DNA extraction kit with a Thermo Fisher KingFisher Flex automated extraction instrument (Thermo

Fisher, U.S.A.) following all manufacturer protocols. DNA concentration was determined for all samples via fluorometry with the Invitrogen Qubit dsDNA HS Assay Kit (Thermo Fisher, U.S.A.).

The extracted DNA template was submitted to the Michigan State University Core Genomics Facility for Illumina bacterial 16S V4 and fungal ITS1 library construction using the Illumina TruSeq Nano DNA library preparation kit and sequencing, and reads were quality filtered and merged using the USEARCH pipeline (https://drive5.com/usearch). Libraries of the bacterial 16S V4 region were prepared using Illumina-compatible, dual-indexed 515Ff/806r primers (Kozich et al. 2013). Libraries of the fungal ITS1 region were prepared using ITS1f/ITS2 primer sequences (Martin and Rygiewicz 2005) in an initial PCR followed by the addition of dual indexed Illumina library adapters in a subsequent PCR. Libraries were batch normalized using Norgen Biotek NGS Normalization Kits, pooled, cleaned, and concentrated using AmpureXP magnetic beads. Libraries were quality checked and quantified using a combination of Qubit dsDNA HS, Agilent 4200 TapeStation HS DNA1000 and Kapa Illumina Library Quantification qPCR assays. 16S and ITS1 amplicons were sequenced independently in a 2x250bp paired end format using independent v2 500 cycle MiSeq reagent cartridges.

To test whether prairie strip microbial communities resemble those of older restored prairies, we compared prairie strip bacterial communities to 10 year old restored prairie bacterial communities using amplicon data collected by Benucci et al. 2022 (NCBI accession no. PRJNA751075). Restored prairies were established in the KBS Great Lakes Bioenergy Research Center (GLBRC) Bioenergy Cropping System Experiment (BCSE) in 2008. For our analysis, we included data from fifteen soil samples collected by Benucci et al. (2022) from the Restored Prairie (G10) treatment at a depth of 0-10 cm in 2018. Bacterial amplicon data from KBS LTER prairie strip soils and GLBRC restored prairie soils were pooled, quality filtered, and analyzed together.

Reads were quality filtered and merged using the USEARCH pipeline (https://drive5.com/usearch). Primers and adapter bases were removed using cutadapt. Bacterial reads were filtered and truncated to 250 bp, clustered into actual sequence variants (ASVs) at 100% sequence similarity then classified against SILVAv123 rRNA database (https://arb-silva.de). Non-bacterial ASVs and samples with fewer than 5000 reads were removed, and remaining samples were rarefied to 5,167 reads, resulting in 118,598 bacterial ASVs and 4,701,970 bacterial reads (Thiéry et al. 2012). Seventy-six samples, of 1,008 total samples, were removed from the bacterial dataset due to low read depth. Fungal sequences were filtered to 250 bp. Fungal reads were clustered into ASVs at 100% sequence similarity and classified against the UNITE 8.3 reference database (https://unite.ut.ee). Non-fungal ASVs and samples with fewer than 5000 reads were removed, and remaining samples were rarefied to 5000 reads, resulting in 12,643 fungal ASVs and 4,415,000 fungal reads. Ninety-one samples, of 1,008 total samples, were removed from the fungal dataset due to low read depth.

We used FUNGuild v1.0 (Nguyen et al. 2016) to assign trophic mode classifications to fungal ASVs. Fungal amplicons were first filtered to include only the ASVs for which trophic modes were assigned with "Probable" or "Highly Probable" classification, and amplicons were further filtered to include only the twenty most abundant ASVs. The filtered dataset comprised 62.1% of total fungal reads. ASVs were grouped by trophic modes (pathotroph, symbiotroph, and saprotroph). If an ASV was categorized into multiple trophic modes, ASV abundance was normalized by dividing the total read number by the number of trophic mode classifications.

Arbuscular mycorrhizal fungi (AMF) colonization rates were quantified using root staining and microscopy according to McGonigle et al. (1990). Briefly, fine roots were collected from each big bluestem and winter wheat plant (1 year * 2 treatments * 6 replicates * 7 distances * 1 transects,

n=84 samples total). Roots were cleared, stained, and examined using a compound microscope at 200X magnification for the presence of AMF structures (arbuscules, vescicles, and hyphae).

Statistical analysis

First, univariate datasets for alpha diversity, fungal trophic mode abundance, and AMF colonization were checked for normality and heterogeneity of variances. If assumptions were not met, data were log transformed to meet assumptions and/or outliers removed. We tested the effects of prairie strips on bacterial and fungal Shannon diversity, richness, and evenness using generalized linear models that included year, cropping system, vegetation (prairie vs. crop), and their interactions as factors. We then measured the effect of prairie strips on fungal trophic mode abundance and AMF colonization. For both trophic mode abundance and AMF colonization, we constructed two separate generalized linear models – one including all soils (prairie strip and cropland) and one including cropland soils only. Models of all soils included year (2 months, 1 year, 2 years, and 3 years post-planting), cropping system (Reduced Input and Organic), and vegetation (prairie and crop) as predictors. Models of cropland soils only included distance from prairie strip as the sole model predictor. Each fungal trophic mode was analyzed separately.

We selected several bacterial and fungal phyla previously shown to change in abundance during prairie restoration. Acidobacteria, Actinobacteria, Planctomycetes, and Verrucomicrobia bacteria, as well as Basidiomycota, Glomeromycota fungi, are generally enriched in soils during restoration, while Ascomycota fungi typically decreases in abundance (Barber et al. 2017, Dutter et al. 2024, Fierer et al. 2013). We analyzed pairwise comparisons in phylum abundance between prairie strip (PS) samples and averaged cropland distance (1m, 5m, 20m) samples within each cropping system. We chose to measure phyla abundances because nearly all literature describing soil microbial communities in remnant and restored prairies report responses at the phylum level

(Allison et al. 2005, Herzberger et al. 2014, Duncan et al. 2016, Barber et al. 2017, Mackelprang et al. 2018, Docherty and Gutknecht 2019, Barber et al 2023). Therefore, while microbial functions are not well conserved at the phylum level, phylum-level analyses are our best approach to determine whether prairie strip community responses resemble those of whole-field prairie restorations. For each phylum, we tested for differential abundance between prairie strip and cropland soils using the 'manyglm' and 'anova' functions in the *MVabund* (version 4.2.1) R package (Wang Y. et al. 2012). We used Tukey's honestly significant difference (HSD) test for post-hoc analysis of significantly different abundance among treatment groups.

To compare prairie strip and cropland microbial community composition, we first ordinated Bray-Curtis distance matrices for rarefied prairie strip and cropland bacterial and fungal communities via Non-metric Multidimensional Scaling (NMDS) using the *ordinate()* function in R *phyloseq* (R version version 4.2.3, R Core Team 2022, *phyloseq* version 1.42.0, McMurdie and Holmes 2013). We then conducted a separate permutational analysis of variance (PERMANOVA) on the distance matrix of each sampling year using the *adonis()* function in the *vegan* R package (*vegan* version 2.6-4) and included cropping system (Reduced Input and Organic), vegetation (prairie and crop), and their interaction as model predictors. We used the cropping system x vegetation interaction in this model to determine whether cropland management legacy controls prairie strip community composition.

We also compared prairie strip bacterial communities in each year with those of 10 year restored prairies. First, we ordinated Bray-Curtis distance matrices for rarefied prairie strip and restored prairie bacterial communities via NMDS. Using these distance matrices, we conducted a PERMANOVA within each sampling year and included prairie type (prairie strips vs. restored prairie) as the sole model predictor. We also calculated average Bray-Curtis dissimilarity between

prairie strip and restored prairie bacterial communities in each year.

To determine if prairie strips change microbial communities of surrounding cropland soils, we tested the impact of prairie strips on overall bacterial and fungal community composition, and abundance of prairie-associated microbial phyla in surrounding cropland. We first ordinated Bray-Curtis distance matrices for rarefied cropland bacterial and fungal communities via NMDS, then performed a PERMANOVA for all cropland samples with year, cropping system, distance from prairie strip, and their interactions included as model predictors. Where significant cropping system x year interactions were observed, we performed secondary PERMANOVA analyses on cropland soils from each cropping system and each year. Next, we constructed a generalized linear model for cropland samples that included year, cropping system, and distance from the prairie strip as model predictors and abundance of prairie-associated phyla as a response variable. We used Tukey's honestly significant difference (HSD) test for post-hoc analysis of significantly different abundance among treatment groups. Finally, to understand whether community similarity decreases with distance from the prairie strip, we calculated average Bray-Curtis dissimilarity between prairie strips (PS) and each cropland distance (1m, 5m, 20m) for each cropping system.

RESULTS

Prairie strip and cropland community divergence

Prairie strips showed higher bacterial Shannon diversity ($p < 0.001$; $R^2 = 0.022$; Table 1.1), richness ($p = 0.018$; $R^2 = 0.005$; Table 1.1), and evenness ($p < 0.001$; $R^2 = 0.029$; Table 1.1) than cropland. One year post-planting, prairie strip soils contained 2.9% higher bacterial and 6.2% higher fungal diversity than cropland soils. Prairie strip bacterial and fungal community composition diverged from cropland community composition starting two months post-planting for fungi ($p = 0.032$; $R^2 = 0.007$; Table 1.2; Figure 1.3) and one year post-planting for bacteria (p

< 0.001; $R^2 = 0.032$; Table 1.2; Figure 1.3), and remained dissimilar from cropland communities each year thereafter in both cropping systems (Figure 1.3; Table 1.2).

Abundance of prairie-associated microbial groups in prairie strips

Prairie-associated bacterial and fungal phyla increased in abundance in prairie strip soils starting one year after planting in both cropping systems (Figure 1.4; Table 1.4). Each phylum showed higher abundance in prairie strips in at least one year and cropping system (Figure 1.4; Table 1.4). Ascomycota was more abundant in prairie strips after one year ($p = 0.028$), two years ($p = 0.009$), and three years ($p = 0.007$; Figure 1.4; Table 1.4). Arbuscular mycorrhizal fungi (AMF) colonization rates in prairie strip big bluestem plants were similar to those in surrounding winter wheat plants (average 57.9% colonization in winter wheat and average 57.0% colonization in big bluestem; $p = 0.848$; Figure 1.5). Fungal trophic modes were present at similar abundances in prairie strips across cropping systems ($p > 0.05$ for each trophic mode in prairie strips; Figure 1.6). Prairie strips contained more saprotrophic fungi ($p = 0.024$) and symbiotrophic fungi ($p = 0.018$) than Organic cropland, and less symbiotrophic fungi than Reduced Input cropland ($p = 0.02$; Figure 1.6).

Prairie strip and restored prairie communities

Prairie strip bacterial community composition did not become more similar to the bacterial composition of 10 year restored prairies (Figure 1.7). Prairie strip community composition was distinct from restored prairie community composition in every year of the study ($p < 0.001$ each year; Figure 1.7) and the magnitude of dissimilarity between prairie strip communities and restored prairie communities also remained consistent across years. Bray-Curtis dissimilarity between restored prairie communities and prairie strip communities (averaged across cropping systems) was 0.899 at 2 months post-planting, 0.889 1 year post-planting, 0.886 2 years post-planting, and

0.888 3 years post-planting.

Land use history effects on prairie strip communities

Prairie strips in Reduced Input plots showed distinct community composition from prairie strips in Organic plots in the latter three sampling years ($p < 0.005$ after 1 year, 2 years, and 3 years; Table 1.2; Figure 1.3). Prairie strip communities in each cropping system were equally dissimilar from 10 year restored prairie communities (Figure 1.7). The abundance of some prairie-associated taxa in prairie strip soils varied among cropland management treatments (Table 1.4). There was no difference in prairie strip big bluestem AMF colonization rates between cropland management treatments ($p = 0.493$; Figure 1.5). Prairie strips in Organic plots showed higher relative abundance of saprotrophic and symbiotrophic fungal groups relative to surrounding cropland ($p = 0.028$; $R^2 = 0.033$ for saprotrophic; $p = 0.018$; $R^2 = 0.012$ for symbiotrophic; Figure 1.6), and prairie strips in Reduced Input plots showed lower relative abundance of symbiotrophic fungi ($p = 022$; $R^2 = 0.007$; Figure 1.6).

Prairie strip effects on cropland microbial communities

Our last hypothesis was not supported; prairie strips had little influence on overall community structure and abundance of prairie-associated phyla in surrounding cropland soils. Cropland soils showed distinct community composition across years ($p < 0.001$ for bacteria; $p < 0.001$ for fungi; Figure 1.3), cropping systems ($p < 0.001$ for bacteria; $p <0.001$ for fungi; Figure 1.3), and distances from the prairie strip edge ($p = 0.008$ for bacteria; $p = 0.005$ for fungi; Figure 1.3). When cropland communities from all four years were analyzed together, year x cropping system interaction ($p = <0.001$ for bacteria and fungi) and cropping system x distance interaction ($p = 0.016$ for bacteria; $p = 0.013$ for fungi) explained cropland community composition, and thus we performed separate PERMANOVA analyses for each cropland management treatment in each

year (Table 1.3).

Cropland proximity to prairie strips did not consistently explain community composition (Table 1.3). Even when distance was a significant predictor of community composition (Table 1.3), pairwise Bray-Curtis dissimilarity between prairie strip and cropland communities did not linearly increase with distance from the prairie strip (Figure 1.8). In the cases where distance from prairie strip was significant (Table 1.3), the greatest community dissimilarity from prairie strips was at an intermediate distance of 5m from the prairie strip (Figure 1.8). Generally, distance influenced fungal communities more often than bacterial communities (Table 1.3). Overall, prairie strips did not change the abundance of prairie-associated phyla in surrounding cropland soils ($p = 0.774$ for bacteria; $p = 0.957$; Table 1.5). AMF colonization rates in winter wheat were higher in Organic plots than in Reduced Input plots ($p = 0.003$, Figure 1.5), and colonization did not vary with distance from prairie strip ($p = 0.47$, Figure 1.5). Relative abundance of saprotrophic fungi, but not symbiotrophic or pathotrophic fungi, was explained by distance from the prairie strip ($p = 0.051$; Figure 1.6), but saprotrophs were not consistently enriched at a particular distance.

DISCUSSION

Integrating prairie strips within annual cropland restores soil physical and chemical measures of soil function, but it has remained unclear how quickly these benefits accrue following prairie strip establishment, and also whether these benefits extend to cropping systems outside of Iowa. Here we find that prairie strip communities diverged from cropland communities one year after establishment in multiple cropping systems and prairie strips showed increased abundance of several microbial taxa commonly found in remnant and older restored prairies. Land use history shaped prairie strip community composition, where prairie strips planted in cropping systems with a history of agrochemical use had distinct communities from

those planted in cropping systems with no history of agrochemical use. Prairie strips did not alter microbial communities in adjacent cropland soils, and cropland fungal communities were more strongly affected by proximity to prairie strips than cropland bacterial communities. Enrichment of prairie-associated microbial phyla in prairie strips suggests that establishing prairie strips may enhance soil functionality in restored areas of multiple cropping systems.

Prairie strip communities diverge rapidly from cropland communities

Early differentiation of prairie strip and cropland communities were generally consistent with microbial responses observed in larger prairie restorations (Griffiths et al. 2011, Nacke et al. 2011, Van Trump et al. 2011, Fierer et al. 2013, Barber et al. 2017, Dutter et al. 2024). For example, fungal phyla Glomeromycota (arbuscular mycorrhizal fungi) and Basidiomycota were enriched in prairie strip soils (Table 1.4; Figure 1.4), likely due to greater abundance and diversity of mycorrhizae-associated plants (Burrows and Pfleger 2002), lower inorganic nitrogen application (Jach-Smith and Jackson 2018), and lower soil disturbance (Allison et al. 2005, Köhl et al. 2014, Säle et al. 2015). Reduced land use intensity typically shifts fungal communities from Ascomycota-dominated to Basidiomycetes-dominated structure (Upton et al. 2018, Dutter et al. 2024), but here we find that Ascomycota fungi are enriched in prairie strip soils. Management activities that take place early in prairie strip establishment – including tillage, mowing, and burning - may select for spore-forming, disturbance-tolerant Ascomycota groups (Holden et al. 2016, Whitman et al. 2019, Figure 1.2).

Prairie strip effects on fungal trophic modes were strongly dependent on the cropping system in which they were established. Prairie strips increased fungal saprotrophs and symbiotrophs when planted in the unfertilized Organic cropping systems, but decreased fungal symbiotrophs when planted in the fertilized Reduced Input cropping systems (Figure 1.6). In

cropping systems with no history of fertilization, fungal decomposers and symbionts were quickly enriched in prairie strips, likely due to an influx of diverse organic matter inputs from perennial plants (Sprunger et al. 2017, Córdova et al. 2018). On the other hand, in systems with a long history of fertilization, the already high nutrient conditions likely curbed the selection for fungal symbionts as prairie strips established.

Prairie strip bacterial communities did not converge on the composition of the mature restored prairie we measured (Figure 1.7). Time discrepancies in sampling may explain some community dissimilarity, as we compared prairie strip soils collected in 2019 through 2022 to restored prairie soils collected in 2018. However, our results also highlight that restored prairie soil microbial communities can vary widely depending on management, even when established on the same soil type and in close proximity. The prairie strips and restored prairies we measured were planted with different seed mixes (Sanford et al. 2016, Kemmerling et al. 2022), and this may have resulted in different assemblages of plant-associated microbes across sites. Also, prairie strips underwent biennial burning, which is known to change the size and composition of soil microbial communities, while restored prairies did not (Kitchen et al. 2009, Dooley and Treseder 2012, Carson and Zeglin 2018, Whitman et al. 2019). It is unclear when prairie strip communities will converge on the composition of nearby restored prairies or remnant prairies, if ever. Restored prairie soils commonly form an "intermediate" microbial community structure distinct from both pre-restoration soils and remnant prairie soils (Jangid et al. 2011, Herzberger et al. 2014, Barber et al. 2017), and over time, we may see the same process occur in prairie strips.

Land use history effects on prairie strip community composition could be due to surrounding cropland management that continued to exert pressure on microbial communities in

strips, but is more likely due to the cropland management that took place prior to prairie strip establishment, which has been shown to control community composition for decades following land use change (Jangid et al. 2011). Prairie strip fungal composition is most strongly affected by cropland management history, consistent with other studies evaluating microbial response to land use history (Turley et al. 2020), potentially due to fungi's slower growth and thus slower recovery following land use change (Rousk and Bååth 2007).

Prairie strips have little effect on microbes in surrounding cropland

Prairie strips generally had minimal effects on bacterial and fungal communities in adjacent annual cropland, and prairie strips are not likely to enrich prairie-associated microbial phyla in surrounding cropland soils. We find limited evidence for successful microbial dispersal from strips, corroborating patterns we observed in older prairie strips in a previous study in Iowa (Dutter and Rutkoski et al.2024). Prairie-associated microbes are likely still dispersing from prairie strips to cropland (Bell and Tylianakis 2016; Yang and Elsas 2018; Chaudhary et al. 2020; Choudoir and DeAngelis 2022), but are inhibited from persisting in adjacent cropland areas due to cropland management activities (Coleman and Wall 2015; Säle et al. 2015). While this does not preclude rare, low-abundance microbes from conferring benefits to croplands through spillover, it does suggest that on the whole, the positive effect of prairie strips on microbes appears limited to areas within the strip.

We also find that proximity to prairie strips did not shape cropland communities in all years and cropping systems, but when it did, it more strongly affected fungi than bacteria (Table 1.3). This may be due to fungi's dispersal limitation and overall higher sensitivity to cropland soil disturbance including rotary hoeing and cultivation (Langenheder and Lindström 2019, Peay et al. 2010). We also found that Bray-Curtis dissimilarity between prairie strip and cropland

communities did not increase linearly with distance from the prairie strip (Figure 1.8), and prairie-cropland community dissimilarity was often greatest at the intermediate distance from the prairie strip (5m; Figure 1.1, Figure 1.8).

Our results are consistent with findings from another prairie strip experiment in Iowa; in an earlier study (Dutter and Rutkoski et al. 2024), we found that environmental filtering by cropland management activities shaped microbial communities in cropland adjacent to prairie strips, resulting in distinct prairie strip and cropland communities. Here, we find a similar result, suggesting that dispersal of prairie microbes from prairie strips to adjacent cropland may be stifled by cropland management activities, regardless of cropping system. While some low abundance microbial taxa in prairie strips may be less dispersal limited, our findings suggest that most microbes in prairie strips are unable to successfully disperse to and colonize adjacent cropland soils. Future studies might focus on particular groups, particularly of fungi, that are known to be harbored by prairies and dispersal limited, but also able to survive cropland management.

CONCLUSION

Our study shows that prairie strip soil microbial communities rapidly differentiated from cropland communities during establishment. Prairie strip fungal communities responded especially quickly, becoming distinct from cropland fungal communities just two months after planting. In two cropping systems, prairie strip soils exhibited higher microbial diversity, and contained increased abundance of microbial groups characteristic of tallgrass prairie soils. Prairie strips also formed unique assemblages of microbial taxonomic and functional groups depending on agricultural land use history, where prairie strips increased abundance of fungal decomposers and symbionts in cropping systems with no chemical inputs, and decreased fungal symbionts in

cropping systems managed with reduced chemical inputs. Overall, prairie strips had little impact on cropland microbial community composition nor the abundance of prairie-associated phyla in adjacent cropland soils, but effects varied among crop rotation phases and cropping systems. Our findings show that prairie strip effects on surrounding cropland microbial communities are limited, but under the strips, prairie strips rapidly restore belowground tallgrass prairie biodiversity.

ACKNOWLEDGEMENTS

We would like to thank Marshall McDaniel and Lisa Schulte Moore for their help with project conceptualization. We thank Holly Vander Stel and Stacey Vanderwulp for their help coordinating sample collection and equipment use for this project. We thank Madaris Serrano-Perez, Ceco Maples, Yahn-Jau Su, Sofie Iwamasa, and Lee Leonard for their assistance with sample collection and processing. We also thank Lukas Bell-Dereske, Jennifer Jones, Tayler Ulbrich, Caitlin Broderick, Glade Bogar, Moriah Young, and Brandon Kristy for their data analysis and manuscript feedback. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. 2235783 and the NSF Long Term Ecological Research Program [Grant ID: DEB 2224712]. This work took place on occupied Anishinaabe land where Hickory Corners, Michigan and KBS sites are now located. We acknowledge the legacies of violence, displacement, migration, and settlement that comes with our use of the land.

LITERATURE CITED

Aguiar, M., Conway, A., Bell, J., Stewart, K. 2023. Agroecosystem edge effects on vegetation, soil properties, and the soil microbial community in the Canadian prairie. PLoS ONE. https://doi.org/10.1371/journal.pone.0283832

Allen, E.B., Allen, M.F., Helm, D.J., Trappe, J.M., James M. Trappe, Trappe, J.M., Molina, R., Rincón, E., 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. Plant and Soil 170, 47–62. https://doi.org/10.1007/bf02183054

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, 1412–1421. https://doi.org/10.2136/sssaj2004.0252

Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences of the United States of America 105. https://doi.org/10.1073/pnas.0801925105

Bach, E.M., Hofmockel, K.S., 2015. Coupled Carbon and Nitrogen Inputs Increase Microbial Biomass and Activity in Prairie Bioenergy Systems. Ecosystems 18, 417–427. https://doi.org/10.1007/s10021-014-9835-8

Bach, E.M., Baer, S.G., Six, J., 2012. Plant and Soil Responses to High and Low Diversity Grassland Restoration Practices. Environmental Management 49, 412–424. https://doi.org/10.1007/s00267-011-9787-0

Bach, E.M., Williams, R.J., Hargreaves, S.K., Yang, F., Hofmockel, K.S., 2018. Greatest soil microbial diversity found in micro-habitats. Soil Biology & Biochemistry 118, 217–226. https://doi.org/10.1016/j.soilbio.2017.12.018

Badger Hanson, E., Docherty, K.M., 2022. Mini-review: Current and Future Perspectives on Microbially Focused Restoration Strategies in Tallgrass Prairies. Microbial Ecology. https://doi.org/10.1007/s00248-022-02150-1

Baer, S.G., Kitchen, D.J., Blair, J.M., Rice, C.W., 2002. Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecological Applications 12, 1688–1701. https://doi.org/10.1890/1051-0761(2002)012[1688:ciesaf]2.0.co;2

Bakker, M.G., Glover, J.D., Mai, J.G., Kinkel, L.L., 2010. Plant community effects on the diversity and pathogen suppressive activity of soil streptomycetes. Applied Soil Ecology 46, 35–42. https://doi.org/10.1016/j.apsoil.2010.06.003

Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A.Y., Gattinger, A., Keller, T., Keller, T., Charles, R., van der Heijden, M.G.A., 2018. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. bioRxiv 416271. https://doi.org/10.1101/416271

Barber, N.A., Chantos-Davidson, K.M., Peralta, R.A., Sherwood, J.P., Swingley, W.D., 2017. Soil microbial community composition in tallgrass prairie restorations converge with remnants across a 27-year chronosequence. Environmental Microbiology 19, 3118–3131. https://doi.org/10.1111/1462-2920.13785

Barber, N., Klimek, D.M., Bell, J., Swingley, W. 2023. Restoration age and re-introduced bison may shape soil bacterial communities in restored tallgrass prairies. FEMS Microbiology Ecology. https://doi.org/10.1093/femsec/fiad007

Bell, T. and Tylianakis, J.M., 2016. Microbes in the Anthropocene: spillover of agriculturally selected bacteria and their impact on natural ecosystems. Proceedings of The Royal Society B: Biological Sciences 283, 20160896. https://doi.org/10.1098/rspb.2016.0896

Benucci, G.M.N., Beschoren da Costa, P., Wang, X. & Bonito, G. 2023. Stochastic and deterministic processes shape bioenergy crop microbiomes along a vertical soil niche. *Environmental Microbiology*, 25(2), 352–366. https://doi.org/10.1111/1462-2920.16269.

Bergner, B., Johnstone, J.F., Treseder, K.K., 2004. Experimental warming and burn severity alter soil CO2 flux and soil functional groups in a recently burned boreal forest. Global Change Biology 10, 1996–2004. https://doi.org/10.1111/j.1365-2486.2004.00868.x

Buckley, D.H., Schmidt, T.J., Schmidt, T.M., 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environmental Microbiology 5, 441–452. https://doi.org/10.1046/j.1462-2920.2003.00404.x

Burrows, R.L. and Pfleger, F.L. 2002. Arbuscular mycorrhizal fungi respond to increasing plant diversity. Botany 80, 120–130. https://doi.org/10.1139/b01-138

Carson, C.M., Zeglin, L.H., 2018. Long-term fire management history affects N-fertilization sensitivity, but not seasonality, of grassland soil microbial communities. Soil Biology & Biochemistry 121, 231–239. https://doi.org/10.1016/j.soilbio.2018.03.023

Chaudhary, V.B., O'Dell, T.E., Thomas E. O'Dell, Rillig, M.C., Johnson, N.C., 2014. Multiscale patterns of arbuscular mycorrhizal fungal abundance and diversity in semiarid shrublands. Fungal Ecology 12, 32–43. https://doi.org/10.1016/j.funeco.2014.06.003

Chaudhary, B., Nolimal, S., Sosa-Hernández, M.A., Egan, C., and Kastens, J. 2020. Trait-based aerial dispersal of arbuscular mycorrhizal fungi. New Phytologist 228:238-252. https://doi.org/10.1111/nph.16667

Choudoir, M.J., Barberán, A., Menninger, H.L., Dunn, R.R. and Fierer, N., 2018. Variation in range size and dispersal capabilities of microbial taxa. Ecology 99(2), pp.322-334. https://doi.org/10.1002/ecy.2094

Choudoir, M.J. and DeAngelis, K.M., 2022. A framework for integrating microbial dispersal modes into soil ecosystem ecology. iScience 103887–103887. https://doi.org/10.1016/j.isci.2022.103887

Coleman, D.C. and Wall, D.H., Soil Fauna: Occurrence, Biodiversity, and Roles in Ecosystem Function in: Paul, E.A. (Ed) Soil Microbiology, Ecology and Biochemistry (Fourth Edition), Academic Press, 2015, pp. 111-149. https://doi.org/10.1016/B978-0-12-415955-6.00005-0

Collins, S.L., Knapp, A.K., Briggs, J.M., Blair, J.M., Steinauer, E.M., 1998. Modulation of Diversity by Grazing and Mowing in Native Tallgrass Prairie. Science 280, 745–747. https://doi.org/10.1126/science.280.5364.745

Coolon, J.D., Jones, K.L., Todd, T.C., Blair, J.M., Herman, M.A., 2013. Long-term nitrogen amendment alters the diversity and assemblage of soil bacterial communities in tallgrass prairie. PLOS ONE 8. https://doi.org/10.1371/journal.pone.0067884

Córdova, S.C., Olk, D.C., Dietzel, R., Mueller, K.E., Archontouilis, S.V., Michael J. Castellano, Castellano, M.J., 2018. Plant litter quality affects the accumulation rate, composition, and stability of mineral-associated soil organic matter. Soil Biology & Biochemistry 125, 115–124. https://doi.org/10.1016/j.soilbio.2018.07.010

Crum, J. R. and H. P. Collins. 1995. KBS Soils. Kellogg Biological Station Long-term Ecological Research Special Publication. Zenodo. http://doi.org/10.5281/zenodo.2581504.

Duncan, D.S., Jewell, K.A., Suen, G., Jackson, R.D., 2016. Detection of short-term cropping system-induced changes to soil bacterial communities differs among four molecular characterization methods. Soil Biology & Biochemistry 96, 160–168. https://doi.org/10.1016/j.soilbio.2016.02.002

de Araújo, A.S.F., Monteiro, R.T.R., Abarkeli, R.B., 2003. Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52, 799–804. https://doi.org/10.1016/s0045-6535(03)00266-2

Docherty, K.M., Gutknecht, J.L.M., 2019. Soil Microbial Restoration Strategies for Promoting Climate-Ready Prairie Ecosystems. Bulletin of The Ecological Society of America 100. https://doi.org/10.1002/bes2.1523

Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109, 49–61. https://doi.org/10.1007/s10533-011-9633-8

Drijber, R.A., Doran, J.W., Parkhurst, A.M., Lyon, D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology & Biochemistry 32, 1419–1430. https://doi.org/10.1016/s0038-0717(00)00060-2

Dutter, C., Rutkoski, C.E., Evans, S.E., McDaniel, M.D., Contour Prairie Strips Alter Soil Microbial Communities and Functioning Both Below the Prairie Strip and in Adjacent Cropland. Available at SSRN: https://ssrn.com/abstract=4542718 or http://dx.doi.org/10.2139/ssrn.4542718

Egidi, E., Megharaj, M., Delgado-Baquerizo, M., Plett, J.M., Wang, J.-T., Eldridge, D.J., Bardgett, R.D., Maestre, F.T., Singh, B.K., 2019. A few Ascomycota taxa dominate soil fungal communities worldwide. Nature Communications 10, 2369. https://doi.org/10.1038/s41467-019-10373-z

Eom, A.H., Hartnett, D.C., Wilson, G.W.T., 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. Oecologia 122, 435–444. https://doi.org/10.1007/s004420050050

Evans, S.E., Martiny, J.B.H., Allison, S.D. 2017. Effects of dispersal and selection on stochastic assembly in microbial communities. The ISME Journal 11:1, 176–185. https://doi.org/10.1038/ismej.2016.96

Ferrero, F., Prencipe, S., Tabacco, E., Ferrocino, I., Borreani, G., Spadaro, D. 2022. Effects of a prothioconazole- and tebuconazole-based fungicide on the yield, silage characteristics, and fungal mycobiota of corn harvested and conserved as whole-crop and high-moisture ear silages. Journal of Applied Microbiology. https://doi.org/10.1093/jambio/lxac033

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103, 626–631. https://doi.org/10.1073/pnas.0507535103

Fierer, N., Ladau, J., Clemente, J.C., Leff, J.W., Owens, S.M., Pollard, K.S., Knight, R., Gilbert, J.A., McCulley, R.L., 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342, 621–624. https://doi.org/10.1126/science.1243768

Frey, S.D., 2007. Spatial distribution of soil organisms In: Eldor, A.P., Ed., Soil Microbiology, Ecology and Biochemistry, 3rd Edition. 283–300. https://doi.org/10.1016/b978-0-08-047514-1.50015-9

Garcia, F.O., Rice, C.W., 1994. Microbial biomass dynamics in tallgrass prairie. Soil Science Society of America Journal 58, 816–823. https://doi.org/10.2136/sssaj1994.03615995005800030026x

Gardi, C., Jeffery, S., Saltelli, A., 2013. An estimate of potential threats levels to soil biodiversity in EU. Global Change Biology 19, 1538–1548. https://doi.org/10.1111/gcb.12159

Geisen, S., Wall, D.H., van der Putten, W.H., 2019. Challenges and Opportunities for Soil Biodiversity in the Anthropocene. Current Biology 29. https://doi.org/10.1016/j.cub.2019.08.007

Gottshall, C. B. 2015. Effects of different agricultural management systems on arbuscular mycorrhizal fungal diversity, community structure, and ecosystem services. Dissertation, University of Louisville, Louisville, Kentucky. https://lter.kbs.msu.edu/pub/3501

Grandy, A.S., Robertson, G.P., 2007. Land-Use Intensity Effects on Soil Organic Carbon Accumulation Rates and Mechanisms. Ecosystems 10, 59–74. https://doi.org/10.1007/s10021-006-9010-y

Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M.J., Whiteley, A.S., 2011. The bacterial biogeography of British soils. Environmental Microbiology 13, 1642–1654. https://doi.org/10.1111/j.1462-2920.2011.02480.x

Hernandez-Santana, V., Xiaobo Zhou, Zhou, X., Helmers, M.J., Asbjornsen, H., Kolka, R., Tomer, M.D., 2013. Native prairie filter strips reduce runoff from hillslopes under annual row-crop systems in Iowa, USA. Journal of Hydrology 477, 94–103. https://doi.org/10.1016/j.jhydrol.2012.11.013

Herzberger, A., Duncan, D.S., Jackson, R.D., 2014. Bouncing Back: Plant-Associated Soil Microbes Respond Rapidly to Prairie Establishment. PLOS ONE 9. https://doi.org/10.1371/journal.pone.0115775

Hill, P., Krištůfek, V., Dijkhuizen, L., Boddy, C.N., Kroetsch, D., van Elsas, J.D., 2011. Land use intensity controls actinobacterial community structure. Microbial Ecology 61, 286–302. https://doi.org/10.1007/s00248-010-9752-0

Holden, S.R., Rogers, B.M., Treseder, K.K., Randerson, J.T., 2016. Fire Severity Influences the Response of Soil Microbes to a Boreal Forest Fire 2016. https://doi.org/10.1088/1748-9326/11/3/035004

Iqbal, J., Parkin, Timothy B., Parkin, T. B., Helmers, M.J., Zhou, X., Castellano, M.J., 2015. Denitrification and Nitrous Oxide Emissions in Annual Croplands, Perennial Grass Buffers, and Restored Perennial Grasslands. Soil Science Society of America Journal 79, 239–250. https://doi.org/10.2136/sssaj2014.05.0221

Jach-Smith, L.C., Jackson, R.D., 2018. N addition undermines N supplied by arbuscular mycorrhizal fungi to native perennial grasses. Soil Biology & Biochemistry 116, 148–157. https://doi.org/10.1016/j.soilbio.2017.10.009

Jangid, K., Williams, M.A., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J., Jenkins, M.B., Endale, D.M., Coleman, D.C., Whitman, W.B., 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biology & Biochemistry 40, 2843–2853. https://doi.org/10.1016/j.soilbio.2008.07.030

Jangid, K., Williams, M.A., Williams, M.A., Franzluebbers, A.J., Blair, J.M., Coleman, D.C., Whitman, W.B., 2010. Development of soil microbial communities during tallgrass prairie restoration. Soil Biology & Biochemistry 42, 302–312. https://doi.org/10.1016/j.soilbio.2009.11.008

Jangid, K., Williams, M.A., Williams, M.A., Franzluebbers, A.J., Schmidt, T.J., Schmidt, T.M., Coleman, D.C., Whitman, W.B., 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology & Biochemistry 43, 2184–2193. https://doi.org/10.1016/j.soilbio.2011.06.022

Jasper, D.A., Abbott, L.K., Robson, A., 1989. Soil disturbance reduces the infectivity of external hyphae of vesicular—arbuscular mycorrhizal fungi. New Phytologist 112, 93–99. https://doi.org/10.1111/j.1469-8137.1989.tb00313.x

Johnsen, K., Jacobsen, C.S., Torsvik, V., Sørensen, J.T., 2001. Pesticide effects on bacterial diversity in agricultural soils – a review. Biology and Fertility of Soils 33, 443–453. https://doi.org/10.1007/s003740100351

Johnson, L.C., Matchett, J.R. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. Ecology 82, 3377–3389. https://doi.org/10.1890/0012-9658(2001)082[3377:fagrbp]2.0.co;2

Jumpponen, A., Jones, K.L., Blair, J.M., 2010. Vertical distribution of fungal communities in tallgrass prairie soil. Mycologia 102, 1027–1041. https://doi.org/10.3852/09-316

Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications 7, 13630–13630. https://doi.org/10.1038/ncomms13630

Kavamura, V.N., Mendes, R., Bargaz, A., Adnane Bargaz, Adnane Bargaz, Mauchline, T.H., 2021. Defining the wheat microbiome: Towards microbiome-facilitated crop production. Computational and structural biotechnology journal 19, 1200–1213. https://doi.org/10.1016/j.csbj.2021.01.045

Kemmerling, L.R., Rutkoski, C.R., Evans, S.E., Helms, J.A., Cordova-Ortiz, E.S., Smith, J.A., Vázquez Custodio, J.A., Vizza, C., Haddad, N.M. Prairie Strips and Lower Land Use Intensity Increase Biodiversity and Ecosystem Services. Frontiers in Ecology and Evolution. https://doi.org/10.3389/fevo.2022.833170

Kemmerling, L., McCarthy, A.C., Brown, C.S., Haddad, N. 2023. Butterfly biodiversity increases with prairie strips and conservation management in row crop agriculture. Insect Conservation and Diversity. https://doi.org/10.1111/icad.12675

Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., Johannes A. van Veen, van Veen, J.A., Kuramae, E.E., 2016. The Ecology of Acidobacteria: Moving beyond Genes and Genomes. Frontiers in Microbiology 7, 744–744. https://doi.org/10.3389/fmicb.2016.00744

Kitchen, D.J., Blair, J.M., Callaham, M.A., 2009. Annual fire and mowing alter biomass, depth distribution, and C and N content of roots and soil in tallgrass prairie. Plant and Soil 323, 235–247. https://doi.org/10.1007/s11104-009-9931-2

Köhl, L., Oehl, F., van der Heijden, M.G.A., 2014. Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. Ecological Applications 24, 1842–1853. https://doi.org/10.1890/13-1821.1

Koziol, L., Bever, J.D., 2017. The missing link in grassland restoration: arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession. Journal of Applied Ecology 54, 1301–1309. https://doi.org/10.1111/1365-2664.12843

Langenheder, S., Lindström, E.S., 2019. Factors influencing aquatic and terrestrial bacterial community assembly. Environmental Microbiology Reports 11:306-315. doi:10.1111/1758-2229.12731

Lekberg, Y., Koide, R. 2014. Integrating physiological, community, and evolutionary perspectives on the arbuscular mycorrhizal symbiosis. Botany. 92(4): 241-251. https://doi.org/10.1139/cjb-2013-0182

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kõljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J. and Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytologist 199: 288-299. doi:10.1111/nph.12243

Liu, F., Hewezi, T., Lebeis, S.L., Pantalone, V., Grewal, P.S., Parwinder S. Grewal, Parwinder S. Grewal, Staton, M., 2019. Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. BMC Microbiology 19, 201–201. https://doi.org/10.1186/s12866-019-1572-x

Mackelprang, R., Grube, A.M., Lamendella, R., da Conceição Jesus, E., Copeland, A., Liang, C., Jackson, R.D., Rice, C.W., Kapucija, S., Parsa, B., Tringe, S.G., Tiedje, J.M., Jansson, J.K., 2018. Microbial Community Structure and Functional Potential in Cultivated and Native Tallgrass Prairie Soils of the Midwestern United States. Frontiers in Microbiology 9, 1775–1775. https://doi.org/10.3389/fmicb.2018.01775

Manzoni, S., Schimel, J.P. and Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93:930-938. https://doi.org/10.1890/11-0026.1

Martin, K.J. and Rygiewicz, P.T., 2005. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiology 5, 28. https://doi.org/10.1186/1471-2180-5-28

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytologist 115, 495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x

Middleton, E.L., Middleton, E.L., Richardson, S., Koziol, L., Palmer, C.E., Yermakov, Z., Henning, J.A., Schultz, P.A., Bever, J.D., 2015. Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere 6, 1–16. https://doi.org/10.1890/es15-00152.1

Millar, N. and G. P. Robertson. 2015. Nitrogen transfers and transformations in row-crop ecosystems. Pages 213-251 in S. K. Hamilton, J. E. Doll, and G. P. Robertson, editors. The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability.

Morris, S.J., Blackwood, C.B., 2007. The Ecology of the Soil Biota and their Function In: Eldor, A.P., Ed., Soil Microbiology, Ecology and Biochemistry, 3rd Edition. 273–309. https://doi.org/10.1016/b978-0-12-415955-6.00010-4

Nacke, H., Thürmer, A., Wollherr, A., Will, C., Hodač, L., Herold, N., Schöning, I., Schrumpf, M., Daniel, R., 2011. Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. PLOS ONE 6. https://doi.org/10.1371/journal.pone.0017000

Paul, E. A., A. Kravchenko, A. S. Grandy, and S. Morris. 2015. Soil organic matter dynamics: Controls and management for sustainable ecosystem functioning. Pages 104-134 in S. K. Hamilton, J. E. Doll, and G. P. Robertson, editors. The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability.

Peay, K.G., Garbelotto, M., Bruns, T.D., 2010. Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree islands. Ecology 91, 3631–3640. https://doi.org/10.1890/09-2237.1

Plassart, P., Vinceslas, M.A., Gangneux, C., Mercier, A., Barray, S., Laval, K., 2008. Molecular and functional responses of soil microbial communities under grassland restoration. Agriculture, Ecosystems & Environment 127, 286–293. https://doi.org/10.1016/j.agee.2008.04.008

Plenchette, C., Fortin, J. André, Furlan, V. 1983. Growth response of several plants species to mycorrhiza in a soil of moderate P fertility. I. Mycorrhizal dependency under field conditions. Plant and Soil. 70. 199-209. 10.1007/BF02374780

Poole, P.S., Ramachandran, V.K., Terpolilli, J., 2018. Rhizobia: from saprophytes to endosymbionts. Nature Reviews Microbiology 16, 291–303. https://doi.org/10.1038/nrmicro.2017.171

Rieke, E.L., Cappellazzi, S., Michael Cope, Daniel Liptzin, G. Mac Bean, Greub, K.L.H., Norris, C.E., Paul W. Tracy, Ezra Aberle, Amanda Ashworth, Oscar Bañuelos Tavarez, Andy I. Bary, R.L. Baumhardt, Alberto Borbón Gracia, Daniel C. Brainard, Jameson R. Brennan, Dolores Briones Reyes, Darren Bruhjell, Carlyle, C.N., Carlyle, C.N., James J.W. Crawford, Cody F. Creech, Steve W. Culman, Deen, B., Curtis J. Dell, Justin D. Derner, Ducey, T.F., Duiker, S.W., Miles F. Dyck, Benjamin H. Ellert, Avelino Espinosa Solorio, Fonte, S.J., Fonteyne, S., Fortuna, A.-M., Foster, J.L., Lisa M. Fultz, Gamble, A.V., Geddes, C.M., LaHue, D.G., John H. Grove, Stephen K. Hamilton, Xiying Hao, Zachary D. Hayden, Honsdorf, N., Howe, J.A., Ippolito, J.A., Gregg A. Johnson, Mark A. Kautz, Newell R. Kitchen, Sandeep Kum ar, Kirsten SM Kurtz, Larney, F.J., Katie L. Lewis, Matt Liebman, Antonio Lopez Ramirez, Stephen Machado, Maharjan, B., Miguel Angel Martinez Gamiño, William E. May, Mitchel P. McClaran, Marshall D. McDaniel, Neville Millar, Jeffrey P. Mitchell, Moore, A., Philip A. Moore, Manuel Mora Gutiérrez, Kelly A. Nelson, Emmanuel C. Omondi, Shannon L. Osborne, Leodegario Osorio Alcalá, Owens, P.R., Eugenia M. Pena-Yewtukhiw, Poffenbarger, H., Brenda Ponce Lira, Jennifer R. Reeve, Timothy M. Reinbott, Mark S. Reiter, Edwin L. Ritchey, Rozeboom, K., Roozeboom, K.L., Yichao Rui, Sadeghpour, A., Sainju, U.M., Gregg R. Sanford, Schillinger, W.F., Robert R. Schindelbeck, Meagan E. Schipanski, Alan J. Schlegel, Kate M. Scow, Lucretia A. Sherrod, Shober, A.L., Sudeep S. Sidhu, Ernesto Solís Moya, Mervin St Luce, Jeffrey S. Strock, Andrew E. Suyker, Sykes, V.R., Haiying Tao, Alberto Trujillo Campos, Laura L. Van Eerd, Verhulst, N., Tony J. Vyn, Yutao Wang, Watts, D.B., Bryan B. William, David L. Wright, Tiequan Zhang, Zhang, T.Q., Cristine L.S. Morgan, Honeycutt, C.W., 2022. Linking soil microbial community structure to potential carbon mineralization: A continental scale assessment of reduced tillage. Soil Biology & Biochemistry 108618–108618. https://doi.org/10.1016/j.soilbio.2022.108618

Robertson, G.P., Klingensmith, K.M., Klug, M.J., Paul, E.A., Crum, J.R., Ellis, B.G., 1997. Soil resources, microbial activity, and primary production across an agricultural ecosystem. Ecological Applications 7, 158–170. https://doi.org/10.1890/1051-0761(1997)007[0158:srmaap]2.0.co;2

Robertson, G. Philip, Robertson, G. P., Gross, K.L., Hamilton, S.K., Landis, D.A., Schmidt, T.J., Schmidt, T.M., Snapp, S.S., Swinton, S.M., 2015. Farming for ecosystem services: An ecological approach to production agriculture. BioScience 33–53. https://doi.org/10.1093/biosci/biu037

Robertson, G. P. and S. K. Hamilton. 2015. Long-term ecological research in agricultural landscapes at the Kellogg Biological Station LTER site: conceptual and experimental framework. Pages 1-32 in S. K. Hamilton, J. E. Doll, and G. P. Robertson, eds. The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability.

Rousk, J., Bååth, E., 2007. Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique. Soil Biology & Biochemistry 39, 2173–2177. https://doi.org/10.1016/j.soilbio.2007.03.023

Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., Urs Zihlmann, Zihlmann, U., van der Heijden, M.G.A., Oehl, F., Oehl, F., 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biology & Biochemistry 84, 38–52. https://doi.org/10.1016/j.soilbio.2015.02.005

Samson, F.B., Knopf, F.L., 1994. Prairie conservation in North America. BioScience 44, 418–421. https://doi.org/10.2307/1312365

Sanford, G.R., Oates, L.G., Jasrotia, P., Thelen, K.D., Robertson, G.P., Jackson, R.D., 2016. Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA. Agriculture, Ecosystems & Environment 216, 344–355. https://doi.org/10.1016/j.agee.2015.10.018

Schulte, L.A., Niemi, J., Helmers, M.J., Liebman, M., Arbuckle, J.G., David E. James, James, D.E., Kolka, R.K., O'Neal, M.E., Tomer, M.D., Tomer, M.D., Tyndall, J.C., Asbjornsen, H., Drobney, P., Neal, J., Van Ryswyk, G., Witte, C., 2017. Prairie strips improve biodiversity and the delivery of multiple ecosystem services from corn–soybean croplands. Proceedings of the National Academy of Sciences of the United States of America 114, 11247–11252. https://doi.org/10.1073/pnas.1620229114

Schmidt, S.K., Costello, E.K., Nemergut, D.R., Cleveland, C.C., Reed, S.C., Weintraub, M.N., Meyer, A.F., Martin, A.M., 2007. Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. Ecology 88, 1379–1385. https://doi.org/10.1890/06-0164

Schmidt, T.J., Schmidt, T.M., Waldron, C., 2015. Microbial diversity in soils of agricultural landscapes and its relation to ecosystem function in S. K. Hamilton, J. E. Doll, and G. P. Robertson, eds. The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability.

Schmidt, R., Gravuer, K., Bossange, A.V., Mitchell, J.P., Scow, K.M., 2018. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLOS ONE 13. https://doi.org/10.1371/journal.pone.0192953

Shade, A., 2017. Diversity is the question, not the answer. The ISME Journal 11, 1–6. https://doi.org/10.1038/ismej.2016.118

Shaw, E.A., Denef, K., de Tomasel, C.M., Cotrufo, M.F., Wall, D.H., 2016. Fire affects root decomposition, soil food web structure, and carbon flow in tallgrass prairie 2, 199–210. https://doi.org/10.5194/soil-2-199-2016

Stephenson, J.A., M. Liebman, J. Niemi, R. Cruse, J. Tyndall, C. Witte, D. James, and M. Helmers. 2024. The influence of prairie strips sown in midwestern corn and soybean fields on sediment discharge throughout the year. Journal of Soil and Water Conservation: In press.

Raksha S., Goodwin, S.B. 2022. Exploring the Corn Microbiome: A Detailed Review on Current Knowledge, Techniques, and Future Direction. PhytoFrontiers. https://doi.org/10.1094/phytofr-04-21-0026-rvw

Smith, T.E., Kolka, R.K., Zhou, X., Kolka, R., Tomer, M.D., Cruse, R. Effects of native perennial vegetation buffer strips on dissolved organic carbon in surface runoff from an agricultural landscape. *Biogeochemistry* 120, 121–132 (2014). https://doi.org/10.1007/s10533-014-9985-y

Sprunger, C.D., Oates, L.G., Jackson, R.D., Robertson, G.P., 2017. Plant community composition influences fine root production and biomass allocation in perennial bioenergy cropping systems of the upper Midwest, USA. Biomass & Bioenergy 105, 248–258. https://doi.org/10.1016/j.biombioe.2017.07.007

Sprunger, C.D., Robertson, G.P., 2018. Early accumulation of active fraction soil carbon in newly established cellulosic biofuel systems. Geoderma 318, 42–51. https://doi.org/10.1016/j.geoderma.2017.11.040

Syswerda, S.P., Corbin, A.T., Mokma, D.L., Kravchenko, A., Robertson, G. Philip, Robertson, G. P., 2011. Agricultural Management and Soil Carbon Storage in Surface vs. Deep Layers. Soil Science Society of America Journal 75, 92–101. https://doi.org/10.2136/sssaj2009.0414

Thiéry, O., Moora, M., Vasar, M., Zobel, M. and Öpik, M., 2012. Inter- and intrasporal nuclear ribosomal gene sequence variation within one isolate of arbuscular mycorrhizal fungus, Diversispora sp. Symbiosis 58, 135–147. doi:10.1007/s13199-012-0212-0

Tsiafouli, M.A., Thébault, E., Sgardelis, S.P., de Ruiter, P.C., van der Putten, W.H., Birkhofer, K., Hemerik, L., de Vries, F.T., Bardgett, R.D., Brady, M., Bjørnlund, L., Jørgensen, H.B., Christensen, S., Hertefeldt, T.D., Hotes, S., Hol, W.H.G., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Josef Stary, Stary, J., Wolters, V., Hedlund, K., 2015. Intensive agriculture reduces soil biodiversity across Europe. Global Change Biology 21, 973–985. https://doi.org/10.1111/gcb.12752

Turley, N.E., Bell-Dereske, L., Evans, S.E., Brudvig, L.A., 2020. Agricultural land-use history and restoration impact soil microbial biodiversity. Journal of Applied Ecology 57, 852–863. https://doi.org/10.1111/1365-2664.13591

United States Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS). 2020. Conservation Reserve Program CP43 Prairie Strips.

United States Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS). 2022. Conservation Practices on Cultivated Cropland: A Comparison of CEAP I and CEAP II Survey Data and Modeling.

Upton, R.N., Bach, E.M., Hofmockel, K.S., 2018. Belowground response of prairie restoration and resiliency to drought. Agriculture, Ecosystems & Environment 266, 122–132. https://doi.org/10.1016/j.agee.2018.07.021

van Elsas, J.D., Trevors, J.T. and van Overbeek, L. S., 1991. Influence of soil properties on the vertical movement of genetically-marked pseudomonas fluorescens through large soil microcosms. Biology and Fertility of Soils, 10(4), 249-255. https://doi.org/10.1007/BF00337375

Van Trump, J.I., Wrighton, K.C., Thrash, J.C., Weber, K.A., Andersen, G.L., Coates, J.D., 2011. Humic Acid-Oxidizing, Nitrate-Reducing Bacteria in Agricultural Soils. Mbio 2. https://doi.org/10.1128/mbio.00044-11

Van Veen, J.A., Paul, E.A., 1981. Organic carbon dynamics in grassland soils. 1. Background information and computer simulation. Canadian Journal of Soil Science 61, 185–201. https://doi.org/10.4141/cjss81-024

Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America 111, 5266–5270. https://doi.org/10.1073/pnas.1320054111

Warmink, J., Nazir, R., Corten, B. and van Elsas, J.D., 2011. Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. Soil Biology and Biochemistry 43:760-765. http://doi.org/10.1016/j.soilbio.2010.12.009

West, J.R. and Whitman, T., 2022. Disturbance by soil mixing decreases microbial richness and supports homogenizing community assembly processes. FEMS Microbiology Ecology 98(9). https://doi.org/10.1093/femsec/fiac089

Whitman, T., Whitman, E., Woolet, J., Flannigan, M.D., Thompson, D.K., Parisien, M.-A., 2019. Soil Bacterial and Fungal Response to Wildfires in the Canadian Boreal Forest Across a Burn Severity Gradient. bioRxiv 512798. https://doi.org/10.1101/512798

Wiegand, S., Jogler, M., Jogler, C., 2018. On the maverick Planctomycetes. Fems Microbiology Reviews 42, 739–760. https://doi.org/10.1093/femsre/fuy029

Wilson, G.W.T., Hartnett, D.C., 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany 85, 1732–1738. https://doi.org/10.2307/2446507

Yang, P., van Elsas, J.D., 2018. Mechanisms and ecological implications of the movement of bacteria in soil. Applied Soil Ecology 129, 112–120. https://doi.org/10.1016/j.apsoil.2018.04.014

Zhang, S., Li, Q., Lü, Y., Zhang, X., Liang, W., 2013. Contributions of soil biota to C sequestration varied with aggregate fractions under different tillage systems. Soil Biology & Biochemistry 62, 147–156. https://doi.org/10.1016/j.soilbio.2013.03.023

Zhu, L., Dickson, T.L., Zhang, Z., Dere, A., Xu, J., Bragg, T.B., Tapprich, W., Lu, G., 2021. Effects of burning and mowing on the soil microbiome of restored tallgrass prairie. European Journal of Soil Science 72, 385–399. https://doi.org/10.1111/ejss.12980

APPENDIX A: CHAPTER 1

Tables

	Bacteria			Fungi		
Factor	Shannon	Richness	Evenness	Shannon	Richness	Evenness
Year	**<0.001** (0.023)	**<0.001** (0.126)	0.569 (<0.001)	**0.031** (0.005)	**<0.001** (0.131)	0.056 (0.004)
Cropping system	**<0.001** (0.043)	**<0.001** (0.027)	**<0.001** (0.041)	0.882 (<0.001)	**0.048** (0.006)	0.586 (<0.001)
Vegetation	**<0.001** (0.022)	**0.018** (0.005)	**<0.001** (0.029)	**<0.001** (0.02)	0.102 (0.003)	**<0.001** (0.023)
Year x Cropping system	0.235 (0.043)	0.048 (0.027)	0.494 (0.041)	**0.003** (<0.001)	**<0.001** (0.006)	**0.045** (<0.001)
Year x Vegetation	0.995 (0.022)	0.347 (0.005)	0.544 (0.029)	**0.044** (0.02)	0.570 (0.003)	**0.041** (0.023)
Cropping system x Vegetation	0.069 (0.054)	0.249 (0.025)	0.057 (0.059)	0.824 (0.009)	0.224 (0.007)	0.367 (0.008)

Table 1.1. Bacterial and fungal alpha diversity in prairie strip and cropland soils. P-values† and R^2 values (in parentheses)‡ from linear models§ .

†Bold values indicate significant difference in diversity metric between treatment groups.at $p < 0.05$. Underlined values indicate significance at $p < 0.1$.

‡R^2 value indicates the percentage of variation in microbial alpha diversity explained by the prairie strip treatment

§Linear models with factors Year (2 months, 1 year, 2 years, and 3 years after prairie strip planting), Cropping system (Reduced Input and Organic), Vegetation (prairie and cropland), and their interactions.

	Bacteria				Fungi			
	2 months	1 year	2 years	3 years	2 months	1 year	2 years	3 years
Cropping system	**<0.001** (0.037)	**<0.001** (0.044)	**<0.001** (0.034)	**<0.001** (0.034)	**<0.001** (<0.001)	**<0.001** (0.038)	**<0.001** (0.073)	**<0.001** (0.082)
Vegetation	0.258 (0.005)	**<0.001** (0.032)	**<0.001** (0.025)	**<0.001** (0.021)	**0.032** (0.007)	**<0.001** (0.102)	**<0.001** (0.042)	**<0.001** (0.046)
Cropping system x Vegetation	0.417 (0.005)	**<0.001** (0.008)	**0.001** (0.006)	**0.001** (0.006)	0.525 (0.528)	**<0.001** (0.015)	**<0.001** (0.011)	**0.005** (0.008)

Table 1.2. Bacterial and fungal community composition in prairie strip and cropland soils. P-values† and R^2 values (in parentheses)‡ from permutational analysis of variance (PERMANOVA§).

†Bold values indicate significant difference in cropland microbial community composition between groups in each year at $p < 0.05$.

‡R^2 value indicates the percentage of variation in microbial beta diversity explained by distance from the prairie strip.

§PERMANOVAs include factor Cropping system (Reduced Input and Organic), Vegetation (prairie strip and cropland), and their interaction.

	Bacteria		Fungi	
	Reduced Input	Organic	Reduced Input	Organic
2 months (wheat)	0.295 (0.014)	<u>0.062</u> (0.011)	0.297 (0.014)	0.389 (0.009)
1 year (corn)	0.429 (0.009)	0.700 (0.013)	0.222 (0.011)	**0.034** (0.022)
2 years (soybean)	0.421 (0.009)	0.686 (0.011)	**0.021** (0.019)	0.768 (0.008)
3 years (wheat)	0.112 (0.011)	**0.038** (0.011)	**0.026** (0.015)	0.578 (0.010)

Table 1.3. Bacterial and fungal community composition in cropland soils. P-values† and $R^{2‡}$ values (in parentheses) from permutational analysis of variance (PERMANOVA§).

†Bold values indicate significant difference in cropland microbial community composition among distances from the prairie strip (1m, 5m, 20m) in each year at $p < 0.05$. Underlined values indicate significance at $p < 0.1$.

‡R^2 value indicates the percentage of variation in microbial beta diversity explained by distance from the prairie strip.

§PERMANOVAs include factor Distance (1m, 5, and 20m from prairie strip).

Reduced Input

Phylum	2 months	1 year	2 years	3 years
Acidobacteria	0.923	**0.041**	**0.001**	0.495
Actinobacteria	0.722	0.052	**0.029**	0.495
Planctomycetes	**0.031**	**0.001**	**0.002**	0.088
Verrucomicrobia	0.963	**0.001**	**0.001**	**0.001**
Ascomycota	0.084	**0.006**	0.609	0.322
Basidiomycota	0.198	0.526	0.609	0.322
Glomeromycota	0.99	**0.001**	**0.002**	0.615

Organic

Phylum	2 months	1 year	2 years	3 years
Acidobacteria	0.966	0.358	**0.001**	0.813
Actinobacteria	0.966	**0.001**	0.82	0.136
Planctomycetes	0.876	**0.001**	0.08	0.294
Verrucomicrobia	0.961	**0.001**	**0.001**	**0.002**
Ascomycota	0.733	0.591	**0.001**	**0.002**
Basidiomycota	0.063	**0.004**	0.07	**0.002**
Glomeromycota	0.225	**0.001**	**0.001**	**0.002**

Table 1.4. Differences in relative abundance of tallgrass prairie microbial taxa in prairie strip and cropland soils. P-values† from two-way ANOVA‡. Cell shading represents the vegetation that contains significantly higher phylum abundance: light grey represents higher abundance in prairie strip soils, and dark grey represents higher abundance in cropland soils.

†Bold values indicate significant difference in abundance between prairie strip and cropland soils at $p < 0.05$. Underlined values indicate significance at $p < 0.1$.

‡Two-way ANOVA includes factor Vegetation (prairie strip and cropland).

	Prairie-associated Bacteria	Prairie-associated Fungi
Factor	P value (R^2)	P value (R^2)
Year	**0.004** (0.003)	**<0.001** (0.007)
Cropping system	0.422 (<0.001)	**<0.001** (0.01)
Distance	0.774 (<0.001)	0.957 (<0.001)
Year x Cropping system	0.935 (<0.001)	0.754 (0.002)
Year x Distance	0.837 (<0.001)	0.539 (<0.001)
Cropping system x Distance	0.629 (<0.001)	0.978 (<0.001)

Table 1.5. Differences in relative abundance of tallgrass prairie microbial taxa in cropland soils. P-values† and R^2 values (in parentheses)‡ from linear model§ of relative abundance of prairie-associated bacterial phyla (Acidobacteria, Actinobacteria, Planctomycetes and Verrucomicrobia) and prairie-associated fungal phyla (Ascomycota, Basidiomycota, and Glomeromycota).

†Bold values indicate significant difference in phyla relative abundance between groups at $p < 0.05$. Underlined values indicate significance at $p < 0.1$.

‡R^2 value indicates the percentage of variation in relative abundance explained by each factor.

§Linear models include factors Year (2 months, 1 year, 2 years, and 3 years after prairie strip planting), Cropping system (Reduced Input and Organic), Distance (1m, 5m, and 20m from the prairie strip), and their interactions.

Figures

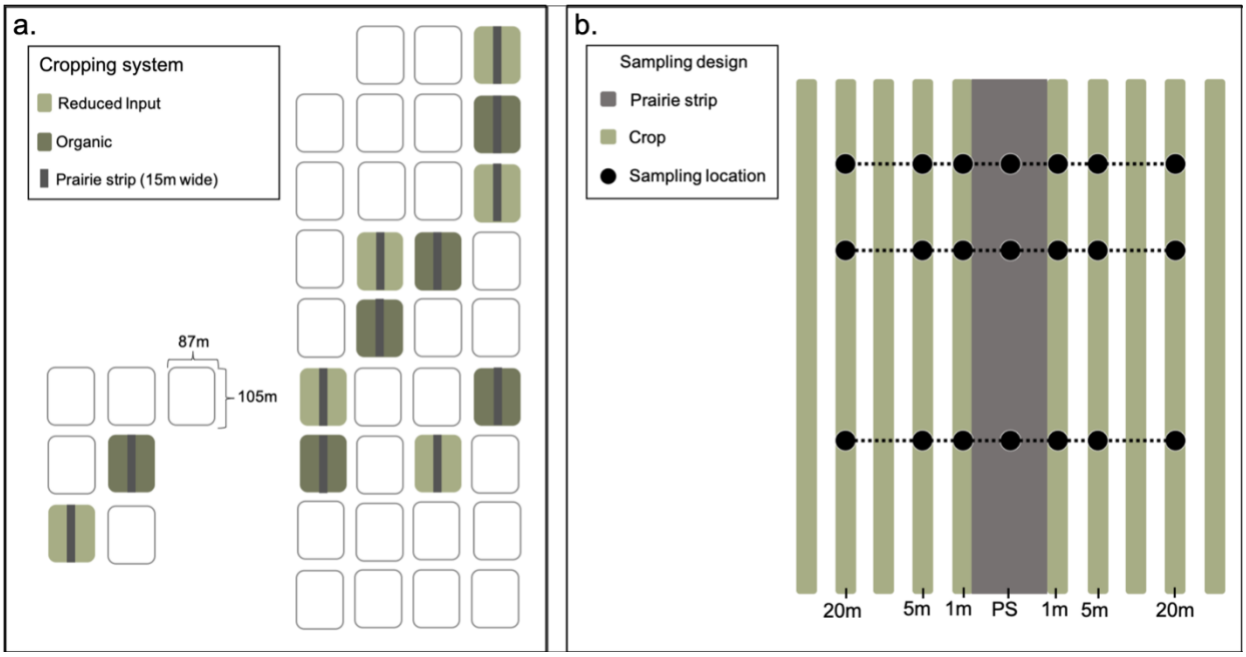

Figure 1.1. a. Schematic of the KBS LTER Main Cropping System Experiment including six replicated 1-ha plots of each crop management treatment with prairie strips. b. Sampling design within each 1-ha plot. Each point represents the sampling location of a 0-10cm soil sample during each annual sampling event.

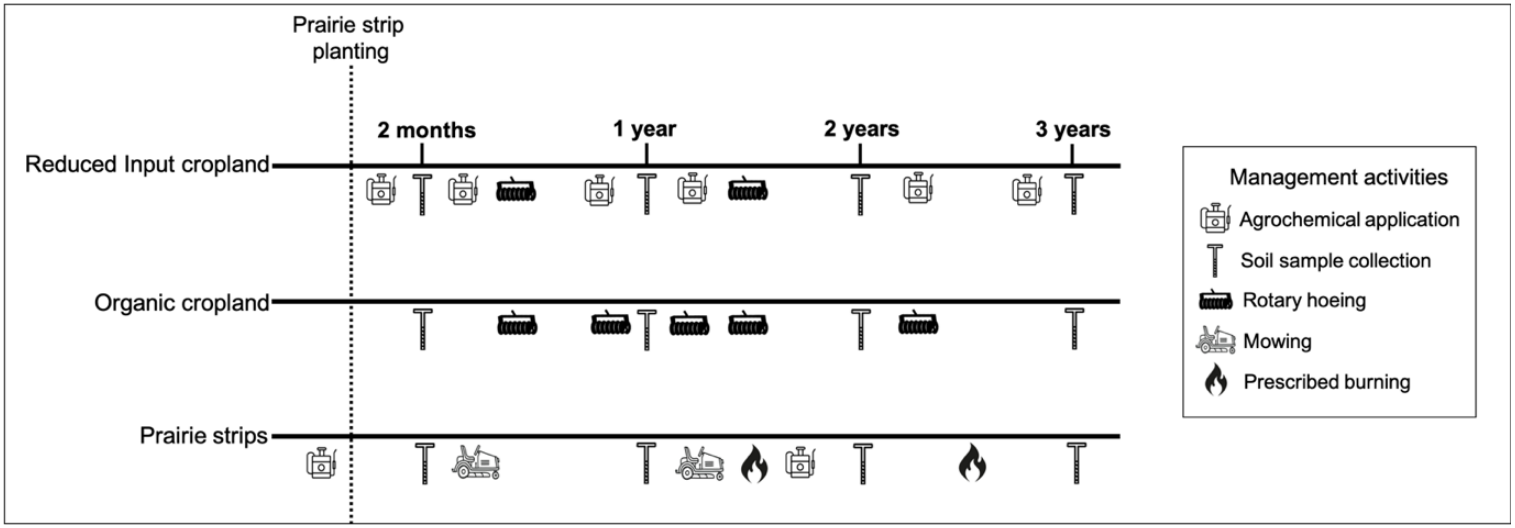

Figure 1.2. Crop management timeline for Reduced Input cropland, Organic cropland, and prairie strips. Note: agrochemical application only occurred in prairie strips within Reduced Input plots.

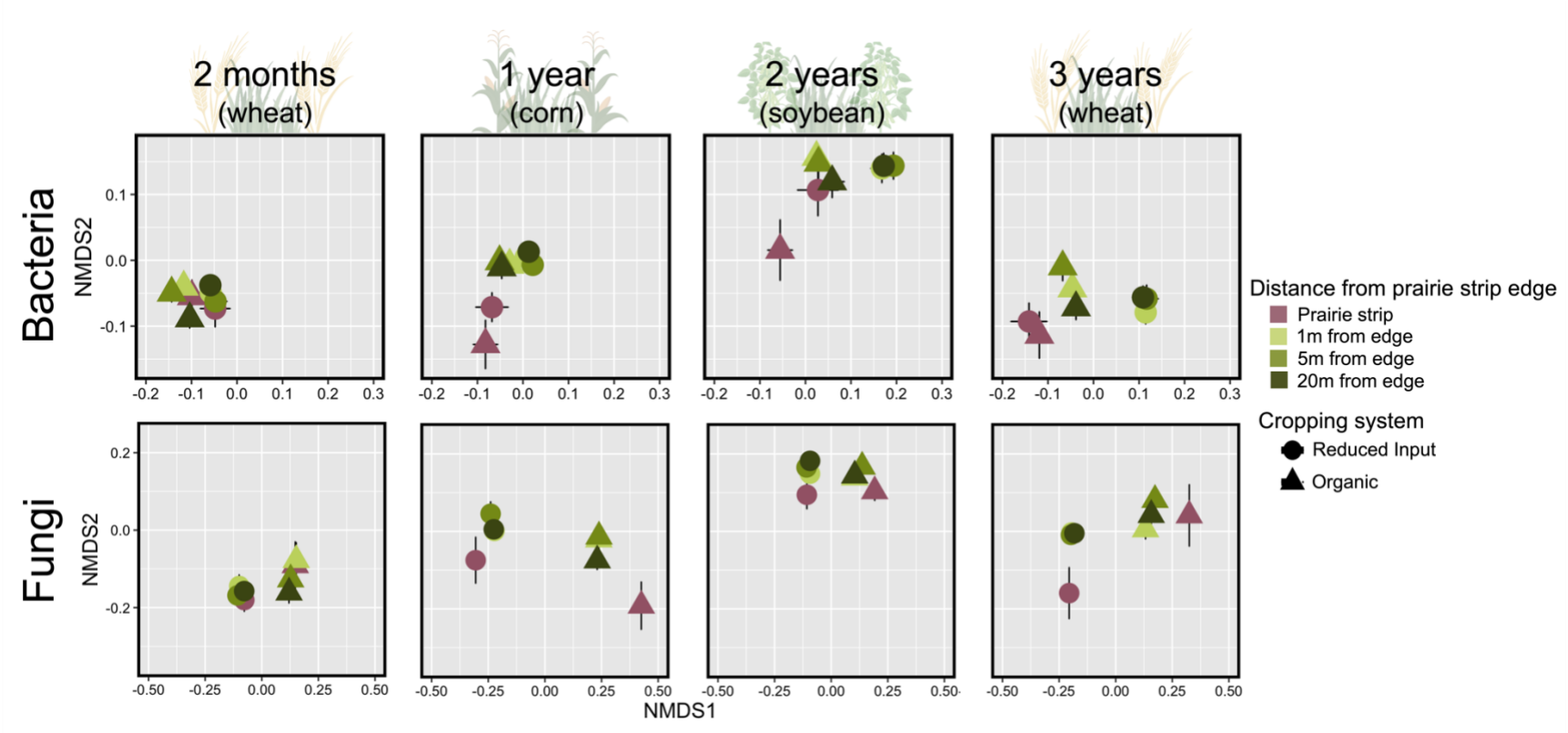

Figure 1.3. Non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarity matrices for prairie strip and cropland bacterial and fungal communities in each year of prairie strip establishment: 2 months post-planting (wheat), 1 year post-planting (corn), 2 years post-planting (soybean), and 3 years post-planting (wheat).

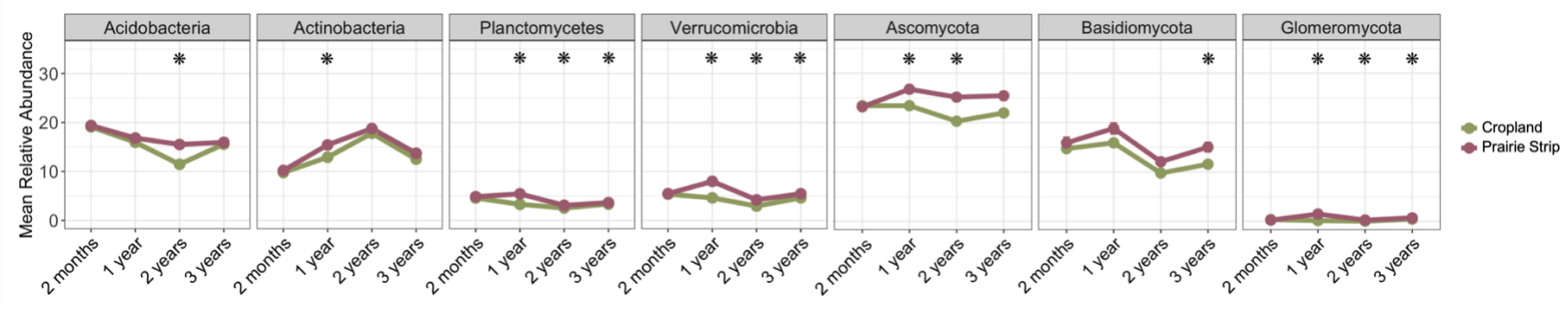

Figure 1.4. Average relative abundance of prairie-associated bacterial and fungal phyla each year, averaged across cropping systems. Asterisks indicate significant ($p < 0.05$) difference in relative abundance between prairie strip and cropland soils in that year of the study.

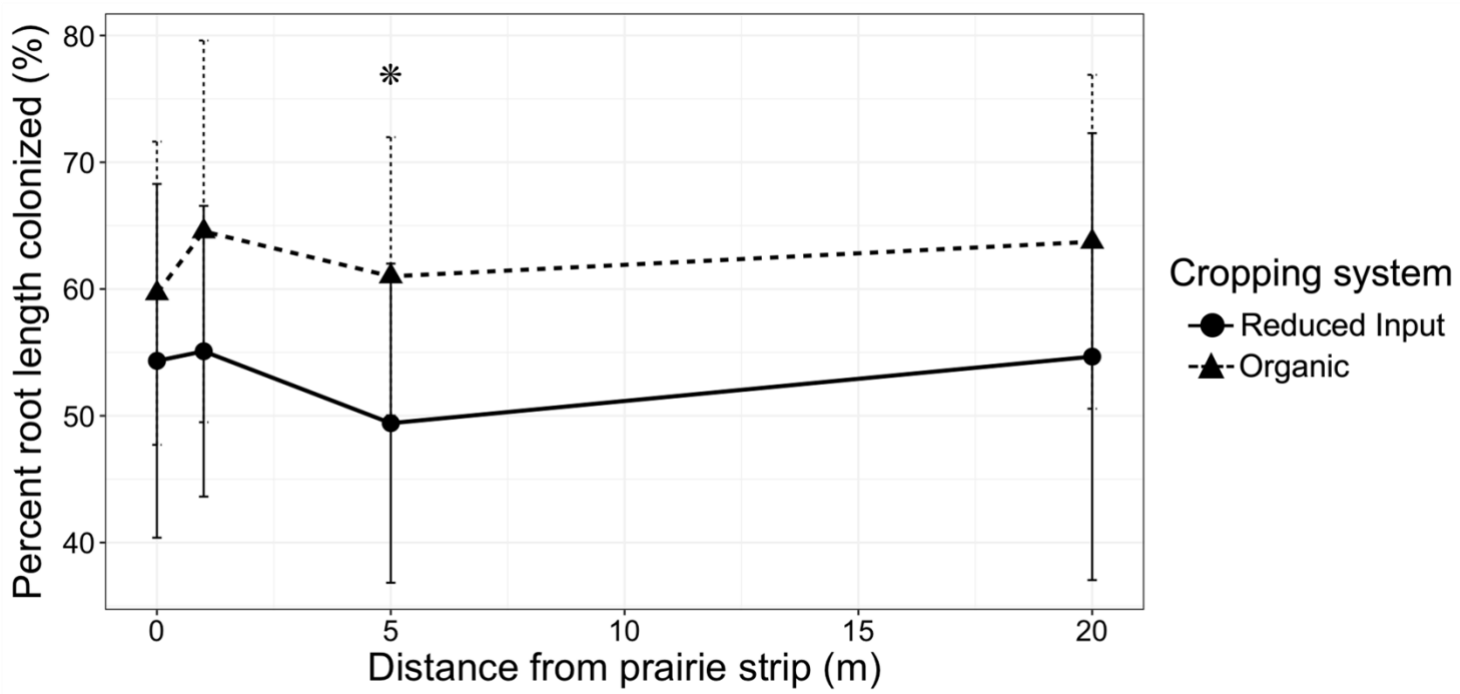

Figure 1.5. Percent root length colonized by arbuscular mycorrhizal fungal (AMF) structures in Reduced Input plots (circles and solid line) and Organic plots (triangles and dashed line) four years after prairie strip planting. In prairie strips (0m distance), AMF structures were quantified in big bluestem roots. In cropland (1m, 5m, 20m distances), AMF structures were quantified in winter wheat roots. Asterisks indicate difference in abundance between cropping systems at $p < 0.05$.

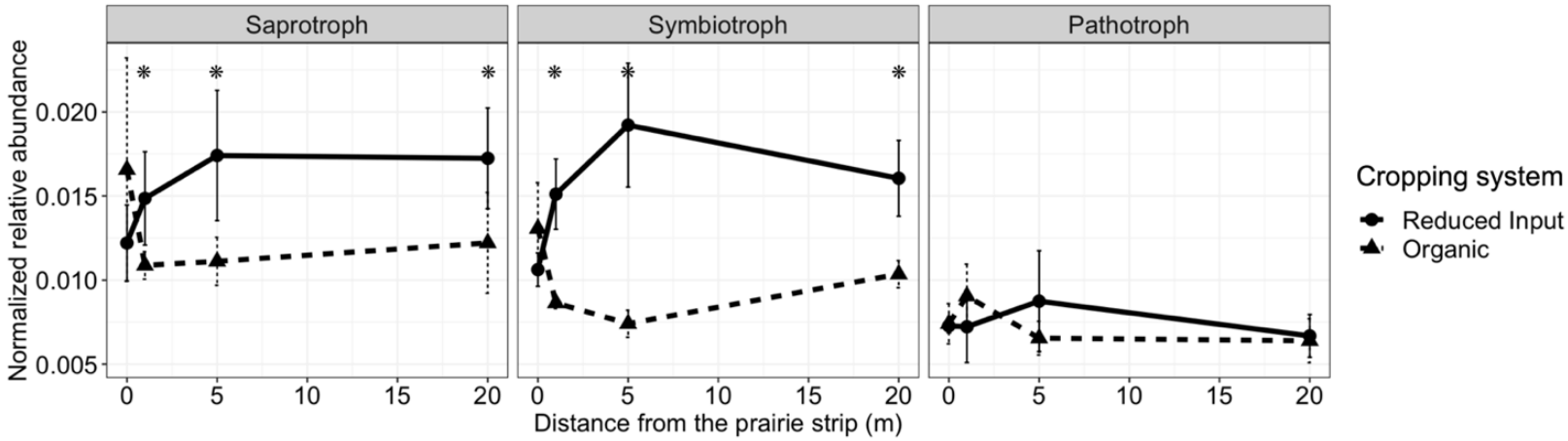

Figure 1.6. Relative abundance of fungal trophic modes in prairie strips (0m) and each cropland distance (1m, 5m, 20m) averaged over each year and cropping system. Asterisks indicate significant difference in abundance between cropping systems at $p < 0.05$.

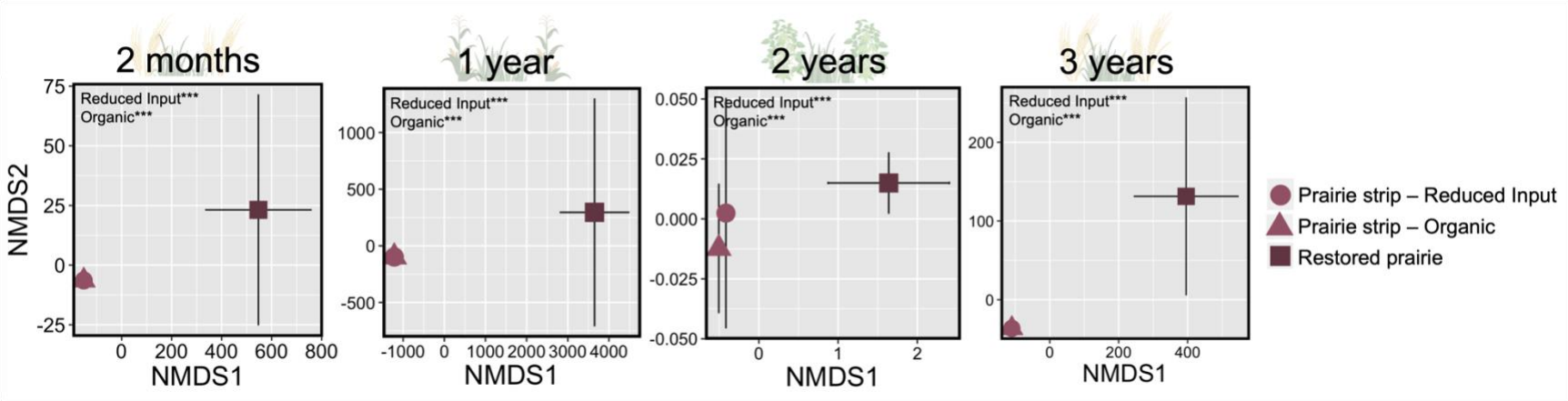

Figure 1.7. Non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarity matrices for bacterial communities in a 10 year old restored prairie (squares), prairie strips in Reduced Input cropping systems (circles) and prairie strips in Organic cropping systems (triangles).

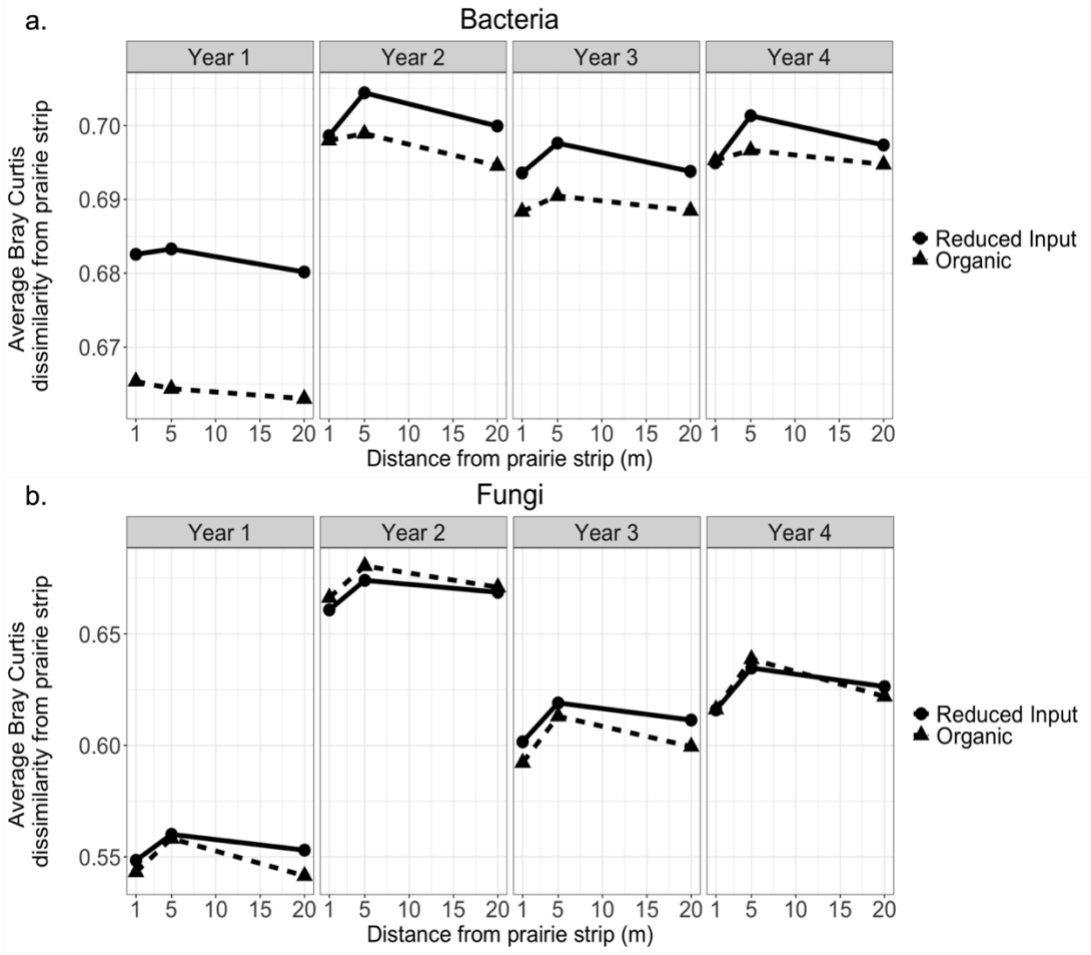

Figure 1.8. Average Bray-Curtis dissimilarity between bacterial (a) and fungal (b) communities in prairie strip (PS) and cropland soil bacterial communities at each distance (1m, 5m, and 20m).

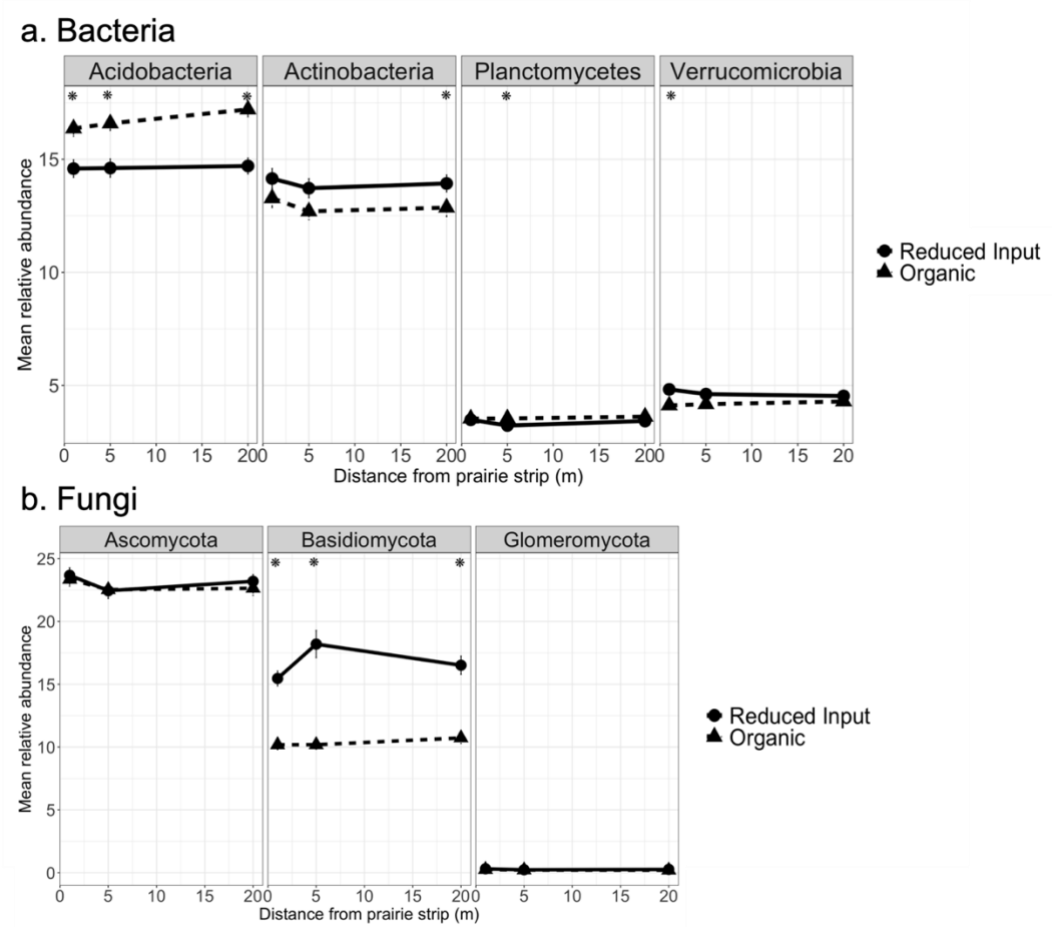

Figure 1.9. Relative abundance of prairie-associated bacterial and fungal phyla in cropland soils at each distance from the prairie strip. Asterisk indicates significant difference in abundance between cropping systems at $p < 0.05$.

CHAPTER 2: CONTOUR PRAIRIE STRIPS ALTER MICROBIAL COMMUNITIES AND FUNCTIONING BOTH BELOW AND IN ADJACENT CROPLAND SOILS[1]

ABSTRACT

Prairie strips are narrow strips of native, perennial vegetation (10-40 m width) integrated within cropped fields to provide benefits for water quality and biodiversity. However, the impact of prairie strips on soil microbial communities and function, both underneath the prairie strips and in the adjacent cropland, is not known. We assessed the effect of restoring native perennial vegetation on soil C and N, potential enzyme activities (PEA), and microbial community composition in the soil directly underneath and cropland adjacent (0.1 to 9 m) to 12-year-old prairie strips integrated within row crop fields. We found that prairie strips consistently increased soil microbial biomass carbon (>56%) and altered PEA in complex ways. Generally, prairie strips increased hydrolase and decreased oxidoreductase PEA. Prairie strips also changed the soil microbial community directly under prairie vegetation, and, contrary to the expectation that greater plant diversity leads to greater soil microbial diversity, prairie strips reduced bacterial and fungal diversity. The prairie strip's effect on adjacent cropland soils depended on year, but it was strong when it occurred and was typically independent of distance from the prairie strip. Prairie strips increased PEA in adjacent soils (<9 m) by as much as 38% and shifted bacterial and fungal beta diversity, but neither showed patterns with distance from the prairie strip, indicating that prairie strips cause field-scale shifts in soil biota and functioning, and these effects are not mediated by proximity to the prairie strip. Understanding the mechanisms underlying prairie strips' impact on soil biota, both underneath and adjacent to the prairie, is key to optimize their agroecosystem benefits.

[1] Originally published as: Dutter, C., C.E. Rutkoski, S.E. Evans & M.D. McDaniel. 2024. Contour prairie strips alter microbial communities and functioning both below and in adjacent cropland soils. *Applied Soil Ecology*.

INTRODUCTION

While intensive agriculture has steadily increased the per-hectare productivity of most grain crops (Cassman and Grassini 2020), it has come with consequences, including the degradation of soil ecosystem services (SESs) regulated by soil biota (Baldwin-Kordick et al. 2022; Gerla 2007). To restore biota-driven SESs and maintain economic viability of agroecosystems, we must find ways to regenerate soil health while maintaining or increasing crop productivity. In the Midwest US, restoring native, perennial vegetation is one effective approach to regenerate SESs (Bach and Hofmockel 2015; Baer et al. 2002; De et al. 2020; McLauchlan et al. 2006). Despite the known improvement in SESs, converting entire fields from annual cropland to perennial grassland is often not economically feasible for individual growers, nor can it meet the global demand for agricultural products.

Integrating prairie strips into cropland is a new conservation practice that offers both the environmental benefits of grassland restoration and the economic benefits of crop production. Instead of taking an entire field out of production, prairie strips are narrow strips of diverse perennial grasses and forbs (10-40 m width and <25% of the field) integrated into agricultural fields to slow overland water flow and minimize sediment and nutrient losses from fields (Figure 2.1). Prairie strips disproportionately benefit ecological function at the catchment scale (Schulte et al. 2017). For example, prairie strips occupying as little as 10% of a given catchment can: reduce sediment export by up to 95% (Helmers et al. 2012; Schulte et al. 2017), reduce total water runoff by up to 29-44% (Gutierrez-Lopez et al. 2014; Hernandez-Santana et al. 2013), increase plant diversity up to 380% and increase wildlife abundance and activity by up to 150-288% (Hirsh et al. 2013; Schulte et al. 2016).

In addition to these catchment-scale benefits, prairie strips should also enhance SESs in

the soil directly underneath, similar to large swaths of native, perennial vegetation. Specifically, restoring perennial grasslands increases microbial biomass (Bach and Hofmockel 2015; Li et al. 2018), increases microbial activity measured as respiration or potential enzyme activities (PEA; Bach and Hofmockel 2015; Raiesi and Salek-Gilani 2018), reduces mobile nitrate-nitrogen (N; Baer et al. 2002; Karlen et al. 1999), increases labile C (De et al. 2020; Hurisso et al. 2014), and increases soil organic C (Li et al. 2017; Munson et al. 2012; Pérez-Suárez et al. 2014). These biochemical measurements often coincide with measures of larger and more stable aggregates (Jastrow 1996), reduced nutrient leaching (Daigh et al. 2015), and lower greenhouse gas emissions (Oates et al. 2016).

Like larger prairie or grassland restoration studies, prairie strips will also alter the soil microbial community composition under the perennial, native vegetation. Over time, the soil microbial communities in restored prairies increasingly resemble those of remnant prairie during the first several years of establishment, and prairie strip communities may follow a similar trajectory (Barber et al. 2017). Restoring prairie vegetation has been shown to increase bacterial diversity in some cases (Bach et al. 2018, Upton et al. 2018), but in other cases, soil bacterial diversity declines as perennial restorations become older and more established (Barber et al. 2017).

While the effects of restored perennial vegetation might be strongest on the underlying soil, the benefits of prairie strips may also extend beyond, causing a "spillover effect" into the adjacent cropland. Indeed, more motile organisms like insects can move between habitats when prairie is integrated into cropland (Kemmerling et al. 2022), but the extent to which this may apply to other, less-motile soil biota is unclear. One line of reasoning is that a landscape comprised of varied habitat types can serve as an important source for microbial colonizers that

would otherwise be absent from a disturbed, simplified landscape of annual crops (Mony et al. 2022; Bell and Tylianakis 2016). Prairie strips could provide a habitat source or refuge by providing a reservoir of novel prairie taxa, especially closer to the strip. In addition, prairie strips have already been shown to alter the adjacent cropland soil environment (Dutter et al. 2023; Figure 2.10). More specifically, prairie strips have been shown to decrease soil water content, decrease nitrate, and accumulate plant-availble phosphorus and potassium in cropland soils up to 9 m distance from the native, perennial vegetation (Dutter et al. 2023). And this, in turn, would likely impact soil biota in the adjacent cropland (Senaviratne et al., 2012); especially cropland soil closer to the prairie strip (Hargreaves et al. 2015).

Alternatively, prairie strips may not affect adjacent cropland soil biota because the intensity of cropland management as an "environmental filter," and may preclude any influence of the prairie strip on cropland soil biota. Many soil microbes are limited in movement, or rely on the movement of air, water, fungal hyphae, and microbivores to migrate (Choudoir et al. 2018, Chaudhary et al. 2020, van Elsas et al. 1991, Warmink et al. 2011, Coleman and Wall 2015). Cropland management is also likely to prevent soil biota from growing and surviving, even if dispersed from the prairie strip. Tillage, agrochemical applications, harvest, and other management practices are known to have strong effects on soil microbial community composition, alter SESs (Manzoni et al. 2012, West and Whitman 2022, Fierer and Jackson 2006, Schmidt et al. 2018), and be stronger drivers than dispersal limitation (Jones et al. 2022). This management-induced environmental filter may generate two distinct soil habitats - prairie and cropland - rather than a gradual integration of prairie and cropland soil biota at the habitat edges.

Our primary research objectives were to quantify prairie strips' effect on soil microbial

biomass, PEA, and microbial community composition and diversity under the prairie strips and in the adjacent cropland (< 9 m). We hypothesize that prairie strips will 1) increase microbial biomass, PEA, and bacterial and fungal diversity under the prairie strip as has been observed in larger grassland and prairie restorations (Bach and Hofmockel 2015, Bach et al. 2018, Upton et al. 2018); and 2) have little-to-no effect on adjacent cropland soil microbial biomass, PEA, and bacterial and fungal diversity because cropland management will be a strong environmental filter of the microbial community (Manzoni et al. 2012, West and Whitman 2022, Fierer and Jackson 2006, Schmidt et al. 2018, Jones et al. 2022), and because of previous inconsistent effects of prairie strips on cropland soil moisture and plant-available nutrients (Dutter et al. 2023). Measuring the direct and indirect effects of prairie strips on soil biota and SESs is critical for understanding how the practice impacts long-term agroecosystem sustainability.

METHODS

Site description and experimental design

The study was located on the Neal Smith National Wildlife Refuge (NSNWR; 41° 33' N;93°16' W), a 3000-ha mosaic of forest, remnant prairie, restored prairie, and cropland managed by the U.S. National Fish and Wildlife Service. NSNWR is in the Walnut Creek watershed in Jasper County, Iowa, which lies on the Iowa southern drift plain (Major Land Resource Area 108C; USDA Natural Resources Conservation Service, 2006). This area consists of steep rolling hills of Wisconsinan loess on pre-Illinoian till (Prior 1991). The soils within the catchments which we are studying prairie strips are classified as Ladoga (Mollic Hapludalf) or Otley (Oxyaquic Argiudolls) soil series with 5 to 14% slopes and are highly erodible (Nestrud and Worster 1979; Soil Survey Staff 2003). The 50-year mean (± standard deviation) annual precipitation is 876 ± 205 mm, and the mean annual temperature is 9.6 ± 0.9 ℃.

In 2007, a catchment-scale prairie strip experiment was established within NSNWR. Prairie strip and control catchments were arranged in a randomized, balanced, incomplete block design on 12 catchments ranging in size from 0.47 to 3.2 ha. Prairie strips were planted such that the prairie covered 0% (control), 10%, and 20% of the catchment area, and the prairie was established within the cropland (usually shoulder or backslope contour position) and at the foot slope of the catchments (Zhou et al. 2010). Before 2007, these fields were in smooth brome (*Bromus inermis* L) cover for at least ten years. Prairie strips were seeded with a tallgrass prairie seed mix containing 32 species in 2007. The seed mix consisted of 27% grasses, 24% forbs, 5% weedy forbs and weedy grasses, and 44% inert material by weight. Since 2007, after the prairie strip establishment, the adjacent cropland was planted in a soybean (*Glycine max* (L.) Merr.) and maize (*Zea mays* L.) rotation with no-till management. During the initial two years, the prairie strips were mowed periodically. In 2019, no fertilizer was added to the cropland before planting soybeans. In 2020, cropland was fertilized with 211 kg N ha^{-1}, 136 kg P ha^{-1}, and 185 kg K ha^{-1} before planting maize. This N rate is typical for maize years, but these P and K rates are applied on a 3-4 year basis depending on soil fertility tests.

For this study, we compared only the paired 10% prairie strip catchments (n=3) to those with 0% prairie (n=3), hereafter referred to as '*control'* (Figure 2.2). We chose to sample the 10% prairie strip catchments because previous research showed that converting 10% of a catchment to prairie was sufficient for environmental benefits (Schulte et al. 2017). Three transects perpendicularly bisecting prairie strips and paired positions in control catchments were chosen based on a digital elevation model, plan curvature and flow accumulation (see Dutter et al. 2023 for more details). Soil samples were collected along transects at ten distances with respect to the prairie strip and paired location in the control catchments: 3, 1, 0.3, 0.1 m upslope; and 0 ,0.1,

0.3, 1, 3, and 9 m downslope. The 0 m distance is in the center of the prairie strips or the equivalent, paired position in the control catchments (Figure 2.2). Transects and sampling locations were marked using the Arrow 100 GNSS® receiver. For more experimental details, see Dutter et al. (2023).

Soil sampling and analysis

Ten soil cores (0-15 cm depth) were taken with a 2-cm-diameter probe from each of the ten transect sampling points and composited (10 distances from strips × 3 transects × 3 catchments × 2 treatments). Soil cores were taken on July 1st in both 2019 and 2020. Samples were sieved to <2 mm for analysis. A 15 g subsample of soil was weighed and dried at 105 °C for 24 h for gravimetric water content (GWC) measurement. To measure microbial biomass C and N, twin replicates from each soil were weighed to ~ 5 g. One replicate was fumigated for 24 h with ethanol-free chloroform and both replicates were extracted with 25 ml of 0.5 M K_2SO_4. Non-purgeable organic carbon and total salt-extractable nitrogen were measured in all samples with a Shimadzu TOC-L analyzer with TN capabilities (Shimadzu Corporation, Kyoto, Japan). Readings were corrected with extraction coefficients (0.45 for C; 0.54 for N) and compared between each replicate (Brookes et al. 1985; Vance et al. 1987; Jenkinson 1988; Joergensen and Mueller 1996). Total salt-extractable N values were corrected for inorganic N. The C and N in salt-extracted but unfumigated samples are hereafter referred to as salt-extractable organic C (SEOC) and organic N (SEON). The non-fumigated extracts were also measured for ammonium-N and nitrate-N, hereafter referred to as salt-extractable inorganic N (SEIN), using a SynergyTM HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT, USA) with Gen5TM software (Doane and Horwáth 2003, Sinsabaugh et al. 2000). Ammonium-N was measured using absorbance at 595 nm wavelength and nitrate-N was measured using absorbance at 540 nm

wavelength. The remainder of the soil was air dried at 24 ℃ until stable weight (~1 month). Soil organic matter (SOM) was measured using loss on ignition for 2 h at 360 ℃ using a Blue M oven and TSI weighing system. Soil pH was measured using a 1:1 (w:w) soil water slurry and measured with a meter (Lignin Probes, Albuquerque, NM, USA). Cation exchange capacity was estimated from ammonium acetate equivalent values of the Mehlich 3 extracted cations.

Potential enzyme activity assays

Five g soil was immediately frozen after sieving and lyophilized within 2-3 months and stored at -20 ℃ before measuring PEA. Freezing soils has been shown to affect potential enzyme activity (Abellan et al. 2011; Peoples and Koide, 2012), but logistical constraints precluded analysis on fresh soils, and any storage effects are consistent for all samples. The potential activities of both hydrolytic and oxidative enzymes were measured according to standard protocols (Deforest 2009; Deng et al. 2011; German et al. 2011a). Hydrolytic enzymes – arylsulfatase (ARSase), β-glucosidase (BGase), cellobiohydrolase (CBHase), β-N-acetylglucosaminidase (NAGase), leucine aminopeptidase (LAPase), phosphatase (PHOSase) (Table 2.1) – were assayed following Deng et al.'s (2011) protocol for fluorescence measured via methylumbelliferyl - or methyl-coumarin-linked substrates in 96-well microplates with some modifications. One gram of freeze-dried soil was weighed and placed in a 200 ml beaker, with 150 ml of distilled (DI) water and stirred for 30 min. Afterward, 200 µl aliquots of soil suspension were incubated for 1 h at 37 ℃ with 50 µl of the substrate. After the incubation, 50 µl of the substrate was added to the control columns, and 50 µl of THAM was added to all columns to terminate enzyme activities. Then pre-incubation and post-incubation suspensions were compared. Autohydrolysis controls were also used for each enzyme, and standard curves for each catchment were prepared. Enzyme activity was calculated from fluorescence with

excitement at 360 nm and emission at 460 nm.

Oxidative enzyme activities – polyphenol oxidase (PPOase) and peroxidase (PERase) – were quantified using the colorimetric assay method in clear 96-well plates (Saiya-Cork et al. 2002). One gram of freeze-dried soil was weighed, put into suspension with 125 ml of Acetate buffer, and incubated with L-DOPA for 18 h at 25 ℃. Activities were calculated from an absorbance of 450 nm, and the standard extinction coefficient of 7.9 was used for these equations (DeForest 2009). All enzyme activities, both fluorometric and colorimetric, were measured using a SynergyTM HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT, USA) with Gen5TM software. Potential enzyme activity was calculated as measured substrate activity in nmol divided by g SOM and time in hours. We normalized for SOM due to known influence of SOM on PEA and increased SOM under the prairie strip (German 2011b; Zhang et al. 2015).

Microbial community analysis

A 5 g subsample from each original composite sample was sieved to <2mm and frozen at -80 ℃ for 4 months before DNA extraction. We characterized microbial communities in all prairie strip and cropland soil samples using a high-throughput amplicon sequencing Illumina MiSeq platform (Illumina, CA, USA). For each soil sample (360 samples total), we extracted genomic DNA using the Qiagen MagAttract KF PowerSoil DNA extraction kit with a Thermo Fisher KingFisher Flex automated extraction instrument (Thermo Fisher, U.S.A.) following all manufacturer protocols. DNA concentration was determined for all samples via fluorometry with the Invitrogen Qubit dsDNA HS Assay Kit (Thermo Fisher, U.S.A.).

Extracted DNA template was submitted to the Michigan State University Core Genomics Facility for Illumina bacterial 16S V4 and fungal ITS1 library construction using the Illumina TruSeq Nano DNA library preparation kit and sequencing, and reads were quality filtered and

merged using the USEARCH pipeline (https://drive5.com/usearch). Libraries of the bacterial 16S V4 region were prepared using Illumina-compatible, dual-indexed 515Ff/806r primers (Kozich et al. 2013). Libraries of the fungal ITS1 region were prepared using ITS1f/ITS2 primer sequences (Martin et al. 2005) in an initial PCR followed by the addition of dual indexed Illumina library adapters in a subsequent PCR. Libraries were batch normalized using Norgen Biotek NGS Normalization Kits, pooled, cleaned up, and concentrated using AmpureXP magnetic beads. The pool was quality checked and quantified using a combination of Qubit dsDNA HS, Agilent 4200 TapeStation HS DNA1000 and Kapa Illumina Library Quantification qPCR assays. 16S and ITS1 amplicons were sequenced independently in a 2x250bp paired end format using independent v2 500 cycle MiSeq reagent cartridges.

Reads were quality filtered and merged using the USEARCH pipeline (https://drive5.com/usearch). Primers and adapter bases were removed using cutadapt. Bacterial reads were filtered and truncated to 250 bp, clustered into actual sequence variants (ASVs, hereafter referred to as ZOTUs) at 100% sequence similarity then classified against SILVAv138 rRNA database (https://arb-silva.de). ZOTUs classified to Chloroplast, Mitochondria, or with less than two reads across all samples were removed (Thiéry et al. 2012) and samples were rarefied to 6,984 reads (all samples included), resulting in 68,703 bacterial ZOTUs and 2,507,256 bacterial reads. Fungal sequences were filtered to 250 bp. Fungal reads were clustered into ZOTUs at 100% sequence similarity and classified against the UNITE 8.3 reference database (https://unite.ut.ee). Non-fungal ZOTUs and ZOTUs with fewer than two reads were removed and samples were rarefied to 5,803 reads, resulting in 12,716 fungal ZOTUs and 2,060,065 fungal reads.

Statistical analysis

First, we divided the data into two groups to be analyzed separately: i) under the prairie strip and paired control catchment locations (0 m), and ii) adjacent cropland locations (-3, -1, -0.3, -0.1, 0.1, 0.3, 0.1, 3, 9). The data were checked for normality and heterogeneity of variances, and if not found, data were log transformed to meet assumptions and/or outliers removed. The two groups of data were then analyzed via separate mixed-effect linear models. Both linear models used the following response variables on the log scale: microbial biomass C and N, salt-extractable inorganic N, salt-extractable organic C and N, ARSase, BGase, CBHase, LAPase, NAGase, PHOSase, PPOase, and PERase. Data were analyzed for normalcy and homoscedasticity using ggResidpanel (version 0.3.0) (Goode and Rey, 2019). The fixed effect for the prairie strip samples was treatment and the random effects were catchment (six levels) and transect within the catchment (3 per catchment). The model equation is given by:

log(response) ~ treatment + (transect | catchment).

The fixed effects for adjacent cropland samples were treatment (control vs. prairie strip), distance (9-level categorical variable) from the prairie strip, and treatment-distance interaction. The random effects were catchment (six levels) and transect within the catchment (3 per catchment). The model equation is given by:

log(response) ~ treatment + distance + treatment × distance + (transect | catchment).

All variables were analyzed separately within years, i.e., the model was fit independently for a given response and year. We chose to separate years because the year variable was confounded with crop type (soybean vs. maize), land management decisions (fertilizer vs. none), and weather conditions in 2019 and 2020. The unknowns were estimated via residual maximum likelihood (REML) using the software defaults in lme4 (version 1.1.31) (Bates et al., 2015) and

emmeans (version 1.8.3) (Lenth, 2021) packages in the statistical software R (version 4.2.2) (R Core Team, 2022).

Univariate microbial diversity measurements - observed richness, Shannon diversity and evenness - were analyzed using two-factor ANOVA. Prairie strip treatment was the sole predictor variable for modeling soil communities under the prairie strip. Treatment, distance, and their interaction were predictors for modeling soil communities in surrounding cropland. Microbial community structure was analyzed using PERMANOVA on Bray–Curtis distance matrices for rarefied bacterial and fungal communities using phyloseq (version 1.42.0) (McMurdie and Holmes 2013) and vegan (version 2.6.4) (Oksanen et al. 2022) in R (version 4.2.2) (R Core Team 2022). Two extreme outliers were removed from Bray-Curtis distance matrices - one prairie strips treatment 9 m downslope sample from the 2019 surrounding cropland dataset and one Control treatment 3 m downslope sample from the 2020 surrounding cropland dataset. We used distance-based redundancy analysis (dbRDA function in vegan, Legendre and Anderson 1999) to determine the correlation of PEA and soil physiochemical properties to bacterial and fungal community structure under the prairie strip and in surrounding cropland (Oksanen et al. 2022). Our dbRDA model included watershed as a conditional factor. We identified phyla with differential abundance among prairie strip treatments and distances from prairie strips using the 'manyglm' and 'anova' functions in the MVabund (version 4.2.1) R package (Wang Y. et al. 2012).

RESULTS

Microbial biomass, functioning, and community composition directly under the prairie strip

Overall, prairie strips did influence soil physiochemical properties under the prairie strip (Figure 2.11). Prairie strips increased SOM by 12%, pH by 7%, and also 17% in soil moisture

content but in just 2020 ($p < 0.05$; Figure 2.11). Prairie strips strongly affected C and N pools under the prairie strip in both years (Figure 2.3, Table 2.2). Prairie strips, on average, increased microbial biomass C (MBC) and microbial biomass N (MBN) by 56% and 133% across 2019 and 2020. Prairie strips did not affect SEOC or SEON. Prairie strips lowered SEIN by 66% across 2019 and 2020 (Figure 2.3) – and, on average, SEIN was composed of about 50% nitrate-N and 50% ammonium-N.

Prairie strips had inconsistent effects on the soil PEA underneath the prairie strips when expressed per gram of SOM (Figure 2.4, Table 2.2). Generally, prairie strips tended to increase hydrolase and decrease oxidoreductase enzymes. Prairie strips had the most consistent positive effects on PEA in 2019, when the early growing season was relatively wet, with 233 more mm in the 2019 growing season than 2020 (Figure 2.12; Dutter et al. 2023). Prairie strips significantly increased hydrolytic PEA of CBHase by 77%, NAGase by 108%, and PHOSase by 46% compared to the control when expressed per gram of SOM. Unlike hydrolytic PEA, however, prairie strips had a negative effect on oxidative PEA. For instance, prairie strips decreased PERase by 28% in 2019 and PPOase by 33% in 2020 (Figure 2.4; Table 2.2).

Prairie strips shifted bacterial and fungal beta diversity compared to cropland control soils in both 2019 and 2020 (Table 2.2). The extent to which prairie strips affected bacterial and fungal alpha diversity measures varied by year, but in general, prairie strips either reduced or had no effect on fungal and bacterial alpha diversity (Table 2.2, Figure 2.5). Prairie strips reduced bacterial and fungal alpha diversity up to 12% compared to the cropland control ($p < 0.085$; Table 2.2, Figure 2.5). Prairie strips also changed the relative abundance of specific microbial phyla (Figure 2.11, Table 2.5). Gemmatimonadetes bacteria ($p=0.018$ in 2019; $p=0.012$ in 2020; Table 2.5), Elusimicrobia bacteria ($p=0.01$ in 2019), Armatimonadetes bacteria ($p=0.026$ in 2019), and

Basidiomycota fungi (p=0.003 in 2019 and p=0.003 in 2020) were more abundant in prairie strip soils. On the other hand, Chytridiomycota fungi (p=0.028 in 2019 and p=0.029 in 2020), Mortierellomycota fungi (p=0.04 in 2019), and Ascomycota fungi (p=0.037 in 2020) were more abundant in cropland soils (Figure 2.11, Table 2.5).

Microbial biomass, functioning, and microbial communities in adjacent cropland soil

In general, prairie strips did not affect soil physiochemical properties in adjacent cropland soils (Figure 2.10). Across both study years, cropland soils in prairie strip and control catchments showed similar SOM, pH, and CEC. Cropland soils in prairie strip catchments showed marginally lower GWC in 2019 but not 2020 (Figure 2.10; Dutter et al. 2023). Prairie strips also had negligible effects on adjacent soils' C and N pools. There were no significant prairie strip effects on the C and N pools besides SEIN. Salt-extractable inorganic N, comprised mostly of nitrate-N (85%), was 33% lower in the soil <1 m from the prairie strip but only in 2019 under soybean (Figure 2.6; Dutter et al. 2023).

Prairie strips more clearly affected PEA in the adjacent cropland, but these effects were highly inconsistent among enzymes and dependent on the year (Figure 2.7, Table 2.3). The effects of prairie strips on adjacent cropland PEA, when they occurred, were largely independent of distance from the prairie strips. In other words, prairie strips affected adjacent soil PEA equally at all distances up to 9 m away from the prairie strip.

Prairie strips had more positive effects in 2019 when cropland was under soybean, mirroring the predominantly positive effects seen directly under the prairie strip (Figure 2.7). Prairie strips significantly increased three hydrolytic PEA in adjacent crop – BGase by 27%, NAGase by 31%, and PHOSase by 38% – in 2019 soybeans, across all distances when expressed per gram of SOM. In 2020 under maize, however, there was a prairie strips Treatment × Distance

interaction on LAPase, whereby the prairie strips increased LAPase PEA by 164% but only 0.3 m downslope from the prairie strips (Figure 2.7). In 2020 maize, there was also a significant positive main effect of prairie strips on PERase, where prairie strips increased adjacent cropland PERase by 29% compared to the control across all distances.

Prairie strips affected bacterial and fungal community composition in surrounding cropland soils in both 2019 soybean and 2020 maize (Figure 2.13, Table 2.3), but neither distance from the prairie strips nor the interaction between distance and prairie strip treatment were significant drivers of bacterial and fungal community composition in either crop year (Table 2.3). Prairie strips only affected microbial alpha diversity measurements in surrounding cropland soils in 2019 soybean (Shannon diversity $p=0.023$, observed richness $p=0.032$, evenness $p=0.033$) and showed no effect on bacterial and fungal alpha diversity measurements in 2020 maize (Figure 2.14, Table 2.3). In 2019 soybean, bacterial and fungal observed richness was 2.85% and 4.87% lower, respectively, in the adjacent cropland soils of prairie strip catchments (Figure 2.14). Distance from the prairie strip correlated with changes in microbial community richness, Shannon diversity, and evenness, but the direction and magnitude of this effect varied among upslope and downslope distances (Figure 2.14). Prairie strips also shifted the relative abundance of several bacterial and fungal phyla in adjacent cropland soils (Figure 2.13, Table 2.4).

Prairie strips explained some, but not all, correlations between soil properties, PEA, and microbial community composition in surrounding cropland soils. Soil microbial biomass C correlated with fungal community composition in both years, likely driven by high microbial biomass C in soils under the prairie strip ($p < 0.002$; Figure 2.8, Table 2.6). Marginally higher soil nitrate in cropland control soils was a significant predictor of bacterial (Table 2.6, $p=0.029$ in

2019 soybean, p=0.003 in 2020 maize) and fungal (Table 2.6, p=0.001 in 2019 soybean, p=0.001 in 2020 maize) community composition across both years. Several other soil properties and one PEA were consistent correlates of bacterial and fungal community composition across both years: GWC, soil pH, clay, SEOC, nitrate, and PERase (Figure 2.8, Table 2.6); however, in this study, only half of these variables in surrounding cropland soils were affected by prairie strips (GWC in 2019, SEIN in 2019 as Treatment × Distance interaction and PERase in 2020, Tables S2 & S4; Figures 5 & S5). In other words, because prairie strips did not significantly affect all of these soil properties and PEAs in surrounding cropland, we can attribute some - but not all - correlations to the presence of prairie strips.

DISCUSSION

Prairie strips had strong effects on soil microbial community composition and function, both underneath the prairie strips and in adjacent cropland, though effects can be highly dependent on the cropping year. These inconsistent effects could be driven by crop and/or weather (Figure 2.12, Smith et al. 2015). This discussion is separated into the effects of prairie strips on soils underneath the prairie strip – which are more analogous to traditional land-use change studies converting cropland to restored grasslands – and the effects of prairie strips on adjacent cropland soil – which draw from studies on ecotone and edge effects at the interface of habitat types.

Prairie strips increased soil microbial biomass and hydrolytic enzyme activity but decreased oxidoreductase enzymes under the prairie strip

In partial support of our 1st hypothesis, prairie strips had positive effects on soil microbial biomass and hydrolytic enzyme activities. More specifically, 12 years of prairie strips increased soil microbial biomass but not salt-extractable organic C and N, and decreased plant-available

inorganic N, suggesting that prairie strips tighten C and N cycling. This supports findings from larger prairie and grassland restoration studies, which show a 100% to 500% increase in microbial biomass following restoration (Bach and Hofmockel 2015; Purakayastha et al. 2009; Rosenzweig et al. 2016). Greater microbial biomass and less leachable, bioavailable N (i.e., SEIN) under the prairie strip is likely due to greater density and duration of living plant roots and greater rhizodeposition rate (Dietzel et al. 2017; Leptin et al. 2021). Prairie strips even increased more static soil properties like soil organic matter (+12%) and pH (+7%) (Figure 2.11). These findings, in support of our first hypothesis, show that narrow strips of prairie have similar impacts on underlying soil inorganic C and N as have been observed in larger prairie restoration studies. Potential enzyme activities, however, were not as consistent.

Generally, prairie strips had positive effects on hydrolytic PEA and negative effects on oxidoreductase PEA but depended on the crop year (Figure 2.4). This incongruence between the consistent increase in microbial biomass and yet inconsistent effect of prairie strips on PEA implies that PEA are not merely increasing or decreasing due to changes in the microbial biomass, but because of year-to-year shifts in bioavailable soil resources. PEA can also be temporally variable within a year, so it is possible that our sampling captured only a snapshot of potential activity amid fluctuations throughout the growing season (Bach and Hofmockel 2015).

Perennial, diverse plant communities often increase hydrolase PEA compared to monoculture cropland (Li et al. 2023; Wallenius et al. 2011; Yu et al. 2017). CBHase degrades cellulose and may increase in the presence of increased substrate availability (i.e., plant residue; Ljungdahl and Eriksson 1985). NAGase degrades chitin and our finding is consistent with other studies that found grasslands increase NAGase activities compared to cropland (Shahariar et al. 2021; Xu et al. 2019). The elevated NAGase activity may be due to the decrease in bioavailable

N (i.e., SEIN, Figure 2.3). In combination with increased microbial biomass C, this may reflect a shift toward mining fungal necromass for C and N acquisition from under prairie strips (Guggenberger et al. 1999; Kallenbach et al. 2015; Manzoni et al. 2008). Prairie strips also increased phosphatase activity in 2019 (Figure 2.4). Phosphatase cleaves phosphate from organic sources and can be secreted by plants and microbes (Utobo and Tewari 2015). Elevated PHOSase activity could be due to increased plant root competition for organic P in the prairie strips as P fertilizer is not added to the prairie strips since planting (Curtright and Tiemann 2021; Margalef et al. 2017).

Prairie strips generally reduced oxidoreductase PEA (PPOase and PERase; Figure 2.4). PPOase can degrade lignin, detoxify phenolic compounds, and metal ions, and be used as an antimicrobial defense (Sinsabaugh 2010). PERase activity can also indicate lignin degradation, detoxification, and oxidative stress (Sinsabaugh 2010). Both oxidoreductase enzymes are thought to be used by fungi for mining N from SOM (Jian et al. 2016; Sinsabaugh 2010). Our study confirms many previous studies that show agricultural management practices that reduce residual inorganic N, increase labile SOM and general microbial activity also decrease oxidoreductase PEA (Bowles et al. 2022; McDaniel & Grandy 2016; Wickings et al 2011). More specifically, our findings align with cropland restoration studies that report decreased oxidoreductase PEA (Sciubba et al. 2021; Wang et al. 2011; Wang B. et al 2012).

Prairie strips decreased microbial community richness and shifted community composition underneath the prairie strip

Contrary to our 1st hypothesis, prairie strips did not increase but instead decreased soil bacterial and fungal diversity (Figure 2.5, Table 2.2). This decrease was more or less consistent across years and multiple diversity metrics. Previous studies have shown inconsistent findings,

where converting agricultural land to prairie has been shown to both increase (Bach et al. 2018, Upton et al. 2018) and decrease (Barber et al. 2017) soil microbial diversity. Our findings challenge the more generally accepted paradigm that restoring physical habitat and restoring diverse plant community will increase microbial diversity (Hilderbrand et al. 2005; Lange et al. 2015). Our contrary finding could be due to increased microbial niche space from introducing additional resources (e.g., fertilizer and pesticide inputs) and creating unique soil microhabitat conditions due to soil disturbance (e.g. machinery compaction and minor disturbance from planting equipment; Schmidt et al. 2018). Alternatively, the 12 years of prairie strip establishment may have increased diversity or connectivity among higher-trophic-level primary consumers, like nematodes and invertebrates that feed on fungi and bacteria (not measured here), which then in turn may have suppressed bacterial and fungal diversity (Wang et al 2022). Because greater soil microbial diversity does not always translate to greater microbial function or resilience, soil microbial diversity in-and-of-itself should not be the ultimate management goal (Shade 2017). Therefore, it is critical to assess the response of taxa and functions in order to better inform our basic understanding but also for evaluation of different management practices.

Changes in bacterial and fungal community composition underneath the prairie strips corresponded with soil physicochemical properties (Figure 2.8). For example, Gemmatimonadetes bacteria were significantly less abundant under prairie strips (Table 2.5, Figure 2.9), and this may be due to the phyla's preference for more acidic conditions under cropland (< 6; Mackelprang et al. 2018). Prairie strips also decreased sporulating fungi and increased filamentous fungi, as is typical under restored prairies (Upton et al. 2018). The reduced soil disturbance and greater plant inputs under perennial prairie likely increased abundance of decomposers like Basidiomycota (Table 2.5, Figure 2.9), while frequent soil disturbance and

agrochemicals in cropland increased spore-forming Ascomycota (Figure 2.9). While prairie restoration often leads to greater abundance of Glomeromycota (arbuscular mycorrhizal fungi; Allison et al. 2005; Cook et al. 1988; Herzberger et al. 2014), we did not observe this in prairie strip soils (Table 2.5, Figure 2.9). Low Glomeromycota abundance may be due to the dispersal limitation of some mycorrhizal groups (Chaudhary et al. 2020), to N fertilizer drift from surrounding cropland (Jach-Smith and Jackson 2018), or to biases in our ITS fungal sequencing method (Lindahl et al. 2013).

Prairie strips affected adjacent cropland soil microbial biomass and potential enzyme activities

Contrary to our 2nd hypothesis, we found prairie strips did affect adjacent cropland soil, but these effects were weaker than on soils directly underneath prairies (Figure 2.6). Prairie strips had decreased bioavailable N by 33% in the adjacent soils, but only in 2019 when cropland was under soybeans (Figure 2.6). Prior work showed that prairie strips altered other plant-available nutrients in adjacent cropland; nitrate was reduced by 23% in soil within 1 m of the prairie strips (Dutter et al. 2023). The change in plant-available nutrients, especially mobile nutrients, might be due to greater uptake of N under prairie strips or prairie strips changing belowground water balance either by increasing evapotranspiration or limiting subsurface flow and transport of nutrients (Zhou et al. 2010; Zhou et al. 2014).

Prairie strips had strong effects on some PEA in adjacent croplands depending on the year, and only in one case this effect was dependent on distance from the prairie strip (Figure 2.7). Three enzymes – BGase, NAGase, and PHOSase – were all greater in cropland adjacent to the prairie strips than in control catchments, regardless of distance from the prairie strip. The latter two hydrolytic enzymes also had greater activities under the prairie strip, but BGase did not. BGase is a C-acquiring enzyme that tends to be elevated in soils with easily decomposable

organic matter (de Almeida et al. 2015). While the specifics of why hydrolytic PEA increases are unclear, it does suggest that prairie strips alter the supply and demand of carbon and nutrients in adjacent soil.

Increased NAGase activity may be due to N scarcity in cropland soils adjacent to prairie strips, or changes in other resources that were not measured in this study (Wang et al. 2013). PHOSase was elevated at all distances in cropland adjacent to prairie strips (Figure 2.7). While PHOSase has been shown to negatively correlate to P availability (Allison et al. 2005; Hernández and Hobbie 2010), our previous study found that prairie strips increased Mehlich-III extractable P <1 m upslope from the prairie strips (Dutter et al. 2023), but phosphatase activity remained elevated across all distances.

Leucine aminopeptidase, an exclusively N-acquiring enzyme that hydrolyzes leucine amino acid from proteins and peptides, increased by 164% 30 cm downslope of the prairie strip during 2020 maize. Leucine aminopeptidase was elevated downslope of the prairie strip, at the same locations where maize plants were N-stressed and bioavailable N was depleted in the previous year (Dutter et al. 2023). This provides some evidence that greater plant and microbial demand for bioavailable N downslope of the prairie strip is driving increased LAPase. PERase activity in 2020 was also elevated across the entire prairie strip treatment (Figure 2.7). Elevated PERase activity in the cropland adjacent to prairie strips could indicate a labile C or N limitation due to N, P, and K fertilizer addition in 2020.

Prairie strips decreased microbial richness and enriched C-degrading taxa in adjacent soybean soils, but not maize soils

Another reason we must reject our second hypothesis that prairie strips would have no effect on surround soil microbiota is that prairie strips reduced bacterial and fungal richness

under surrounding soils in one out of two years (Figure 2.14, Table 2.3). The difference between years could be because maize and soybean may have different filtering effects on microbial communities, or because cropland fertilization varied across years. N additions in 2020, but not 2019, may have quenched N demand across all distances from the prairie strip, thus homogenizing microbial communities across cropland soils to a greater extent than in 2019. This is also evidenced by the lower NAGase activity in surrounding cropland and was a stronger predictor of fungal community composition in 2019 than in 2020 (Figure 2.7, Table 2.4).

The prairie strip effect seems to have linked structure and function because changes in particular organisms aligned with changes in soil PEA. For example, in 2019 under soybean, prairie strips increased microbial phyla capable of degrading complex C substrates (Figure 2.13, Table 2.5), such as Firmicutes and Planctomycetes (Reguera and Leschine 2001; Wiegland et al. 2018); and this also corresponded to increases in BGase and NAGase that year. Second, in 2020 under maize, prairie strips enriched decomposer fungi (Basidiomycota; Figure 2.13, Table 2.3) and increased PERase in adjacent cropland soils (Table 2.3 & Table 2.6). Taken together, these two independent lines of evidence suggests that prairie strips are affecting microbial structure and functioning in subtle, consistent ways but depends on the crop year.

Our findings suggest that environmental filtering is the dominant mechanism shaping soil microbial communities adjacent to prairie strips. Across both years, neither alpha diversity nor beta diversity showed a significant Treatment × Distance interaction, suggesting that bacterial and fungal communities were controlled by the slope position of the soil within the cropland rather than the soil's proximity to the prairie strips (Table 2.3). The lack of a distance-based spillover effect suggests that either prairie strip microbes are not dispersing outward from the prairie strips, or more likely, dispersal is present, but cropland environmental filtering is shaping

community composition at each catchment slope position. Cropland disturbance and fertilization (Fierer and Jackson 2006; Manzoni et al. 2012; Schmidt et al. 2018; West and Whitman 2022), in combination with soil physiochemical heterogeneity across the field (Figure 2.10; Table 2.6), appear to be a stronger control on microbial communities than proximity to the prairie strip. These results also indicate that prairie strips do not introduce beneficial microbial taxa to adjacent cropland soils; however, rare taxa that we may not have detected can have a disproportionate effect on function (Shade et al. 2014), so this possibility should not be ruled out.

Microbial community composition was associated with several soil properties and PEA, some as a result of prairie strip establishment, and others as a function of abiotic heterogeneity across the landscape. Inorganic N was a primary driver of differences in bacterial and fungal community composition across treatments, as evidenced by the strong influence of soil nitrate on community composition across years (Table 2.6, Figure 2.8); and specifically evidenced by the enrichment of particular microbial phyla like Planctomycetes which are capable of oxidizing ammonium (Figure 2.13, Table 2.5, Shively et al. 2014). The emergence of microbial biomass C as a significant correlate of fungal, but not bacterial, community composition in surrounding cropland soils may have resulted from the enrichment of Basidiomycota, a filamentous, high C:N fungal group, in cropland soils surrounding prairie strips (Figure 2.13, Table 2.6 & S3, Zhang and Elser 2017).

Several soil measurements (nitrate, GWC, soil pH, clay, SEOC, and potassium) and microbial biomass C were consistent correlates with bacterial and fungal community composition (Figure 2.8, Table 2.4). The majority of these variables were not affected by prairie strips (Table 2.3 & S5), varied widely across slope positions (Figures 5 & 6), and did not affect community composition in consistent directions (Figure 2.8). The exceptions to this were

microbial biomass C and nitrate, variables whose correlations with microbial communities were clearly mediated by the presence of a prairie strip (Figure 2.8, Table 2.4). Our study showed that prairie strips do have some effects (albeit some more consistent than others) on soil biota and SESs in both prairie strip and adjacent cropland soils that may be important for implementation and management.

CONCLUSION

Prairie strips are a conservation practice aimed at increasing biodiversity on the landscape, reducing agricultural runoff, and regenerating soil health. We found that the oldest prairie strips in Iowa (12 years old) had many effects on soil microbial community structure and functioning underneath the prairie strips and even in the adjacent cropland, though effects were more inconsistent and complex in the latter. These prairie strips – like large swaths of restored perennial vegetation – increased soil microbial biomass, hydrolytic PEA, and C-degrading microbial taxa, and decreased salt-extractable inorganic N, oxidative PEA, and bacterial and fungal richness in soils directly underneath the prairie vegetation. In adjacent cropland soils, prairie strips had little effect on C and N pools but did have strong positive effects on several hydrolytic and oxidative PEA (BGase, NAGase, PHOSase, and PERase) and microbial community structure, depending on the crop year. Overall, we find strong evidence for prairie strips' affecting soil biota and SESs both underneath and adjacent to them, but effects are strongly dependent on localized abiotic conditions, crop species, and crop-specific management activities.

Future studies might improve the predictability of prairie strips' effects on adjacent soil biota by monitoring more frequently within one year, exploring the role of prairie plant species composition, and testing the interaction with other cropping systems other than maize-soybean

rotation. Doing so will help to understand the complex interactive effects of prairie strips have on shaping soil biota and SESs in adjacent cropland. A greater understanding of these complex interactions between cropland and prairie strips will help improve agriculture management for maximizing ecosystem services.

ACKNOWLEDGMENTS

We would like to thank Neal Smith National Wildlife Refuge and the U.S. Fish and Wildlife Service for using their land, Scott Gilje, Karen Viste-Sparkman, Pauline Drobney, and other members of the FWS staff for their help in this research. We thank Gary Van Ryswyk for his collaboration and management of the crops. We would also like to thank the McDaniel Lab and Evans Lab for their help with soil sampling and analysis: Theresa Brehm, Carrie Dodd, Terry West, Haley Wilson, Jean Etnyre, Malcom St Cyr, Christar Kin, Perla Carmenate, Valeria Cano Camacho, Stephen Potter, Mriganka De, Holly Vander Stel, Robert Logan, Ceco Maples, Tayler Ulbrich, and Lukas Bell-Derekse. We would like to thank Omar de Kok-Mercado for his photos used in this publication. This is W.K. Kellogg Biological Station Contribution No. 2363. Research reported in this publication was supported by the Foundation for Food and Agriculture Research [Grant ID: CA18-SS-0000000278], the USDA Farm Service Agency [Contract ID: 19CPT0010516], the NSF Long Term Ecological Research Program [Grant ID: DEB 1832042] and the NSF Graduate Research Fellowship Program. This article/paper is a product of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. IOW03717 is supported by USDA/NIFA and State of Iowa funds. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of the Foundation for Food and Agriculture Research.

LITERATURE CITED

Abellan, M.A., Baena, C.W., Morote, F.G., Cordoba, M.P., Perez, D.C. and Lucas-Borja, M.E., 2011. Influence of the soil storage method on soil enzymatic activities. Forest Systems, 20(3), pp.379-388.

Allison, S.D. and Vitousek P.M., 2005. Responses of Extracellular Enzymes to Simple and Complex Nutrient Inputs. Soil Biology and Biochemistry 37(5):937–44. doi: 10.1016/j.soilbio.2004.09.014.

Bach, E.M. and Hofmockel K.S., 2015. Coupled Carbon and Nitrogen Inputs Increase Microbial Biomass and Activity in Prairie Bioenergy Systems. Ecosystems 18(3):417–27. doi: 10.1007/s10021-014-9835-8.

Bach, E.M., Williams R.J., Hargreaves S.K., Yang F., and Hofmockel K.S., 2018. Greatest soil microbial diversity found in micro-habitats. Soil Biology and Biochemistry 118:217-226. doi:10.1016/j.soilbio.2017.12.018.

Baer, S.G., Kitchen D.J., Blair J.M., and Rice C.W., 2002. Changes in Ecosystem Structure and Function along a Chronosequence of Restored Grasslands. Ecological Applications 12(6):1688–1701. doi: 10.1890/1051-0761(2002)012[1688:CIESAF]2.0.CO;2.

Baldwin-Kordick, R., De, M., Lopez, M.D., Liebman, M., Lauter, N., Marino, J., and McDaniel, M.D., 2022. Comprehensive Impacts of Diversified Cropping on Soil Health and Sustainability. Agroecology and Sustainable Food Systems 46(3):331–63. doi: 10.1080/21683565.2021.2019167.

Bates, D., Maechler, M., Bolker, B., and Walker, S., 2015. Fitting Linear Mixed-Effects Models Using Lme4. Journal of Statistical Software 67(1):1–48. doi: doi:10.18637/jss.v067.i01.

Barber, N.A., Chantos-Davidson, K.M., Peralta, R.A., Sherwood, J.P., and Swingley, W.D., 2017. Soil microbial community composition in tallgrass prairie restorations converge with remnants across a 27-year chronosequence. Environmental Microbiology 19(8):3118–3131. doi: 10.1111/1462-2920.13785

Bell, T. and Tylianakis, J.M., 2016. Microbes in the Anthropocene: spillover of agriculturally selected bacteria and their impact on natural ecosystems. Proceedings of the Royal Society B: Biological Sciences 283(1844), p.20160896. doi: 10.1098/rspb.2016.0896.

Bowles, T.M., Jilling, A., Morán-Rivera, K., Schnecker, J. and Grandy, A.S., 2022. Crop rotational complexity affects plant-soil nitrogen cycling during water deficit. Soil Biology and Biochemistry, 166, p.108552.

Brookes, P. C., Landman, A., Pruden, G., and Jenkinson, D. S., 1985. Chloroform Fumigation and the Release of Soil Nitrogen: A Rapid Direct Extraction Method to Measure Microbial Biomass Nitrogen in Soil. Soil Biology and Biochemistry 17(6):837–42. doi: 10.1016/0038-0717(85)90144-0.

Cassman, K.G., and Grassini, P., 2020. A Global Perspective on Sustainable Intensification Research. Nature Sustainability 3(4):262–68. doi: 10.1038/s41893-020-0507-8.

Chaudhary, B., Nolimal, S., Sosa-Hernández, M.A., Egan, C., and Kastens, J.. 2020. Trait-based aerial dispersal of arbuscular mycorrhizal fungi. New Phytologist 228:238-252. doi: 10.1111/nph.16667.

Choudoir, M.J., Barberán, A., Menninger, H.L., Dunn, R.R. and Fierer, N., 2018. Variation in range size and dispersal capabilities of microbial taxa. Ecology 99(2), pp.322-334. doi: 10.1002/ecy.2094.

Coleman, D.C. and Wall, D.H., Soil Fauna: Occurrence, Biodiversity, and Roles in Ecosystem Function in: Paul, E.A. (Ed) Soil Microbiology, Ecology and Biochemistry (Fourth Edition), Academic Press, 2015, pp. 111-149. doi.org/10.1016/B978-0-12-415955-6.00005-0.

Cook, B.D., Jastrow, J.D. and Miller, R.M. 1988. Root and mycorrhizal endophyte development in a chronosequence of restored tallgrass prairie. New Phytologist, 110: 355-362. https://doi.org/10.1111/j.1469-8137.1988.tb00272.x

Curtright, A.J., and Tiemann, L.K.. 2021. Intercropping Increases Soil Extracellular Enzyme Activity: A Meta-Analysis. Agriculture, Ecosystems and Environment 319(May):107489. doi: 10.1016/j.agee.2021.107489.

Daigh, A.L.M., Zhou, X., Helmers, M.J., Pederson, C.H., Horton, R., Jarchow, M., and Liebman, M., 2015. Subsurface Drainage Nitrate and Total Reactive Phosphorus Losses in Bioenergy-Based Prairies and Corn Systems. Journal of Environmental Quality 44(5):1638–46. doi: 10.2134/jeq2015.02.0080.

de Almeida, R.F., Naves, E.R. and da Mota, R.P., 2015. Soil quality: Enzymatic activity of soil β-glucosidase. Global Journal of Agricultural Research and Reviews, 3(2), pp.146-450. Fierer, Noah and Robert B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103(3):626–631. doi:10.1073/pnas.0507535103.

De, M., Riopel, J.A., Cihacek, L.J., Lawrinenko, M., Baldwin-Kordick, R., Hall, S.J., and McDaniel, M.D., 2020. Soil Health Recovery after Grassland Reestablishment on Cropland: The Effects of Time and Topographic Position. Soil Science Society of America Journal 84(2):568–86. doi: 10.1002/saj2.20007.

DeForest, J.L., 2009. The Influence of Time, Storage Temperature, and Substrate Age on Potential Soil Enzyme Activity in Acidic Forest Soils Using MUB-Linked Substrates and l-DOPA. Soil Biology and Biochemistry 41(6):1180–86. doi: 10.1016/j.soilbio.2009.02.029.

Deng, S.P., Kang, H., and Freeman, C., 2011. Microplate Fluorimetric Assay of Soil Enzymes. in Dick, R.P. (Ed) Methods of Soil Enzymology, Madison, WI, USA: Soil Science Society of America, Inc. pp. 311–18.

Dietzel, R., Liebman, M., and Archontoulis, S., 2017. A Deeper Look at the Relationship between Root Carbon Pools and the Vertical Distribution of the Soil Carbon Pool. Soil 3(3):139–52. doi: 10.5194/soil-3-139-2017.

Doane, T.A., and Horwáth, W.R., 2003. Spectrophotometric Determination of Nitrate with a Single Reagent. Analytical Letters 36 (12): 2713–22. https://doi.org/10.1081/AL-120024647.

Dutter, C., Damiano, L.A., Niemi, J., Miller, B.A., Schulte L.A., Liebman, M., Helmers, M., Cruse, R., and McDaniel, M.D., 2023. Contour Prairie Strips Affect Surrounding Soil but Only Have Slight Effects on Crop Yield. Field Crops Research 296 (March): 108905. https://doi.org/10.1016/j.fcr.2023.108905.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A. and Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6(5), pp.1007-1017.

Gerla, Philip J. 2007. Estimating the Effect of Cropland to Prairie Conversion on Peak Storm Run-Off. Restoration Ecology 15(4):720–30. doi: 10.1111/j.1526-100X.2007.00284.x.

German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L. and Allison, S.D., 2011a. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry, 43(7), pp.1387-1397.

German, D.P., Chacon, S.S. and Allison, S.D., 2011b. Substrate concentration and enzyme allocation can affect rates of microbial decomposition. Ecology 92(7), pp.1471-1480.

Goode K., Rey K., 2019. _ggResidpanel: Panels and Interactive Versions of Diagnostic Plots using 'ggplot2'_. R package version 0.3.0, <https://CRAN.R-project.org/package=ggResidpanel>.

Grass Development Team. 2021. Geographic Resources Analysis Support System (GRASS) Software. http://grass.osgeo.org.

Guggenberger, G., Frey, S.D., Six, J., Paustian, K. and Elliott, E.T., 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Science Society of America Journal 63(5), pp.1188-1198.

Gutierrez-Lopez, J., Asbjornsen, H., Helmers, M. and Isenhart, T., 2014. Regulation of soil moisture dynamics in agricultural fields using strips of native prairie vegetation. Geoderma 226, pp.238-249.

Hargreaves, S.K., Williams, R.J. and Hofmockel, K.S., 2015. Environmental filtering of microbial communities in agricultural soil shifts with crop growth. PLoS One 10(7), p.e0134345.

Helmers, M.J., Zhou, X., Asbjornsen, H., Kolka, R., Tomer, M.D. and Cruse, R.M., 2012. Sediment removal by prairie filter strips in row-cropped ephemeral watersheds. Journal of Environmental Quality 41(5), pp.1531-1539.

Hernandez-Santana, V., Zhou, X., Helmers, M.J., Asbjornsen, H., Kolka, R. and Tomer, M., 2013. Native prairie filter strips reduce runoff from hillslopes under annual row-crop systems in Iowa, USA. Journal of Hydrology 477, pp.94-103.

Hernández, D.L. and Hobbie, S.E., 2010. The effects of substrate composition, quantity, and diversity on microbial activity. Plant and Soil 335, pp.397-411.

Hilderbrand, R.H., Watts, A.C. and Randle, A.M., 2005. The myths of restoration ecology. Ecology and Society, 10(1).

Hirsh, S.M., Mabry, C.M., Schulte, L.A. and Liebman, M., 2013. Diversifying agricultural catchments by incorporating tallgrass prairie buffer strips. Ecological Restoration 31(2), pp.201-211.

Hurisso, T.T., Norton, J.B. and Norton, U., 2014. Labile soil organic carbon and nitrogen within a gradient of dryland agricultural land-use intensity in Wyoming, USA. Geoderma 226, pp.1-7.

Jastrow, J.D., 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology and Biochemistry 28(4-5), pp.665-676.

Jenkinson, D.S., 1988. Determination of microbial biomass carbon and nitrogen in soil. Advances in Nitrogen Cycling pp.368-386.

Jian, S., Li, J., Chen, J.I., Wang, G., Mayes, M.A., Dzantor, K.E., Hui, D. and Luo, Y., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. Soil Biology and Biochemistry, 101, pp.32-43.

Joergensen, R.G. and Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the kEN value. Soil Biology and Biochemistry 28(1), pp.33-37.

Jones, J.M., Boehm, E.L., Kahmark, K., Lau, J. and Evans, S., 2022. Microbial community response to drought depends on crop. Elementa: Science of the Anthropocene 10(1), p.00110.

Kallenbach, C.M., Grandy, A.S., Frey, S.D. and Diefendorf, A.F., 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biology and Biochemistry 91, pp.279-290.

Karlen, D.L., Rosek, M.J., Gardner, J.C., Allan, D.L., Alms, M.J., Bezdicek, D.F., Flock, M., Huggins, D.R., Miller, B.S. and Staben, M.L., 1999. Conservation Reserve Program effects on soil quality indicators. Journal of Soil and Water Conservation 54(1), pp.439-444.

Kemmerling, L.R., Rutkoski, C.E., Evans, S.E., Helms, J.A., Cordova-Ortiz,E.S., Smith, J.D., Vázquez Custodio, J.A., Vizza, C. and Haddad, N.M., 2022. Prairie Strips and Lower Land Use Intensity Increase Biodiversity and Ecosystem Services. Frontiers in Ecology and Evolution 10(May):1–18. doi: 10.3389/fevo.2022.833170.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. and Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology 79(17):5112-20. doi: 10.1128/AEM.01043-13.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels,C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E. and Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications 6:6707. doi:10.1038/ncomms7707.

Legendre, P. and Anderson, M.J., 1999. Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. Ecological Monographs. 69(1). doi: 10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2.

Lenth, R.V. 2021., Emmeans: Estimated Marginal Means, Aka Least-Squares Means.

Leptin, A., Whitehead, D., Anderson, C.R., Cameron, K.C. and Lehto, N.J., 2021. Increased Soil Nitrogen Supply Enhances Root-Derived Available Soil Carbon Leading to Reduced Potential Nitrification Activity. Applied Soil Ecology 159: 103842. https://doi.org/10.1016/j.apsoil.2020.103842.

Li, C., Fultz, L.M., Moore-Kucera, J., Acosta-Martínez, V., Horita, J., Strauss, R., Zak, J., Calderón, F. and Weindorf, D., 2017. Soil Carbon Sequestration Potential in Semi-Arid Grasslands in the Conservation Reserve Program. Geoderma 294:80–90. doi: 10.1016/j.geoderma.2017.01.032.

Li, C., Fultz, L.M., Moore-Kucera, J., Acosta-Martínez, V., Kakarla, M., and Weindorf, D.C., 2018. Soil Microbial Community Restoration in Conservation Reserve Program Semi-Arid Grasslands. Soil Biology and Biochemistry 118:166–77. doi: 10.1016/j.soilbio.2017.12.001.

Li, Q., Shi, J., Li, G., Hu, K. and Ma, R., 2023. Extracellular Enzyme Stoichiometry and Microbial Resource Limitation Following Various Grassland Reestablishment in Abandoned Cropland. Science of the Total Environment 870 (January): 161746. https://doi.org/10.1016/j.scitotenv.2023.161746.

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kõljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J. and Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytologist 199: 288-299. doi:10.1111/nph.12243.

Liu, X.J.A., Finley, B.K., Mau, R.L., Schwartz, E., Dijkstra, P., Bowker, M.A. and Hungate, B.A., 2020. The soil priming effect: Consistent across ecosystems, elusive mechanisms. Soil Biology and Biochemistry, 140, p.107617.

Ljungdahl, L.G., and Eriksson, K.E., 1985. Ecology of Microbial Cellulose Degradation. In Advances in Microbial Ecology, 8th ed., 237–99.

Luo, L., Meng, H. and Gu, J.D., 2017. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. Journal of Environmental Management, 197, pp.539-549.

Mackelprang, R., Grube, A.M., Lamendella, R., da C. Jesus, E., Copeland, A., Liang, C., Jackson, R.D., Rice, C.W., Kapucija, S., Parsa, B., Tringe, S.G., Tiedje, J.M., Jansson, J.K., 2018. Microbial Community Structure and Functional Potential in Cultivated and Native Tallgrass Prairie Soils of the Midwestern United States. Frontiers in Microbiology. 9. doi: 10.3389/fmicb.2018.01775.

Manzoni, S., Jackson, R.B., Trofymow, J.A. and Porporato, A., 2008. The Global Stoichiometry of Litter Nitrogen Mineralization. Science 321:684-686. doi:10.1126/science.1159792

Manzoni, S., Schimel, J.P. and Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93:930-938. doi:10.1890/11-0026.1.

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P. Goll, D., Richter, A., Obersteiner, M., Asensio, D., and Peñuelas, J., 2017. Global Patterns of Phosphatase Activity in Natural Soils. Scientific Reports 7(1):1–13. doi: 10.1038/s41598-017-01418-8.

Martin, K.J. and Rygiewicz, P.T., 2005. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiology 5, 28. doi:10.1186/1471-2180-5-28.

McLauchlan, K.K., Hobbie, S.E. and Post, W.M., 2006. Conversion from Agriculture to Grassland Builds Soil Organic Matter on Decadal Timescales. Ecological Applications 16 (1): 143–53. https://doi.org/10.1890/04-1650.

McMurdie, P.J. and Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One 8(4):e61217. doi: 10.1371/journal.pone.0061217.

Miller, B.A., 2014. Semantic Calibration of Digital Terrain Analysis Scale. Cartography and Geographic Information Science 41(2):166–76. doi: 10.1080/15230406.2014.883488.

Mony, C., Uroy, L., Khalfallah, F., Haddad, N. and Vandenkoornhuyse, P., 2022. Landscape connectivity for the invisibles. Ecography. https://doi.org/10.1111/ecog.06041

Munson, S. M., Lauenroth, W.K. and Burke, I.C., 2012. Soil Carbon and Nitrogen Recovery on Semiarid Conservation Reserve Program Lands. Journal of Arid Environments 79:25–31. doi: 10.1016/j.jaridenv.2011.11.027.

Nestrud, L.M., and Worster, J.R., 1979. Soil Survey of Jasper County, Iowa.

Oates, L.G., Duncan, D.S., Gelfand, I., Millar, N., Robertson, G.P., and Jackson, R.D., 2016. Nitrous Oxide Emissions during Establishment of Eight Alternative Cellulosic Bioenergy Cropping Systems in the North Central United States. GCB Bioenergy 8(3):539–49. doi: 10.1111/gcbb.12268.

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Peter, S., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Antoniazi Evangelista, H.B., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.H., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C.J.F. and Weedon, J., 2022. Vegan: Community Ecology Package. https://cran.r-project.org/package=vegan.

Peoples, M.S. and Koide, R.T., 2012. Considerations in the storage of soil samples for enzyme activity analysis. Applied soil ecology, 62, pp.98-102.

Pérez-Suárez, M., Castellano, M.J., Kolka, R., Asbjornsen, H. and Helmers, M., 2014. Nitrogen and Carbon Dynamics in Prairie Vegetation Strips across Topographical Gradients in Mixed Central Iowa Agroecosystems. Agriculture, Ecosystems and Environment 188(3):1–11. doi: 10.1016/j.agee.2014.01.023.

Prior, Jean. C., 1991. Landforms of Iowa. University of Iowa Press.

Purakayastha, T. J., Smith, J.L. and Huggins, D.R., 2009. Microbial Biomass and N Cycling under Native Prairie, Conservation Reserve and No-Tillage in Palouse Soils. Geoderma 152(3–4):283–89. doi: 10.1016/j.geoderma.2009.06.013.

R Core Team., 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Raiesi, F., and Salek-Gilani, S., 2018. The Potential Activity of Soil Extracellular Enzymes as an Indicator for Ecological Restoration of Rangeland Soils after Agricultural Abandonment. Applied Soil Ecology 126(February):140–47. doi: 10.1016/j.apsoil.2018.02.022.

Reguera, G. and Leschine, S.B., 2001. Chitin degradation by cellulolytic anaerobes and facultative aerobes from soils and sediments. FEMS Microbiology Letters 204(2):367–374. doi: 10.1111/j.1574-6968.2001.tb10912.x.

Rosenzweig, S.T., Carson, M.A., Baer, S.G. and 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 100:186–94. doi: 10.1016/j.apsoil.2016.01.001.

Saiya-Cork, K. R., Sinsabaugh, R. L. and Zak, D. R., 2002. The Effects of Long Term Nitrogen Deposition on Extracellular Enzyme Activity in an Acer Saccharum Forest Soil. Soil Biology and Biochemistry 34(9):1309–15. doi: 10.1016/S0038-0717(02)00074-3.

Schmidt, R., Gravuer, K., Bossange, A.V. Mitchell, J. and Scow, K., 2018. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLoS One. 13(2):e0192953. doi: 10.1371/journal.pone.0192953.

Schulte, L.A., MacDonald, A.L., Niemi, J.B. and Helmers, M.J., 2016. Prairie Strips as a Mechanism to Promote Land Sharing by Birds in Industrial Agricultural Landscapes. Agriculture, Ecosystems and Environment 220:55–63. doi: 10.1016/j.agee.2016.01.007.

Schulte, L.A., Niemi, J.B., Helmers, M.J., Liebman, M., Arbuckle, J.G., James, D.E., Randall, K., ONeal, M.E., Tomer, M.D., Tyndall, J.C., Drobney, P., Neal, J., Van Ryswyk, G. and Witte, C., 2017. Prairie Strips Improve Biodiversity and the Delivery of Multiple Ecosystem Services from Corn–Soybean Croplands. Proceedings of the National Academy of Sciences of the United States of America 114(50):E10851. doi: 10.1073/pnas.1719680114.

Sciubba, L., Mazzon, M., Cavani, L., Baldi, E., Toselli, M., Ciavatta, C. and Marzadori, C., 2021. Soil Response to Agricultural Land Abandonment: A Case Study of a Vineyard in Northern Italy. Agronomy 11 (9). https://doi.org/10.3390/agronomy11091841.

Senaviratne, G.M.M.M.A., Udawatta, R. P., Nelson, K. A., Shannon, K. and Jose, S., 2012. Temporal and Spatial Influence of Perennial Upland Buffers on Corn and Soybean Yields. Agronomy Journal, 104(5), 1356–1362. https://doi.org/10.2134/agronj2012.0081

Shade, A., Jones, S.E., Caporaso, J.G., Handelsman, J., Knight, R., Fierer, N. and Gilbert, J.A., 2014. Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. mBio 5(4). doi: 10.1128/mBio.01371-14.

Shade, A., 2017. Diversity is the question, not the answer. ISME 11:1–6. doi: 10.1038/ismej.2016.118.

Shahariar, S., Helgason, B., Soolanayakanahally, R. and Bedard-Haughn, A., 2021. Soil Enzyme Activity as Affected by Land-Use, Salinity, and Groundwater Fluctuations in Wetland Soils of the Prairie Pothole Region. Wetlands 41(2). doi: 10.1007/s13157-021-01431-8.

Sinsabaugh, R.L., Reynolds, H. and Long, T.M., 2000. Rapid Assay for Amidohydrolase (Urease) Activity in Environmental Samples. Soil Biology and Biochemistry 32 (14): 2095–97. https://doi.org/10.1016/S0038-0717(00)00102-4.

Sinsabaugh, R.L., 2010. Phenol Oxidase, Peroxidase and Organic Matter Dynamics of Soil. Soil Biology and Biochemistry 42(3):391–404. doi: 10.1016/j.soilbio.2009.10.014.

Smith, J.L., Collins, H.P., Crump, A.R. and Bailey, V.L., 2015. Chapter 18: Management of Soil Biota and Their Processes. In E.A. Paul (Ed.) Soil Microbiology, Ecology and Biochemistry 4: 539-572. Academic Press. doi: 10.1016/B978-0-12-415955-6.00018-9.

Soil Survey Staff., 2003. Keys to Soil Taxonomy. Washington DC.

Thiéry, O., Moora, M., Vasar, M., Zobel, M. and Öpik, M., 2012. Inter- and intrasporal nuclear ribosomal gene sequence variation within one isolate of arbuscular mycorrhizal fungus, Diversispora sp. Symbiosis 58, 135–147. doi:10.1007/s13199-012-0212-0.

Upton, R.N., Bach, E.M., Hofmockel, K.S., 2018. Belowground response of prairie restoration and resiliency to drought. Agriculture, Ecosystems & Environment. 266:122-132. doi:10.1016/j.agee.2018.07.021.

USDA-NRCS, 2006. Land resource regions and major land resource areas of the United States, the Caribbean, and the Pacific Basin. USDA Handbook 296. US Department of Agriculture, Natural Resources Conservation Service, Washington, DC, USA.

Utobo, E. B. and Tewari, L., 2015. Soil Enzymes as Bioindicators of Soil Ecosystem Status. Applied Ecology and Environmental Research 13(1):147–69. doi: 10.15666/aeer/1301_147169.

van Elsas, J.D., Trevors, J.T. and van Overbeek, L. S., 1991. Influence of soil properties on the vertical movement of genetically-marked pseudomonas fluorescens through large soil microcosms. Biology and Fertility of Soils, 10(4), 249-255. doi:10.1007/BF00337375

Vance, E.D., Brookes, P.C. and Jenkinson, D.S., 1987. An Extraction Method for Measuring Soil Microbial Biomass C. Soil Biology and Biochemistry 19(6):703–7.

Wallenius, K., Rita, H., Mikkonen, A., Lappi, K., Lindström, K., Hartikainen, H., Raateland, A. and Niemi, R.M., 2011. Effects of Land Use on the Level, Variation and Spatial Structure of Soil Enzyme Activities and Bacterial Communities. Soil Biology and Biochemistry 43 (7): 1464–73. https://doi.org/10.1016/j.soilbio.2011.03.018.

Wang, B., Liu, G.B., Xue, S. and Zhu, B., 2011. Changes in Soil Physico-Chemical and Microbiological Properties during Natural Succession on Abandoned Farmland in the Loess Plateau. Environmental Earth Sciences 62(5):915–25. doi: 10.1007/s12665-010-0577-4.

Wang, B., Xue, S., Liu, G.B., Zhang, G.H., Li, G. and Ren, Z.P., 2012. Changes in Soil Nutrient and Enzyme Activities under Different Vegetations in the Loess Plateau Area, Northwest China. Catena 92:186–95. doi: 10.1016/j.catena.2011.12.004.

Wang, B., Zhu, Y., Chen, X., Chen, D., Wu, Y., Wu, L., Liu, S., Yue, L., Wang, Y., Bai, Y., 2022. Even short-term revegetation complicates soil food webs and strengthens their links with ecosystem functions. Journal of Applied Ecology 59:1721– 1733. doi: 10.1111/1365-2664.14180.

Wang, Q.Y., Wang, Y., Wang, Q.C., Liu, Q., Lv, D.A., Guan, J.N. and Liu, J.S., 2013. Effects of Land Use Changes on the Spectroscopic Characterization of Hot-Water Extractable Organic Matter along a Chronosequence: Correlations with Soil Enzyme Activity. European Journal of Soil Biology 58:8–12. doi: 10.1016/j.ejsobi.2013.05.003.

Wang, Y., Naumann, U., Wright, S.T. and Warton, D.I., 2012. mvabund: An R package for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution 3:471-474. doi: 10.1111/j.2041-210X.2012.00190.

Warmink, J., Nazir, R., Corten, B. and van Elsas, J.D., 2011. Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. Soil Biology and Biochemistry 43:760-765. doi: 10.1016/j.soilbio.2010.12.009.

West, J.R. and Whitman, T., 2022. Disturbance by soil mixing decreases microbial richness and supports homogenizing community assembly processes. FEMS Microbiology Ecology 98(9). doi:10.1093/femsec/fiac089.

Wickings, K., Stuart Grandy, A., Reed, S. and Cleveland, C., 2011. Management intensity alters decomposition via biological pathways. Biogeochemistry, 104, pp.365-379.

Xu, C., Xiao-zeng, H., Meng-yang, Y., Jun, Y., Xin chun, L., Horwath, W.R. and Wen-xiu, Z., 2019. Soil Macroaggregates and Organic-Matter Content Regulate Microbial Communities and Enzymatic Activity in a Chinese Mollisol. Journal of Integrative Agriculture 18(11):2605–18. doi: 10.1016/S2095-3119(19)62759-0.

Yu, P., Liu, S., Han, K., Guan, S. and Zhou, D., 2017. Conversion of Cropland to Forage Land and Grassland Increases Soil Labile Carbon and Enzyme Activities in Northeastern China. Agriculture, Ecosystems and Environment 245 (December 2016): 83–91. doi:10.1016/j.agee.2017.05.013.

Zhang, J. and Elser, J.J., 2017. Carbon:Nitrogen:Phosphorus Stoichiometry in Fungi: A Meta-Analysis. Frontiers in Microbiology 8. doi:10.3389/fmicb.2017.01281.

Zhang, X., Dong, W., Dai, X., Schaeffer, S., Yang, F., Radosevich, M., Xu, L., Liu, X. and Sun, X., 2015. Responses of absolute and specific soil enzyme activities to long term additions of organic and mineral fertilizer. Science of the Total Environment, 536, pp.59-67.

Zhou, X., Helmers, M.J., Asbjornsen, H., Kolka, R. and Tomer, M.D., 2010. Perennial Filter Strips Reduce Nitrate Levels in Soil and Shallow Groundwater after Grassland-to-Cropland Conversion. Journal of Environmental Quality 39(6):2006–15. doi: 10.2134/jeq2010.0151.

Zhou, X., Helmers, M., Asbjornsen, H., Kolka, R., Tomer, M.D. and Cruse, R.M., 2014. Nutrient Removal by Prairie Filter Strips in Agricultural Landscapes. Journal of Soil and Water Conservation 69(1):54–64. doi: 10.2489/jswc.69.1.54.

APPENDIX B: CHAPTER 2

Tables

Enzyme	Enzyme Commission Number	Abbreviation	Substrate
Arylsufatase	EC 3.1.6.1	ARSase	4-MUF-sulfate
β-1,4-glucosidase	EC 3.2.1.21	BGase	4-MUF-β-D-glucoside
Cellobiohydrolase	EC 3.2.1.91	CBHase	4-MUF-β-D-cellobioside
β-N-acetylglucosaminidase	EC 3.2.1.14	NAGase	4-MUF-N-acetyl-β-D-glucosaminise
Leucyl aminopeptidase	EC 3.4.11.1	LAPase	L-Leucine-7-amido-4-methylcoumarin
Acid (alkaline) Phosphatase	EC 3.1.3.1	PHOSase	4-MUF-phosphate
Polyphenol oxidase	EC 1.10.3.2	PPOase	L-3,4-dihydroxyphenylalanine
Peroxidase	EC 1.11.1.7	PERase	L-3,4-dihydroxyphenylalanine and H_2O_2

Abbreviation: MUF = methylumbelliferyl

Table 2.1. Extracellular enzymes assayed in this study and the corresponding substrates for potential enzyme activity (PEA) measurements.

	2019	2020
Soil Biochemical Properties (Figure 2.2)		
Microbial Biomass C	**0.03**	**0.021**
Microbial Biomass N	**0.014**	**0.02**
Salt-extractable Organic C	0.98	0.259
Salt-extractable Inorganic N	**0.029**	**0.018**
Salt-extractable Organic N	0.131	0.135
Potential Enzyme Activities (Figure 2.3)		
Arylsulfatase	0.348	0.257
β-glucosidase	0.135	0.477
Cellobiohydrolase	**0.01**	0.925
Leucine Aminopeptidase	0.345	0.646
N-acetyl-β-glucosaminidase	**0.023**	0.534
Phosphatase	**0.026**	0.291
Polyphenol Oxidase	0.137	**0.029**
Peroxidase	**0.025**	0.667
Microbial beta diversity‡		
Bacterial community composition	<**0.001** (R=0.14)	<**0.001** (R=0.13)
Fungal community composition	<**0.001** (R=0.17)	<**0.001** (R=0.2)
Microbial alpha diversity (Figure 2.4)		
Bacteria Shannon diversity	0.484	**0.045**
Bacteria observed richness	0.107	**0.012**
Bacteria evenness	0.798	<u>0.085</u>
Fungi Shannon diversity	**0.011**	**0.012**
Fungi observed richness	0.129	0.149
Fungi evenness	**0.008**	**0.010**

Table 2.2. P-values from One-way ANOVA† comparing soil biological and chemical parameters underneath the 12-year prairie strips and the control.

†Bold values indicate significance at $p < 0.05$. Underlined values indicate significance at $p < 0.1$.

‡ R value indicates the percentage of variation in microbial beta diversity explained by the prairie strip treatment.

	2019 Soybean			2020 Maize		
	Treatment	**Distance**	**Treatment: Distance**	**Treatment**	**Distance**	**Treatment: Distance**
			Soil Biochemical Properties (Figure 2.6)			
Microbial Biomass C	0.477	**0.002**	0.154	0.605	0.401	0.888
Microbial Biomass N	0.453	0.205	0.446	0.976	0.064	0.979
Salt-extractable Organic C	0.554	0.691	0.167	0.528	0.177	0.637
Salt-extractable Inorganic N	0.065	0.231	**0.05**	0.346	0.078	0.861
Salt-extractable Organic N	0.434	0.095	0.584	0.463	0.117	0.881
			Potential Enzyme Activities (Figure 2.7)			
Arylsulfatase	0.186	0.782	0.789	0.316	0.907	0.305
β-glucosidase	0.071	0.985	0.683	0.642	0.969	0.425
Cellobiohydrolase	0.196	0.902	0.915	0.663	0.934	0.736
Leucine Aminopeptidase	0.253	0.349	0.158	0.710	0.847	0.067
N-acetyl-β-glucosaminidase	**0.047**	0.935	0.416	0.573	0.566	0.315
Phosphatase	**0.033**	0.910	0.769	0.509	0.756	0.188
Polyphenol Oxidase	0.942	0.524	0.715	0.379	0.615	0.524
Peroxidase	0.753	0.978	0.433	**<0.001**	0.470	0.334
			Microbial beta diversity‡			
Bacterial community composition	**<0.001** (R=0.03)	0.765	0.973	**<0.001** (R=0.03)	1.000	1.000
Fungal community composition	**<0.001** (R=0.04)	0.2213	0.9901	**<0.001** (R=0.04)	0.726	0.593
			Microbial alpha diversity			
Bacteria Shannon diversity	0.1001	**0.023**	0.899	0.29	0.393	0.801
Bacteria observed richness	**0.01**	**0.032**	0.948	0.454	0.501	0.628
Bacteria evenness	0.364	**0.033**	0.844	0.286	0.305	0.818
Fungi Shannon diversity	0.396	0.064	0.664	0.423	0.611	0.554
Fungi observed richness	**0.032**	0.071	0.455	0.148	0.665	0.175
Fungi evenness	0.987	0.078	0.763	0.699	0.597	0.676

Table 2.3. P-values from Two-way ANOVAs† comparing soil biological and chemical parameters in adjacent cropland soils to the 12-year prairie strips (Treatment) and the control.

†Two-Way ANOVA with factors of Treatment (Prairie Strip = Yes, No) and Distance from prairie strip (Distances = -3, -1, -0.3, -0.1, 0.1, 0.3, 1, 3, and 9) and interaction. Bold values indicate significance at $p < 0.05$. Underlined values indicate significance at $p < 0.1$.

‡ ‡ R value indicates the percentage of variation in microbial beta diversity explained by the prairie strip treatment.

		2019			2020		
	Phylum	**Control**	**Prairie Strip**	**p-value**	**Control**	**Prairie Strip**	**p-value**
		Mean relative abundance (%)			Mean relative abundance (%)		
Bacteria	Planctomycetes	4.784	5.542	**0.024**	3.691	4.252	NS
	Firmicutes	0.999	1.377	**0.038**	1.155	1.274	NS
	Alphaproteobacteria	11.814	10.664	**0.03**	14.072	12.627	NS
Fungi	Monoblepharomycota	0.036	0.002	**0.006**	0.011	0.004	NS
	Kickxellomycota	0.589	1.521	**0.001**	0.693	1.312	**0.048**
	Basidiomycota	11.179	14.758	0.073	10.065	12.111	NS

Table 2.4. Mean relative abundance, and ANOVA p-values (n =18)†, for select soil microbial taxa that differed between surrounding cropland soil of Control and Prairie Strip watersheds.

† Bold values indicate significance at $p < 0.05$. Underlined values indicate significance at $p < 0.1$.

		2019			2020		
	Phylum	**Control**	**Prairie Strip**	**p-value**	**Control**	**Prairie Strip**	**p-value**
		Mean Relative Abundance (%)			Mean Relative Abundance (%)		
Bacteria	Gemmatimonadetes	3.124	1.942	**0.018**	3.844	2.288	**0.012**
	Elusimicrobia	0.498	0.175	**0.010**	0.251	0.121	NS
	Armatimonadetes	0.669	0.278	**0.026**	0.487	0.274	NS
Fungi	Basidiomycota	13.047	52.218	**0.003**	14.408	51.755	**0.003**
	Chytridiomycota	2.928	0.871	**0.028**	4.084	1.076	**0.029**
	Mortierellomycota	13.097	4.582	**0.04**	11.038	5.796	NS
	Ascomycota	25.213	18.025	NS	27.07	15.58	**0.037**

Table 2.5. Mean relative abundance, and ANOVA p-values (n = 9)†, for select soil microbial taxa that differed between Control and soil under the Prairie Strip vegetation.

† Bold values indicate significance at $p < 0.05$. Underlined values indicate significance at $p < 0.1$.

		2019 Soybean				2020 Maize		
Community	**Soil Properties**	**Vector Magnitude**	**R^2**	**p-value**	**Soil Properties**	**Vector Magnitude**	**R^2**	**p-value**
Bacteria	**Soil pH**	**0.397247**	**0.62**	**0.007**	**SEOC**	**0.3706784**	**0.019**	**0.001**
	GWC	**0.3145357**	**0.69**	**0.001**	**Zinc**	**0.307493**	**0.013**	**0.002**
	Clay	**0.2807785**	**0.68**	**0.001**	**Soil pH**	**0.2595039**	**0.014**	**0.002**
	SEOC	**0.2747628**	**0.73**	**0.001**	**Potassium**	**0.2099002**	**0.009**	**0.051**
	Nitrate	**0.258258**	**0.59**	**0.025**	**Clay**	**0.1741649**	**0.016**	**0.001**
	PERase	0.2005677	0.67	0.001	Nitrate	0.1687116	0.013	0.003
	Potassium	0.1319459	0.63	0.008	ARSase	0.1650093	0.013	0.002
	PPOase	0.1233884	0.61	0.015	MBN	0.132576	0.01	0.025
	Phosphorus	0.1009331	0.57	0.053	Phosphorus	0.1295336	0.012	0.007
	Calcium	0.0709824	0.69	0.001	BGase	0.1122515	0.012	0.007
	BGase	0.0689229	0.6	0.016	PERase	0.063423	0.013	0.004
	PHOSase	0.0239232	0.62	0.007	SWHC	0.0614732	0.013	0.002
					MBC	0.048536	0.013	0.004
					Ammonium	0.0391297	0.01	0.04
					Calcium	0.0187584	0.012	0.01
					GWC	0.0060799	0.01	0.03
Fungi	**GWC**	**0.4610992**	**0.02**	**0.001**	**MBC**	**0.4889082**	**0.013**	**0.001**
	Clay	**0.3923759**	**0.02**	**0.001**	**Potassium**	**0.3405654**	**0.01**	**0.001**
	Nitrate	**0.3751424**	**0.02**	**0.002**	**ARSase**	**0.3128338**	**0.007**	**0.001**
	MBC	**0.32235**	**0.01**	**0.002**	**SEOC**	**0.2944516**	**0.019**	**0.002**
	SEOC	**0.2911179**	**0.02**	**0.001**	**Nitrate**	**0.231135**	**0.016**	**0.002**
	Ammonium	0.2471166	0.01	0.003	GWC	0.2174641	0.021	0.002
	Soil pH	0.2000376	0.01	0.054	Phosphorus	0.2169568	0.009	0.002
	Potassium	0.1700604	0.01	0.046	BGase	0.2018854	0.009	0.009
	PPOase	0.1568697	0.01	0.005	Soil pH	0.1829476	0.01	0.011
	PERase	0.128591	0.01	0.003	SWHC	0.1447008	0.008	0.006
	Calcium	0.0683806	0.02	0.001	NAGase	0.1262598	0.012	0.015
	NAGase	0.0386576	0.01	0.006	Clay	0.1001841	0.021	0.001
	PHOSase	0.0211009	0.01	0.019	Calcium	0.0253847	0.017	0.005
					Zinc	0.0232526	0.009	0.004
					PERase	0.0153274	0.014	0.002

Table 2.6. Vector magnitude, R2, and p-values for top drivers of microbial communities across both 2019 and 2020.

Soil Properties	2019 Soybean			2020 Maize		
	Treatment	**Distance**	**Treatment: Distance**	**Treatment**	**Distance**	**Treatment: Distance**
Soil Organic Matter	0.167	0.331	0.744	0.270	**0.048**	0.868
Gravimetric Water Content	0.811	**0.020**	<u>0.074</u>	0.581	<u>0.088</u>	0.888
Soil pH	0.726	0.408	0.769	0.668	0.382	0.587
Cation Exchange Capacity	0.136	0.514	0.378	0.831	0.628	0.803

Table 2.7. P-values from Two-way ANOVAs† comparing soil biological and chemical parameters in adjacent cropland soils to the 12-year prairie strips (Treatment) and the control.

† Two-Way ANOVA with factors of Treatment (Prairie Strip = Yes, No) and Distance from prairie strip (Distances = -3, -1, -0.3, -0.1, 0.1, 0.3, 1, 3, and 9 m) and interaction. Bold values indicate significance at $p < 0.05$. Underlined values indicate significance at $p < 0.1$.

Figures

Figure 2.1. Overhead photograph of prairie strip planted in a soybean field in Eastern Iowa, USA. Photo Credit: Iowa State University.

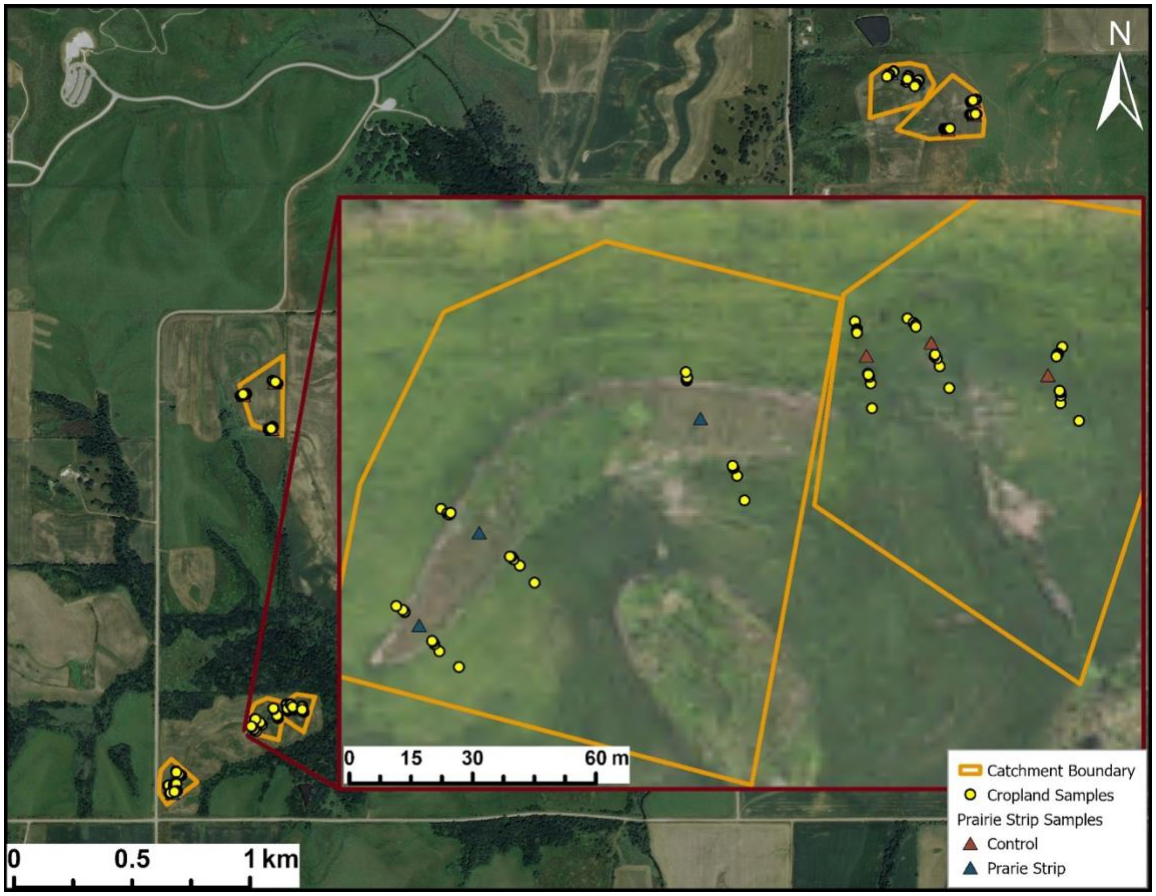

Figure 2.2. Map of prairie strip (n=3) and control catchments (n=3) and transects (n=3) with measurements at -3, -1, -0.3, -0.1, 0.1, 0.3, 1, 3, and 9 m (yellow dots). Inset: close-up of prairie strip and control catchment showing transects used for soil sampling. Paired sampling locations for prairie strips (blue) and control (red) 0m locations are indicated by triangles. Map Credit: Dr. Haliegh Summers.

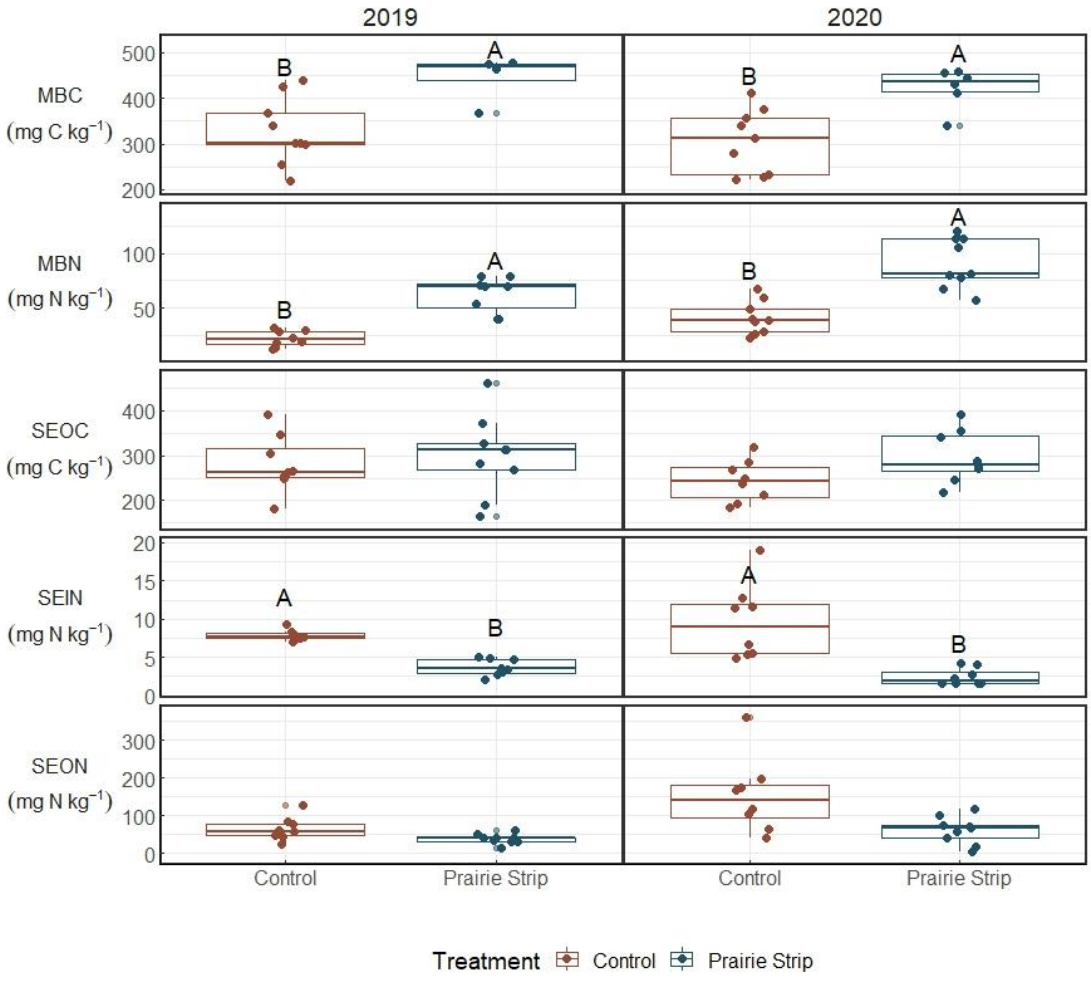

Figure 2.3. Soil carbon (C) and nitrogen (N) pools under prairie strips and paired control locations in 2019 and 2020. Boxplots of prairie strip and paired control catchment samples (n=9) sampled across three treatment catchments. Letters indicate significance of p-value <0.05. Abbreviations: MBC = microbial biomass C, MBN = microbial biomass N, SEOC = salt-extractable organic C, SEIN = salt-extractable inorganic N, SEON = salt-extractable organic N.

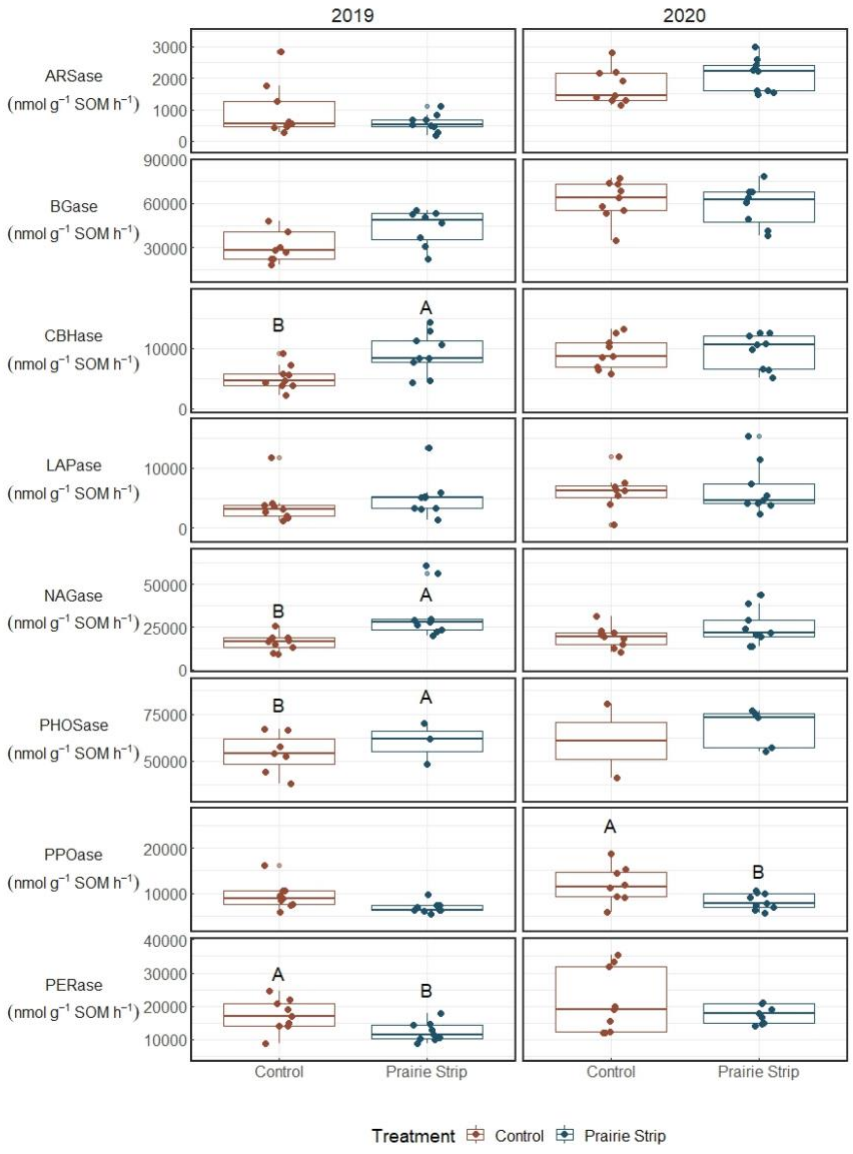

Figure 2.4. Potential enzyme activities, expressed per g of soil organic matter (SOM), under the prairie strips and paired control locations in 2019 and 2020. Boxplots of prairie strip and paired control catchment samples (n=9) sampled across three treatment catchments. Letters indicate significance at p-value <0.05. Abbreviations: ARSase= arylsulfatase,BGase=β-glucosidase, CBHase=cellobiohydrolase,LAPase=leucine aminopeptidase,NAGase=N-acetylglucosiminidase, PHOSase=phosphatase,PPOase=polyphenol oxidase,PERase=peroxidase.

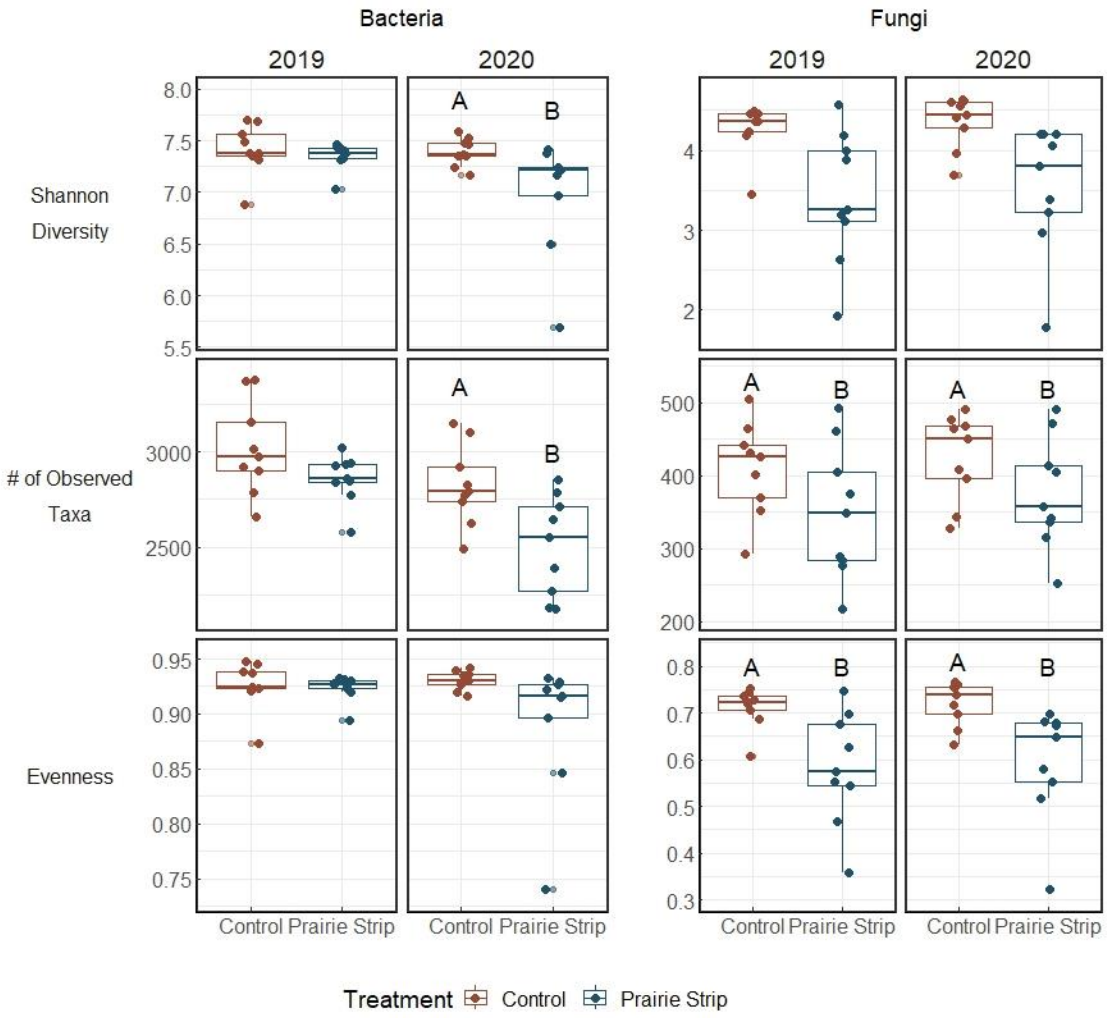

Figure 2.5. Bacterial and fungal alpha diversity under prairie strips and paired control locations in 2019 and 2020. Boxplots of prairie strip and paired control catchment samples (n=9) sampled across three catchments. Letters indicate significance at p-value <0.05.

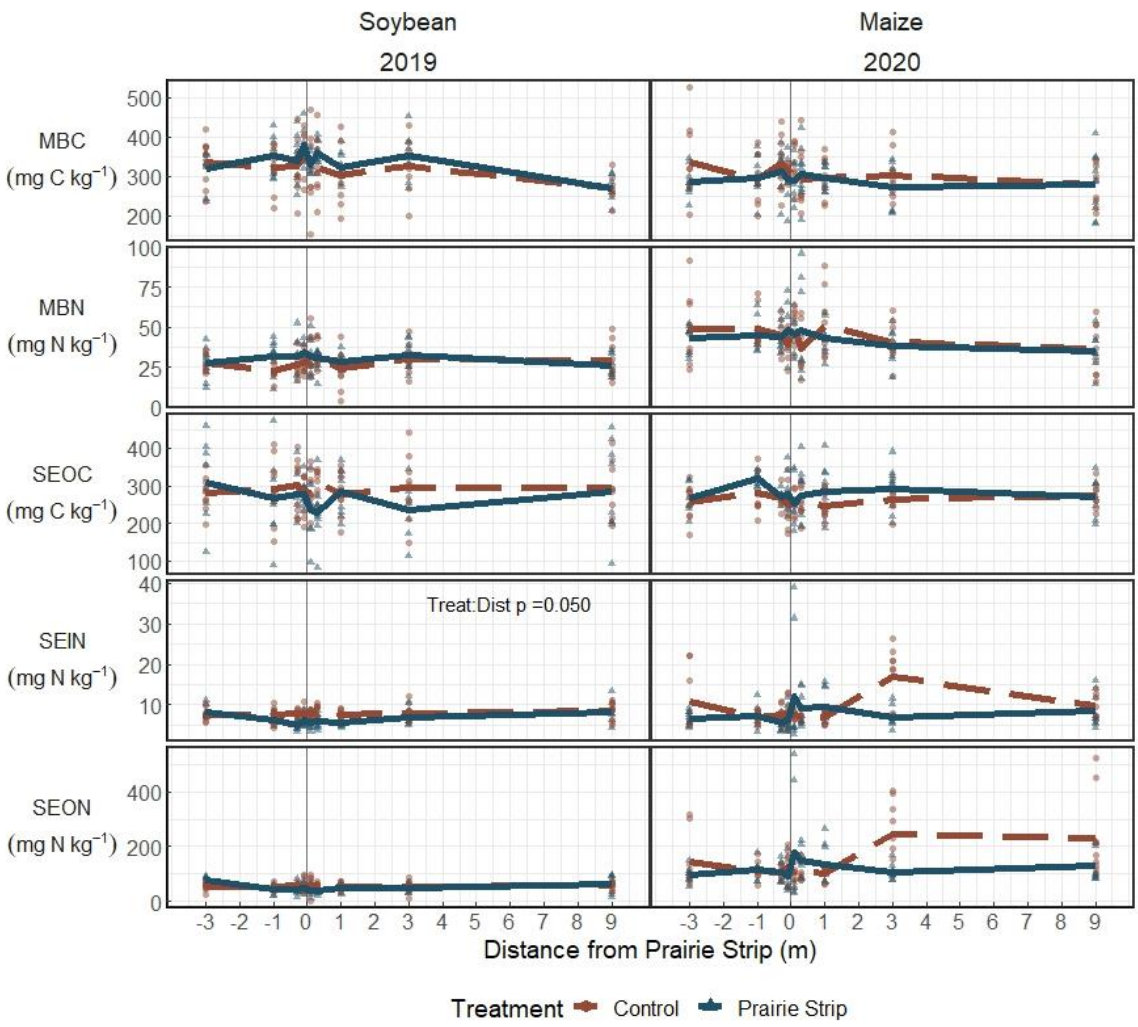

Figure 2.6. Soil carbon (C) and nitrogen (N) pools within cropland adjacent to the prairie strip and paired control locations in 2019 and 2020. Significant treatment or treatment × distance interaction and p-values shown within graph panels ($p < 0.1$). Individual samples shown and lines drawn through mean (n=9) at each distance soil samples were collected from the prairie strip, prairie strip samples (0 m) not included. Thin vertical line indicates the placement of the prairie strip. Abbreviations: MBC = microbial biomass C, MBN = microbial biomass N, SEOC = salt-extractable organic C, SEIN = salt-extractable inorganic N, SEON = salt-extractable organic N.

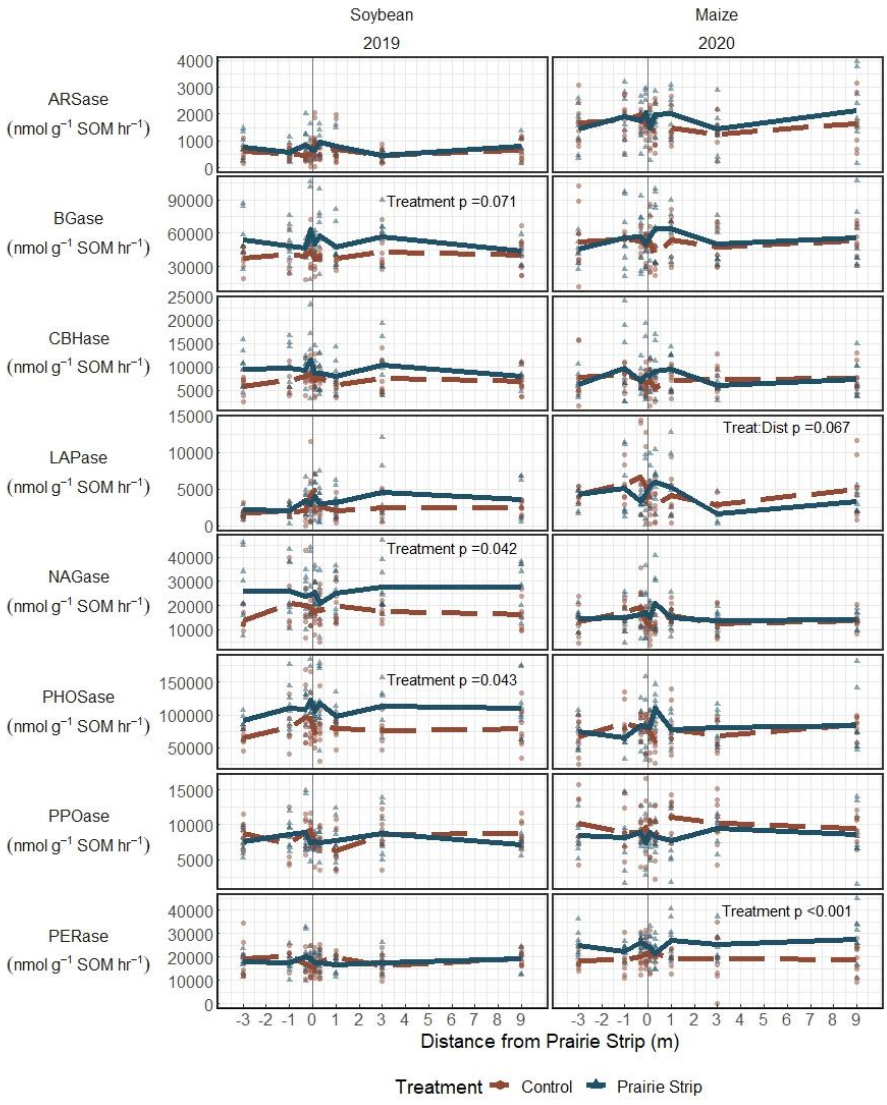

Figure 2.7. Potential enzyme activities, expressed per gram of soil organic matter (SOM), in cropland surrounding the prairies strip and paired control locations in 2019 and 2020. Significant treatment or treatment × distance interaction and p-values shown within graph panels ($p < 0.1$). Individual samples shown and lines drawn through mean (n=9) at each distance soil samples were collected from the prairie strip, prairie strip samples (0 m) not included. Thin vertical line indicates the placement of the prairie strip. Abbreviations: ARSase= arylsulfatase, BGase=β-glucosidase, CBHase=cellobiohydrolase, LAPase=leucine aminopeptidase, NAGase=N-acetyl-glucosiminidase, PHOSase=phosphatase, PPOase=polyphenol oxidase, PERase=peroxidase.

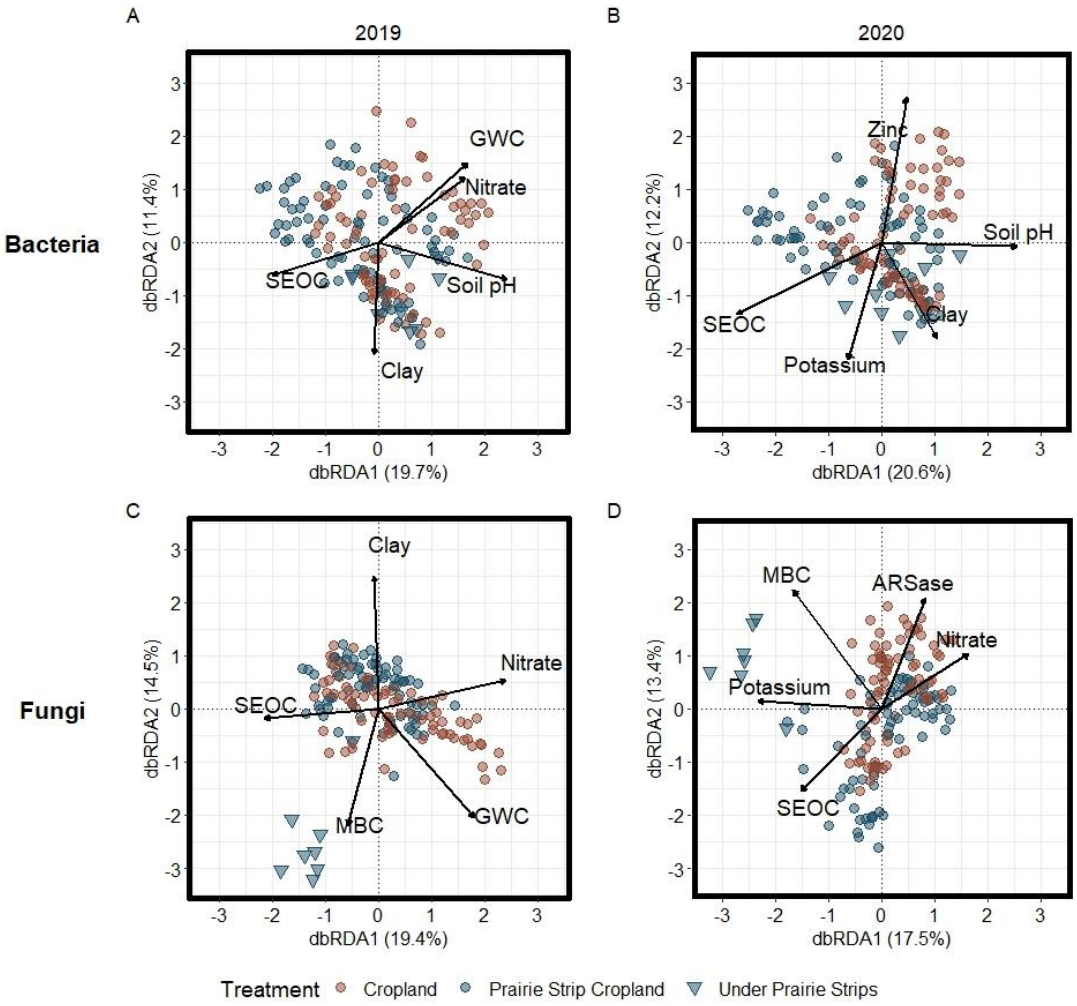

Figure 2.8. 1st and 2nd dimensions from distance-based Redundancy Analysis (dbRDA) on soil bacteria communities in 2019 (A) and 2020 (B), and fungal communities in 2019 (C) and 2020 (D). Vectors show the top five strongest predictors of community composition are shown in each panel. All significant predictors of microbial community are in Table 2.6.

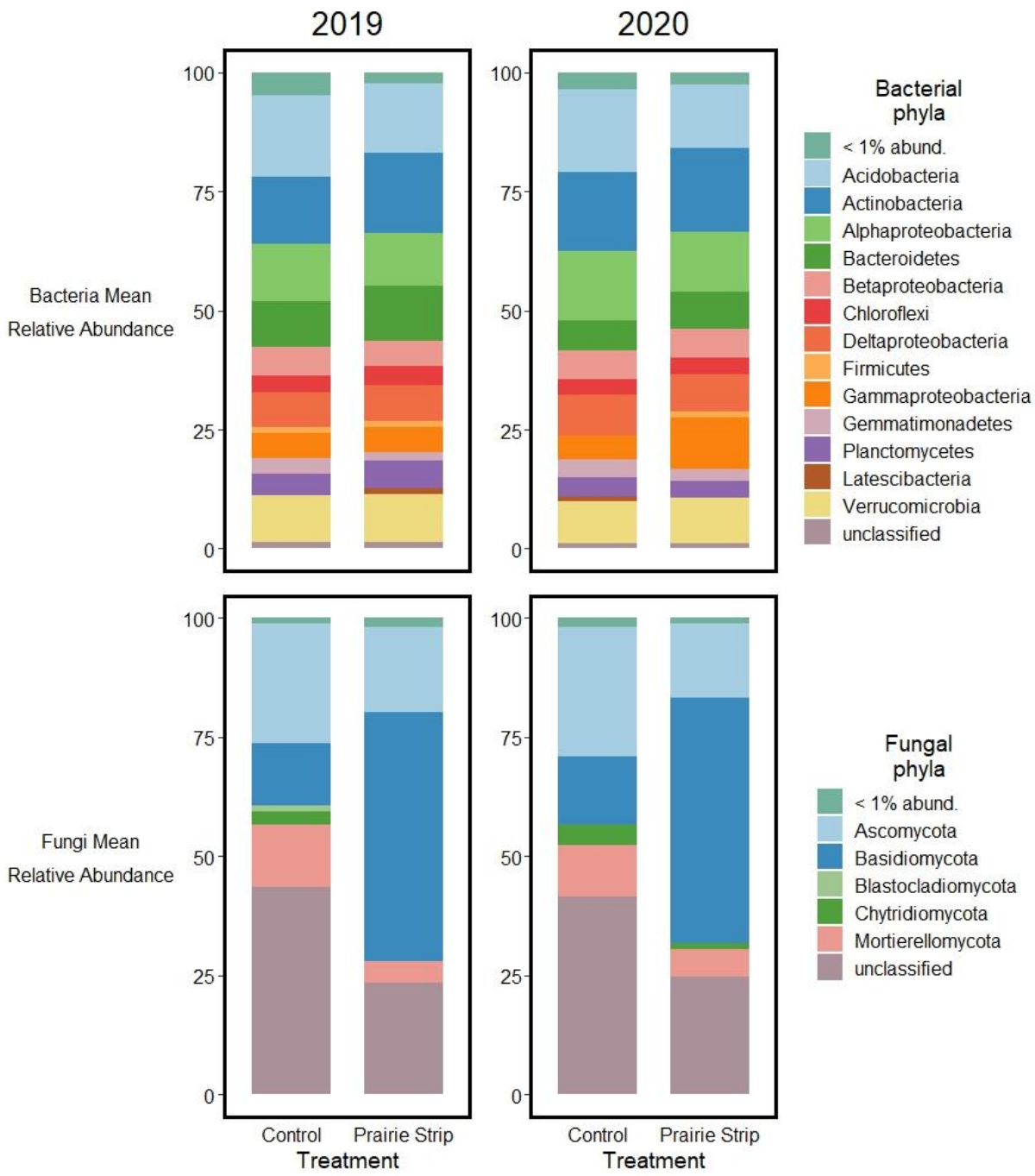

Figure 2.9. Relative abundance of bacterial and fungal phyla under prairie strip vegetation and paired control locations in 2019 and 2020. Means shown (n=9) sampled across three catchments.

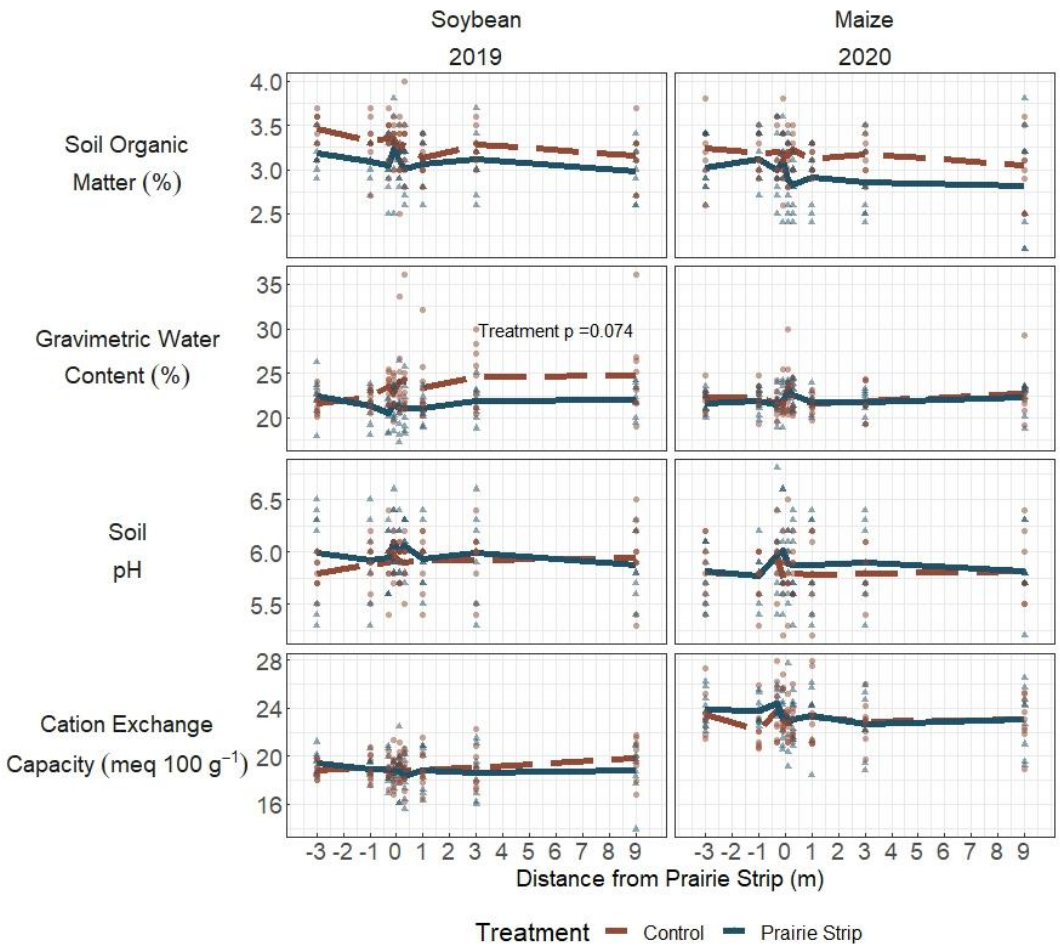

Figure 2.10. Soil properties and water content within cropland adjacent to prairie strips in 2019 and 2020. Significant treatment or treatment × distance interaction and p-values shown within graph panels ($p < 0.1$). Individual samples shown and lines drawn through mean (n=9) at each distance soil samples were collected from the prairie strip, prairie strip samples (0m) not included. Data previously published in Dutter et al. (2023)*.

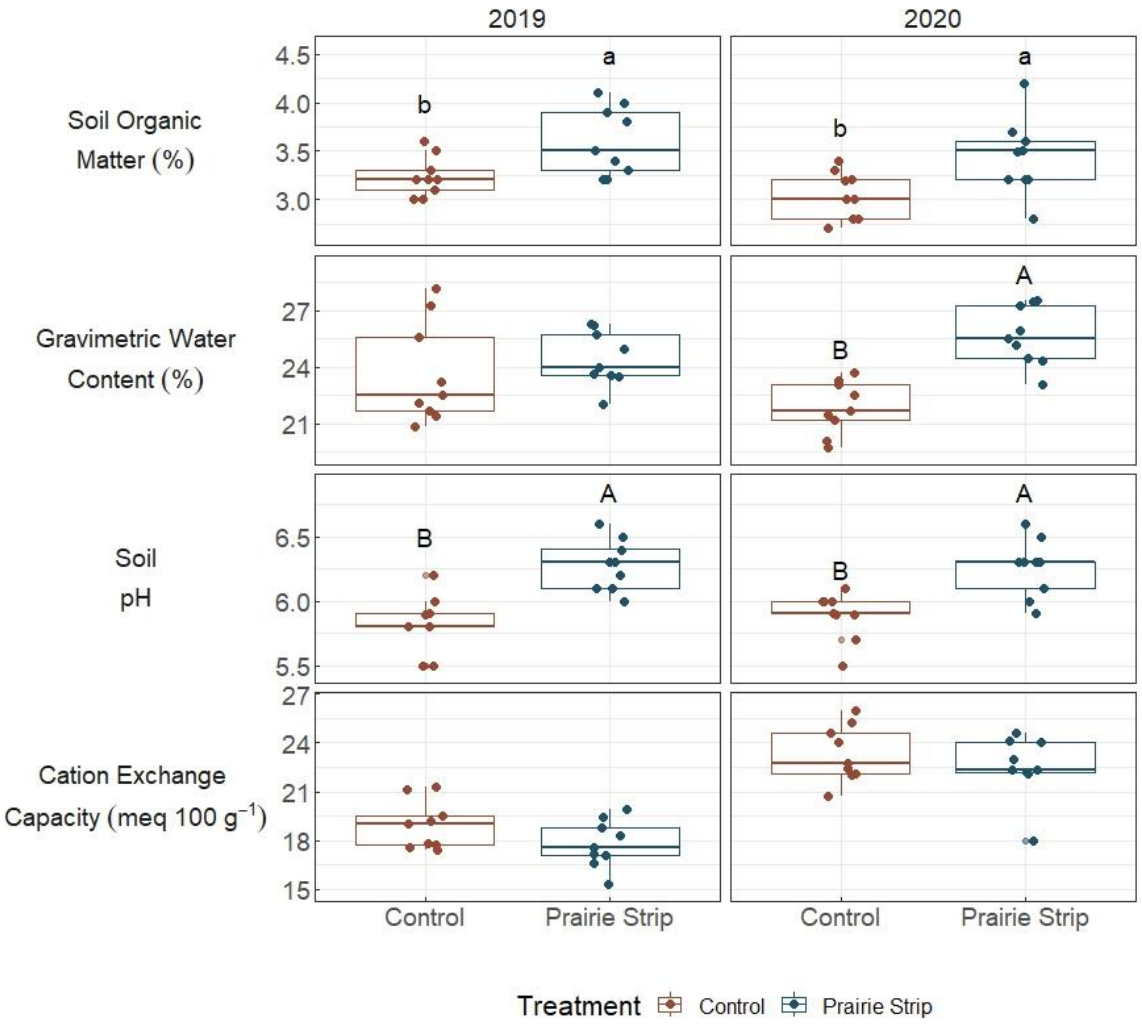

Figure 2.11. Soil properties under the prairie strips in 2019 and 2020. Boxplots of prairie strip and paired control samples (n=9) sampled across three treatment catchments. Lower case a, b indicates p-value < 0.1, capital A, B indicates p-value <0.05.

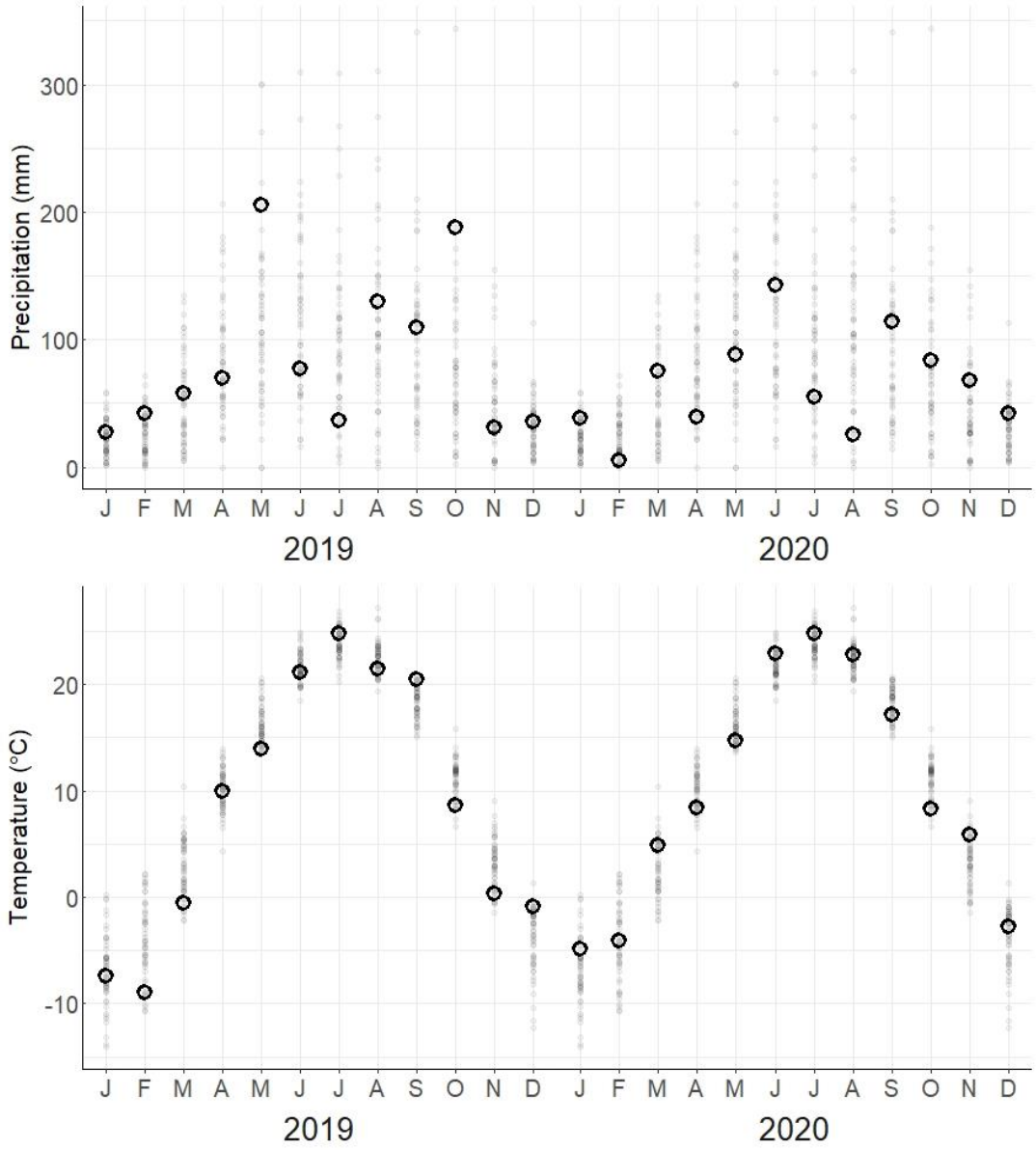

Figure 2.12. Mean monthly temperature and precipitation for the two years of the intensive study (open circles) and the prior 50-year historical record for each month (filled, shaded smaller dots). Precipitation totals during the experiment were 1010 mm in 2019 and 777 mm in 2020. Average temperatures were 8.6 ℃ in 2019 and 9.8 ℃ in 2020. Data are from Iowa Mesonet station in Jasper County, IA weather station that is 30 km from the site (IA Mesonet 2021). Data previously published in Dutter et al. (2023).

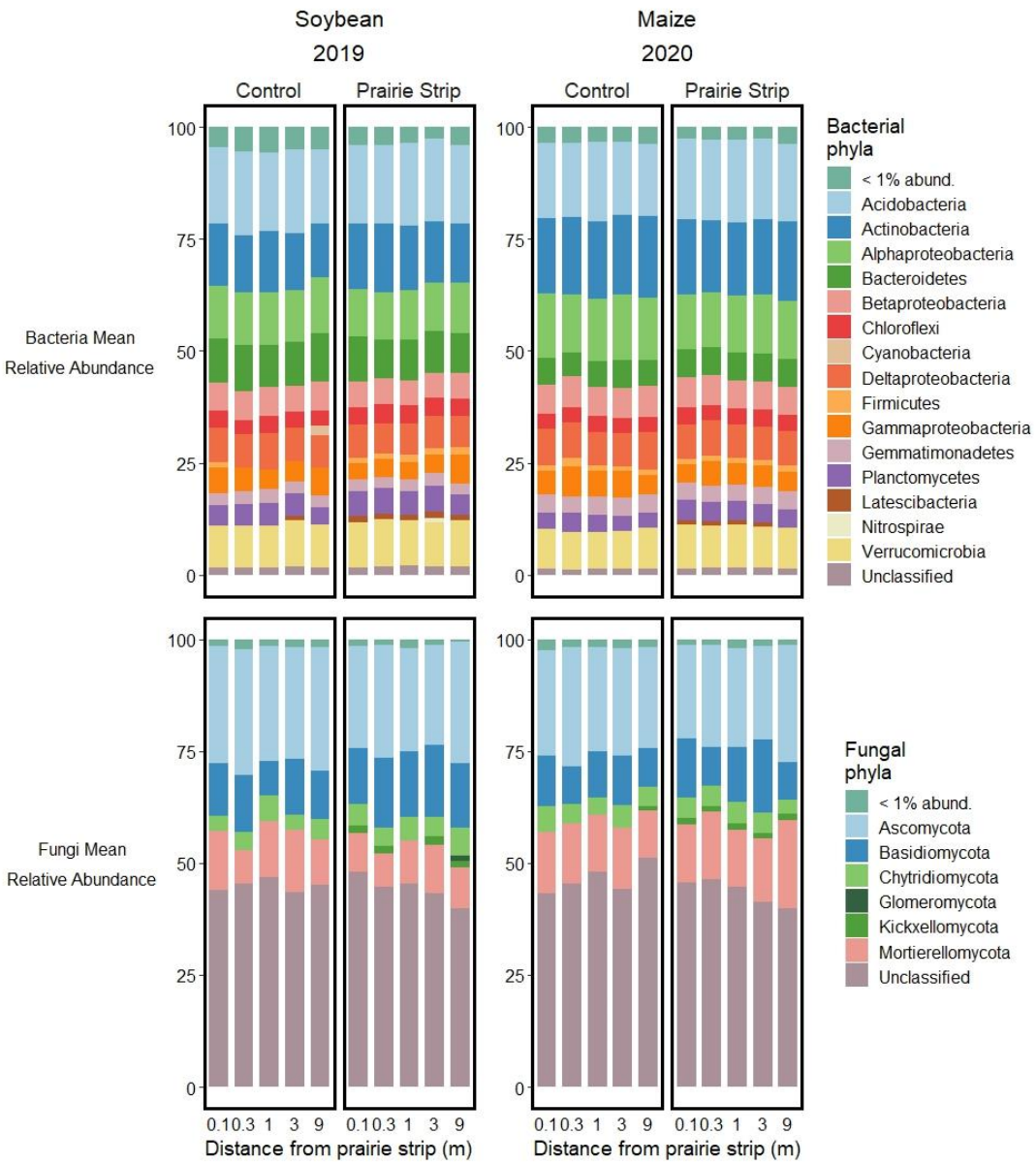

Figure 2.13. Relative abundance of bacterial and fungal phyla under cropland at five distances from the prairie in Soybean 2019 and Maize 2020. Means shown (n=18 for all except n=9 for 9m distance) sampled across three catchments.

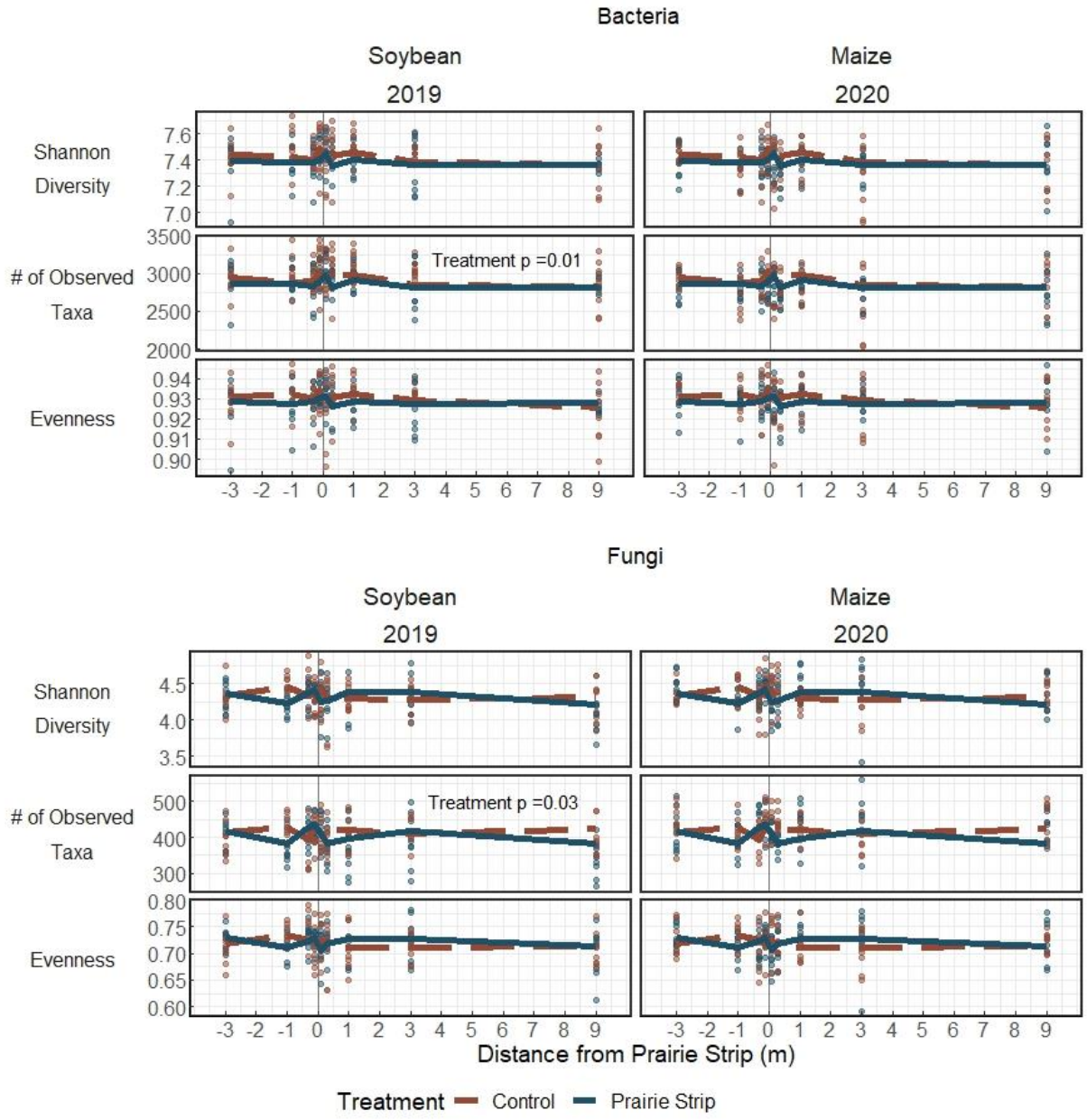

Figure 2.14. Bacterial and fungal alpha diversity in cropland soil surrounding the prairie strip in 2019 and 2020. Significant treatment or treatment × distance interaction and p-values shown within graph panels ($p < 0.1$). Individual samples shown and lines drawn through mean (n=9) at each distance soil samples were collected from the prairie strip, prairie strip samples (0 m) not included. Thin vertical line indicates the placement of the prairie strip.

CHAPTER 3: PRAIRIE STRIPS SHOW HIGHER ACTIVE C THAN CROPLAND SOILS DURING THEIR FIRST THREE YEARS OF ESTABLISHMENT

ABSTRACT

Prairie strips, zones of agricultural land converted to perennial vegetation, have the potential to sequester soil carbon and improve soil health. In this study, we introduced prairie strips to two cropping systems that had been maintained with reduced chemical inputs for the previous 32 years. We evaluated soil carbon within newly established prairie strips and in adjacent cropland, measuring measured microbial biomass carbon (MBC), permanganate oxidizable carbon (POXC), and mineralizable carbon (MinC) in each of the first 3 years of prairie strip establishment. We also measured C stocks in particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions 3 years after prairie strip planting. We found that prairie strips increased soil MBC and MinC in some years, depending on recent prairie and cropland management activities, and did not increase C in POM or MAOM fractions after 3 years. We also found no evidence that prairie strips increased soil C in adjacent cropland soils. Our results show that while prairie strips do not increase C in organic matter fractions or increase soil C in adjacent cropland soils, prairie strips increase sensitive indicators of soil active C relative to cropland soils, suggesting that prairie strips could likely accumulate C later in their establishment.

INTRODUCTION

Expansion and intensification of agriculture in the U.S. has led to rapid soil carbon loss, exacerbating climate change and compromising food security (Grandy and Robertson 2006; Poeplau et al. 2011; Sanderman et al. 2017; Dinerstein et al. 2019; IPBES 2019; FAO et al. 2021; UNEP and IUCN 2021). As of 2017, agriculture comprised over 40% of U.S. land area (USDA NASS 2017), and therefore, agricultural management practices control the fate of soil C

over a large spatial extent (Grandy and Robertson 2007, Syswerda et al. 2011). An emerging strategy for soil C accrual on cropland is perennialization, a suite of practices involving the establishment of perennial forbs and grasses within row crop systems (Mosier et al. 2021; Asbjornsen et al. 2013; Decré 2021). On-farm perennial areas provide an array of ecosystem services, including increased diversity of native plants, insects, and birds (Schulte et al. 2017; Kemmerling et al. 2022; Zhang et al. 2023), bioenergy crop production (Robertson et al. 2017), pollination services (Albrecht et al. 2020), and reduced nutrient losses (Crews and Brookes 2014). Perennial areas also increase soil C (Sprunger and Robertson 2018; Mosier et al. 2021; Dass et al. 2018), and are therefore a candidate management strategy to offset historical soil C losses.

The tallgrass prairie ecosystem that once dominated the Midwest, U.S. is an ideal ecosystem to integrate within farms for enhanced soil C storage. Tallgrass prairies harbor plants with high belowground biomass allocation (Sprunger et al. 2018), soil fauna with high biomass C content (Kallenbach et al. 2015), and high soil organic matter stocks amassed from centuries-old fire and grazing regimes (Anderson 2006; Helzer 2009; McClain et al. 2021), making tallgrass prairie an ideal ecosystem to integrate within farms for enhanced soil C storage. One form of farm perennialization is prairie strips, a management practice in which linear zones within or along the margin of crop fields are converted to tallgrass prairie vegetation. Prairie strips have unique features that position them to increase soil C, including increased biodiversity (Schulte et al. 2017; Kemmerling et al. 2022), reduced nutrient runoff (Gutierrez-Lopez et al. 2014; Hernandez-Santana et al. 2013), reduced soil erosion (Schulte et al. 2017), and increased soil biological function (Dutter et al. 2024). Still, prairie strips' capacity to build soil C remains relatively unexplored.

On-farm prairie strips may respond similarly to large scale prairie restorations that are known to alter soil physical and chemical properties that impact soil C. Physically, perennial polycultures exhibit optimal pore space for microbial C decomposition (Kravchenko et al. 2019), high root-to-shoot ratios (Baer et al. 2002; Mackelprang et al. 2018; Sprunger et al. 2018; Kavdir and Smucker 2005), soil aggregation (Grandy and Robertson 2006), and physical protection of soil C on mineral surfaces (Lavallee et al. 2020). Prairie strips are likely to incur similar physical changes. Land use change from annual cropland to perennial prairie also changes the stoichiometry of soils and organic inputs (Manzoni et al. 2008; Sprunger and Robertson 2018) depending on whether prairies are fertilized (Bach and Hofmockel 2015; Ludwig et al. 2011; Sprunger et al. 2020), mowed (Szymanski et al. 2019; Robertson and Groffman 2015; Córdova et al. 2018), and/or burned (Garcia and Rice 1994; Johnson and Matchett 2001; Jangid et al. 2010; Dooley and Treseder 2012; Kitchen et al. 2009). Both mowing and burning also stimulate higher prairie plant diversity (Collins et al. 1998), which has been shown to correspond with greater C storage (Kravchenko et al. 2019; Sprunger et al. 2020). We may see similar soil carbon responses in prairie strips.

Soil carbon changes under prairie strips have the potential to extend to adjacent croplands. Yet, there are mechanisms in cropping systems that could both facilitate and inhibit prairie strips' ability to build cropland soil C. Prairie strips also have the potential to build and stabilize C in surrounding cropland soils. Prairie roots and fungal hyphae may extend into cropland (Sprunger et al. 2018; Middleton et al. 2018), but this outward growth may be inhibited by cropland tillage (Säle et al. 2015; Warmink et al. 2011) or N fertilization (Jeske et al. 2018). Soil biota with C-rich biomass also have the potential to disperse from prairie strips to surrounding cropland (Fierer et al. 2013; Chaudhary et al. 2020; Kemmerling et al. 2022), but

cropland management may limit their ability to survive and establish (Johnsen et al. 2001; Ferrero et al. 2022; Dutter et al. 2024). Agrochemical application in cropland could either promote or inhibit soil C accrual at the prairie strip interface by altering soil properties that control soil C, including pH (Weil and Brady 2017), soil moisture (Maher et al. 2010), and cation exchange capacity (Stewart et al. 2007). Prairie strips may require decades build soil C in prairie and adjacent cropland areas (Kemmerling et al. 2022; Dutter et al. 2024), if at all, and year-to-year management practices in prairie strips and adjacent cropland are likely to affect soil C accrual.

The active fraction of soil C is likely to shift during early years of prairie strip establishment, and changes in this fraction can indicate longer term patterns of C storage in prairie strips. The active pool of C consists of living biomass of soil fauna, unprotected particulate organic matter, and microbe-derived biomolecules that are available for microbial decomposition, and, eventually, long term C stabilization (Weil and Brady 2017). Unlike slower cycling soil C pools that respond to land management changes after years or decades, active C shows detectable changes within weeks to months after land use change. Thus, the size of the active C pool in newly established prairie strips can provide an early indication of potential future C storage (Culman et al. 2012; Hurisso et al. 2016; Sprunger et al. 2020). Prairie strip establishment may also shift physically-defined soil C fractions: particulate organic matter (POM) and mineral-associated organic matter (MAOM; Cotrufo et al. 2015; Lavallee et al. 2020; Cotrufo and Lavallee 2022). Organic matter inputs in prairie strips, including burning (Kitchen et al. 2009), mowing (Johnson and Matchett 2001), enhanced soil aggregation (Grandy and Robertson 2007; Trivedi et al. 2015), and gradual dominance of high root-to-shoot C4 grasses (Helzer et al. 2009) are all likely to increase active C and C in POM compared to cropland soils.

We established experimental prairie strips in two cropping systems managed with little to no chemical inputs and cover crops for 32 years. We measured soil C accrual in prairie strips and adjacent cropland during their first 3 years of establishment in two cropping systems. We measured early indicators of soil C accrual - microbial biomass C (MBC), permanganate oxidizable carbon (POXC), and mineralizable C (MinC) - annually for 3 years after planting, as well as C and N stocks in POM and MAOM fractions 3 years after planting. We hypothesize that in prairie strip soils, soil C indicators, and C in organic matter fractions will increase due to cessation of soil disturbance. We also hypothesize that prairie strips will increase early indicators of soil C in nearby cropland soils due to outward movement of root biomass and organic matter inputs from the prairie strip edge.

METHODS

Site description and experimental design

This study was conducted at the W.K. Kellogg Biological Station (KBS) Long Term Ecological Research (LTER) Main Cropping System Experiment (MCSE) site located in Hickory Corners, Michigan, United States (occupied Anishinaabe land, 85° 24'W, 42° 24' N). A complete site description is available in Robertson and Hamilton (2015). Briefly, the MCSE was established in 1987 and includes a matrix of annual, perennial and unmanaged successional cropping systems situated in a randomized complete block design with 1 ha (90 x 110 m) plots each replicated in six blocks. Our study took place in two annual cropping systems: Reduced Input and Biologically Based (Organic, Figure 3.1). Both cropping systems are planted with a corn-soybean-winter wheat rotation that includes a red clover cover crop between wheat and corn phases, and a rye cover crop between corn and soybean phases. Reduced Input plots are managed with fertilizer, herbicide, and fungicide, each applied on 33% of the cropland field area.

Organic plots receive no chemical inputs, compost, or manure. Both cropping systems receive post-planting cultivation each year, and the Organic cropping system receives additional rotary-hoeing as needed for weed control (Figure 3.2).

Prairie strips were planted in both cropping systems in April 2019. A prairie seed mix was sown in a 15 m wide strip running down the center of each Reduced Input and Organic plot, parallel to crop rows and comprising five percent of each plot area (Figure 3.1). The seed mix included 4 grass species and 18 forb species (sourced from Native Connections in Kalamazoo, Michigan, United States; plant species are listed in Kemmerling et al. 2022). Prairie strips were mowed in June 2019 and July 2020, and burned in March 2021 and April 2022 (Figure 3.2). Prairie strips in Reduced Input plots were treated with potash and phosphate fertilizer in May 2020 and May 2021 (Figure 3.2). Our study took place from June 2020 to June 2022, capturing a 3-year rotational period with prairie strips planted at the site: corn in 2020, soybean in 2021, and wheat in 2022 (Figure 3.2).

We compared soil carbon across crop management treatments and across distances from the prairie strip using a replicated transect design within each plot (Figure 3.1). Soil samples were collected from 3 transects perpendicular to each prairie strip with sampling locations at four distances: 0 m, 1 m, 5 m, and 20 m from the prairie strip (the station at 0 m was located in the center of the prairie strip), such that 12 individual soil samples were collected from each plot. In each plot, individual soil samples were combined across transects to form a single composite sample for each distance (Figure 3.1). Soil samples were collected in mid to late July in each sampling year, when differences in soil carbon between cropland and prairie systems are usually most pronounced (Bach and Hofmockel 2015; Hargreaves and Hofmockel 2014). Samples were collected approximately 1 year after prairie strip planting on July 20, 2020, approximately 2

years post-planting on July 22, 2021, and approximately 3 years post-planting on July 25, 2022 (3 years * 2 treatments * 6 replicates * 4 distances, n=144 samples total). Each sample was sieved to <2mm and a subsample was stored at 4°C prior to chloroform fumigation. The remainder of each sample was air dried for further carbon analysis.

Soil carbon analysis

Permanganate oxidizable carbon

We determined permanganate oxidizable carbon (POXC) in each composite soil sample using a protocol adapted from Weil et al. (2003) and Culman et al. (2012). POXC reflects soil C that has been processed and mineralized by microbes (Hurisso et al. 2016). First, 2 mL of 0.2 M KMnO4 (potassium permanganate) and 18 mL deionized water were added to 2.5 g of each air-dried soil sample in a 50 mL conical tube and samples were shaken for 2.0 minutes at 180 rpm. After settling for 10 minutes, 0.5 mL supernatant of each mixture was diluted in 49.5 mL deionized water in a separate 50 mL conical tube. A 200 µL aliquot of each diluted sample was pipetted in triplicate into a clear 96-well flat-bottom plate for spectrophotometric analysis. Each plate included a set of internal standards with analytical replicates, including a deionized water blank, four standard solutions (0.00005, 0.0001, 0.00015, and 0.0002 mol L^{-1} KMnO4), and a standard soil analyzed on all plates. Absorbance (optical density) values were analyzed using a Biotek Synergy microplate reader (Winooski, VT) at 550 nm. Sample POXC values were calculated by converting raw absorbance values to the quantity of KMnO4 reduced during the assay using a formula provided in Weil et al. (2003).

Mineralizable carbon

Mineralizable carbon (MinC) was measured for each composite soil using a protocol adapted from Franzluebbers et al. 2000. MinC reflects labile soil C that is available for microbial

mineralization and comprises ~20% of total soil organic carbon (Hurisso et al. 2016; Weil and Brady 2017). First, 5 g of each soil sample was added to a 125 mL Wheaton serum bottle and samples were rewetted to 70% water holding capacity with ultrapure water (Córdova et al. 2018). Bottles were sealed using phenolic screw caps fitted with Chlorobutyl septa and incubated at room temperature for 24 hr prior to first sample collection. Gas samples were collected from bottle headspace at two time points following the incubation period (0 and 24 hr). CO_2 samples were collected in overpressurized 6 mL glass vials (Exetainers, Labco Ltd., Lampeter, Wales) flushed with N_2. Gas samples were analyzed using a gas chromatograph (Agilent 7890A) coupled to an autosampler (Gerstel MPS2XL; Shcherbak and Robertson 2019). Short-term mineralizable C was calculated as the difference between 0 and 24 hr CO_2 measurements and reported as μg CO_2 g^{-1} dry soil.

Microbial biomass carbon and nitrogen

We measured microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) in each composite soil using a chloroform fumigation and extraction method according to Brookes et al. (1985). First, we added 6 g of each fresh soil sample to two 50 mL conical tubes. We added 2 mL chloroform to one tube and incubated for 24 hr at room temperature. We added 30 mL of K_2SO_4 (potassium sulfate) to all sample tubes and shook samples at 120 rpm for 1 hr. Mixed samples were filtered using Whatman 202 filter paper, transferred into 25 mL scintillation vials, and stored at -20°C until analysis. Samples were analyzed using a TOC analyzer (TOC-Vcph carbon analyzer, Shimadzu, Kyoto, Japan). MBC and MBN values were corrected by dividing values by 0.45 (Wu et al. 1990) and 0.54 (Brookes et al. 1985), respectively. We removed 11 samples from the dataset that contained less than -200 μg C g^{-1} soil MBC.

Particulate and mineral associated organic matter

We quantified particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions in soils collected three years after prairie strip planting using a protocol adapted from Poeplau et al. (2018) and Mosier et al. (2019). We physically separated POM and MAOM fractions by adding 35 mL of 0.5% sodium hexametaphosphate and 12 3 mm glass beads to a 50 mL centrifuge tube containing 10 g sieved, dried soil and shaking samples at 120 rpm for 18 hr. Samples were separated into POM (> 53 μm) and MAOM (< 53 μm) fractions by passing samples through a 53 μm sieve using ultrapure water and separating fractions into separate aluminum pans. We dried pans of POM and MAOM fractions at 60°C for 5 days and weighed each fraction when completely dry. Sample recovery was within ±5% of initial mass. We ground bulk soil and POM and MAOM fractions to 250 μm and analyzed samples for C and N concentration using a Costech Elemental Combustion System (ECS 4010; Costech Analytical Technologies; Valencia, CA).

Soil physiochemical properties

Soil samples were analyzed for a suite of physiochemical properties expected to explain patterns in soil C: soil moisture, inorganic N, pH, and cation exchange capacity. Percent soil moisture was measured for each individual sample included in soil C analyses. Additional soil properties were measured from supplemental soils collected from different sampling locations within each plot and different time points within each year of the study. Supplemental soils were collected in June 2020, June 2021, and July 2022 from four sampling stations in each plot (separate from the transect sampling locations) which corresponded approximately with prairie strip distances: prairie strip center (0m), ~1 m, ~5 m, and ~20 m from the prairie strip edge (see https://lter.kbs.msu.edu/research/long-term-experiments/main-cropping-system-experiment/ for a

complete description of sampling locations and protocols). Each supplemental soil was analyzed for extractable nitrate and extractable ammonium via an extraction, filtration, and colorimetric quantification method (see https://lter.kbs.msu.edu/protocols/33 for a complete protocol). Finally, all supplemental soils were submitted to the Michigan State University Soil and Plant Nutrient Lab (SPNL; East Lansing, MI, USA, http://www. spnl.msu.edu/) for pH and cation exchange capacity (CEC) analysis.

Statistical analysis

To determine whether prairie strips increased soil active C relative to cropland, we constructed linear models using the lm() function in R (R version 4.2.3, R Core Team 2022). Each model included year, cropping system, vegetation type (prairie vs. crop) and their interactions as predictor variables, and each soil C metric (POXC, MinC, MBC) as a response variable. To understand whether prairie strips contained greater stabilized soil C, we constructed additional linear models for samples collected 3 years after prairie strip planting that included cropping system, vegetation type (prairie vs. crop) and their interaction as predictor variables, and either POM C or MAOM C the response variable.

To determine whether prairie strips increased soil active C in nearby cropland soils, we constructed linear models that included cropland soils only. We included year, cropping system, distance from the prairie strip, and their interactions as predictor variables, and each soil C metric as a response variable. To test whether prairie strips increased stabilized soil C in nearby cropland soils, we constructed additional linear models for cropland samples collected 3 years after planting that included cropping system, distance from prairie strip, and their interaction as predictor variables, and either POM C or MAOM C the response variable.

We tested the effect of prairie strips on soil N and physiochemical properties using two-

way ANOVAs. Each model included year, cropping system, vegetation type (prairie vs. crop) and their interactions as predictor variables, and each soil N metric (MBN, NO_3- , NH_4+ , N mineralization, nitrification, ammonification) or physiochemical property (soil moisture, pH and CEC) as a response variable. To determine whether cropland soil N metrics and physiochemical properties varied across distances from the prairie strip, we used two-way ANOVAs that included cropland soils only. We included year, cropping system, distance from the prairie strip, and their interactions as predictor variables, and each soil N metric or physiochemical property as a response variable.

RESULTS

Permanganate oxidizable carbon

POXC in prairie strips and cropland soils did not differ (Table 3.1; Figure 3.3). In both prairie strip and cropland soils, soil POXC increased over time ($p < 0.001$; Table 3.1; Figure 3.3). POXC in prairie strips in Reduced Input and Organic cropping systems did not differ (Figure 3.3). Soil POXC did not change significantly with distance from the prairie strip (Table 3.2; Figure 3.4).

Mineralizable carbon

Prairie strip soils contained greater MinC than cropland soils ($p < 0.001$; average 42.7 μg CO_2 g^{-1} soil in prairie strips; average 36.2 μg CO_2 g^{-1} soil in cropland; Table 3.1; Figure 3.3). There was a significant year x vegetation interaction, where prairie strip soils contained significantly greater MinC than cropland soils after 1 year ($p < 0.001$) and after 3 years ($p = 0.027$), but not after 2 years (Table 3.1; Figure 3.3). Prairie strips MinC remained consistent from 1 year to 3 years post-planting (Figure 3.3). Reduced Input and Organic cropping systems contained similar amounts of MinC in both prairie strips and cropland (Table 3.1; Figure 3.3).

Cropland soil MinC varied over time ($p < 0.001$; Table 3.2), but not linearly (2 years post-planting, under soybeans, was highest, Figure 3.3). Cropland soil MinC did not significantly vary with distance from the prairie strip ($p = 0.395$; Table 3.2; Figure 3.4).

Microbial biomass carbon

Prairie strips and cropland contained similar soil MBC overall (average 140.2 μg C g^{-1} soil in prairie strips over 3 years and average 143.6 μg C g^{-1} soil in cropland over 3 years; Table 3.1; Figure 3.3). There was a significant year x vegetation interaction, where prairie strips contained higher MBC than cropland after 1 year, but not 2 or 3 years (Table 3.1), driven by MBC in the Organic cropping system (Figure 3.3). Soil MBC varied among years ($p < 0.001$; Table 3.1), and was lowest after 2 years (Figure 3.3). Organic cropping systems contained twofold greater soil MBC than Reduced Input cropping systems across both prairie strip and cropland areas (Figure 3.3). Cropland soil MBC was not affected by distance from the prairie strip (Table 3.2; Figure 3.4).

Particulate and mineral associated organic matter

Three years after planting, prairie strips and cropland contained similar C and N in soil particulate organic matter (POM) and mineral associated organic matter (MAOM) fractions ($p > 0.05$; Table 3.3; Figure 3.5). Across prairie strip and cropland areas, Organic cropping systems contained higher POM C and POM N than Reduced Input systems ($p < 0.001$; Table 3.3; Figure 3.5), while Reduced Input systems contained higher MAOM C and MAOM N ($p < 0.1$; Table 3.3; Figure 3.5). Distance from the prairie strip did not explain C and N in POM or MAOM fractions 3 years after planting (Table 3.4).

Soil N and physiochemical properties

Prairie strip soils contained significantly lower NO_3- and NH_{4+} than cropland soils ($p <$

0.001; Table 3.5; Figure 3.6). Prairie strips and cropland exhibited similar MBN and CEC values (Table 3.5). Prairie strip soils showed lower soil moisture ($p = 0.008$; Table 3.5) and marginally higher soil pH ($p = 0.092$; Table 3.5) than cropland soils after 3 years ($p = 0.008$; Table 3.5). Organic cropping system soils contained twofold greater MBN than Reduced Input soils in both prairie strip and cropland areas ($p < 0.001$; Table 3.5; Figure 3.6). Prairie strips showed similar soil moisture, pH, and CEC across cropping systems (Table 3.5). Distance from the prairie strip was not an important predictor of any soil N or physiochemical measurement, and was only marginally significant control of soil NH_{4+}, where soils nearest to the prairie strip edge contained greater NH_{4+} than soils further from the prairie strip edge ($p = 0.094$; average 3.06 mg NH_{4+} g^{-1} soil at 1m; average 2.42 NH_{4+} g^{-1} soil at 5 m; average 2.16 NH_{4+} g^{-1} soil at 20 m; Table 3.6; Figure 3.6).

DISCUSSION

Prairie strips are a form of on-farm perennialization with the potential to restore soil C by reintroducing C storage mechanisms of the tallgrass prairie ecosystem. Here we find that prairie strips show observable increases in soil active C (mineralizable C and microbial biomass C) relative to surrounding cropland in two of the first three years of their establishment. Prairie strips did not increase soil active C in even the nearest cropland soils during the first three years of establishment, nor did they increase C stocks in organic matter fractions after three years. In both prairie and cropland areas, soil active C reflected annual crop rotations and management practices that occurred shortly before sampling. Early increases in prairie strip soil active C suggest that in coming years, prairie strips will respond similarly to larger prairie restorations, showing more pronounced increases in soil C stocks relative to cropland.

Prairie strips increase soil active C , but not organic matter C stocks, during first three years of establishment

Soil active C within prairie and cropland soils varied significantly among study years, and prairie strip soils contained greater MinC and MBC than adjacent cropland soils in two of the three years of the study when cropland was planted with corn and wheat (Figure 3.3). Enhanced MinC, MBC, and POM C pools are often observed in maturing perennial systems (Camill et al. 2004; Maher et al. 2010; Schmer et al. 2011; Perry et al. 2023), including prairie strips (Pérez-Suárez et al. 2014), due to increases in ANPP and belowground biomass allocation. The variable nature of increased MinC and MBC in KBS LTER prairie strips (Figure 3.3) may be a product of mowing and burning, management practices associated with labile carbon gains and losses, respectively, in restored prairies (Garcia and Rice 1994; Johnson and Matchett 2001; Christensen 2001; Jangid et al. 2010, Dooley and Treseder 2012; Merino et al. 2021). Unfertilized and recently-mowed prairie strips also contained little to no inorganic N, which may have limited microbial decomposition of POM (Stewart et al. 2015) and led to a short-term buildup of MinC (Manzoni et al. 2008; Robertson and Groffman 2015; Córdova et al. 2018). Our study suggests that as long as prairie strips undergo regular mowing and burning, sensitive soil C indicators will change with time since these practices occurred, regardless of the cropping system. Still, changes in POM and MAOM C stocks are usually not detectable until 5 or more years after establishment (Sprunger and Robertson 2018; Perry et al. 2023), and we expect that amid continual fluctuations of sensitive active C measures, prairie strips will increase C stocks in POM and MAOM in coming years.

Soil POXC increased over the course of the study in both prairie strips and cropland (Figure 3.3), but by year 3, prairie strip POXC values remained closer to those of annual

cropping systems than of decades-old perennial polyculture systems (Sprunger et al. 2020; Martin and Sprunger 2022). Several studies have shown increased POXC within 3 years after cessation of tillage (Quincke et al. 2007; Lewis et al. 2011; López-Garrido et al. 2011; Culman et al. 2012), perennial vegetation establishment (DuPont et al. 2010; Inwood et al. 2015), and prescribed burning (Demisie et al. 2014; Hurisso et al. 2016). However, the sandy loam soils at our study site are less likely to show POXC changes within 3 years, as they contain fewer fine mineral particles to which processed C can be adsorbed (Tiemann and Grandy 2014; Lucas and Weil 2012). Prairie strips may have a greater effect on POXC in cropping systems with finer texture and greater potential for C stabilization via mineral association (Six et al. 2002; Stewart et al. 2007; Schmidt et al. 2011; Cotrufo et al. 2015; Lavallee et al. 2020; Cotrufo and Lavallee 2022; Guillaume et al. 2022). Still, we expect that in coming years, prairie strip POXC values will increase, following the trajectory of other restored prairie and native grassland systems at the KBS LTER site (Sprunger et al. 2020).

Prairie strips do not increase soil active C in surrounding cropland

Prairie strips did not increase soil active C, nor C stocks in POM and MAOM fractions, in cropland adjacent to prairie strips in either cropping system (Figure 3.5). Reduced Input and Organic cropping systems harbored unique C and N cycling regimes before prairie strips were planted due to differences in agrochemical application (Robertson et al. 2014; Kallenbach et al. 2015), and we show that these regimes still persist. For instance, high MAOM C and N and low POM C and N in Reduced Input plots can be attributed to N fertilization and subsequent decomposition of POM (Stewart et al. 2007). Differences among cropping systems, however, were not explained by distance from prairie strips, suggesting that long-term fertilizer, insecticide, and fungicide application does not enhance nor inhibit prairie strips' capacity to

build soil C in neighboring cropland in early establishment. While agrochemicals did not affect prairie strips' contributions of soil C to cropland, no-till management may increase prairie strips' ability to contribute soil C to surrounding cropland, as prairie roots and fungal hyphae would be better able to grow outward and provide inputs to nearby soils (Warmink et al. 2011; Säle et al. 2015; Sprunger et al. 2018; Middleton et al. 2018).

Other recent prairie strip studies show mixed results for C accrual in adjacent cropland. Kemmerling et al. (2022) measured KBS LTER prairie strips and found that just a few months after prairie planting, cropland soils near prairie strips contained higher MinC than cropland farther away in tilled cropping systems. Here we show that this pattern does not hold in the following 3 years. Similarly, in an earlier study of mature (12 year old) prairie strips planted in untilled cropland, we found that prairie strips did not affect C and N pools in adjacent cropland soils (Dutter and Rutkoski et al. 2024). Taken together, these findings suggest that prairie strips alter active soil C in surrounding cropland during initial establishment, perhaps as soil aggregates are broken near the prairie edge during site preparation (Grandy and Robertson 2007) or as active C is introduced from newly establishing prairie plants (Córdova et al. 2018), but increases in active C in adjacent cropland soils do not persist beyond the first year, and are unlikely to result in cropland C sequestration long term.

Soil active C measurements are sensitive to recent management activities in prairie and cropland

Active C measurements are more sensitive to land use change than total C measurements, providing a useful early indication of future soil C sequestration (Culman et al. 2012; Hurisso et al. 2016; Sprunger et al. 2020). Many of the differences we observed in soil C, both between prairie strips and cropland, and between cropping systems, can be attributed to management

activities that occurred shortly before soil sample collection. For instance, MBC was higher in prairie strips than cropland after 1 year, when cropland soils were intensively tilled shortly before soil sampling (Figure 3.2; Figure 3.7). Soil disturbance generally reduces soil MBC (Dorodnikov et al. 2009; Trivedi et al. 2015; Nie et al. 2014), and the tillage in cropland prior to soil sampling may have magnified the difference between prairie strip and cropland MBC. As another example, prairie strip soil MinC was higher than cropland soil MinC at 1 year post-planting, shortly after prairie strips had undergone 2 mowing treatments that contributed a significant amount of organic matter to prairie strip soils (Figure 3.2; Figure 3.7). Management activities immediately preceding soil collection in prairie and cropland areas appear to have a strong effect on the soil C indicators we measured. The short-term sensitivity of active C measurements – and their resulting temporal heterogeneity – may be greater than their mean changes with land use, questioning their utility in studies comparing land use practices. Soil MBC, MinC, and POXC have the potential to give valuable short-term feedback to land managers, but future studies should validate these indicators across soil types with high-frequency measurements to disentangle long-term (annual) responses vs. short-term (weeks) responses, especially when they are being used to predict longer term patterns in soil C.

CONCLUSION

During their first 3 years after planting, prairie strips increased soil mineralizable C and microbial biomass C in two cropping systems, but did not show consistently higher soil active C than surrounding cropland. Changes in soil C only occurred under prairie strips and did not extend to surrounding cropland soils in either cropping system. The timing of soil active C responses suggests that prairie and cropland management activities within each year could be a more important control of sensitive soil C indicators than prairie strip age. While prairies strips

did not contain higher C stocks in organic matter fractions after 3 years, increases in soil active C indicate that, like in other diverse perennial systems, prairie strips will likely build total C stocks in coming years as they mature.

ACKNOWLEDGEMENTS

We would like to thank Marshall McDaniel, Lisa Schulte Moore, Christine Sprunger, and Phil Robertson for their help with project conceptualization. We thank Holly Vander Stel, Stacey Vanderwulp, and Kevin Kahmark for their help coordinating sample collection and equipment use for this project. We thank Lindsey Kemmerling, Madaris Serrano-Perez, Ceco Maples, Yahn-Jau Su, Sofie Iwamasa, and Lee Leonard for their assistance with sample collection and processing. We also thank Lukas Bell-Dereske, Jennifer Jones, Tayler Ulbrich, Caitlin Broderick, Glade Bogar, Moriah Young, and Brandon Kristy for their data analysis and manuscript feedback. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. 2235783 and the NSF Long Term Ecological Research Program [Grant ID: DEB 2224712]. This work took place on occupied Anishinaabe land where Hickory Corners, Michigan and KBS sites are now located. We acknowledge the legacies of violence, displacement, migration, and settlement that comes with our use of the land.

LITERATURE CITED

Albrecht, M., Kleijn, D., Williams, N.M., Tschumi, M., Blaauw, B.R., Bommarco, R., Campbell, A.J., Dainese, M., Drummond, F.A., Entling, M.H., Ganser, D., de Groot, G.A., Goulson, D., Grab, H., Hamilton, H., Herzog, F., Isaacs, R., Jacot, K., Jeanneret, P., Jonsson, M., Knop, E., Kremen, C., Landis, D.A., Loeb, G.M., Marini, L., McKerchar, M., Morandin, L.A., Pfister, S.C., Potts, S.G., Maj Rundlöf, Sardiñas, H.S., Sciligo, A.R., Thies, C., Tscharntke, T., Venturini, E., Veromann, E., Vollhardt, I.M.G., Wäckers, F.L., Ward, K.L., Wilby, A., Woltz, J.M., Wratten, S.D., Sutter, L., 2020. The effectiveness of flower strips and hedgerows on pest control, pollination services and crop yield: a quantitative synthesis. Ecology Letters 23, 1488–1498. https://doi.org/10.1111/ele.13576

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, 1412–1421. https://doi.org/10.2136/sssaj2004.0252

Anderson, R.C., 2006. Evolution and origin of the Central Grassland of North America: climate, fire, and mammalian grazers. Journal of The Torrey Botanical Society 133, 626–647. https://doi.org/10.3159/1095-5674(2006)133[626:eaootc]2.0.co;2

Asbjornsen, H., V. Hernandez-Santana, M. Liebman, J. Bayala, J. Chen, M. Helmers, C.K. Ong, and L.A. Schulte. "Targeting Perennial Vegetation in Agricultural Landscapes for Enhancing Ecosystem Services." *Renewable Agriculture and Food Systems* 29, no. 2 (2014): 101-25. doi:10.1017/S1742170512000385.

Bach, E.M., Hofmockel, K.S., 2015. Coupled Carbon and Nitrogen Inputs Increase Microbial Biomass and Activity in Prairie Bioenergy Systems. Ecosystems 18, 417–427. https://doi.org/10.1007/s10021-014-9835-8

Barber, N.A., Chantos-Davidson, K.M., Peralta, R.A., Sherwood, J.P., Swingley, W.D., 2017. Soil microbial community composition in tallgrass prairie restorations converge with remnants across a 27-year chronosequence. Environmental Microbiology 19, 3118–3131. https://doi.org/10.1111/1462-2920.13785

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17, 837–842. https://doi.org/10.1016/0038-0717(85)90144-0

Camill, P., McKone, M.J., Sturges, S.T., William J. Severud, Severud, W.J., Ellis, E., Limmer, J., Martin, C.B., Navratil, R.T., Purdie, A.J., Sandel, B., Talukder, S., 2004. Community and ecosystem-level changes in a species-rich tallgrass prairie restoration. https://doi.org/10.1890/03-5273

Chassé, M., Lutfalla, S., Cécillon, L., Baudin, F., Abiven, S., Chenu, C., Barré, P., 2021. Long-term bare-fallow soil fractions reveal thermo-chemical properties controlling soil organic carbon dynamics. Biogeosciences 18, 1703–1718. https://doi.org/10.5194/bg-18-1703-2021

Chaudhary, B., Nolimal, S., Sosa-Hernández, M.A., Egan, C., and Kastens, J. 2020. Trait-based aerial dispersal of arbuscular mycorrhizal fungi. New Phytologist 228:238-252. https://doi.org/10.1111/nph.16667

Christensen, B.T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science, 52: 345-353. https://doi.org/10.1046/j.1365-2389.2001.00417.x

Collins, S.L., Knapp, A.K., Briggs, J.M., Blair, J.M., Steinauer, E.M., 1998. Modulation of Diversity by Grazing and Mowing in Native Tallgrass Prairie. Science 280, 745–747. https://doi.org/10.1126/science.280.5364.745

Córdova, S.C., Olk, D.C., Dietzel, R., Mueller, K.E., Archontouilis, S.V., Michael J. Castellano, Castellano, M.J., 2018. Plant litter quality affects the accumulation rate, composition, and stability of mineral-associated soil organic matter. Soil Biology & Biochemistry 125, 115–124. https://doi.org/10.1016/j.soilbio.2018.07.010

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Eleanor E. Campbell, 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, 776–779. https://doi.org/10.1038/ngeo2520

Cotrufo, M.F. and J.M. Lavallee. 2022. Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration. Advances in Agronomy 1–66. https://doi.org/10.1016/bs.agron.2021.11.002

Crews, T.E., Crews, T.E., Philip C. Brookes, Brookes, P.C., 2014. Changes in soil phosphorus forms through time in perennial versus annual agroecosystems. Agriculture, Ecosystems & Environment 184, 168–181. https://doi.org/10.1016/j.agee.2013.11.022

Crum, J. R. and H. P. Collins. 1995. KBS Soils. Kellogg Biological Station Long-term Ecological Research Special Publication. Zenodo. http://doi.org/10.5281/zenodo.2581504.

Culman, Steven W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S., Lee, J., Six, J., Maul, J.E., Mirksy, S.B., Spargo, J.T., Wander, M.M., 2012. Permanganate Oxidizable Carbon Reflects a Processed Soil Fraction that is Sensitive to Management. Soil Science Society of America Journal 76, 494–504. https://doi.org/10.2136/sssaj2011.0286

Decré, Barbara. Under-Stories of Perennial Agriculture: Emerging Narratives among Upper-Midwestern Perennial Farmers, The University of Wisconsin - Madison, United States -- Wisconsin, 2021. ProQuest, https://ezproxy.msu.edu/login?url=https://www.proquest.com/dissertations-theses/under-stories-perennial-agriculture-emerging/docview/2552049523/se-2.

Demisie, W., Liu, Z., Mingkui Zhang, Zhang, M., 2014. Effect of biochar on carbon fractions and enzyme activity of red soil. Catena 121, 214–221. https://doi.org/10.1016/j.catena.2014.05.020

Docherty, K.M., Gutknecht, J.L.M., 2019. Soil Microbial Restoration Strategies for Promoting Climate-Ready Prairie Ecosystems. Bulletin of The Ecological Society of America 100. https://doi.org/10.1002/bes2.1523

Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Fangmeier, A., Kuzyakov, Y., 2009. Stimulation of r- vs. K-selected microorganisms by elevated atmospheric CO2 depends on soil aggregate size. FEMS Microbiology Ecology 69, 43–52. https://doi.org/10.1111/j.1574-6941.2009.00697.x

Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109, 49–61. https://doi.org/10.1007/s10533-011-9633-8

Drijber, R.A., Doran, J.W., Parkhurst, A.M., Lyon, D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology & Biochemistry 32, 1419–1430. https://doi.org/10.1016/s0038-0717(00)00060-2

Duncan, D.S., Jewell, K.A., Suen, G., Jackson, R.D., 2016. Detection of short-term cropping system-induced changes to soil bacterial communities differs among four molecular characterization methods. Soil Biology & Biochemistry 96, 160–168. https://doi.org/10.1016/j.soilbio.2016.02.002

DuPont, S.T., Culman, S.W., Ferris, H., Buckley, D.H., Glover, J.D. 2010. No-tillage conversion of harvested perennial grassland to annual cropland reduces root biomass, decreases active carbon stocks, and impacts soil biota. Agriculture, Ecosystems & Environment 137, 25–32. https://doi.org/10.1016/j.agee.2009.12.021

Dutter, C., Damiano, L.A., Niemi, J., Miller, B.A., Schulte L.A., Liebman, M., Helmers, M., Cruse, R., and McDaniel, M.D., 2023. Contour Prairie Strips Affect Surrounding Soil but Only Have Slight Effects on Crop Yield. Field Crops Research 296: 108905. https://doi.org/10.1016/j.fcr.2023.108905.

Dutter, C., Rutkoski, C.E., Evans, S.E., McDaniel, M.D., Contour Prairie Strips Alter Soil Microbial Communities and Functioning Both Below the Prairie Strip and in Adjacent Cropland. Available at SSRN: https://ssrn.com/abstract=4542718 or http://dx.doi.org/10.2139/ssrn.4542718

Inwood, S.E.E., Bates, G.E., Butler, D.M. 2015. Forage Performance and Soil Quality in Forage Systems under Organic Management in the Southeastern United States. Agronomy Journal 107, 1641–1652. https://doi.org/10.2134/agronj14.0472

Ferrero, F., Prencipe, S., Tabacco, E., Ferrocino, I., Borreani, G., Spadaro, D. 2022. Effects of a prothioconazole- and tebuconazole-based fungicide on the yield, silage characteristics, and fungal mycobiota of corn harvested and conserved as whole-crop and high-moisture ear silages. Journal of Applied Microbiology. https://doi.org/10.1093/jambio/lxac033

Fierer, N., Ladau, J., Clemente, J.C., Leff, J.W., Owens, S.M., Pollard, K.S., Knight, R., Gilbert, J.A., McCulley, R.L., 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342, 621–624. https://doi.org/10.1126/science.1243768

Franzluebbers, A.J., Haney, R.L., C. W. Honeycutt, Honeycutt, C.W., Schomberg, H.H., Hons, F.M., 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Science Society of America Journal 64, 613–623. https://doi.org/10.2136/sssaj2000.642613x

FAO, ITPS, GSBI, SCBD, and EC. 2020. State of knowledge of soil biodiversity - Status, challenges and potentialities, Report 2020. Rome, FAO.

Garcia, F.O., Rice, C.W., 1994. Microbial biomass dynamics in tallgrass prairie. Soil Science Society of America Journal 58, 816–823. https://doi.org/10.2136/sssaj1994.03615995005800030026x

Gutierrez-Lopez, J., Asbjornsen, H., Helmers, M.J., Isenhart, T.M., 2014. Regulation of soil moisture dynamics in agricultural fields using strips of native prairie vegetation. Geoderma 226, 238–249. https://doi.org/10.1016/j.geoderma.2014.02.013

Hargreaves, S.K., Hofmockel, K.S., 2014. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochemistry 117, 67–79. https://doi.org/10.1007/s10533-013-9893-6

Helzer, Chris. The Ecology and Management of Prairies in the Central United States. 1 ed. University of Iowa Press, 2009. Project MUSE, https://doi.org/10.1353/book3566.

Hernandez-Santana, V., Xiaobo Zhou, Zhou, X., Helmers, M.J., Asbjornsen, H., Kolka, R., Tomer, M.D., 2013. Native prairie filter strips reduce runoff from hillslopes under annual row-crop systems in Iowa, USA. Journal of Hydrology 477, 94–103. https://doi.org/10.1016/j.jhydrol.2012.11.013

Herzberger, A., Duncan, D.S., Jackson, R.D., 2014. Bouncing Back: Plant-Associated Soil Microbes Respond Rapidly to Prairie Establishment. PLOS ONE 9. https://doi.org/10.1371/journal.pone.0115775

Hurisso, T.T., Culman, S.W., Horwath, W.R., Wade, J., Cass, D., Beniston, J.W., Bowles, T.M., Grandy, A.S., Franzluebbers, A.J., Schipanski, M.E., Lucas, S., Ugarte, C.M., 2016. Comparison of Permanganate-Oxidizable Carbon and Mineralizable Carbon for Assessment of Organic Matter Stabilization and Mineralization. Soil Science Society of America Journal 80, 1352–1364. https://doi.org/10.2136/sssaj2016.04.0106

Jach-Smith, L.C., Jackson, R.D., 2018. N addition undermines N supplied by arbuscular mycorrhizal fungi to native perennial grasses. Soil Biology & Biochemistry 116, 148–157. https://doi.org/10.1016/j.soilbio.2017.10.009

Jangid, K., Williams, M.A., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J., Jenkins, M.B., Endale, D.M., Coleman, D.C., Whitman, W.B., 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biology & Biochemistry 40, 2843–2853. https://doi.org/10.1016/j.soilbio.2008.07.030

Jangid, K., Williams, M.A., Williams, M.A., Franzluebbers, A.J., Schmidt, T.J., Schmidt, T.M., Coleman, D.C., Whitman, W.B., 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology & Biochemistry 43, 2184–2193. https://doi.org/10.1016/j.soilbio.2011.06.022

Jeske, E.S., Tian, H., Hanford, K., Walters, D.T., Drijber, R.A., 2018. Long-term nitrogen fertilization reduces extraradical biomass of arbuscular mycorrhizae in a maize (Zea mays L.) cropping system. Agriculture, Ecosystems & Environment 255, 111–118. https://doi.org/10.1016/j.agee.2017.11.014

Johnson, L.C., Matchett, J.R. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. Ecology 82, 3377–3389. https://doi.org/10.1890/0012-9658(2001)082[3377:fagrbp]2.0.co;2

Johnsen, K., Jacobsen, C.S., Torsvik, V., Sørensen, J.T., 2001. Pesticide effects on bacterial diversity in agricultural soils – a review. Biology and Fertility of Soils 33, 443–453. https://doi.org/10.1007/s003740100351

Kemmerling, L.R., Rutkoski, C.R., Evans, S.E., Helms, J.A., Cordova-Ortiz, E.S., Smith, J.A., Vázquez Custodio, J.A., Vizza, C., Haddad, N.M. Prairie Strips and Lower Land Use Intensity Increase Biodiversity and Ecosystem Services. Frontiers in Ecology and Evolution. https://doi.org/10.3389/fevo.2022.833170

Kitchen, D.J., Blair, J.M., Callaham, M.A., 2009. Annual fire and mowing alter biomass, depth distribution, and C and N content of roots and soil in tallgrass prairie. Plant and Soil 323, 235–247. https://doi.org/10.1007/s11104-009-9931-2

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, 261–273. https://doi.org/10.1111/gcb.14859

Lewis, D.B., Kaye, J.P., Jabbour, R., Barbercheck, M.E., 2011. Labile Carbon and Other Soil Quality Indicators in Two Tillage Systems during Transition to Organic Agriculture. Renewable Agriculture and Food Systems 26, 342–353. https://doi.org/10.1017/s1742170511000147

López-Garrido, R., Madejón, E., Madejón, Murillo, J.M., Moreno, F., 2011. Soil quality alteration by mouldboard ploughing in a commercial farm devoted to no-tillage under Mediterranean conditions. Agriculture, Ecosystems & Environment 140, 182–190. https://doi.org/10.1016/j.agee.2010.12.001

Lucas, S.T., Weil, R.R., 2012. Can a Labile Carbon Test be Used to Predict Crop Responses to Improve Soil Organic Matter Management. Agronomy Journal 104, 1160–1170. https://doi.org/10.2134/agronj2011.0415

Ludwig, B., Daniel Geisseler, Geisseler, D., Kerstin Michel, Michel, K., Michel, K., Joergensen, R.G., Schulz, E., Merbach, I., Raupp, J., Rauber, R., Hu, K., Niu, L., Liu, X., X. Liu, Liu, Xuejun, 2011. Effects of fertilization and soil management on crop yields and carbon stabilization in soils. A review. Agronomy for Sustainable Development 31, 361–372. https://doi.org/10.1051/agro/2010030

Mackelprang, R., Grube, A.M., Lamendella, R., da Conceição Jesus, E., Copeland, A., Liang, C., Jackson, R.D., Rice, C.W., Kapucija, S., Parsa, B., Tringe, S.G., Tiedje, J.M., Jansson, J.K., 2018. Microbial Community Structure and Functional Potential in Cultivated and Native Tallgrass Prairie Soils of the Midwestern United States. Frontiers in Microbiology 9, 1775–1775. https://doi.org/10.3389/fmicb.2018.01775

Maher, R.M., Asbjornsen, H., Kolka, R.K., Cynthia A. Cambardella, Cambardella, C.A., Raich, J.W., 2010. Changes in soil respiration across a chronosequence of tallgrass prairie reconstructions. Agriculture, Ecosystems & Environment 139, 749–753. https://doi.org/10.1016/j.agee.2010.09.009

Manzoni, S., Jackson, R.B., Trofymow, J.A. and Porporato, A., 2008. The Global Stoichiometry of Litter Nitrogen Mineralization. Science 321:684-686. doi:10.1126/science.1159792

Martin, T. and Sprunger, C., 2022. Sensitive Measures of Soil Health Reveal Carbon Stability Across a Management Intensity and Plant Biodiversity Gradient. Frontiers in Soil Science. https://doi.org/10.3389/fsoil.2022.917885

Merino, A., García-Oliva, F., Fontúrbel, M.T., Vega, J.A., 2021. The high content of mineral-free organic matter in soils increases their vulnerability to wildfire in humid-temperate zones. Geoderma 395, 115043. https://doi.org/10.1016/j.geoderma.2021.115043

McClain, W.E., Charles M. Ruffner, Ruffner, C.M., Ebinger, J.E., Spyreas, G., 2021. Patterns of Anthropogenic Fire within the Midwestern Tallgrass Prairie 1673–1905: Evidence from Written Accounts. Natural Areas Journal 41, 283–300. https://doi.org/10.3375/20-5

Middleton, E.L., Richardson, S., Koziol, L., Palmer, C.E., Yermakov, Z., Henning, J.A., Schultz, P.A., Bever, J.D., 2015. Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere 6, 1–16. https://doi.org/10.1890/es15-00152.1

Mosier, S.L., Paustian, K., Davies, C., Kane, M., Cotrufo, M.F., 2019. Soil organic matter pools under management intensification of loblolly pine plantations. Forest Ecology and Management 447, 60–66. https://doi.org/10.1016/j.foreco.2019.05.056

Neal, A.L., Hughes, D., Clark, I.M., Jansson, J.K., Hirsch, P.R., 2021. Microbiome Aggregated Traits and Assembly Are More Sensitive to Soil Management than Diversity. mSystems 6. https://doi.org/10.1128/msystems.01056-20

Nie, M., Pendall, E., Bell, C.W., Wallenstein, M.D., 2014. Soil aggregate size distribution mediates microbial climate change feedbacks. Soil Biology & Biochemistry 68, 357–365. https://doi.org/10.1016/j.soilbio.2013.10.012

Paul, E.A., Kravchenko, A., Grandy, A.S., Morris, S.J., 2015. Soil organic matter dynamics: Controls and management for sustainable ecosystem functioning 104–134.

Paetsch, L., Mueller, C.W., Rumpel, C., Angst, Š., Wiesheu, A.C., Girardin, C., Ivleva, N.P., Niessner, R., Kögel-Knabner, I., 2017. A multi-technique approach to assess the fate of biochar in soil and to quantify its effect on soil organic matter composition. Organic Geochemistry 112, 177–186. https://doi.org/10.1016/j.orggeochem.2017.06.012

Pérez-Suárez, M., Castellano, M.J., Kolka, R.K., Asbjornsen, H., Helmers, M.J., 2014. Nitrogen and carbon dynamics in prairie vegetation strips across topographical gradients in mixed Central Iowa agroecosystems. Agriculture, Ecosystems & Environment 188, 1–11. https://doi.org/10.1016/j.agee.2014.01.023

Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D.K., Chenu, C., Chenu, C., Cotrufo, M.F., Derrien, D., Gioacchini, P., Grand, S., Gregorich, E.G., Marco Griepentrog, Griepentrog, M., Gunina, A., Gunina, A., Haddix, M.L., Kuzyakov, Y., Kühnel, A., Macdonald, L.M., Soong, J.L., Trigalet, S., Marie Liesse Vermeire, Vermeire, M.-L., Rovira, P., van Wesemael, B., Wiesmeier, M., Yeasmin, S., Yevdokimov, I.V., Nieder, R., 2018. Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils – A comprehensive method comparison. Soil Biology & Biochemistry 125, 10–26. https://doi.org/10.1016/j.soilbio.2018.06.025

Quincke, J.A., Charles S. Wortmann, Wortmann, C.S., Martha Mamo, Mamo, M., Thomas G. Franti, Franti, T.G., Rhae A. Drijber, Drijber, R.A., 2007. Occasional Tillage of No-Till Systems: Carbon Dioxide Flux and Changes in Total and Labile Soil Organic Carbon. Agronomy Journal 99, 1158–1168. https://doi.org/10.2134/agronj2006.0317

Robertson, G. Philip, Robertson, G. P., D C Coleman, Coleman, D.C., Bledsoe, C.S., Sollins, P., 1999. Standard soil methods for long-term ecological research.

Robertson, G.P., Groffman, P.M., 2015. Chapter 14 – Nitrogen Transformations 421–446. https://doi.org/10.1016/b978-0-12-415955-6.00014-1

Robertson, G. P. and Hamilton, S.K., 2015. Long-term ecological research in agricultural landscapes at the Kellogg Biological Station LTER site: conceptual and experimental framework. Pages 1-32 in S. K. Hamilton, J. E. Doll, and G. P. Robertson, eds. The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability.

Robertson, G.P., Hamilton, S.K., Barham, B.L., Dale, B.E., Izaurralde, Roberto C., Izaurralde, R. Cesar, R. Cesar Izaurralde, Jackson, R.D., Landis, D.A., Scott M. Swinton, Swinton, S.M., Thelen, K.D., James M. Tiedje, Tiedje, J.M., 2017. Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. Science 356. https://doi.org/10.1126/science.aal2324

Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., Urs Zihlmann, Zihlmann, U., van der Heijden, M.G.A., Oehl, F., Oehl, F., 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biology & Biochemistry 84, 38–52. https://doi.org/10.1016/j.soilbio.2015.02.005

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

Schmer, M.R., Liebig, M.A., Vogel, K.P. and Mitchell, R.B., 2011. Field-scale soil property changes under switchgrass managed for bioenergy. GCB Bioenergy, 3: 439-448. https://doi.org/10.1111/j.1757-1707.2011.01099.x

Schmidt, M., Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Johannes Lehmann, 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, 49–56. https://doi.org/10.1038/nature10386

Schmidt, R., Gravuer, K., Bossange, A.V., Mitchell, J.P., Scow, K.M., 2018. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLOS ONE 13. https://doi.org/10.1371/journal.pone.0192953

Schmidt, T.J., Schmidt, T.M., Waldron, C., 2015. Microbial diversity in soils of agricultural landscapes and its relation to ecosystem function 135–157.

Shcherbak, I., Robertson, G.P., 2019. Nitrous Oxide (N2O) Emissions from Subsurface Soils of Agricultural Ecosystems. Ecosystems 22, 1650–1663. https://doi.org/10.1007/s10021-019-00363-z

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241, 155–176. https://doi.org/10.1023/a:1016125726789

Sprunger, C.D., Oates, L.G., Jackson, R.D., Robertson, G.P., 2017. Plant community composition influences fine root production and biomass allocation in perennial bioenergy cropping systems of the upper Midwest, USA. Biomass & Bioenergy 105, 248–258. https://doi.org/10.1016/j.biombioe.2017.07.007

Sprunger, C.D., Robertson, G.P., 2018. Early accumulation of active fraction soil carbon in newly established cellulosic biofuel systems. Geoderma 318, 42–51. https://doi.org/10.1016/j.geoderma.2017.11.040

Sprunger, C.D., Martin, T.K., Mann, M., 2020. Systems with greater perenniality and crop diversity enhance soil biological health. Agricultural & Environmental Letters 5. https://doi.org/10.1002/ael2.20030

Stephenson, J.A., M. Liebman, J. Niemi, R. Cruse, J. Tyndall, C. Witte, D. James, and M. Helmers. 2024. The influence of prairie strips sown in midwestern corn and soybean fields on sediment discharge throughout the year. Journal of Soil and Water Conservation: In press.

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2007. Soil carbon saturation: concept, evidence and evaluation. Biogeochemistry 86, 19–31. https://doi.org/10.1007/s10533-007-9140-0

Stewart, C.E., Follett, R.F., Pruessner, E.G., Varvel, G.E., Vogel, K.P. and Mitchell, R.B. (2016), N fertilizer and harvest impacts on bioenergy crop contributions to SOC. GCB Bioenergy, 8: 1201-1211. https://doi.org/10.1111/gcbb.12326

Szymanski, L.M., Sanford, G.R., Katherine Heckman, Heckman, K., Jackson, R.D., Marin-Spiotta, E., 2019. Conversion to bioenergy crops alters the amount and age of microbially-respired soil carbon. Soil Biology & Biochemistry 128, 35–44. https://doi.org/10.1016/j.soilbio.2018.08.025

Tiemann, L.K., Grandy, A.S., 2015. Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. Gcb Bioenergy 7, 161–174. https://doi.org/10.1111/gcbb.12126

Trivedi, P., Rochester, I.J., Trivedi, C., Van Nostrand, J.D., Zhou, J., Karunaratne, S., Anderson, I.C., Singh, B.K., 2015. Soil aggregate size mediates the impacts of cropping regimes on soil carbon and microbial communities. Soil Biology & Biochemistry 91, 169–181. https://doi.org/10.1016/j.soilbio.2015.08.034

United Nations Environment Programme and International Union for Conservation of Nature. 2021. Nature-based solutions for climate change mitigation. Nairobi and Gland. https://www.unep.org/resources/report/nature-based-solutions-climate-change-mitigation

United States Department of Agriculture National Agricultural Statistics Service. 2017. *U.S. Census of Agriculture*. [Web Archive] Retrieved from the Library of Congress. https://www.nass.usda.gov/Publications/AgCensus/2017/

Warmink, J., Nazir, R., Corten, B. and van Elsas, J.D., 2011. Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. Soil Biology and Biochemistry 43:760-765. http://doi.org/10.1016/j.soilbio.2010.12.009

Weil, R.R., Islam, Kandikar R., Islam, Khandakar Rafiq, Stine, M.A., Gruver, J.B., Samson-Liebig, S., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. Renewable Agriculture and Food Systems 18, 3–17. https://doi.org/10.1079/ajaa200228

Weil, R. and N. Brady. 2017. The Nature and Properties of Soils. 15th edition.

Wood, S.A., Bradford, M.A., Gilbert, J.A., McGuire, K.L., Palm, C.A., Tully, K.L., Zhou, J., Naeem, S., 2015. Agricultural intensification and the functional capacity of soil microbes on smallholder African farms. Journal of Applied Ecology 52, 744–752. https://doi.org/10.1111/1365-2664.12416

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. Soil Biology & Biochemistry 22, 1167–1169. https://doi.org/10.1016/0038-0717(90)90046-3

Zhang, G. Caroline J. Murray, Ashley L. St. Clair, Randall P Cass, Adam G. Dolezal, L. Schulte, A. Toth, M. O'Neal, 2023. Native vegetation embedded in landscapes dominated by corn and soybean improves honey bee health and productivity. Journal of Applied Ecology. https://doi.org/10.1111/1365-2664.14397

APPENDIX C: CHAPTER 3

Tables

	POXC (R^2 = 0.24)			**MinC** (R^2 = 0.46)			**MBC** (R^2 = 0.38)		
Factor	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**
Year	75079	23.338	**<0.001**	2923.8	39.679	**<0.001**	845842	27.031	**<0.001**
Cropping system	494	0.307	0.581	24.7	0.671	0.414	302596	19.34	**<0.001**
Vegetation	2420	1.504	0.222	1080.2	29.319	**<0.001**	2	<0.001	0.992
Year * Cropping system	4774	1.484	0.231	31.1	0.422	0.656	152967	4.889	0.009
Year * Vegetation	2290	0.712	0.493	622.2	8.444	**<0.001**	135100	4.318	0.015
Cropping system * Vegetation	674	0.419	0.519	23.7	0.643	0.424	46	0.003	0.957

Table 3.1. Summary† of results from linear models‡ of soil carbon indicators in prairie strip and cropland soils.

†Bold values indicate significant difference in soil carbon between treatment groups at $p < 0.05$. Underlined values indicate significance at $p < 0.1$. R^2 value indicates the percentage of variation in soil carbon explained by model factors.

‡ Linear models with factors Year (2 months, 1 year, 2 years, and 3 years after prairie strip planting), Cropping system (Reduced Input and Organic), Vegetation (prairie and cropland), and their interactions.

	POXC (R^2 = 0.28)			**MinC** (R^2 = 0.59)			**MBC** (R^2 = 0.35)		
Factor	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**
Year	62833	21.103	**<0.001**	3474.8	76.809	**<0.001**	628774	18.331	**<0.001**
Cropping system	41	0.028	0.868	5.3	0.232	0.631	207404	12.093	**<0.001**
Distance	2471	0.829	0.439	42.5	0.939	0.395	14679	0.856	0.357
Year * Cropping system	6192	2.079	0.131	46.1	1.019	0.365	164261	4.789	0.011
Year * Distance	916	0.154	0.961	119.9	1.325	0.267	12058	0.352	0.705
Cropping system * Distance	5901	1.982	0.144	52.4	1.159	0.319	8522	0.497	0.483

Table 3.2. Summary† of results from linear models‡ of soil carbon indicators in cropland soils only.

†Bold values indicate significant difference in soil carbon between treatment groups at $p < 0.05$. Underlined values indicate significance at $p < 0.1$. R^2 value indicates the percentage of variation in soil carbon explained by model factors.

‡ Linear models with factors Year (2 months, 1 year, 2 years, and 3 years after prairie strip planting), Cropping system (Reduced Input and Organic), Distance from prairie strip (1m, 5, and 20m), and their interactions.

	POM C (R^2 = 0.26)			MAOM C (R^2 = 0.09)			POM N (R^2 = 0.34)			MAOM N (R^2 = 0.04)		
Factor	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**
Cropping system	151.40	17.185	**<0.001**	24.035	4.214	**0.047**	0.914	23.619	**<0.001**	0.169	3.339	0.076
Vegetation	1.29	0.146	0.704	14.449	2.533	0.119	0.004	0.115	0.737	0.076	1.493	0.229
Cropping system * Vegetation	2.92	0.331	0.568	2.115	0.371	0.546	0.027	0.688	0.412	0.002	0.038	0.847

Table 3.3. Summary† of results from linear models‡ performed on soil POM and MAOM fractions in prairie strip and cropland soils 3 years after prairie strip planting.

†Bold values indicate significant difference in soil carbon or nitrogen between treatment groups at $p < 0.05$. Underlined values indicate significance at $p < 0.1$. R^2 value indicates the percentage of variation in soil carbon or nitrogen explained by model factors.

‡ Linear models with factors Cropping system (Reduced Input and Organic), Vegetation (prairie and cropland), and their interactions.

	POM C (R^2 = 0.29)			MAOM C (R^2 = 0.002)			POM N (R^2 = 0.37)			MAOM N (R^2 = 0.001)		
Factor	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**	**Sum of Sq.**	**F value**	**P value**
Cropping system	134.474	14.831	**<0.001**	13.901	2.297	0.141	0.837	21.016	**<0.001**	0.122	2.327	0.138
Distance	7.366	0.812	0.375	2.822	0.466	0.500	0.057	1.436	0.241	0.013	0.247	0.623
Cropping system * Distance	0.320	0.035	0.852	0.888	0.147	0.705	0.003	0.082	0.777	0.024	0.466	0.501

Table 3.4. Summary† of results from linear models‡ performed on soil POM and MAOM fractions in cropland soils only 3 years after prairie strip planting.

†Bold values indicate significant difference in soil carbon or nitrogen between treatment groups at $p < 0.05$. Underlined values indicate significance at $p < 0.1$. R^2 value indicates the percentage of variation in soil carbon or nitrogen explained by model factors.

‡ Linear models with factors Cropping system (Reduced Input and Organic), Distance from prairie strips (1m, 5m, and 20m), and their interactions

	All samples								2022 samples only			
	MBN (R^2 = 0.55)		**NO_3^-** (R^2 = 0.81)		**NH_4^+** (R^2 = 0.65)		**Soil moisture** (R^2 = 0.49)		**pH** (R^2 = 0.42)		**CEC** (R^2 = 0.04)	
Factor	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**
Year	9353.2	**<0.001**	346.5	**<0.001**	334.03	**<0.001**	0.027	**<0.001**	-	-	-	-
Cropping system	6897.0	**<0.001**	3.83	0.126	4.65	0.106	0.000	0.369	0.248	**<0.001**	0.304	0.459
Vegetation	214.2	0.162	326.06	**<0.001**	28.95	**<0.001**	0.002	0.008	0.047	0.092	0.245	0.506
Year * Cropping system	219.0	0.367	11.63	0.030	12.39	0.032	0.002	0.007	-	-	-	-
Year * Vegetation	279.8	0.279	144.73	**<0.001**	42.08	**<0.001**	0.002	0.017	-	-	-	-
Cropping system * Vegetation	385.1	0.062	1.81	0.292	1.03	0.445	0.000	0.895	0.180	0.002	0.340	0.434

Table 3.5. Summary† of results from linear models‡ performed on soil N and physiochemical properties in prairie strip and cropland soils.

†Bold values indicate significant difference in soil carbon between treatment groups at $p < 0.05$. Underlined values indicate significance at $p < 0.1$. R^2 value indicates the percentage of variation in soil carbon explained by model factors.

‡ Linear models with factors Year (2 months, 1 year, 2 years, and 3 years after prairie strip planting), Cropping system (Reduced Input and Organic), Vegetation (prairie and cropland), and their interactions.

	All samples								2022 samples only			
	MBN (R^2 = 0.58)		**NO_3^-** (R^2 = 0.71)		**NH_4^+** (R^2 = 0.64)		**Soil moisture** (R^2 = 0.45)		**pH** (R^2 = 0.48)		**CEC** (R^2 = 0.14)	
Factor	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**	**Sum of Sq.**	**P value**
Year	8082.0	**<0.001**	476.94	**<0.001**	345.77	**<0.001**	0.022	**<0.001**	-	-	-	-
Cropping system	3784.1	**<0.001**	7.33	0.069	4.99	0.140	0.000	0.502	0.422	**<0.001**	0.600	0.262
Distance	72.6	0.675	0.67	0.856	10.97	0.094	0.000	0.781	0.003	0.913	0.064	0.932
Year * Cropping system	354.1	0.153	14.44	0.040	15.73	0.035	0.003	0.013	-	-	-	-
Year * Distance	515.0	0.242	4.70	0.703	4.83	0.709	0.001	0.603	-	-	-	-
Cropping system * Distance	18.4	0.905	8.12	0.159	0.59	0.878	0.000	0.596	0.013	0.649	0.132	0.865

Table 3.6. Summary† of results from linear models‡ performed on soil N and physiochemical properties in cropland soils only.

†Bold values indicate significant difference in soil carbon between treatment groups at $p < 0.05$. Underlined values indicate significance at $p < 0.1$. R^2 value indicates the percentage of variation in soil carbon explained by model factors.

‡ Linear models with factors Year (2 months, 1 year, 2 years, and 3 years after prairie strip planting), Cropping system (Reduced Input and Organic), Distance from prairie strip (1m, 5, and 20m), and their interactions.

Figures

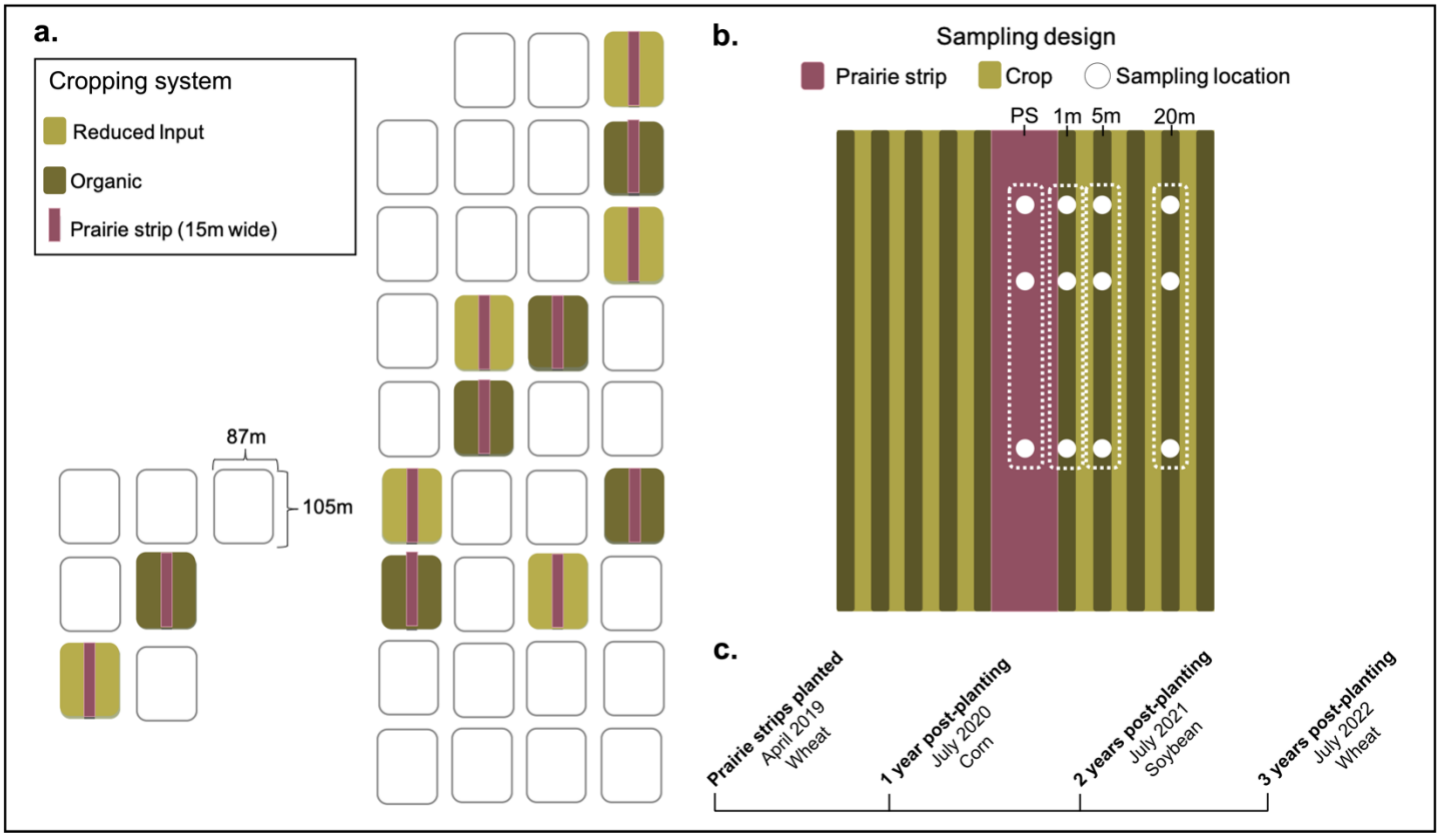

Figure 3.1. a. Schematic of the KBS LTER Main Cropping System Experiment including six replicated 1-ha plots of each cropmanagement treatment with prairie strips. b. Sampling design within each 1-ha plot. Each point represents the sampling location of an individual 0-10cm soil sample during each annual sampling event. Dotted lines represent individual samples combined to form a single composite sample at each distance. c. Study timeline and crop rotation phase in each cropping system.

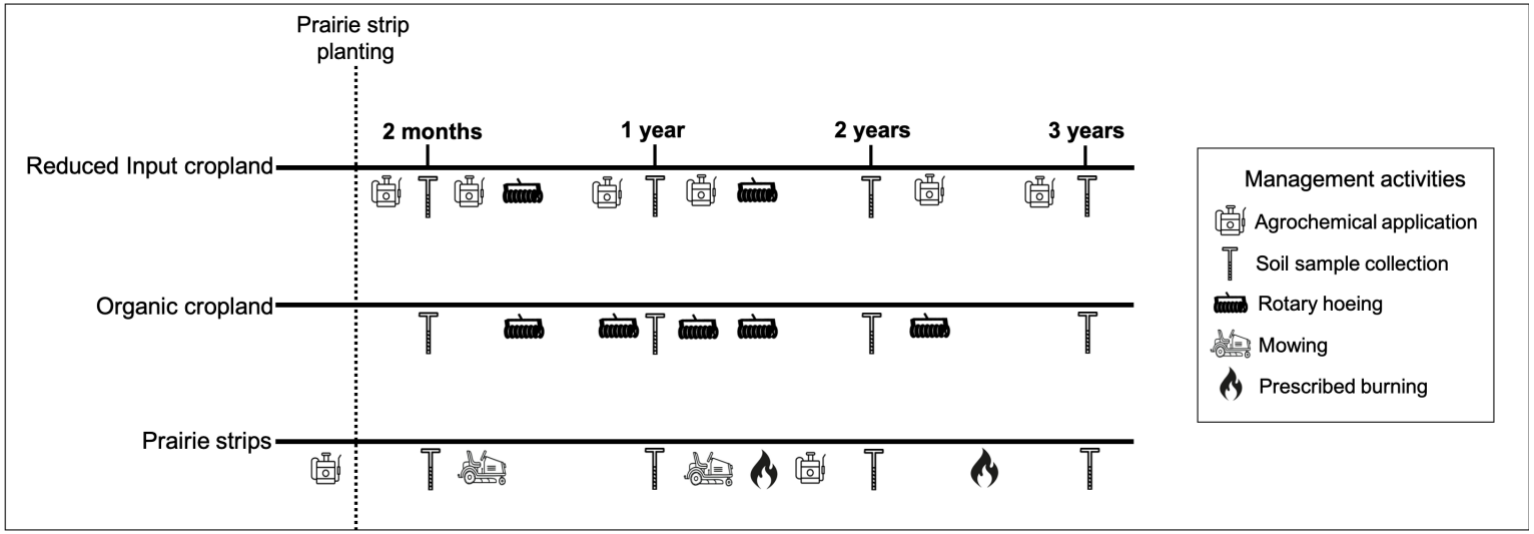

Figure 3.2. Crop management timeline for Reduced Input cropland, Organic cropland, and Prairie Strips. Note: agrochemical application only occurred in Prairie Strips within Reduced Input treatment plots.

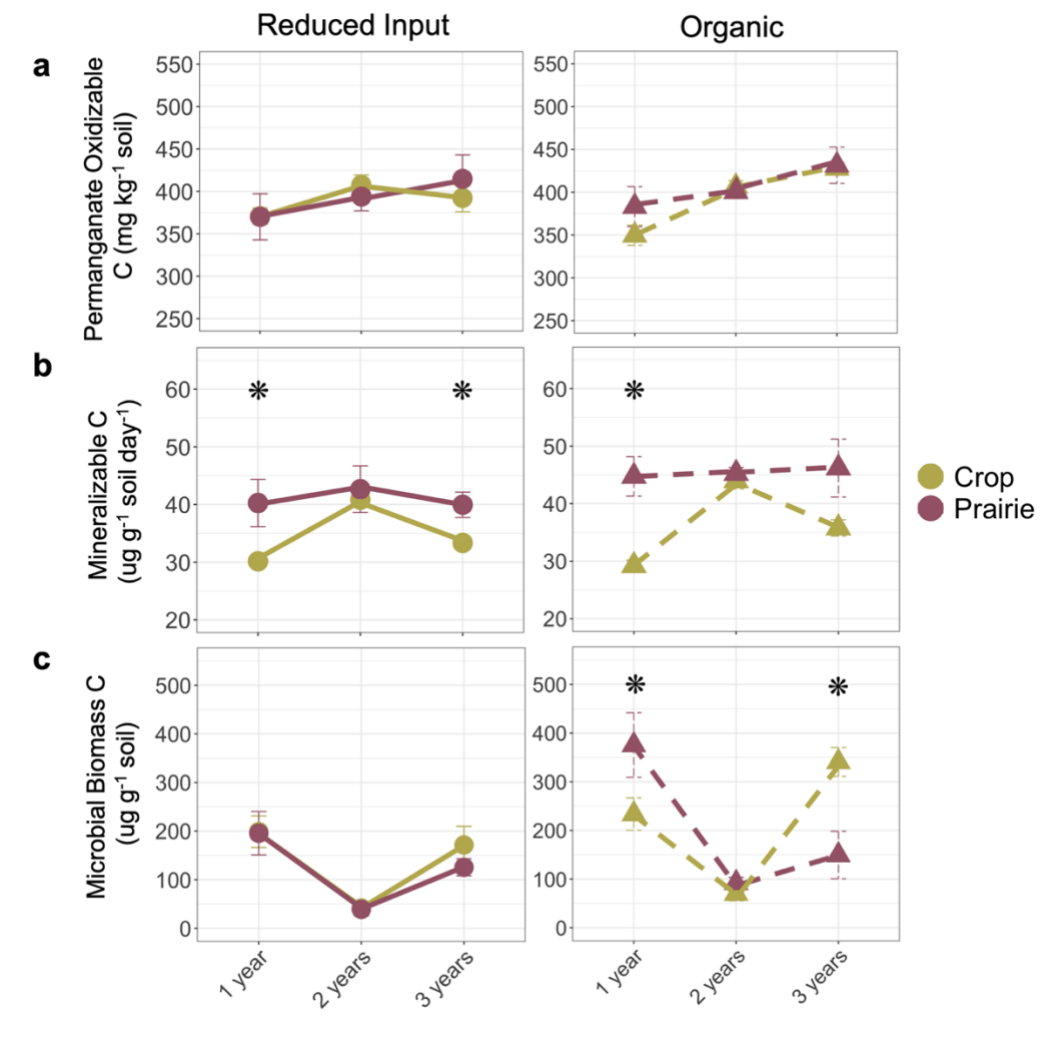

Figure 3.3. Soil a) permanganate oxidizable carbon, b) mineralizable carbon, and c) microbial biomass carbon in cropland (yellow) and prairie strips (pink) in each cropping system. Cropland values include all distances from prairie strips (1m, 5m, 20m). Asterisks represent a significant difference in soil C between prairie strip and cropland soils.

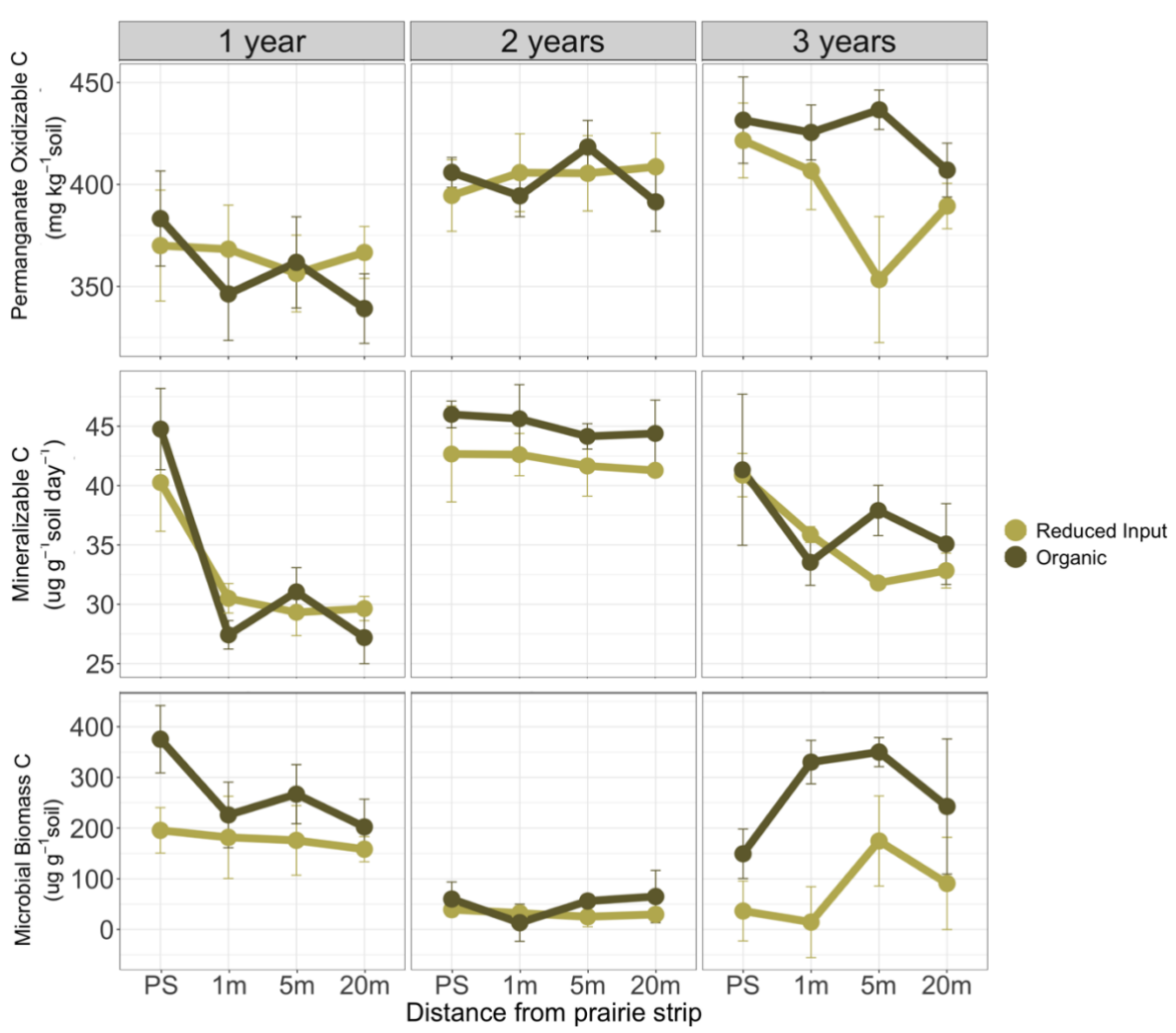

Figure 3.4. Soil a) microbial biomass carbon (MBC), b) permanganate oxidizable carbon (POXC), and c) mineralizable C (MinC) in prairie strips (P) and each cropland distance (1m, 5m, 20m).

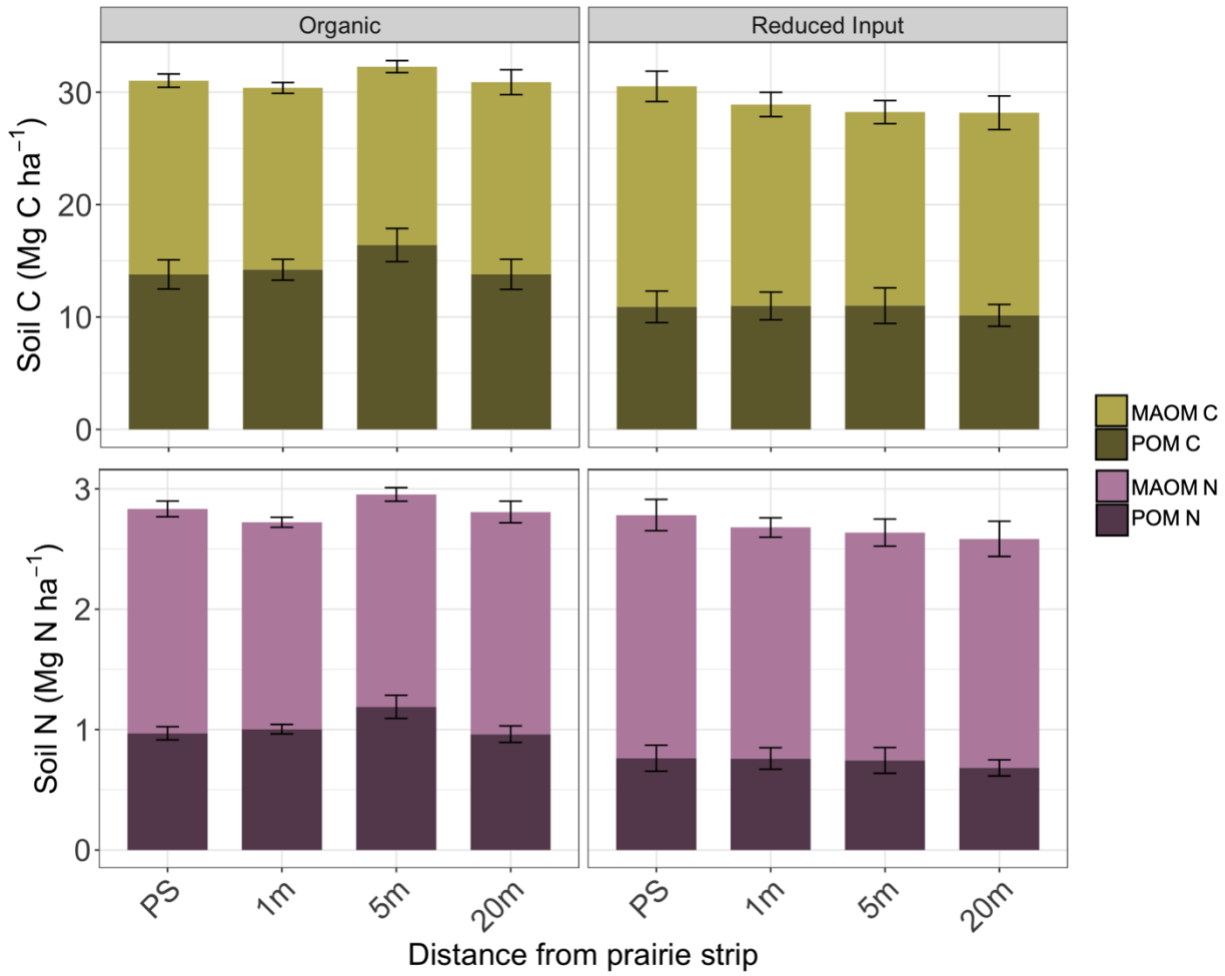

Figure 3.5. Total C and N in soil particulate organic matter (POM) and soil mineral-associated organic matter (MAOM) in prairie strip and cropland soils 3 years after prairie strip planting.

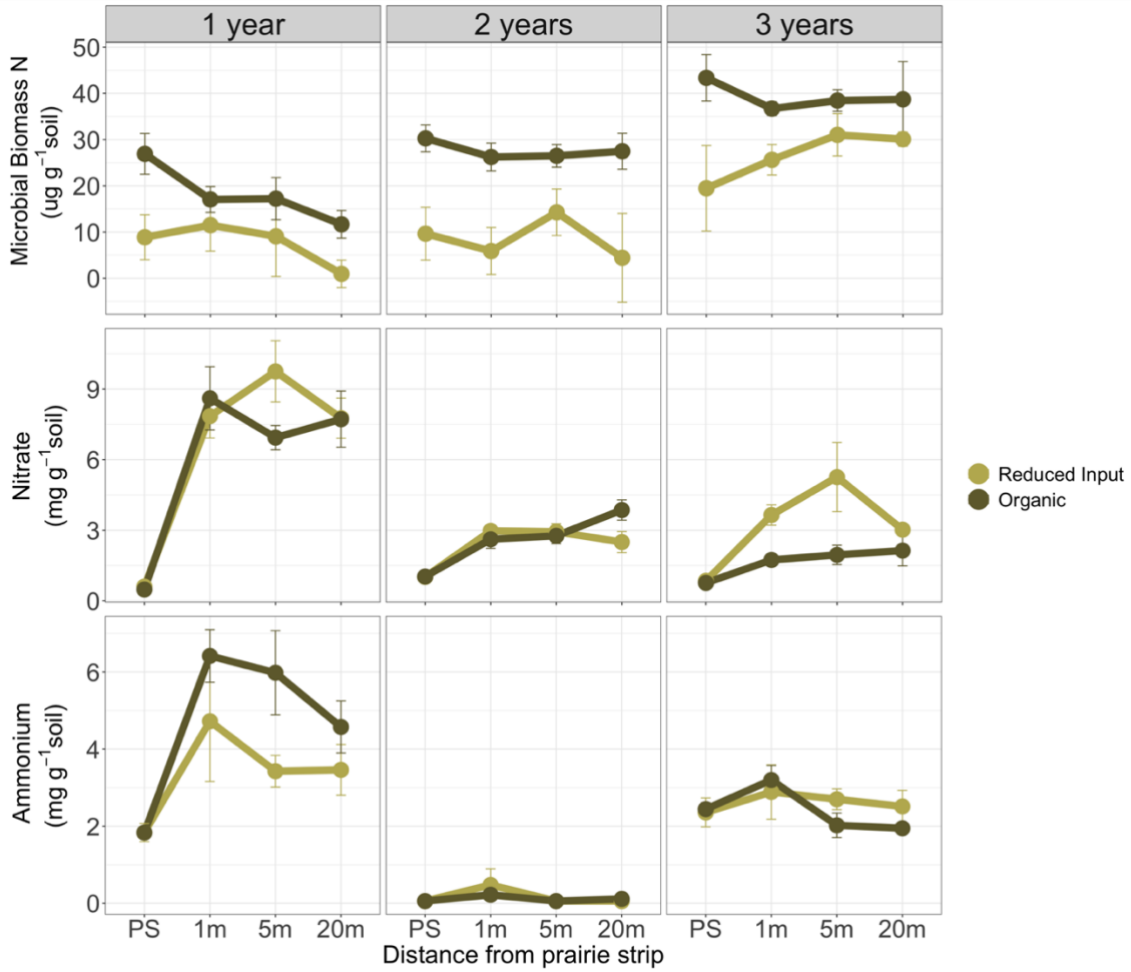

Figure 3.6. Soil a) microbial biomass nitrogen (MBN), b) nitrate (NO_3-), and c) ammonium (NH_4+) in prairie strips (PS) and each cropland distance (1m, 5m, 20m).

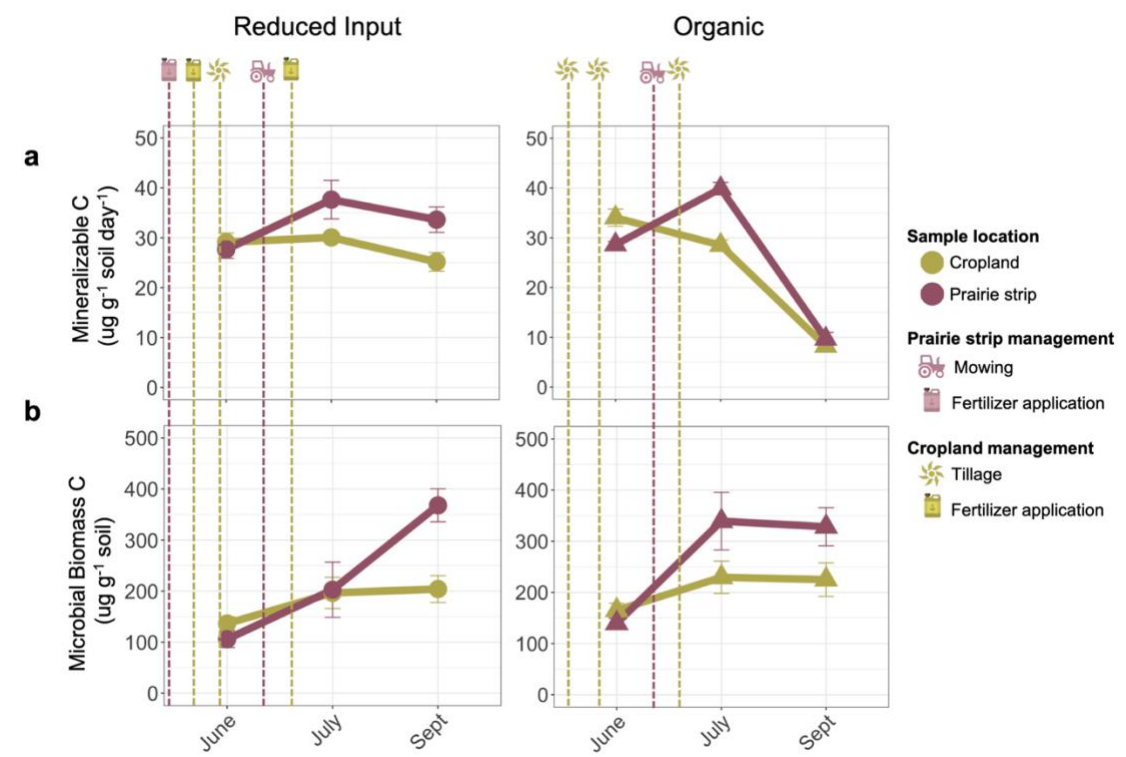

Figure 3.7. Soil a) mineralizable carbon and b) microbial biomass carbon in cropland (yellow) and prairie strips (pink) in each cropping system in 2020, 1 year after prairie strip planting. Cropland values include all distances from prairie strips (1m, 5m, 20m). Icons represent the timing of prairie strip and cropland management activities relative to sample collection.

CHAPTER 4: NEONICOTINOID RETENTION AND TRANSPORT IN A MAIZE CROPPING SYSTEM WITH CONTOUR PRAIRIE STRIPS[2]

ABSTRACT

Conservation areas established in agricultural fields can provide habitat for native organisms, but they also have the potential to accumulate and expose organisms to insecticides. Prairie strips are zones of cropland that have been converted to native prairie vegetation. Prairie strips increase biodiversity and reduce nutrient runoff, but they may also accumulate insecticides that endanger visiting organisms or facilitate the movement of insecticides across the landscape. In a study of paired catchments with ongoing neonicotinoid inputs, we measured the impact of prairie strips (10% of cropland) on the accumulation and movement of the neonicotinoid insecticide clothianidin (CLO) into surface soil, deep soil, plant tissue, and groundwater across multiple slope positions during three phases of a maize growing season. While CLO accumulates in maize leaf tissue midseason following neonicotinoid application, we did not find evidence that prairie plant species in prairie strips accumulate CLO at concentrations lethal to pollinator insects. We also found that downslope soils contained the highest CLO concentrations in both catchments, showing that prairie strips did not eliminate downslope insecticide runoff. Our study adds to the existing literature examining prairie strip effects on downslope agrochemical transport, showing that when prairie strips are planted in cropland with ongoing neonicotinoid inputs, they can provide safe, low-insecticide habitat for visiting organisms amidst their other services, but may not reduce offsite insecticide runoff.

---

[2] Originally published as: Rutkoski, C.E., S.E. Evans, & L.A. Schulte (Accepted). Neonicotinoid Retention and Transport in a Maize Cropping System with Contour Prairie Strips. *Agriculture, Ecosystems & Environment.*

INTRODUCTION

Conservation areas in agricultural fields may provide valuable habitat for native wildlife, but they run the risk of exposing native wildlife to harmful insecticides associated with adjacent cropland (Bass et al. 2015, Gibbons et al 2015, Morrissey et al. 2015, Mogren and Lundgren 2016, Forister et al. 2016, Wood and Goulson 2017, Wagner et al. 2021). Neonicotinoid insecticides are broad-spectrum insecticides applied as an aerial spray or as an ingredient on coated seeds. They impair the growth, reproduction, foraging, and navigation of both pest insects and non-target native insects (Lundin et al. 2015, Wood and Goulson 2017, Crall et al. 2018). The majority of applied neonicotinoid compounds are not taken up by crop plants (Sur and Stork 2003), but are mobilized into non-target "compartments" of the landscape, including air (Nuyttens et al. 2013), water (Smalling et al. 2018, Shaafsma et al. 2015), crop leaf tissue and nectar (Hall et al. 2022, Mogren and Lundgren 2016), deep soil horizons (Radolinski et al. 2019), groundwater (Hladik et al. 2014, Thompson et al. 2021), and adjacent habitat areas (Hladik et al. 2018, Gibbons et al. 2015, Morrissey et al. 2015). The high mobility of neonicotinoids makes them particularly likely to accumulate in or be transported through farmland conservation features (Gibbons et al 2015; Morrissey et al. 2015; Mogren and Lundgren 2016; Wood and Goulson 2017).

Prairie strips are perennial conservation areas that are established on farms to reduce soil movement and nutrient runoff from farmland, and to provide habitat for a suite of native insects and pollinators without disproportionately reducing crop yields (Schulte et al. 2017, Hernandez-Santana et al. 2013, Pérez-Suárez et al. 2014, Gutierrez-Lopez et al. 2014, Kemmerling et al. 2022, Kemmerling et al. 2023). The conservation benefits of prairie strips may be undermined if they accumulate insecticides in plant tissue and soils or facilitate the transport of neonicotinoids within

the landscape. Prairie strips, which comprise 10% of a row crop catchment in strips ≥30 feet wide, have been shown to reduce downslope runoff of sediment, nitrogen, and phosphorus (Zhou et al. 2014), and may inhibit neonicotinoid transport through similar mechanisms. For example, prairie strips may increase neonicotinoids in deep soil and groundwater layers via infiltration and leaching, as their dense perennial root systems change soil structure and can create channels for preferential flow (Smalling et al. 2018, Radolinski et al. 2019, Henning et al. 2024). Prairie strips may also increase plant neonicotinoid uptake due to the density of perennial roots (Hall et al. 2022, Mogren and Lundgren 2016). At a site formerly planted with neonicotinoid-treated seeds, Hladik et al. (2017) report that converting 10% of a row crop catchment to prairie strips reduced neonicotinoid runoff to downslope soils, but prairie strip effects have not yet been measured in cropping systems with ongoing neonicotinoid inputs where insecticide runoff is likely to be greatest. Soil biochemical changes that accompany conversion to perennial vegetation may promote or inhibit accumulation of neonicotinoids. Compared to cropland soils, prairie strip soils have higher soil organic carbon (SOC) that can protect neonicotinoids from degradation (Smith et al. 2014, Zhang et al. 2018, Satowski et al. 2018, Morrison et al. 2022), but at the same time, higher dissolved organic carbon, higher soil moisture, and lower oxygen availability may also enhance neonicotinoid degradation (via denitrification and dehalogenation pathways; Iqbal et al. 2014, Smith et al. 2014, Mitchell et al. 2015, Parte and Kharat 2019).

Here we seek to determine how prairie strips affect the transport of the most widely used neonicotinoid insecticide in U.S. maize cropping systems, clothianidin (CLO, Simon-Delso et al. 2015). We measured CLO residues in surface soil, deep soil, plant tissue, and groundwater at multiple times of year and at multiple slope positions during a single maize growing season in two agricultural catchments: one with prairie strips and one without. We hypothesized that prairie strips

planted in cropping systems with ongoing neonicotinoid inputs promote neonicotinoid transport into deep soil, plant tissue, and groundwater compartments via leaching and plant uptake (Hall et al. 2022, Radolinski et al. 2019). Thus, we predict overall greater CLO levels in plant leaf tissue, deep soil, and groundwater in prairie strips, and that greater plant accumulation and leaching in prairie strips will result in reduced soil CLO runoff downslope in the prairie strip catchment (Hladik et al. 2017).

METHODS

Site description

We collected surface soil, deep soil, plant tissue, and groundwater samples from a farm in Story County, Iowa that is part of the Iowa State University Science-based Trials of Rowcrops Integrated with Prairie Strips project (STRIPS; https://www.nrem.iastate.edu/research/STRIPS/). The 12-hectare field area is managed with a maize-soybean (MSMMSM) rotation, grass waterways, and 15-30% crop residue retention, a management regime typical of the region (USDA 2023; Table 4.2). The site is divided into two catchments: one catchment with 10% prairie strip cover (78% *Zea maize* + 10% prairie strips + 12% grass waterway) and one catchment with no prairie strips (88% maize + 12% grass waterway, Figure 4.2, Table 4.2). Five prairie strips were planted perpendicular to the prairie strip catchment slope in 2015, sown with a mix of 33 forbs and 8 grasses (Hall et al. 2022; English 2020; Table 4.3). Prior to 2015, prairie strip areas were managed identically to the surrounding cropland, including the consistent use of neonicotinoid-treated maize and soybean seed for at least 5 years. We sampled in 2021, a maize year in which catchments were planted with Hoegemeyer 8009AM hybrid maize seed treated with a LumiGEN portfolio, including approximately 0.25 mg clothianidin (CLO) neonicotinoid per kernel (DeVries and Wright 2021).

Surface soil and deep soil collection

Surface soil (0-10 cm) and deep soil (90-100 cm) samples were collected on three days during the 2021 growing season to quantify soil CLO residues one week pre-planting (April 21), five weeks post-planting (June 1), and eleven weeks post-planting (July 16). Soils were collected from six sampling locations within each catchment, arranged from summit to footslope, and were collected from both crop and prairie areas within the prairie strip catchment (Figure 4.2). We selected sampling locations that represented the path of maximum soil movement within the catchments following rain events (Stephenson et al. 2024). Deep soils were collected to 100 cm depth using a hydraulic sampling probe (Giddings #25-TS Model HDGSRTS). To avoid damaging crop and prairie vegetation, deep soil samples were collected at the edge of the grass waterway, slightly offset from surface soil sampling locations (Figure 4.2). Soils were stored in Whirlpak bags at 4°C immediately following collection, and within 48 hours, sieved to 2 mm to homogenize soil and remove gravel and litter fragments, and then stored at -20°C.

Plant tissue collection

Leaf tissue was collected from maize in rowcrop areas of both catchments and from black-eyed susan (*Rudbeckia hirta*) and common milkweed (*Ascleipas syriaca*) in prairie strips on June 1 and July 16. Black-eyed susan and milkweed were selected to represent short-term and long-term bloom durations, respectively, as neonicotinoid uptake rate varies with plant phenology (Mogren and Lundgren 2016). Milkweed was also selected because monarch butterflies (*Danaus plexippus*), a pollinator species of conservation concern, feed directly on milkweed leaf tissue during their larval stage. We sampled one individual of each plant species nearest to its corresponding surface soil sample (Figure 4.2). At each rowcrop sampling location, one maize leaf was collected from the plant at the midpoint of the plant's stem. At each prairie strip sampling

location, we collected one leaf from one black-eyed susan basal rosette and one leaf from the midpoint of one milkweed stem. All leaf tissue samples were stored in Whirlpak bags at 4°C immediately following collection, then at -20°C within 48 hours of sample collection.

Groundwater collection

Groundwater samples were collected on two sampling dates, April 21 and June 2, 2021. Wells were installed at the foot slope of each catchment, constructed using 50 mm i.d. polyvinyl chloride tubes with 0.6-m well screens and a depth of 4.5 m. Groundwater wells were purged prior to sample collection. On each sampling date, one groundwater sample was collected from each catchment by connecting 6.35 mm diameter new nylon tubing to a new 2 L amber HDPE filter flask and inserting the tube into the well. Using a hand pump, suction was applied to the flask, and the sample was transferred to the HDPE bottle and stored at 4°C immediately following collection. There was not sufficient surface water runoff for sample collection during the 2021 growing season. Rainfall during the study period averaged 2.48 mm per day, significantly lower than the 30-year average rainfall of 3.99 mm per day during the same period (Iowa Environmental Mesonet, ISU 2024).

Soil physiochemical analysis

Surface soil samples collected in April 2021 were submitted to the Michigan State University Soil and Plant Nutrient Lab (East Lansing, MI, USA, http://www.spnl.msu.edu/) for analysis of physical and chemical properties known to influence neonicotinoid transport and degradation: pH, cation exchange capacity (CEC), and % clay (Ousely 2017; Zhang et al. 2018).

Neonicotinoid analysis

CLO concentrations were quantified in all field-collected surface soil, deep soil, plant tissue, and groundwater samples using previously published extraction and LC-MS/MS analysis

methods (Hall et al. 2020, Hall et al. 2022) performed by the Iowa State University Veterinary Diagnostic Lab (Ames, IA, USA, https://vetmed.iastate.edu/vdl). CLO was quantified using a method detection limit ranging from 1 ng/g to 20 ng/g for soil, 0.5 ng/g to 20 ng/g for plant tissue, and 0.5 ng/g to 50 ng/g for groundwater. Calibration curves and quality control replicates were prepared using neonicotinoid-free soil, milkweed leaf tissue, and water. No compounds were detected in matrix blanks. For calibration standards, the measured limit of quantification (LOQ) was ≤ 20% RSD of the nominal concentration, and the measured concentration of the remainder of the calibrants was ≤ 15% of the nominal concentration.

Data analysis

Data were checked for normality and heterogeneity of variances. All data were then analyzed with linear models using CLO (ppb) as a response variable. Linear models were checked for normal residuals and homogeneous variance using the Shapiro–Wilks normality test (Zuur et al. 2009). We tested the effects of catchment (maize cropping system with and without 10% prairie strips), slope position (six positions from summit to footslope), sampling date (April 21, June 1, and July 16), and their interactions on CLO in each landscape compartment using a two-factor analysis of variance (ANOVA) in R (version 4.2.3; R Core Team 2022). We performed a separate ANOVA within each sampling date (April, June, July) to determine the effects of prairie strips and slope position on CLO within each phase of the growing season. We also performed a separate two-way ANOVA on samples from the prairie strip catchment to determine the effect of vegetation (maize vs. prairie), slope position, sampling date, and their interactions on CLO in each landscape compartment. Finally, to determine the effects of soil physiochemical properties (pH, CEC, % clay) on surface soil CLO, we performed a two-way ANOVA performed an additional two-factor

ANOVA to evaluate the effects of prairie strips and slope position on significant soil physiochemical predictors of CLO.

RESULTS AND DISCUSSION

We hypothesized that in cropping systems with ongoing neonicotinoid inputs, prairie strips accumulate CLO from adjacent cropland via increased preferential flow into deep soils (Jørgensen et al. 2002, Alaoui 2015, Radolinski et al. 2018) and increased CLO uptake into plant tissue (Ferchaud and Mary 2016); however, we found no evidence for either of these accumulation mechanisms (Figure 4.1). CLO was detected in all maize leaf tissue samples across both catchments, but only in two prairie forb leaf tissue samples (two black-eyed susan plants), and average CLO concentrations were 10-fold greater in maize tissue (average 24.33 ppb) than in prairie forbs (average 0.22 ppb, Figure 4.1, Table 4.6). As is typical for neonicotinoid-treated seedlings, plant tissue CLO decreased from June to July as translocated CLO was diluted into growing plant biomass (Balfour et al. 2016, Figure 4.1). CLO concentrations in prairie strip forbs were well below acute lethal doses for pollinator species studied to date (Wood and Goulson 2017, Krishnan et al. 2021), consistent with findings from a recent plant tissue neonicotinoids survey across prairie strip sites by Hall et al. (2022). While these findings suggest that prairie strips do not accumulate and expose pollinators to acutely toxic levels of neonicotinoids, neonicotinoid exposure has only been studied for a limited number of species (EFSA 2012). Repeated exposure of insects to low-level neonicotinoids in prairie strips may produce more subtle, long-term effects on individual and population health (Feltham et al. 2014, Sandrock et al. 2014, Spurgeon et al. 2016).

Within the prairie strip catchment, prairie strip areas contained lower soil and plant CLO compared to adjacent maize areas (surface soil $p = 0.011$, deep soil $p = 0.072$, plant tissue $p <$

0.001, Table 4.4). Low CLO in prairie strip surface soils may be due simply to lower CLO inputs, as neonicotinoid insecticides are not applied in prairie strip areas. However, because prairie strip soils do receive CLO inputs from upslope cropland areas (Table 4.6; Figure 4.1), lower CLO in prairie strips could be explained by physiochemical soil properties like higher soil pH (average 5.73 prairie strip soil pH, average 5.48 cropland soil pH, $p = 0.051$, Table 4.5), which controls neonicotinoid sorption and degradation (Ousley et al. 2017, Zhang et al. 2018, Parte and Kharat 2019), but future work that experimentally manipulates chemistry of field-collected soils would better elucidate controls on neonicotinoid residence time under different land management regimes. Low CLO concentrations in prairie strip soils, plants, and groundwater may also be a function of low precipitation at the site (Figure 4.3) and/or the presence of grass waterways in the center of each catchment (Figure 4.2). April 2021 was the 3rd most severe April drought recorded in Story County, Iowa since 1895 (Palmer Z-index -3.05; NOAA 2024), and drought conditions remained in the county through August. In a wetter year with more frequent rain events, we might expect that prairie strips would receive more neonicotinoid transport from upslope crop soils and show higher CLO concentrations in all compartments (Radolinski et al. 2019). Grass waterways at our study site may have also directed what little rainfall did occur into surface flow downslope rather than vertical flow into deep soil horizons, and because our deep soil sampling locations were positioned nearer to each grass waterway to mitigate crop damage, we may have captured deep soils with relatively more surface flow and less infiltration.

We also expected that increased neonicotinoid leaching and plant uptake in prairie strips would result in reduced neonicotinoid runoff in the prairie strip catchment; however, at this site, prairie strips did not reduce neonicotinoid runoff through these mechanisms. Footslope surface soils contained the highest CLO levels in both catchments (41.10 ppb in no prairie strip catchment

in June, 20.3 ppb in prairie strip catchment in July, Figure 4.1, Table 4.1). These findings contrast those of Hladik et al. 2017, who reported nondetectable neonicotinoids in footslope soils of 10% prairie strip catchments where neonicotinoid application had ceased 2-3 years prior. This suggests that when 10% prairie strips are integrated into catchments with active neonicotinoid use, they do not remove all CLO via intermittent filtration along the catchment slope. Again, however, the downslope runoff we observed may be due to the downslope mobilization of neonicotinoids in grass waterways.

Our study finds that when neonicotinoid inputs are ongoing, and when prairie strips are planted in tandem with grass waterways, prairie strips may not be a sufficient management strategy to reduce insecticide runoff. Yet, even under continual neonicotinoid inputs in surrounding cropland, prairie strips accumulate little to no neonicotinoids in their plant tissue, indicating that prairie strips *can* provide habitat without exposing visiting native organisms to acute harm. To conclusively determine which conditions may make prairie strip plantings either safe havens, neutral vegetation, ecological sinks, or even ecological traps, we recommend continued measurements of neonicotinoids in cropland-adjacent prairie plantings, and exposure studies with field-representative neonicotinoid levels. Future studies might also measure neonicotinoids in soil, plant, and water immediately following a rain event to determine if prairie strips and grass waterways facilitate downslope neonicotinoid transport at a finer temporal scale than we captured in our study.

ACKNOWLEDGEMENTS

We would like to thank several scientists at Iowa State University, including members of the Science-Based Trials of Rowcrops Integrated with Prairie Strips (STRIPS) team, for their assistance in planning and carrying out this project: Matt Liebman, Darrin Thomson, Joel Coates,

Matthew Helmers, Tim Youngquist, Jessica A. Stephenson, Cole Dutter, Jacob Studt, Christopher Witte, Laura Burns, Dwayne Shrunk, and Maura Hall. We also thank David Cwiertny and Michelle Hladik for their assistance with early project conceptualization. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. 2235783 and the NSF Long Term Ecological Research Program [Grant ID: DEB 1832042]. This is W.K. Kellogg Biological Station Contribution No. 2381.

LITERATURE CITED

Alaoui A. 2015. Modelling susceptibility of grassland soil to macropore flow. Journal of Hydrology: 525:536-546. https://doi.org/10.1016/j.jhydrol.2015.04.016.

Balfour, N.J., Carreck, N.L., Blanchard, H.E., Ratnieks, F.L.W., 2016. Size matters: Significant negative relationship between mature plant mass and residual neonicotinoid levels in seed-treated oilseed rape and maize crops. Agriculture, Ecosystems & Environment 215, 85–88. https://doi.org/10.1016/j.agee.2015.09.020

Bass C., Denholm I., Williamson M.S., Nauen R. 2015. The global status of insect resistance to neonicotinoid insecticides. Pestic Biochem Physiol. 121:78-87. https://doi:10.1016/j.pestbp.2015.04.004.

Berens M.J., Capel P.D., Arnold W.A. 2020. Neonicotinoid Insecticides in Surface Water, Groundwater, and Wastewater Across Land-Use Gradients and Potential Effects. Environmental Toxicology and Chemistry 40(4):1017-1033. https://doi.org/10.1002/etc.4959.

Brandes, E., McNunn, G.S., Schulte, L.A., Muth, D.J., VanLoocke, A. and Heaton, E.A. 2018. Targeted subfield switchgrass integration could improve the farm economy, water quality, and bioenergy feedstock production. GCB Bioenergy, 10: 199-212. https://doi.org/10.1111/gcbb.12481

Burchfield, E., Nelson, K.S., Spangler, K., 2019. The impact of agricultural landscape diversification on U.S. crop production. Agriculture, Ecosystems & Environment 285, 106615. https://doi.org/10.1016/j.agee.2019.106615

Crall J.D., Switzer C.M., Oppenheimer R.L., Ford Versypt A.N., Dey B., Brown A., Mackay E., Guérin C., Pierce N.E., Combes S.S., de Bivort B.L. 2018. Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation. Science 362:683-686. https://doi:10.1126/science.aat1598.

DeVries, T.A. and Wright, R.J. 2021. Evaluation of Traited Corn Rootworm and Refuge Corn Hybrids in Combination With Aztec HC at Planting for Larval Corn Rootworm Control, 2020. Arthropod Management Tests. https://doi.org/10.1093/amt/tsab084

Dolezal A.G., St. Clair A.L., Zhang G., Toth A.L., O'Neal M.E. 2019. Native habitat mitigates feast–famine conditions faced by honey bees in an agricultural landscape. PNAS 116(50): 25147-25155. https://doi.org/10.1073/pnas.1912801116.

Douglas M.R. and Tooker J.F. 2015. Large-Scale Deployment of Seed Treatments Has Driven Rapid Increase in Use of Neonicotinoid Insecticides and Preemptive Pest Management in U.S. Field Crops. Environmental Science & Technology 49(8): 5088-5097. https://doi.org/10.1021/es506141g.

English, L., 2020. Understanding the variation in vegetation composition of prairie restorations within crop fields [Master's thesis, Iowa State University]. Iowa State University Digital Repository. https://dr.lib.iastate.edu/handle/20.500.12876/94275

European Food Safety Authority (EFSA). 2013. Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. European Food Safety Authority Journal 11:3066. https://doi.org/10.2903/j.efsa.2013.3066

Feltham, H., K. Park, and D. Goulson. 2014. Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. Ecotoxicology 23(3):317-23. https://doi.org/10.1007/s10646-014-1189-7.

Ferchaud, F. and Mary, B. 2016. Drainage and Nitrate Leaching Assessed During 7 Years Under Perennial and Annual Bioenergy Crops. Bioenergy Research. 9:656–670. https://doi.org/10.1007/s12155-015-9710-2

Gibbons, D., Morrissey, C. and Mineau, P. 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environmental Science and Pollution Research 22:103–118. https://doi.org/10.1007/s11356-014-3180-5

Goulson D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology, 50: 977-987. https://doi.org/10.1111/1365-2664.12111

Gutierrez-Lopez, J., Asbjornsen, H., Helmers, M.J., Isenhart, T.M., 2014. Regulation of soil moisture dynamics in agricultural fields using strips of native prairie vegetation. Geoderma 226, 238–249. https://doi.org/10.1016/j.geoderma.2014.02.013

Hall M.J., Dang V., Bradbury S.P., Coats J.R. 2020. Optimization of QuEChERS Method for Simultaneous Determination of Neonicotinoid Residues in Pollinator Forage. Molecules. 25(12):2732. https://doi.org/10.3390/molecules25122732

Hall M.J., Zhang G., O'Neal M.E., Bradbury S.P., Coats J.R. 2022. Quantifying neonicotinoid insecticide residues in milkweed and other forbs sampled from prairie strips established in maize and soybean fields. Agriculture, Ecosystems & Environment 325:107723. https://doi.org/10.1016/j.agee.2021.107723

Henning, E.J., Kolka, R.K.,Helmers, M. 2024. Infiltration within native prairie vegetative strips embedded in row crop fields across Iowa. Journal of Soil and Water Conservation. https://doi.org/10.2489/jswc.2024.00146

Hernandez-Santana, V., Xiaobo Zhou, Zhou, X., Helmers, M.J., Asbjornsen, H., Kolka, R., Tomer, M.D., 2013. Native prairie filter strips reduce runoff from hillslopes under annual row-crop systems in Iowa, USA. Journal of Hydrology 477, 94–103. https://doi.org/10.1016/j.jhydrol.2012.11.013

Hladik M.L., Kolpin D.W., Kuivila K.M. 2014. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. Environmental Pollution 193:189-196. https://doi.org/10.1016/j.envpol.2014.06.033

Hladik M.L., Bradbury S., Schulte L.A., Helmers M., Witte C., Kolpin D.W., Garrett J.D., Harris M. 2017. Neonicotinoid insecticide removal by prairie strips in row-cropped watersheds with historical seed coating use. Agriculture, Ecosystems & Environment 241:160-167. https://doi.org/10.1016/j.agee.2017.03.015

Hladik M.L., Main A.R., and Goulson D. 2018. Environmental Risks and Challenges Associated with Neonicotinoid Insecticides. Environmental Science & Technology 52(6):3329-3335 https://doi: 10.1021/acs.est.7b06388

Hussain S., Hartley C.J., Shettigar M., Pandey G. 2016. Bacterial biodegradation of neonicotinoid pesticides in soil and water systems. FEMS Microbiol Lett 363(23). https://doi.org/10.1093/femsle/fnw252

Intergovernmental Platform on Biodiversity and Ecosystem Services [IPBES] (2019). The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production.

Imperatriz-Fonseca, V. and H. T. Ngo (eds). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. https://doi.org/10.5281/zenodo.3402856

Iqbal, J., Parkin, Timothy B., Parkin, T. B., Helmers, M.J., Xiaobo Zhou, Xiaobo Zhou, Zhou, X., Castellano, M.J., 2015. Denitrification and Nitrous Oxide Emissions in Annual Croplands, Perennial Grass Buffers, and Restored Perennial Grasslands. Soil Science Society of America Journal 79, 239–250. https://doi.org/10.2136/sssaj2014.05.0221

ISU [Iowa State University]. 2024. Iowa Environmental Mesonet. https://mesonet.agron.iastate.edu/(accessed 15 February 2024).

Jeschke P., Nauen R. 2008. Neonicotinoids-from zero to hero in insecticide chemistry. Pest Manag Sci. 64(11):1084-98. https://doi.org/10.1002/ps.1631

Jørgensen P.R., Hoffmann M., Kistrup J.P., Bryde C., Bossi R. and Villholth K. G. 2002. Preferential flow and pesticide transport in a clay-rich till: Field, laboratory, and modeling analysis, Water Resour. Res. 38(11):1246. https://doi.org/10.1029/2001WR000494

Kemmerling, L.R., Rutkoski, C.E., Evans, S.E., Helms, J.A., Cordova-Ortiz, E.S., Smith, J.A., Vázquez Custodio, J.A., Vizza, C., Haddad, N.M. Prairie Strips and Lower Land Use Intensity Increase Biodiversity and Ecosystem Services. Frontiers in Ecology and Evolution. https://doi.org/10.3389/fevo.2022.833170

Kemmerling, L.R., McCarthy, A.C., Brown, C.S., Haddad, N.M. 2023. Butterfly biodiversity increases with prairie strips and conservation management in row crop agriculture. Insect Conservation and Diversity, 16(6), 828–837. Available from: https://doi.org/10.1111/icad.12675

Kordbacheh F., Liebman M., Harris M. 2020. Strips of prairie vegetation placed within row crops can sustain native bee communities. PLOS ONE 15(10). https://doi.org/10.1371/journal.pone.0240354

Kremen, C., Merenlender, A.M., 2018. Landscapes that work for biodiversity and people. Science 362. https://doi.org/10.1126/science.aau6020

Lundin O., Rundlöf M., Smith H.G., Fries I., Bommarco R. 2015. Neonicotinoid Insecticides and Their Impacts on Bees: A Systematic Review of Research Approaches and Identification of Knowledge Gaps. PLoS ONE 10(8). doi:10.1371/journal.pone.0136928

Lithourgidis, A., Dordas, C., Damalas, C. A., Damalas, Christos A., Vlachostergios, D.N., 2011. Annual intercrops: an alternative pathway for sustainable agriculture. Australian Journal of Crop Science 5(4):396-410. https://api.semanticscholar.org/CorpusID:55665222

Mackert, M. 2019. Strategies to improve native bee (Hymenoptera: Apoidea) habitat in agroecosystems [Master's thesis, Iowa State University]. Iowa State University Digital Repository. https://www.proquest.com/docview/2296357266

Mitchell, D.C., Xiaobo Zhou, Zhou, X., Parkin, T.B., Helmers, M.J., Castellano, M.J., 2015. Comparing nitrate sink strength in perennial filter strips at toeslopes of cropland watersheds. Journal of Environmental Quality 44, 191–199. https://doi.org/10.2134/jeq2014.05.0201

Mogren, C. and Lundgren, J. 2016. Neonicotinoid-contaminated pollinator strips adjacent to cropland reduce honey bee nutritional status. Sci Rep 6:29608. https://doi.org/10.1038/srep29608

Morrissey C.A., Mineau P., Devries J.H., Sanchez-Bayo F., Liess M., Cavallaro M.C., Liber K. 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. Environ Int 74:291-303. https://doi: 10.1016/j.envint.2014.10.024

Morrison, B. A., Xia, K. and Stewart, R. D. 2022. Soil organic matter can delay—but not eliminate—leaching of neonicotinoid insecticides. Journal of Environmental Quality 51:1270– 1281. https://doi.org/10.1002/jeq2.20402

Stephenson, J.A., M. Liebman, J. Niemi, R. Cruse, J. Tyndall, C. Witte, D. James, and M. Helmers. 2024. The influence of prairie strips sown in midwestern corn and soybean fields on sediment discharge throughout the year. Journal of Soil and Water Conservation: In press.

NOAA National Centers for Environmental information, Climate at a Glance: County Rankings, published February 2024, retrieved on February 15, 2024 from https://www.ncei.noaa.gov/access/monitoring/climate-at-a-glance/county/rankings

Nuyttens D., Devarrewaere W., Verboven P., Foqué D. 2013. Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review. Pest Manag Sci 69(5):564-75. https://doi: 10.1002/ps.3485

Ousely, M. N. Abiotic fate mechanism: Hydrolysis study on selected neonicotinoids [Master's thesis, California State University]. California State University Digital Repository. https://scholarworks.calstate.edu/concern/theses/4j03d099c?locale=en

Pang S., Lin Z., Zhang W., Mishra S., Bhatt P. and Chen S. 2020. Insights Into the Microbial Degradation and Biochemical Mechanisms of Neonicotinoids. Front. Microbiol. 11:868. https://doi.org/10.3389/fmicb.2020.00868

Parte S.G. and Kharat A.S. Aerobic Degradation of Clothianidin to 2-Chloro-methyl Thiazole and Methyl 3-(Thiazole-yl) Methyl Guanidine Produced by Pseudomonas stutzeri smk. 2019. J Environ Public Health. https://doi.org/10.1155/2019/4807913

Pérez-Suárez, M., Castellano, M.J., Kolka, R.K., Asbjornsen, H., Helmers, M.J., 2014. Nitrogen and carbon dynamics in prairie vegetation strips across topographical gradients in mixed Central Iowa agroecosystems. Agriculture, Ecosystems & Environment 188, 1–11. https://doi.org/10.1016/j.agee.2014.01.023

Pietrzak D., Kania J., Kmiecik E., Malina G., Wątor K. 2020. Fate of selected neonicotinoid insecticides in soil–water systems: Current state of the art and knowledge gaps. Chemosphere 255. https://doi.org/10.1016/j.chemosphere.2020.126981

Potts, S., Imperatriz-Fonseca, V., Ngo, H. et al. Safeguarding pollinators and their values to human well-being. Nature 540, 220–229 (2016). https://doi.org/10.1038/nature20588

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Radolinski J., Wu J., Xia K., Stewart R. 2018. Transport of a neonicotinoid pesticide, thiamethoxam, from artificial seed coatings. Sci Total Environ 618:561-568. https://doi: 10.1016/j.scitotenv.2017.11.031

Radolinski J., Wu J., Xia K., Hession W.C., Stewart R.D. 2019. Plants mediate precipitation-driven transport of a neonicotinoid pesticide. Chemosphere 222:445-452. https://doi.org/ 10.1016/j.chemosphere.2019.01.150

Sandrock, C., Tanadini, L. G., Pettis, J. S., Biesmeijer, J. C., Potts, S. G. and Neumann, P. 2014. Sublethal neonicotinoid insecticide exposure reduces solitary bee reproductive success. Agricultural and Forest Entomology 16(2):119-128. http://doi.org/10.1111/afe.12041

Schaafsma, A.W., Limay-Rios, V., Baute, T.S., Smith, J.L., Xue, Y., 2015. Neonicotinoid insecticide residues in surface water and soil associated with commercial maize (corn) fields in southwestern Ontario. PLOS ONE 10. https://doi.org/10.1371/journal.pone.0118139

Schulte L.A., Niemi J., Helmers M.J., Liebman M., Arbuckle J.G., James D.E., Kolka R.K., O'Neal M.E., Tomer M.D., Tyndall J.C., Asbjornsen H., Drobney P., Neal J., Van Ryswyk G., Witte C. 2017. Prairie strips improve biodiversity and the delivery of multiple ecosystem services from corn-soybean croplands. PNAS 114(42):11247-11252. https://doi.org/10.1073/pnas.1620229114

Simon-Delso, N. V. Amaral-Rogers, L. P. Belzunces, J. M. Bonmatin, M. Chagnon, C. Downs, L. Furlan, D. W. Gibbons, C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. H. Krupke, M. Liess, E. Long, M. McField, P. Mineau, E. A. D. Mitchell, C. A. Morrissey, D. A. Noome, L. Pisa, J. Settele, J. D. Stark, A. Tapparo, H. Van Dyck, J. Van Praagh, J. P. Van der Sluijs, P. R. Whitehorn and M. Wiemers. 2015. Systemic Insecticides (Neonicotinoids and Fipronil): Trends, Uses, Mode of Action and Metabolites. Environmental Science and Pollution Research 22 (1):5–34. https://doi:10.1007/s11356-014-3470-y

Smalling, K.L., Hladik, M.L., Sanders, C., Kuivila, K.M., 2018. Leaching and sorption of neonicotinoid insecticides and fungicides from seed coatings. Journal of Environmental Science and Health Part B-pesticides Food Contaminants and Agricultural Wastes 53, 176–183. https://doi.org/10.1080/03601234.2017.1405619

Smith, T.E., Kolka, R.K., Zhou, X., Helmers M.J., Cruse R.M. and Tomer M.D. 2014. Effects of native perennial vegetation buffer strips on dissolved organic carbon in surface runoff from an agricultural landscape. Biogeochemistry 120:121–132. https://doi.org/10.1007/s10533-014-9985y

Spurgeon D, H. Hesketh, E. Lahive, C. Svendsen, J. Baas, A. Robinson, A. Horton and M. Heard. 2016. Chronic oral lethal and sub-lethal toxicities of different binary mixtures of pesticides and contaminants in bees (Apis mellifera, Osmia bicornis and Bombus terrestris). EFSA supporting publication 13(9). http://doi.org/10.2903/sp.efsa.2016.EN-1076

Sur, R. and Stork, A. 2003. Uptake, translocation and metabolism of imidacloprid in plants. Bulletin of Insectology 56:35–40

Thompson, D.A., Kolpin, D.W., Hladik, M.L., Barnes, K.K., Vargo, J.D., Field, R.W., 2021. Prevalence of neonicotinoids and sulfoxaflor in alluvial aquifers in a high corn and soybean producing region of the Midwestern United States. Science of The Total Environment 782, 146762. https://doi.org/10.1016/j.scitotenv.2021.146762

United States Department of Agriculture. 2023. Farm Service Agency Conservation Reserve Program Monthly Summary October 2023. https://www.fsa.usda.gov/Assets/USDA-FSA-Public/usdafiles/Conservation/PDF/CRPMonthlyOctober2023WithPageNumbers.pdf

vanEngelsdorp D., Evans J.D., Saegerman C., Mullin C., Haubruge E., Nguyen B.K., Maryann F., Frazier J., Cox-Foster D., Chen Y., Underwood R., Tarpy D.R., Pettis J.S. 2009. Colony Collapse Disorder: A Descriptive Study. PLOS ONE 4(8)

Wagner, D. L., Grames, E. M., Forister, M. L., Berenbaum, M. R., & Stopak, D. (2021). Insect decline in the Anthropocene: Death by a thousand cuts. Proceedings of the National Academy of Sciences, 118(2), e2023989118.https://doi.org/10.1371/journal.pone.0006481

Zhou X., Helmers M.K., Asbjornsen H., Kolka R., Tomer M.D. and Cruse R.M. 2014. Nutrient removal by prairie filter strips in agricultural landscapes. Journal of Soil and Water Conservation 69(1): 54-64 https://doi.org/10.2489/jswc.69.1.54.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., Smith, G. M., 2009. Mixed effects models and extensions in ecology with R 574. Springer.

APPENDIX D: CHAPTER 4 PART 1

Tables

	Surface soil (0-10cm) R^2 = 0.503			Deep soil (90-100cm) R^2 = 0.086			Plant leaf tissue (maize & prairie) R^2 = 0.534			Maize leaf tissue R^2 = 0.698		
Factor	Sums of Sq.	F Value	P Value	Sums of Sq.	F Value	P Value	Sums of Sq.	F Value	P Value	Sums of Sq.	F Value	P Value
Catchment	575.60	14.0987	**0.0012**	0.0685	0.2331	0.6344	2239.9	10.0168	**0.006**	28.0	0.1005	0.7605
Slope position	1165.54	5.7098	**0.0019**	2.7152	1.8491	0.1489	1263.4	1.1300	0.3843	864.2	0.6201	0.6904
Month	180.06	2.2052	0.1363	1.2490	2.1264	0.1454	4788.5	21.4137	**0.0003**	7943.9	28.5004	**0.001**
Catchment * Slope position	332.44	1.6285	0.1983	2.9649	2.0192	0.1195	2272.4	2.0324	0.1284	5.3	0.0191	0.8939
Catchment * Month	217.83	2.6678	0.0939	0.1311	0.2232	0.8019	1534.2	6.8609	**0.0186**	648.6	1.1635	0.3662

Table 4.1. Results from ANOVA evaluating predictors of clothianidin (CLO) in surface soil (0-10 cm), deep soil (90-100 cm), all plant leaf tissue (both maize and prairie forbs), and maize leaf tissue only (excluding prairie strip forbs). Groundwater data are omitted as CLO was below detection limit in all groundwater samples. Bold values indicate significance at $p < 0.05$. Underlined values indicate significance at $p < 0.1$. R2 values represent percent variation in CLO explained by each model.

	No prairie strips	10% prairie strips
Total area (ha)	6.0	6.0
Maize area (ha)*	5.3	4.7
Prairie area (ha)*	0	0.6
Grass waterway area (ha)	0.7	0.7
Crop rotation	MSMMSM	MSMMSM
Mean slope (%)*	4.9	4.5
2021 maize planting date	April 25	April 25
Soil type	Sandy Clay Loam	Sandy Clay Loam

Table 4.2. Characteristics of each catchment at the study site. Asterisks indicate differences between catchments. Soil type was originally reported in Hall et al. 2022 and catchment slope was originally reported in Stephenson et al. 2024.

Forbs	Grasses	Additions
Blackeyed Susan 0.08lb	Little bluestem 0.5lb	Butterfly milkweed 1pls oz
Gray-headed coneflower 0.2lb	Big bluestem 15lb	Whorled milkweed 1pls oz
Prairie Mimosa 0.15lb	Prairie junegrass 0.04lb	Swamp milkweed 3pls oz
Purple prairie clover 0.5lb	Prairie dropseed 0.04lb	Common milkweed 1pls oz
Partridge Pea 0.5lb	Fox sedge 0.09lb	Canada wild rye 0.095 pls lb
Roundheaded Bush 0.03lb	Sideoats grama 0.1lb	Indian grass 0.190 pls lb
White prairie clover 0.12lb		
Compass plant 0.1lb		
Smooth blue aster 0.09lb		
Showy tick trefoil 0.1lb		
Tall thimbleweed 0.01lb		
Butterfly milkweed 0.1lb		
Sky blue aster 0.05lb		
Wild white indigo 0.1lb		
Pale coneflower 0.1lb		
Rattlesnake master 0.1lb		
Wild bergamot 0.08lb		
Common evening primrose 0.05lb		
White heath aster 0.02lb		
Stiff goldenrod 0.14lb		
Golden Alexander’s 0.06lb		
Culver’s root 0.01lb		
Alumroot 0.01lb		
Tall blazingstar 0.03lb		
Ox-eye 0.1lb		
Prairie cinquefoil 0.02lb		
Red Columbine 0.04lb		
Flowering Spurge 0.01lb		
Wild Petunia 0.1lb		

Table 4.3. Species sown in prairie strips, adapted from Hall et al. 2022.

	Surface soil (0-10 cm) $R^2 = 0.623$			Deep soil (90-100 cm) $R^2 = 0.181$			Plant tissue (maize and prairie) $R^2 = 0.869$		
	Sums of Sq.	F Value	P Value	Sums of Sq.	F Value	P Value	Sums of Sq.	F Value	P Value
Vegetation	126.405	10.877	**0.011**	1.602	4.298	0.072	2022.92	50.189	< **0.001**
Slope position	232.147	4.994	**0.026**	2.053	1.377	0.324	369.28	2.291	0.131
Month	12.076	0.519	0.614	0.389	0.521	0.613	831.08	20.619	**0.001**
Vegetation * Month	60.504	2.603	0.135	0.707	0.949	0.427	1626.52	40.355	< **0.001**

Table 4.4. Results from ANOVA evaluating predictors of clothianidin (CLO) in surface soil (0-10 cm), deep soil (90-100 cm), and plant tissue (both maize and prairie forbs) in the prairie strip catchment only. Groundwater data are omitted as CLO was below detection limit in all groundwater samples. Bold values indicate significance at $p < 0.05$. Underlined values indicate significance at $p < 0.1$. R^2 values represent the percent variation in CLO explained by each model.

	Factor	Sums of Sq.	F Value	P Value
Surface soil (0-10cm) $R^2 = 0.178$	pH	757.50	9.801	**<0.001**
	Cation exchange capacity	31.24	0.405	0.529
	% Clay	28.15	0.365	0.550

Table 4.5. Results from ANOVA evaluating soil physiochemical predictors of clothianidin (CLO) in surface soil (0-10 cm) samples across all sampling dates. Bold values indicate significance at $p < 0.05$. R^2 values represent the percent variation in CLO explained by each model.

	No prairie strips	10% prairie strips
	Average CLO (ppb) (+/- SE)	Average CLO (ppb) (+/- SE)
Surface soil (0-10 cm)	**16.318** (2.674)	**8.321** (1.309)
Deep soil (90-100 cm)	0.441 (0.130)	0.354 (0.159)
Maize leaf tissue	25.208 (7.874)	22.562 (10.376)
Milkweed leaf tissue	NA	0
Black-eyed susan leaf tissue	NA	0.147 (0.105)
Groundwater	0	0

Table 4.6. Average CLO (ppb) and standard error (SE) of each landscape compartment within each catchment. Values are averaged across sampling dates and slope positions.

Figures

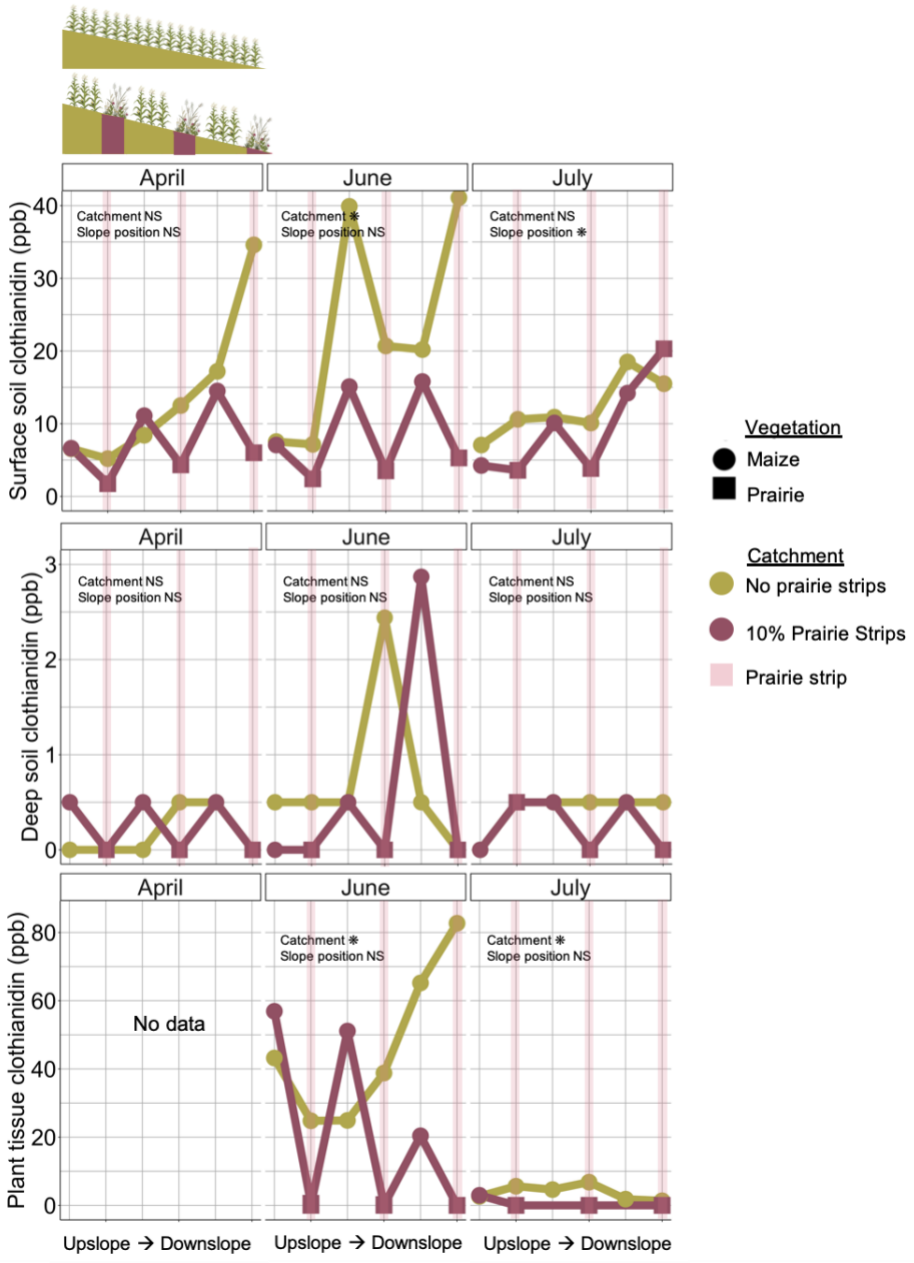

Figure 4.1. Clothianidin (CLO) concentrations in surface soils (0-10cm), deep soils (90-100cm) and plant leaf tissue (prairie forbs and maize) at each slope position. Line color represents catchment, and vertical pink lines represent prairie strip locations within the prairie strip catchment only. ANOVA results represent significant ($p < 0.05$, ❋) and not significant ($p > 0.05$, NS) differences between treatment groups. CLO was last applied to cropland on April 25, 2021.

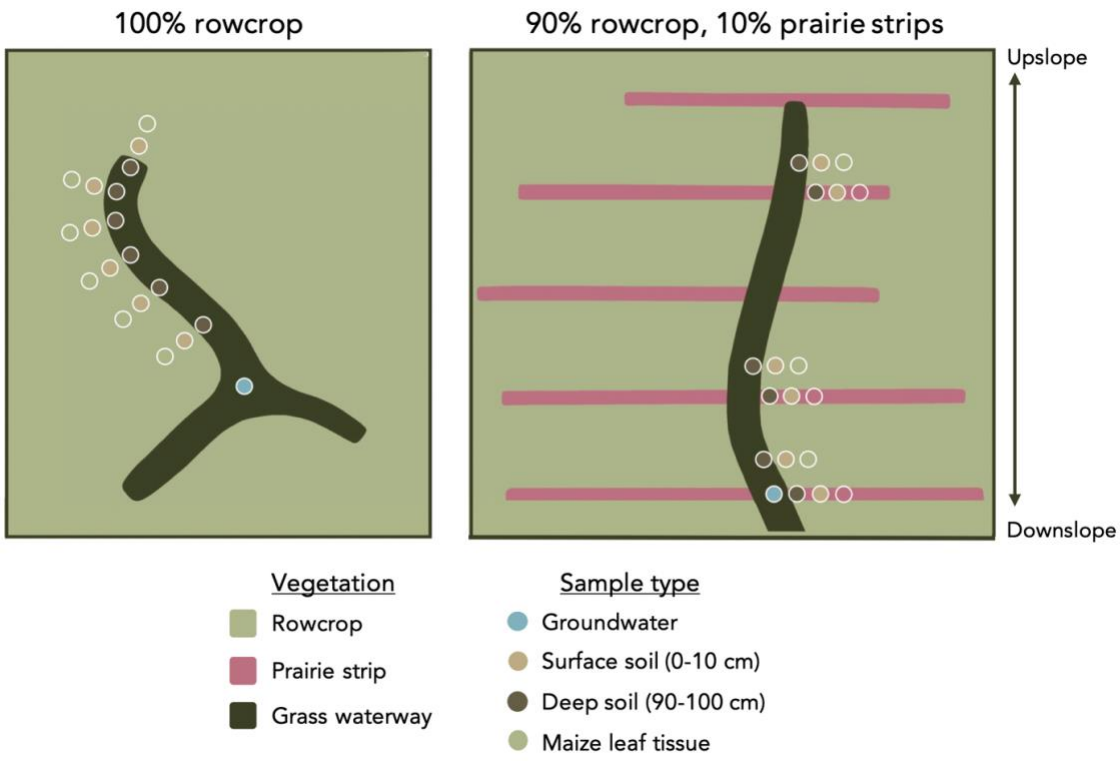

Figure 4.2. Aerial diagram of sampling site and sampling locations.

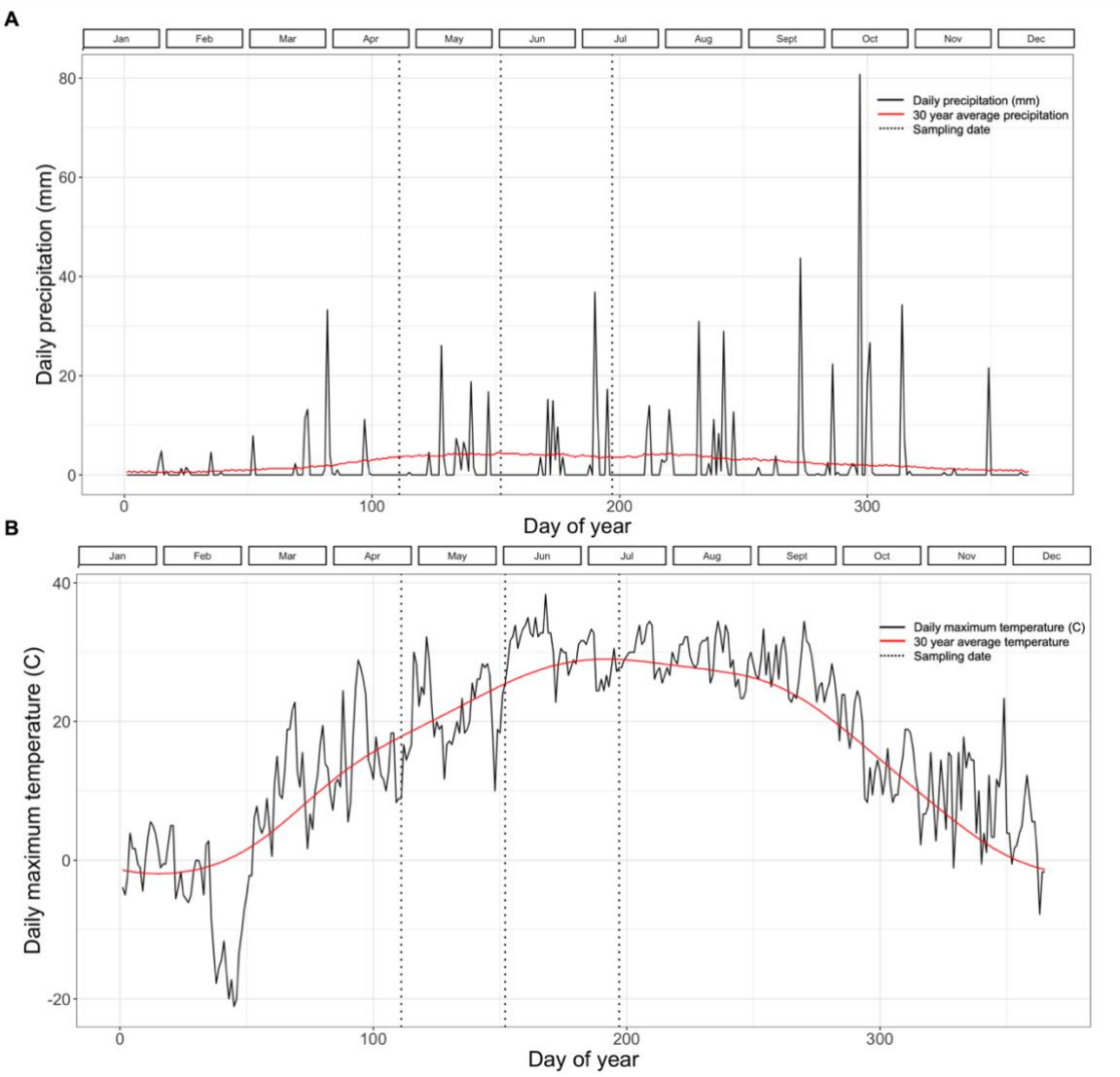

Figure 4.3. (A) Daily precipitation (inches) from January-December in 2021 (solid black) and 30-year average (red). (B) Maximum daily air temperature (F) from January-December in 2021 (solid black) and 30-year average (red). Sampling dates in this study shown in dashed vertical lines. Daily precipitation and temperature measurements were obtained from the Iowa Environmental Mesonet weather station (AMW) in Ames, Iowa located at 41.99044, -93.61852 (ISU 2024).

APPENDIX E: CHAPTER 4 PART 2

INTRODUCTION

In *Chapter 3: Neonicotinoid retention and transport in a maize cropping system with contour prairie strips*, we found that while prairie strips received neonicotinoid inputs from downslope movement of cropland soil, prairie strip surface soils still contained significantly lower neonicotinoid levels (clothianidin or CLO) than cropland surface soils. We found no evidence that CLO in prairie strip soils is reduced via increased accumulation of CLO in prairie plant tissue, increased CLO leaching into prairie strips' deep soil layers, or enhanced chemical degradation in prairie strip soils. We performed a separate study to assess whether lower CLO in prairie strip soils is driven by enhanced biodegradation of CLO by soil microbes. In this study, we measured CLO degradation by prairie strip and cropland soil microbial communities, hypothesizing that prairie strip soil microbial communities degrade CLO at a faster rate than cropland soil microbial communities. Here we describe the methods used for this experiment, the complications we encountered with these methods, and recommendations for use of this method in future studies.

METHODS

Soil sample collection

We collected surface soil samples according to methods described *in Chapter 3: Neonicotinoid retention and transport in a maize cropping system with contour prairie strips* and used soils to prepare microbial inocula. Briefly, we collected six soil samples from a 12-hectare maize cropping system divided into two catchments: one catchment with 10% prairie strip cover (78% Zea maize + 10% prairie strips + 12% grass waterway) and one catchment with no prairie strips (88% maize + 12% grass waterway. Crop areas were planted with maize seed treated with a

LumiGEN portfolio, including approximately 0.25 mg clothianidin (CLO) neonicotinoid per kernel. Surface soil (0-10 cm) samples were collected five weeks after maize planting on June 1, 2021. Soils were collected from three sampling locations within each catchment arranged from summit to footslope. In the catchment with prairie strips, samples were collected from three prairie strip areas: one upslope, one midslope, and one footslope prairie strip. In the catchment without prairie strips, samples were collected from three cropland areas: one upslope, one midslope, and one footslope crop area. Soils were stored in Whirlpak bags at 4C immediately following collection, and within 48 hours, sieved to 2 mm to homogenize soil and remove gravel and litter fragments, and then stored at -20C.

Microbial inocula

First, we prepared nutrient broth to serve as inoculum media as suggested by Mulligan et al. 2016 by dissolving 5 g pancreatic digest of gelatin and 3 g beef extract in 1 L nanopore water. Broth was autoclaved to sterilize. We prepared a soil slurry inoculum from each soil sample by adding 2.5 g each fresh soil to 250 mL nutrient broth. Slurries were shaken at 100 rpm at room temperature for 72 hrs. Slurries were stored at 4C. We determined each slurry's cell count by spreading 100 uL of each slurry diluted 108 on triplicate petri dishes containing nutrient broth and 15% agar. Plates were incubated at 37C for 24 hours and colony forming units (CFUs) were counted on each plate. CFU counts were averaged across slurry triplicates. All slurries were diluted with nutrient broth to a standard concentration of 2.3 x 106 CFUs per mL.

Degradation microcosms

We prepared degradation microcosms containing soil slurry, nutrient broth, and a standard concentration of neonicotinoid compound clothianidin (CLO). First, we prepared a stock solution of CLO analytical standard at a concentration of 0.125 mg per mL. We added 1ml of CLO stock

solution to 240 mL nanopure water for a concentration of 0.5 ug per mL in each microcosm. Three control microcosms were prepared with nutrient broth and CLO only with no soil slurry. Each microcosm was sealed with Breathe Easier tape to minimize contamination and allow air flow, and wrapped in aluminum foil to minimize photodegradation. Microcosms were placed on an orbital shaker table set to 150 rpm and to 35C placed inside of a laminar flow hood to minimize contamination. We collected a 1 mL aliquot from each microcosm on day 0, 2, 5, 10, 20 and 40 and stored aliquots at -20C prior to CLO quantification.

Neonicotinoid analysis

CLO was quantified in all microcosm samples using the LC-MS/MS analysis method described in Chapter 3: Neonicotinoid retention and transport in a maize cropping system with contour prairie strips. Briefly, CLO was quantified using a method detection limit ranging from 1 ng/g to 20 ng/g. Calibration curves and quality control replicates were prepared using neonicotinoid-nutrient broth. No compounds were detected in matrix blanks. For calibration standards, the measured limit of quantification (LOQ) was ≤ 20% RSD of the nominal concentration, and the measured concentration of the remainder of the calibrants was ≤ 15% of the nominal concentration.

RESULTS

We performed the experiment twice and encountered issues with photodegradation, microbial contamination, and evaporation in experimental microcosms, limiting our ability to assess biodegradation in prairie strip and cropland soils. In the first experiment (Figure 5.1), all microcosms exhibited CLO degradation at a similar rate, regardless of the inoculum source.

In the second experiment (Figure 5.2), we limited CLO photodegradation by wrapping all microcosm bottles in aluminum foil, and limited airborne contaminants by performing the

experiment inside of a biosafety cabinet. In this iteration, microcosms exhibited contamination, uneven starting concentrations of CLO, and patterns of increasing CLO over time.

DISCUSSION

In the first experiment of the study, microcosm bottles were not wrapped in aluminum foil, and therefore, CLO in each microcosm was subject to photodegradation from ambient UV. Microcosm controls that contained no soil inoculum also exhibited microbial contamination, evidenced by biofilm formation, inhibiting us from disentangling the effects of photodegradation from microbial degradation. Despite siting the experiment inside of a biosafety cabinet, microcosm controls that contained no soil inoculum still exhibited microbial contamination, though the extent of biofilm formation in microcosm controls was reduced from the previous experiment. Uneven starting concentrations of CLO on Day 0 of the experiment may have been due to an accretion of CLO in the stock CLO solution bottle or microcosm bottles from which aliquots were collected on Day 0, or may have been due to pipetting measurement error when transferring stock CLO solution into each microcosm replicate or collecting aliquots. It is unclear what caused an increase in CLO over time, but this pattern may be due to increased evaporation of microcosm liquid within the biosafety cabinet and subsequent distillation of CLO into higher concentrations over time within each microcosm.

We recommend that if this method is used in future projects, microcosms are prepared with an alternative diluent, rather than nutrient broth, that is less prone to microbial contamination from airborne sources. We recommend that microcosms are sealed with a sealant other than Breathe Easier sealing tape that allows for less air flow and evaporation while still maintaining an aerobic environment. Alternatively, microcosm volume could be measured at each aliquot sampling event, so that CLO values can be standardized per unit volume and any evaporation and distillation of

CLO is accounted for. Performing a similar experiment using sterilized soils inoculated with microbial communities, rather than liquid cultures, would also reduce the likelihood of contamination and evaporation issues.

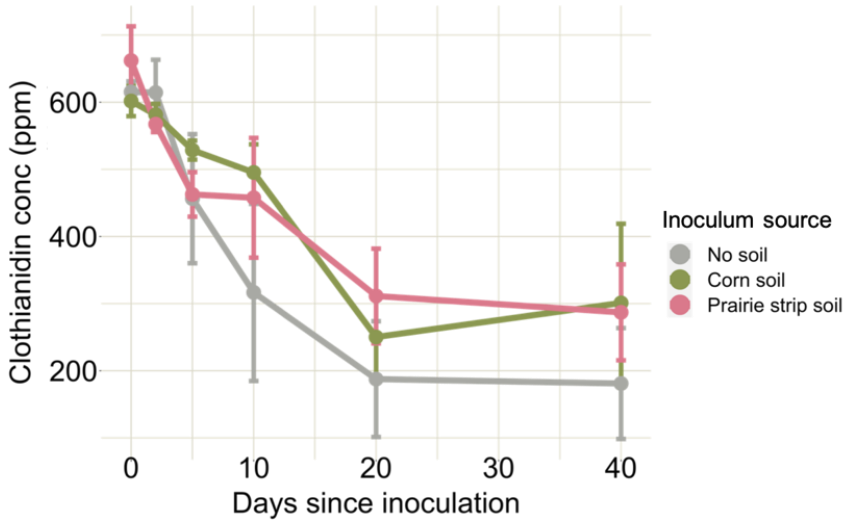

Figure 4.4. Results from the first clothianidin degradation experiment.

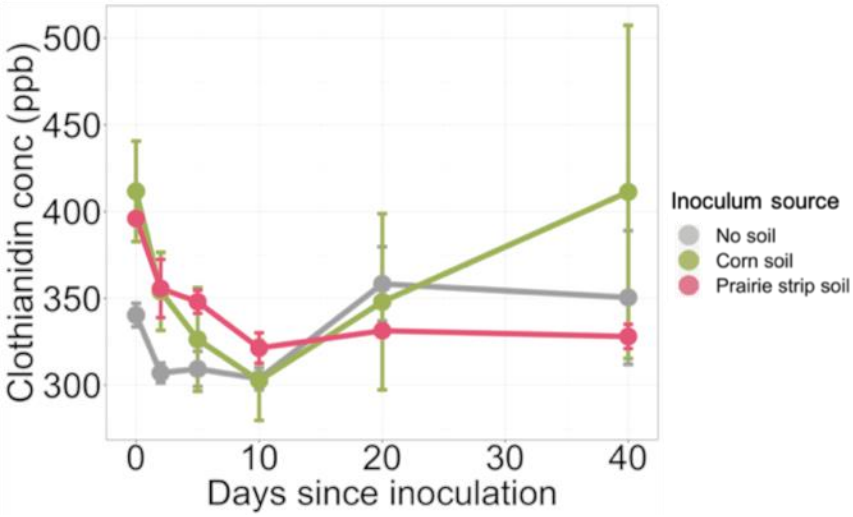

Figure 4.5. Results from the second clothianidin degradation experiment.

CONCLUSION

This dissertation addresses key knowledge gaps in prairie strip research and identifies additional benefits of prairie strips that can inform their adoption in row crop fields of the U.S. Midwest. I show that, in addition to increasing the diversity of plants, insects, and other wildlife in row crop landscapes, prairie strips also increase the diversity and function of soil microbial communities early in their establishment. As early as two months after prairie strips are planted, soils under strips exhibit increased abundance of soil microbial phyla emblematic of remnant tallgrass prairie soils, fungal decomposers, and fungal symbionts. More mature prairie strips also contain higher abundance of prairie bacterial and fungal groups, but show lower soil microbial diversity than adjacent cropland soils.

I also find that compositional shifts in soil communities correspond with greater active soil carbon in both newly-established and mature prairie strips. Prairie strips rapid accrual of active soil C suggests that prairie strips will eventually increase total C stocks similar to larger prairie restorations as they continue to mature, making prairie strips a candidate strategy for C sequestration in emerging agricultural carbon markets. Belowground benefits under prairie strips occur regardless of the cropping system they are established in, and benefits can be seen whether or not surrounding cropland is treated with agrochemical inputs. Prairie strips' belowground benefits only occur beneath the strip, as prairie strips do not affect soil microbes or soil C in adjacent cropland, at least in their first few years.

Finally, this work sheds light on an ongoing concern about prairie strips' interactions with insecticides in surrounding cropland. I show that when prairie strips are planted amid neonicotinoid insecticide-treated cropland, prairie strip plants do accumulate neonicotinoid compounds, but not at levels lethal to pollinator insects. Although prairie strips do not pose a risk

of acute harm to visiting insects, repeated low-dose exposure of pollinators to neonicotinoids in prairie strip plants may still have deleterious impacts on visiting organisms. Prairie strips planted amid treated cropland also showed an accumulation of neonicotinoid insecticides in downslope soils, and thus, prairie strips should not be expected to mitigate runoff of insecticides applied to adjacent row crop fields with on-going insecticide treatment. Altogether, this dissertation demonstrates that while prairie strips extend minimal benefits to surrounding cropland soils, prairie strips quickly increase diversity and function of belowground soil communities beneath the prairie strip.