

Soil physical, chemical, and microbial community responses to two years of perennial
intermediate wheatgrass versus annual maize/soybean management systems

A THESIS
SUBMITTED TO THE FACULTY OF
THE UNIVERSITY OF MINNESOTA BY

Emma Link

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

Dr. Jessica Gutknecht, co-advisor

Dr. Jacob Jungers, co-advisor

2023

ACKNOWLEDGEMENTS

This thesis would not have been possible without the work of many people. I would like to thank Katherine Bohn and Jesse Puka-Beals for managing the experimental plots and agronomic sampling, and for helping collect soil samples. Thanks to Cheyenne Neess and Alex Kincaid-Beal for their dedicated work collecting field data and analyzing samples in the lab, and also Carol Loopstra, Emily Locke, Matthew Leung, Nathan Lund, and Hilary Major for running sample analysis. Thank you to my advisors, Jessica Gutknecht and Jacob Jungers, and my committee members, Christopher Fernandez and Peter Kennedy, for their teaching, guidance and support throughout my graduate program and writing process. Finally, thank you to the faculty, staff, and students of the Graduate Program in Land and Atmospheric Sciences for all of your teaching, support, and friendship.

I also acknowledge the Minnesota Supercomputing Institute (MSI) at the University of Minnesota for providing resources that contributed to the research results reported within this paper. URL: <http://www.msi.umn.edu>. I thank the University of Minnesota Genomics Center Facility (Minneapolis, MN) for providing sequencing and library preparation services.

This project was funded by Foundational Knowledge of Agricultural Production Systems grant no. 2019-67013-29201/project accession no. 1018552 from the USDA National Institute of Food and Agriculture.

ABSTRACT

Intermediate Wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey; IWG) is a perennial grain crop with a dense root system which has the potential to facilitate improvements in soil physical structure, fertility, and potentially soil carbon storage. An experiment was established in Rosemount, MN, USA to assess changes in soil physical, chemical, and microbial community responses to IWG system vs. annual corn/soy system management after two growing seasons. This experiment aims to 1) assess soil quality under four systems representing a gradient of perenniality, cropping system diversity and soil disturbance intervals and, 2) investigate relationships between soil microbial community characteristics and desired soil chemical and physical quality outcomes to better understand the mechanisms behind desired outcomes. In the first chapter of this study, we report the agronomic outcomes of the first two years of the experiment and examine how IWG vs. annual crop management affect soil chemical and physical properties over the course of two growing seasons. After two years, we find that the proportion of large water stable soil aggregates at 15-30 cm soil depth increased significantly in IWG but not IWG-alf intercropped systems compared to annual systems. We also find evidence of increased water use deep in the soil profile by IWG systems under drought conditions. In the second chapter, we investigate the responses of soil microbial community composition and potential function to two years of IWG vs. annual management. We find that after two years, fungal community composition varied significantly by cropping system and IWG systems are associated with increased arbuscular mycorrhizal fungi biomass and AMF indicator species. We also find evidence of greater extracellular enzyme activity in IWG systems and an annual system with cover cropping.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	I
ABSTRACT	II
TABLE OF CONTENTS	III
CHAPTER 1: EARLY IMPROVEMENTS IN SOIL AGGREGATION UNDER PERENNIAL GRAIN INTERMEDIATE WHEATGRASS IN A MIDWEST MOLLISOL 1	
1.1 ABSTRACT.....	1
1.2 INTRODUCTION	2
1.3 MATERIALS AND METHODS	8
1.3.1 Study Site Description and Experimental Design	8
1.3.2 Agronomic Methods	12
1.3.3 Agronomic yield and aboveground biomass	12
1.3.4 Soil Moisture Profile	14
1.3.5 Water Sorptivity	14
1.3.6 Soil and root biomass sampling and storage	15
1.3.7 Bulk Density	16
1.3.8 Basic Soil Properties	16
1.3.9 Total C&N.....	17
1.3.10 POXC	17
1.3.11 ACE Protein	18
1.2.12 Aggregate Stability.....	19
1.2.13 Data Analysis	20
1.4 RESULTS	21
1.4.1 Agronomic Yields	21
1.4.2 Belowground biomass	22
1.4.3 Soil Moisture	24
1.4.4 Soil Physical Properties	28
1.4.5 Soil Chemical Properties	31
1.5 DISCUSSION	35
1.5.1 Crop Yield	36
1.5.2 Root biomass	39
1.5.3 Soil Moisture Profile.....	40
1.5.4 Soil Physical Properties	42
1.5.5 Soil Chemical Properties	44
1.5.6 Soil property differences in IWG vs. IWG-alf treatments	47
1.6 CONCLUSION.....	48

1.8 REFERENCES	49
1.7 SUPPLEMENTARY TABLES AND FIGURES.....	66
CHAPTER 2: SURFACE SOIL BACTERIAL AND FUNGAL COMMUNITY COMPOSITION AND POTENTIAL FUNCTION AFTER TWO YEARS UNDER ANNUAL AND PERENNIAL GRAIN CROP MANAGEMENT	72
2.1 ABSTRACT.....	72
2.2 INTRODUCTION	73
2.3 METHODS.....	77
2.3.1 <i>Experimental Design</i>	77
2.3.2 <i>Soil sampling and storage</i>	78
2.3.3 <i>Phospholipid Fatty Acid /Neutral Lipid Fatty Acid</i>	79
2.3.4 <i>Litter Decomposition</i>	80
2.3.5 <i>Extracellular Enzyme Activity</i>	81
2.3.6 <i>DNA Extraction</i>	82
2.3.7 <i>Amplicon sequencing</i>	83
2.3.8 <i>Data Analysis: Extracellular Enzymes, Litter Decomposition, PLFA/NLFA</i>	84
2.3.8 <i>Bioinformatic data analysis</i>	84
2.3.9 <i>Covariability of microbial community and environmental variables</i>	85
2.4 RESULTS	86
2.4.1 <i>Litter decomposition - microbial community function</i>	86
2.4.2 <i>Extracellular enzyme activity - microbial community function</i>	86
2.4.3 <i>PLFA/NLFA - Microbial biomass and community composition</i>	87
2.4.4 <i>Bacterial and Fungal diversity and community composition</i>	88
2.5 DISCUSSION	94
2.5.1 <i>Enhanced microbial activity under continuous living cover systems after two years.</i> .	94
2.5.2 <i>AMF biomass greater than annual systems' in IWG but not IWG-alf systems</i>	97
2.5.3 <i>Soil bacterial and fungal diversity and community composition responses to annual vs perennial management</i>	98
2.5.4 <i>Covariability of soil microbial community characteristics and function and desired soil quality outcomes</i>	100
2.6 CONCLUSION.....	101
2.7 REFERENCES	102
2.8 SUPPLEMENTARY TABLES AND FIGURES.....	120
APPENDIX 1 - DIFFERING MICROBIAL COMMUNITIES FROM QIAGEN POWERSOIL AND POWERSOIL PRO EXTRACTION KITS	127
APPENDIX 2: DATA AND CODE.....	135

CHAPTER 1: EARLY IMPROVEMENTS IN SOIL AGGREGATION UNDER PERENNIAL GRAIN INTERMEDIATE WHEATGRASS IN A MIDWEST MOLLISOL

1.1 Abstract

Global food production is challenged by increasing soil degradation and climate change, making it essential that new cropping systems be developed which rebuild the soil and perform in changing climates. Intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Buckworth & Dewey), a perennial grass which produces the wheat-like grain called Kernza®, has potential to improve soil health compared to annual grain counterparts. However, there is little information on how IWG affects soil physical and chemical qualities over time, especially compared to annual grains grown with and without existing soil health practices and on rotational timescales where IWG produces significant grain yields and the IWG system is profitable. We evaluated the impact of two IWG cropping systems (IWG monoculture [IWG] and IWG intercropped with alfalfa [IWG-alf]) and two maize/soybean rotation systems (with conventional tillage [annual] and with conservation tillage and winter cover crops [annual-cc]) on agronomic yields, soil physical and chemical properties, and soil water content over the course of two growing seasons in southeastern Minnesota, USA. In general, we observed the most differences between IWG and annual, and we observed no differences between IWG and IWG-alf or annual and annual-cc treatments, respectively. Twenty-four months after establishment, the mean weight diameter of soil aggregates in deeper soil (15-30 cm) was larger in IWG (1.93 mm) vs. annual (1.55 mm), and small macroaggregates (250 µm) in shallow soil (0-15 cm) were a lower proportion of total soil aggregates in IWG (11%) vs annual-cc (14%). In June and August 2021, during a historic drought, IWG had lower soil moisture content than

annual at 30, 60, and 100 cm, suggesting more complete utilization of water at depth. In 2021, there was lower total soil N (weight %) in IWG (0.25%) compared to annual (0.27%) system at 0-15 cm depth, which supports previous findings that IWG is effective at assimilating residual soil N. While we observed these few changes in soil properties in just two years of IWG cropping systems, three or more years of IWG production may be required to potentially see significant improvements in soil physical and chemical quality in relatively fertile soils such as those in our experiment.

1.2 Introduction

Climate change is projected to significantly alter US Midwest precipitation regimes and increase summer extreme temperature frequency and severity, which is expected to affect agriculture by increasing soil erosion (Borrelli et al., 2021), soil GHG emissions, and soil organic carbon loss (Black et al., 2017), and result in regional yield declines of up to 25% in maize and soybeans by 2050 (Angel et al., 2018). These challenges will compound non-climate factors already impacting agricultural productivity such as existing high soil erosion rates (Thaler et al., 2022) and reduced soil fertility (Fixen et al., 2010) caused by current and historic agricultural management practices (Van Oost et al., 2006; Tamburini et al., 2020). A key change that would improve agricultural productivity and reduce losses under climate extremes is the adoption of sustainable agricultural management practices that improve soil health (McGuire et al., 2022; Huang & Sim, 2021; Lal 2020; Lal, 2016). Soil health can generally be defined as a soil's ability to perform a desired function and is often operationally defined using a suite of quantifiable soil physical, chemical, and biological qualities called soil health indicators (Wander et al., 2019; Norris et al., 2020). Healthy soils have been shown to improve overall agronomic yield (e.g.

Oldfield et al., 2019), enhance yield stability under extreme weather conditions (e.g. Gaudin et al., 2015; Williams et al., 2016; Kane et al., 2021; Renwick et al., 2021; Lotter et al., 2003; Bowles et al., 2020), and act as a terrestrial carbon sink (Bossio et al., 2020).

Current recommended best-management strategies for improving soil physical and chemical quality and reducing erosion include reducing or eliminating tillage, using organic amendments, diversifying crop rotations and implementing intercropping, and adopting cover-crops and intercrops (Lenhart and Peterson, 2017; Crystal-Ornelas, 2021). Significant research has established the short and long-term effects of these individual improved management practices on soil properties. For example, relative to high-intensity tillage, reducing tillage over time improves soil aggregate structure, increases SOC storage (Liu et al., 2021) and soil organic N (Farmaha et al., 2022), reduces erosion (Nearing et al., 2017) and slightly reduces bulk density and soil penetration resistance over time (Nunes et al., 2020). Similarly, use of winter cover crops can over time result in larger SOC stocks (Poeplau and Don, 2015) and organic N (Farmaha et al., 2022), greater soil aggregation (Blanco-Canqui and Jasa, 2019), and greater soil water infiltration (Stewart et al., 2018). Though they offer improvements compared to standard practices, these existing best-management strategies are not enough to completely stop loss of soil quality over time or improve soil quality relative to when they were implemented, especially in high-fertility soils like Mollisols and Alfisols (Sanford et al., 2012; Ernst et al., 2018; Rubio et al., 2021; Rui et al., 2022). Many of these strategies are also not always feasible; cover crops, for example, are challenging to incorporate in the short growing seasons of the upper Midwest (Kaye and Quemada, 2017). Finally, these practices often come with additional monetary and time costs and real or feared negative impacts on cash crop yield, which limit farmer willingness

to adopt them (Wallander et al., 2021). Clearly, additional management tools for rebuilding soil health while maintaining profitability in agricultural production are needed.

Perennial grain crops such as intermediate wheatgrass (IWG; *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) are one such emerging tool that could improve soil health and therefore agricultural climate resilience (Ryan et al., 2018; Nunes et al., 2020) as part of a profitable grain production system (Law et al., 2022b). IWG currently produces much lower grain yields than annual counterparts; in MN, it produces 15-20% of the yield of winter wheat in years one and two (Law et al., 2022b). However, in IWG systems managed for dual-use grain and forage production, the revenue from hay can equal that of grain (Law et al., 2022b), and for the first two years the systems can achieve total revenues matching or exceeding those of organic annual wheat systems due to 50% higher forage production than annual wheat and higher IWG grain prices (Law et al., 2022b). Perennial grain crops may improve soil physical and chemical quality relative to annual grains due to their deep and dense root systems, continuous living cover on the soil surface, and the reduced soil tillage/disturbance intervals inherent to the systems (Glover et al., 2010). IWG systems allocate more root biomass at depth than annual grains including wheat and maize, which leads to increased N nutrient use efficiency (Sprunger et al., 2018, Rakkar et al., 2023) and well-documented drastically lower nitrate leaching rates (Culman et al., 2013; Jungers et al., 2019; Reilly et al., 2022a; Huddell et al., 2023). If these large root systems and perennial, undisturbed cover impact soil in similar ways to perennial forage crops and grassland systems managed for hay and forage, they may improve soil physical structure (Culman et al., 2010; DuPont et al., 2014), reduce erosion (Nearing et al., 2017), and increase SOC accumulation and stabilization (Glover et al., 2010; Scott et al., 2017) relative to

annual crops. Eddy flux experiments have shown that IWG fixes ~50% more carbon across the year than it respire and suggest that IWG may act as a carbon sink, sequestering relatively more carbon than maize/soybean rotations (de Oliveira et al., 2020). In addition to the benefits of perenniality and belowground biomass allocation, perennial legume intercrops such as alfalfa and red clover can be incorporated into IWG grain and forage production systems for the additional environmental, nutrient and soil health benefits associated with crop functional diversity. Legume intercrops can reduce IWG grain yield (Tautges et al., 2018), especially in the first year of establishment (Reilly et al., 2022b), but productivity outcomes have been variable (Dick et al., 2018). IWG-legume intercropped systems yield higher quality and generally higher forage biomass than IWG monocultures (e.g. Hayes et al., 2017; Pugliese et al., 2019a), and they may be better able to mobilize unavailable nutrients such as P (Duchene et al., 2020), enhance soil C stabilization (Peixoto et al., 2022) and nutrient cycling (Pugliese et al., 2019a), and maximize deep water use (Clément et al., 2022).

One challenge of growing IWG is that it currently exhibits significant yield decline beginning two to three years after establishment. Thus, growers may decide to rotate the crop out of production after the third grain production year. In order to assess significant high-profile claims of soil quality improvements (eg. Kane et al., 2016) and accurately describe the potential of these systems, research to determine how IWG cropping systems affect soil physical and chemical quality metrics on two to three year timescales are needed (Rakkar et al. 2023).

In practice, a small body of initial research investigating the soil health impacts of IWG monocultures and IWG-legume intercrops have shown varied results, with at most modest improvements in active C and N pool size and aggregate structure (Rakkar et al., 2023) achieved

across different soil and climate contexts. For example, Audu et al. (2022) found decreased surface total SOC but increased subsoil SOC in IWG relative to annuals two years after establishment. In other studies, there have been no documented total SOC gains relative to annual crops two (Rakkar et al., 2023) and four (Sprunger and Robertson., 2018; Sprunger et al., 2020) years post-establishment. Previous research has also found mixed responses in labile carbon and particulate organic matter pools, with some studies documenting increases (Sprunger et al., 2019; Culman et al., 2013; van der Pol et al., 2022) and others no change (Rakkar et al., 2023; Sprunger et al., 2018) after two to four years. The larger body of literature from grassland, forage, and bioenergy crop systems suggests that early in system establishment, soils mostly see changes in active C and N pools (Sprunger and Robertson, 2018; Martin and Sprunger, 2022; Audu et al., 2022), and significant changes in total C driven by aggregation and microbial communities are seen on a decadal scale (Liebig et al., 2005; Cates et al., 2015; Scott et al., 2017; Nunes et al., 2020). Clearly, additional research is necessary to better understand the degree to which IWG managed for grain or dual-use production can improve soil quality across different soils and sites (Stewart et al., 2018), and the timescale on which these improvements may occur.

In this study, we measured the agronomic productivity and soil chemical and physical properties under two annual and two perennial IWG cropping systems over two years in order to assess the relative agronomic outcomes and the initial soil quality impacts of cropping systems representing a gradient in perenniality/continuous living cover, cropping system diversity, and soil disturbance intervals. The four cropping systems were 1) IWG grown with alfalfa (*Medicago sativa*) as an intercrop, 2) IWG grown as a monoculture, 3) a strip-tilled annual maize (*Zea*

mays) and soybean (*Glycine max*) rotation with winter annual cover crops, and 4) a conventionally tilled maize/soybean rotation. We measured agronomic productivity (grain yield, aboveground and belowground biomass) to assess the establishment and performance of cropping systems and crop biomass allocation. We hypothesized that IWG systems would produce lower grain yields and higher belowground biomass compared with annual systems, and that the highest belowground biomass would be in IWG-alf system. We measured soil physical (aggregate stability, soil moisture depth profile, soil sorptivity) and soil chemical (pH, macro- and micronutrients, total organic C, total organic N, POXC and ACE protein) qualities to assess whether commonly used soil health indicators were affected by cropping systems. We hypothesized that as a result of reduced tillage and deep and dense perennial rooting systems, IWG and IWG-alf system soils would display improved water infiltration capacity, larger labile C and N pools, and improved soil aggregate structure compared to annual systems with and without cover crops after two years of the cropping systems. We also expected that soil quality improvements would be greatest under IWG intercropped with a legume compared to IWG monocrop because root biomass from a intercropping legume would facilitate nutrient cycling associated with biological nitrogen fixation properties, have different soil structure affects from differences in root architecture, and lead to synchronized nutrient demand and uptake through complementary plant phenologies. In contrast, cover cropped and conservation tilled annual systems would not significantly improve soil physical or chemical qualities relative to a conventionally managed annual system on a two-year timescale, as seen in previous short-term studies (Farmaha et al., 2021; Cates et al., 2019; Blanco-Canqui and Ruiz, 2020).

1.3 Materials and Methods

1.3.1 Study Site Description and Experimental Design

The experiment was conducted at the University of Minnesota's Rosemount Research and Outreach Center in Rosemount, MN, USA (44°42'42.3"N 93°06'51.1"W). Before the experiment was established, the field was under cultivation in alfalfa for two growing seasons. Alfalfa was terminated and experiment was fallowed in fall 2018, and planted to oats (*Avena sativa*) for the 2019 growing season. The experimental design was a split-plot, randomized complete block design with five blocks. The split-plot design allowed for climate treatments to be imposed on main plots in 2022 and 2023, after establishment of the cropping system treatments that were imposed on the sub plots. However, the 2020 and 2021 data reported here were collected from sub plots prior to main plot treatment application. Therefore, the experiment as described for this study was conducted as a randomized block design. Each block contained four replicates of each cropping system grown in 6.1 by 6.1 m plots, for a total of 20 replicates per cropping system. The soil type is Waukegan silt loam with 0 to 1 percent slopes (Fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludoll) (Soil Survey Staff, accessed June 8, 2022). From 1991-2020, the mean annual precipitation at the site was 87 cm and mean annual temperature was 6.7 °C (Arguez et al., 2010). Historical average monthly precipitation and growing degree days (GDD) and annual cumulative precipitation and GDD over the course of the experiment are shown in Figure 1.1. Growing degree days for IWG were calculated using the following Equation 1:

$$\text{GDD}_i = \frac{T_{\max}(i) + T_{\min}(i)}{2} - T_{\text{base}}$$

where T_{\max} and T_{\min} are maximum and minimum temperatures for day i and T_{base} is 0°C , which is generally used as the base temperature for IWG (Locatelli et al., 2021; Jungers et al., 2018). We began GDD accumulation after five consecutive days of average daily temperature exceeding 0°C and ended it after eight consecutive days of average daily temperature below -2.2°C . Monthly average temperatures and accumulated precipitation are also shown in Table 1.1. Of note, the field site experienced below average precipitation during the first growing season (2020; total annual accumulation 21% below 30-year average), and a historic drought during the second growing season (2021; total annual accumulation 31% below 30-year average).

Most analyses in this study were performed on all replicates ($n=80$). However, a subset of 10 replicates of each cropping system (two per block) were analyzed for measurements that were especially resource-intensive including soil moisture, soil sorptivity, root biomass, and aggregate stability.

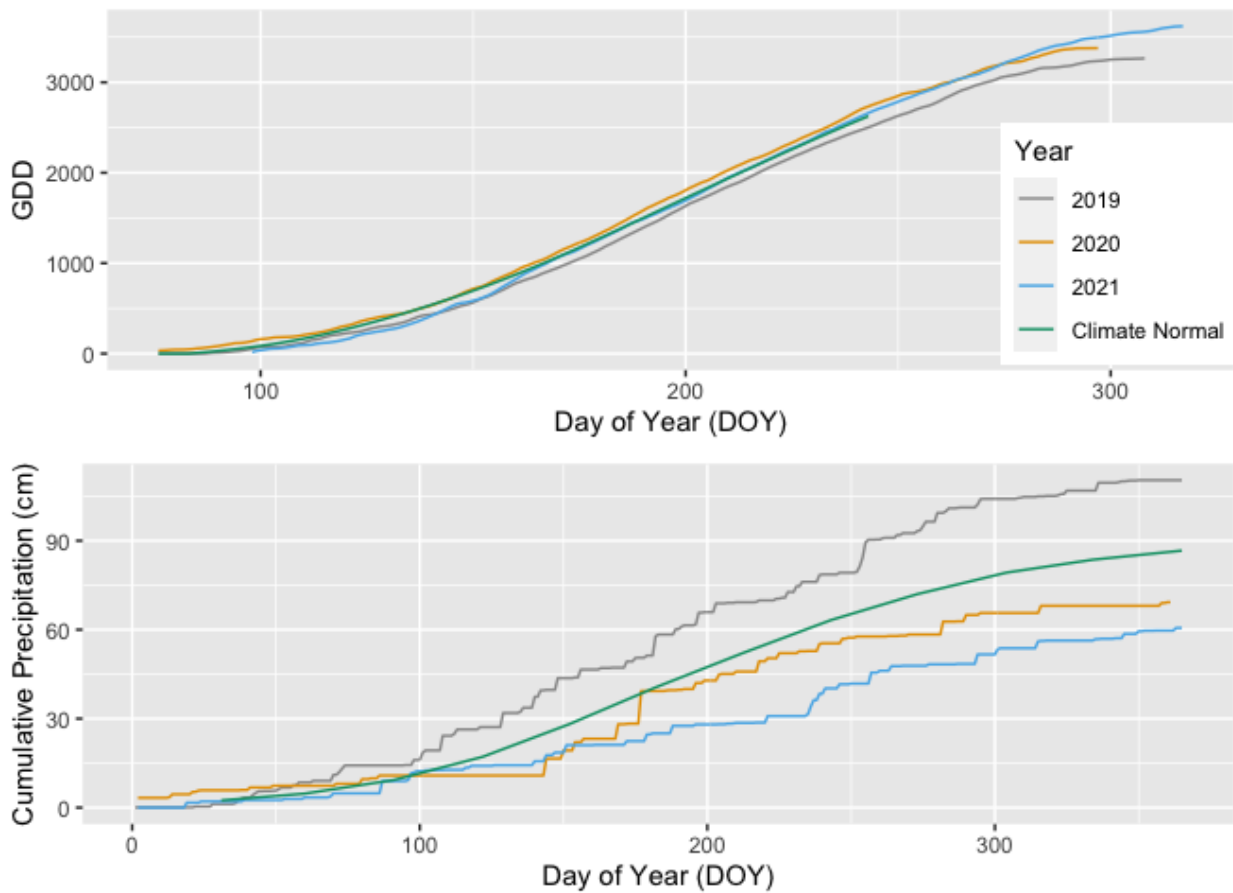


Figure 1.1: Cumulative growing degree days (GDD) and precipitation in 2019 (IWG/alfalfa establishment year), 2020, 2021 (Menne et al., 2012, accessed 10/19/2022), and 1980-2010 climate normals at the Rosemount Research and Outreach Station (NOAA station no. USC00217107)(Arguez et al., 2010, accessed 10/19/2022). Growing degree days were calculated using base temperature 0°C and began accumulating when average daily temperature exceeded base temperature for 5 days.

Month	Average (°C)	2019	2020	2021	30-Year Normal
January	High	-	-3.3	-2.8	-5.4
	Low	-	-11.7	-11.1	-15.9
February	High	-	-2.2	-8.3	-2.8
	Low	-	-15	-17.8	-13.9
March	High	-	4.4	8.9	4.4
	Low	-	-3.9	-1.7	-6.4
April	High	-	17.8	12.8	13.2
	Low	-	5	1.7	0.9
May	High	-	18.9	20	19.9
	Low	-	8.3	8.3	8.1
June	High	-	27.2	30	25.2
	Low	-	15	16.7	13.9
July	High	-	17.2	16.1	16.1
	Low	-	28.9	28.9	27.4
August	High	-	27.2	28.3	26.1
	Low	-	15.6	15.6	14.7
September	High	22.8	20.6	23.9	22.3
	Low	12.2	8.9	10	10.1
October	High	11.7	10	17.8	14.8
	Low	1.7	-0.6	6.1	2.7
November	High	1.1	8.3	-	5.3
	Low	-5.6	-2.8	-	-4.8
December	High	-1.7	0	-	-2.4
	Low	-11.1	-10	-	-11.8

Table 1.1: Monthly average temperatures at Rosemount Research and Outreach Station (NOAA Station # USC00217107) for the duration of the experiment (Menne et al. 2012) and 30-year climate normal temperature at Rosemount Research and Outreach Station from 1991-2020 (Arguez et al., 2010).

1.3.2 Agronomic Methods

We assessed agronomic yields and belowground biomass from the 2020 and 2021 growing seasons. Prior to establishing crops for this study, termination of alfalfa was accomplished using inversion tillage in early August, 2019, followed by discing and field cultivation. IWG, alfalfa, and winter cereal rye (*Secale cereale* L.) were planted on Aug. 5, 2019 with a 1.5 m wide no-till drill (Truax Flex 2, New Hope, MN, USA). Annual systems were in soybean in 2020 and maize in 2021. The winter cover crops were winter rye (*Secale cereale*) after maize and hairy vetch (*Vicia villosa*) after soybean. Annual treatments were tilled using a JD 2305 rototiller and seeded with a walk-behind seeder. Annual-cc treatments were strip tilled each fall and spring for cover crop and maize/soybean establishment using a walk-behind rototiller (30.5 cm width in 2020, 23 cm width in 2021) and seeded with a walk-behind seeder. In the fall, annual crop residue was removed after sampling for yield. Cover crops were terminated with glyphosate application followed by strip-tillage. Row spacing, planting details, and other agronomic management information are shown in Table S1.1. All systems were conventionally managed with annual applications of fertilizer for fertility and herbicides and pesticides for weed and pest management (Tables S1.2a and S1.2b).

1.3.3 Agronomic yield and aboveground biomass

To assess perennial crop establishment, plant counts were taken after fall seedling emergence and spring regrowth; two separate 1-m long sections of planted crop rows in each plot were surveyed. Planted species and weeds were counted in each row section.

Cover crops were harvested for dry matter biomass prior to spring termination. Two 60 cm x 60 cm quadrats covering three rows of cover crop were cut at 7.5 cm height and combined

from each plot. Biomass was dried at 30°C for five days and consistent dry matter weight was obtained. IWG grain and biomass was harvested at physiological maturity (when grain moisture content was approximately 20 to 30%), on August 4 in 2020 and July 21 in 2021 (S1) (Gamble et al., 2015; Heineck et al., 2022). For IWG monoculture, two 81 x 81 cm quadrats covering two rows of the crop were harvested. Within each quadrat, IWG seed heads were cut approximately 3 cm below the lowest spikelet (see Heineck et al. 2022 for description of seed head structure) and aggregated. The remaining vegetative biomass was harvested to a stubble height of 7.5 cm and aggregated by plot. In the intercropping treatments, IWG seed heads were harvested similarly to the IWG monocultures and alfalfa biomass was separated from the remaining IWG biomass in the field. Weed biomass was separated and discarded in the field. Seed heads were weighed at field moisture then dried at 30°C for five days or until we obtained consistent dry matter weight. Grain was removed from the dried seed heads and dehulled using a laboratory scale thresher (Wintersteiger Inc., Salt Lake City, Utah). Threshed grain was separated from chaff using sieves and a fractionating aspirator (Carter Day, Fridley, MN) then weighed for dry matter yield determination. Separated alfalfa and IWG biomass were dried at 30°C for five days, then reweighed for dry matter yield. Harvest index was calculated as the ratio of IWG grain biomass to the sum of IWG grain and vegetative biomass (total aboveground biomass). For soybean yield assessment, two 81 x 81 cm quadrats covering two rows of the crop were harvested and combined from each plot using the following method. Soybeans were harvested at physiological maturity on Sept. 22. Soybean biomass and grain was bagged together in the field and dried at 30°C for five days. Soybean grain was threshed from pods and stem biomass by hand. Remaining biomass was removed by hand and discarded. In 2021, maize was

harvested on Aug. 12 for silage due to drought conditions. For yield assessment, three 1.5 m sections of different rows were harvested, weighed at field moisture, chipped, and a ~500 g sample of chipped biomass was taken for moisture content determination following the same biomass drying protocol as for IWG.

1.3.4 Soil Moisture Profile

Soil moisture along a 1 m depth profile in 10 reps of each treatment was measured every two weeks from July-Nov 2020 and May-Aug 2021 using a Delta-T Devices Ltd PR2 Profile Probe. The probe takes measurements at 10, 20, 30, 40, 60, and 100 cm depths. Soil moisture access tubes were installed between cropping system rows vertically following manufacturer instructions (Delta-T Devices, 2016). To account for soil moisture variability around the moisture access tube, the probe was rotated and three measurements were taken at each tube and averaged. Unfortunately, access tubes could not be installed until July 2020, tubes had to be removed in late August 2021 for field maintenance and tillage, and July 2021 measurements were lost due to equipment malfunction, resulting in uneven sampling across years. Though we are aware of issues with accuracy of soil moisture estimates using default coefficients describing the relationship between soil permittivity as measured by the PR2 Profile probe and soil water content at our field site, we determined that default coefficients were sufficient to describe relative differences between cropping systems and proceeded with default coefficients (Delta-T Devices 2016; Dhakal et al. 2019).

1.3.5 Water Sorptivity

Surface soil water sorptivity was measured in the subset plots on June 22, 2021 to assess the impact of cropping systems on infiltration (Smith, 1999). This method was chosen as it

quickly measures the early stage of water infiltration, when the effect of gravity is negligible, where traditional infiltration flux measurements would have been prohibitively long and water-intensive to take at all replicates. Briefly, a metal ring with a diameter of 15.24 cm was inserted 3 cm deep into the ground between crop rows. 444 ml or 2.54 cm depth of water were added to the ring, and the time it took for water to infiltrate was recorded. This process was repeated twice for each ring location, and measurements were performed in duplicate at each plot. Water sorptivity was calculated as the following, Equation 2:

$$\text{Water Sorptivity (cm/sec}^{0.5}\text{)} = D/T^{0.5}$$

Where D is depth of water (cm) and T is time water takes to infiltrate (seconds).

1.3.6 Soil and root biomass sampling and storage

In 2020, bulk soil samples were taken as baseline data from each plot at 0-15 cm and 15-30 cm depths using a hand soil push probe. 10 subsamples were taken from throughout the crop aisles and inter row and row spaces on August 20 and 25th. Probes were wiped of visible soil between plots. Root biomass and bulk density samples were taken in a randomly chosen subset of half of the plots on Sept. 29 using a hydraulic soil probe (Giddings Machine Co, Windsor, CO) with an internal diameter of 1.9 cm diameter. Samples were taken at depths 0-15, 15-30, 30-45, and 45-60 cm, with three subsamples taken in row, interrow, and shoulder spaces for root samples, and 2 subsamples taken for bulk density samples.

In 2021, the Giddings probe was used to collect bulk soil samples and root biomass samples on August 12 and 13th. For bulk soils, three subsamples from 0-15 and 15-30 cm increments were collected for each plot, one from in-row, row shoulder, and crop aisle. In the

subset of half of the plots, three additional subsamples were taken at 0-15, 15-30, 30-45, 45-60 cm increments for total root biomass.

In both 2020 and 2021, bulk soils were homogenized by hand and refrigerated until subsampled for soil gravimetric moisture; 20-25g subsamples were oven dried at 105°C for at least 24 hours, and weight was used to calculate gravimetric water content (Gardner 2018). The remaining bulk sample was sieved to 8mm, and air-dried at 35°C for several days until reaching a stable weight. A subsample was taken for aggregate stability, and the remainder of the dried sample was ground to 2 mm for total C/N, POX-C, ACE protein, and soil properties assessment by Agvise.

Root samples were frozen at -20°C until processed. Root samples were sorted from soil using a hydropneumatic elutriation system, dried (35°C), cleaned of sand, gravel, and non-root organic material by hand, and weighed (Smucker et al., 1982)

1.3.7 Bulk Density

After sampling with the Giddings probe, baseline bulk density samples were weighed, homogenized, and 20-25 g were subsampled, dried at 105°C for at least 24 h, and weighed again to calculate percent moisture. Moisture content of this subsample was used to calculate the dry weight of the whole bulk density soil samples, and the probe volume (1.9 cm diameter x 15 cm depth = 44.74 cm³) was used to calculate bulk density. Bulk density was measured only in 2020 as a baseline measurement for future years of the experiment.

1.3.8 Basic Soil Properties

Baseline soils sampled and combined from throughout the experimental site before system establishment in May, 2019 had 0.22 % N, 2.04 % C, 18 mg/kg P, 4.3% OM, pH of 6.3,

7.21 mg/kg nitrate-N, 0.03 % S, bulk density 0.91 g/cm³ to 5.2 cm depth and 1.02 g/cm³ to 10.4 cm depth.

In 2020 and 2021, air-dried soil samples from 0-15 and 15-30 cm depth fractions were ground to 2 mm and Agvise labs (Benson, MN) performed basic soil tests including macronutrient, micronutrient, base cations, and soil properties following standard soil testing procedures for the north central region (NCERA, 2015). Soil P was determined via Bray method for pH <7.7 or Olsen method for pH > 7.5.

1.3.9 Total C&N

Dry combustion was used to quantify total soil C and N at 0-15 and 15-30 cm depths fractions (Nelson and Sommers 2018). Soils dried at 35°C and sieved to 2mm were transferred to 1.5 ml microcentrifuge tubes with 3mm steel balls and ground at 1500 rpm for 5 minutes using a 2010 Geno/Grinder (SPEX Sample Prep LLC, NJ, USA). An elemental analyzer was used to assess ~10 mg of ground soil for total C and total N (varioPYRO cube, Elementer Inc, NJ, USA).

1.3.10 POXC

We measured the labile fraction of soil carbon with potential oxidizable carbon (POXC) (Weil et al. 2003; Hurisso et al., 2016). POXC measures a relatively processed pool of labile soil C and is management sensitive (Culman et al., 2012). Briefly, 2.5 g of soil ground to 2mm were added to 0.02 L of 0.2 M KMnO₄ solution, shaken for 2 minutes at 120 rpm, and incubated for an additional 10 minutes. Immediately, 0.5 ml of supernate was sampled and diluted in 49.5 ml water. Absorbance was read at 550 nm using a spectrophotometer plate reader (BioTek® Synergy HT microplate reader). A standard curve of known concentrations of KMnO₄ was created with each batch and used to calculate the mg kg⁻¹ POX-C in soil, assuming 9000 mg C

are oxidized by 1 mole of KMnO_4 changing from Mn^{7+} to Mn^{4+} (Equation 2). A soil standard of similar pH and soil type to the experiment samples were run in each batch to control for batch variability. POXC was calculated using the following, Equation 3:

$$\text{POXC (mg/kg soil)} = [0.02 \text{ M/L} - (a + b \times \text{Abs})] \times (9000 \text{ mg C/M}) \times (0.02 \text{ L} / 0.0025 \text{ kg soil})$$

Where 0.02 M/L = initial solution concentration, a = intercept of the standard curve, b = slope of the standard curve, Abs = sample absorbance, 9000 mg C/M = mg of C oxidized by 1 mole of KMnO_4 changing from $\text{Mn}^{7+} \rightarrow \text{Mn}^{4+}$, and 0.02 L = volume of solution reacted.

1.3.11 ACE Protein

Autoclaved-citrate extractable (ACE) protein is a measure of a broad soil protein pool that potentially indicates the amount of organic N/potentially available organic N in the soil (Hurisso et al. 2018b; Geisseler et al. 2019) and is a commonly used soil health indicator sensitive to crop management practices (Sainju et al., 2022). We assessed ACE protein using a protocol based on Wright and Upadhyaya (1996) with adaptations from Hurisso et al. (2018b). 24 mL 20 mM sodium citrate (pH=7) was added to 6 g of dried soil ground to 2 mm. Samples were shaken at 180 rpm for 5 minutes then autoclaved for 30 minutes on the liquid cycle (121°C). Samples were cooled, sediment was resuspended by vortexing, and 1.75 ml of solution was removed and centrifuged for 3 min at 10,000 xg/RCF. 1 mL of supernate was transferred to cluster tubes and frozen at -20°C for longer term storage. Quantification was performed using a microplate assay method with a Pierce BCA Protein Assay Kit (Thermo Scientific, Catalog number 23227). 10 ul of sample was added to 200 ul Pierce BCA protein reagent, and bovine serum albumin (BSA) standards were run in each plate. Plates were incubated for 1 hr at 61.5°C and read at 562 nm on a spectrophotometer plate reader (BioTek® Synergy HT microplate

reader). A separate quadratic standard curve was created for each plate. ACE protein was calculated as a function of standard curve coefficients, extractant volume, sample weight, and absorbance corrected for background color of the solutions (see Equation 4 and R code available in Appendix). All samples were run in duplicate and a soil standard of similar pH and soil type to our field samples was run in each batch to control for batch variability. ACE protein was calculated using the following, Equation 4:

$$\text{ACE Protein (mg/g soil)} = [(ax^2 + bx + c) \times y \times 1 \text{ mg}] / (z \times 1000 \text{ } \mu\text{g})$$

Where a = coefficient of the x^2 term from the standard curve, b = coefficient of the x term from the standard curve, c = intercept of the standard curve using stock standard values, x = average sample absorbance, y = volume extractant (24 mL in this protocol), and z = grams of soil extracted.

1.2.12 Aggregate Stability

All “aggregates” referred to in results of this study are water-stable aggregates. Water stability of soil aggregates was performed following Soil Health NRCS Technical Note 450-03 (Stott, 2019). In 2020, water stability of soil aggregates was determined only on 0-15 cm soils, while in 2021 we assessed water stability of soil aggregates on both 0-15 and 15-30 cm soils. Briefly, 25 g of air-dried soil sieved to 8mm were pre-wet with capillary action and then run in a Yoder-style wet sieving apparatus with sieves of sizes 2.00 mm, 500 μm , 250 μm , and 53 μm for 10 minutes. Aggregates remaining in each sieve were oven dried at 50°C degrees, weighed, and then rinsed through their respective sieve size. Any sand and gravel not passing through the sieve were collected, dried, weighed, and used to correct the aggregate weight. We present the data both as the weight fraction, the weight of each aggregate size class as a fraction of the original

sample weight, and as the mean weight diameter (MWD) of aggregates. We calculated the MWD using Equation 5:

$$\text{MWD} = \sum_{i=1}^n \underline{X}_i w_i$$

where X_i is the mean diameter of each size fraction (mm) and w_i is the weight fraction of the aggregate size. MWD of the sample is calculated as the sum of MWD of each fraction.

1.2.13 Data Analysis

All analyses were conducted with R version 4.0.2 (R Core Team, 2022). All data and R code are available on [Github](#). Data were assessed for normality of distribution and homogeneity of variance by inspecting histograms and qq-plots and conducting Levene's test using the `car` package (Fox and Weisberg, 2019). IWG system grain yields and all root biomass yields were log-transformed to obtain greater normality. Means in tables referenced in text refer to untransformed values.

Analysis of variance (ANOVA) was performed on agronomic and soil responses using the `lme` command of the `nlme` package followed by a type III/marginal sum of squares ANOVA using `nlme`'s `anova` command (Pinheiro et al., 2022). In agronomic datasets, data were analyzed separately by year to account for differences in annual crops within treatments. Crop system was treated as a fixed effect and block as a random effect. For soils datasets, cropping system, year, and the interaction between cropping system and year were treated as fixed effects and block as a random effect. Where multiple depths were investigated in soils data, each depth was modeled separately because there are well established significant differences in soil physical/chemical properties at depth and relationships between depths were not of primary interest in this study.

Repeated measures ANOVAs were performed on soil moisture data separated by year, with cropping system and sampling date treated as fixed effects and plot nested within block as a random effect. For all datasets, Tukey's post-hoc analysis was performed to determine differences in distributions between cropping systems nested by year using estimated marginal means via the emmeans package (Lenth, 2021). Significant differences for all analyses were determined at $p < 0.05$, and marginally significant differences at $p < 0.10$.

1.4 Results

1.4.1 Agronomic Yields

Perennial crop treatment (IWG vs IWG-alf) significantly affected IWG grain yield and IWG straw yield in both 2020 and 2021 and harvest index and total aboveground biomass in 2020 (Table 1.2.1). IWG grain and total biomass yields were higher in 2021 than in 2020 across both IWG and IWG-alf systems, and mean alfalfa yield was 122% higher in 2020 compared to 2021. In 2020, grain yield in the IWG-alf system was 12% of that of the IWG monoculture system grain yield. In contrast, total aboveground biomass of the IWG-alf was 150% greater than the IWG monoculture biomass yield. In 2021, IWG grain yield was again higher in the IWG monoculture than in the IWG-alf (Table 1.3.1). However, total system biomass was similar across the systems due to the biomass inputs of alfalfa.

In the annual systems, crop treatment (annual vs. annual-cc) affected soybean yield in 2020 and marginally contributed to variance in silage yields in 2021 (Table 1.2.2). However, there were no differences in the means of 2020 soybean yield or 2021 maize silage yield between treatments (Table 1.3.2; Table S1.5). In 2021, silage moisture content was not influenced by crop treatment.

1.4.2 Belowground biomass

Depth ($F = 54.77$, $p < 0.0001$), crop ($F = 35.8243$, $p < 0.0001$), year ($F = 203.86$, $p < 0.0001$), and the interaction between depth and crop ($F = 3.45$, $p = 0.0005$) all affected log-transformed root weight. In 2020, mean root biomass at 0-15 cm depth was four times greater in IWG and IWG-alf compared to annual and annual-cc (Table 1.4). In 2021, root biomass was highest in IWG and IWG-alf compared with the annual and annual-cc. Below the 0-15 cm depth, there were no differences in mean root biomass by cropping system.

		Harvest Index (%)		IWG Grain Yield (kg/ha)		System Total Biomass (kg/ha)		IWG Straw Yield (kg/ha)	
Year	Predictor	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
2020	Crop	18.42	0	5.51	0.025	22.84	0	17.75	0
2021	Crop	0.96	0.334	12.37	0.001	0.84	0.366	19.33	0

Table 1.2.1: Results of mixed-effects ANOVAs with Year and Crop as fixed effects and Block as a random effect. Separate ANOVAs were performed for each year.

Predictor	Soybean Yield (kg/ha)		Silage Yield (kg/ha)		Silage Moisture (%)	
	F-value	p-value	F-value	p-value	F-value	p-value
Crop	3.05	0.09	0.03	0.86	0.15	0.699

Table 1.2.2: Annual systems results of mixed-effects ANOVAs with Crop as a fixed effect and Block as a random effect.

	2020						2021					
	IWG			IWG-alf			IWG			IWG-alf		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	
Harvest Index (%)	10.3	2.1	b	2.8	0.7	a	9.9	1.2	a	9.2	1.8	a
IWG Grain Yield (kg/ha)	361.2	88	b	43.1	12.4	a	466.3	67.2	b	303.5	57.3	a
IWG Straw Yield (kg/ha)	2837.6	408	b	1253.4	170	a	4752.9	431.2	b	3276.2	312.4	a
IWG System Total Biomass (kg/ha)	2837.6	408	a	4281.2	215.1	b	4752.9	431.2	a	4639.6	310.2	a

Table 1.3.1: Means and standard errors of IWG system agronomic metrics. Letters represent significant differences shown by Tukey’s HSD test on estimated marginal means.

	2020						2021					
	Annual			Annual-cc			Annual			Annual-cc		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	
Soybean Yield (kg/ha)	3848.0	267.5		4251.6	130.8		NA	NA		NA	NA	
Silage Biomass (kg/ha)	NA	NA		NA	NA		31710.4	4538.9		36645.8	2617.7	
Silage Moisture Content (%)	NA	NA		NA	NA		76	1		76	1	

Table 1.3.2: Means and standard errors of annual system agronomic metrics. There were no differences in groups shown by Tukey’s HSD.

Year	Depth	Annual			Annual-cc			IWG			IWG-alf		
		Root Biomass	SE		Root Biomass	SE		Root Biomass	SE		Root Biomass	SE	
2020	0-15	0.22	0.02	a	0.35	0.05	a	1.23	0.06	b	1.19	0.1	b
	15-30	0.37	0.05	a	0.15	0.01	a	0.38	0.02	a	0.35	0.02	a
	30-45	0.09	0.01	a	0.15	0.01	a	0.22	0.02	a	0.16	0.01	a
	45-60	0.06	0.01	a	0.12	0.01	a	0.12	0.01	a	0.13	0.01	a
2021	0-15	0.77	0.04	a	0.94	0.03	a	3.37	0.14	c	2.24	0.15	b
	15-30	0.5	0.05	a	0.51	0.02	a	0.92	0.02	a	0.76	0.03	a
	30-45	0.28	0.01	a	0.39	0.02	a	0.64	0.04	a	0.56	0.04	a
	45-60	0.24	0.01	a	0.28	0.01	a	0.37	0.01	a	0.44	0.02	a

Table 1.4: Mean root biomass (Mg/ha) by cropping system and results of Tukey's HSD post-hoc tests. Pairwise comparisons calculated for each depth and year individually

1.4.3 Soil Moisture

Soil moisture varied by depth and sampling date in both years of the experiment (Figure 1.2). Soil moisture varied by sampling date at all depths except 100 cm in both years (Supplementary Table 1.2). In 2020, soil moisture content at 10-30 cm depth varied throughout the season while at 40-60 cm it generally trended lower from June to November. In 2021, soil moisture at all depths except 100 cm trend lower from May to August reflecting little precipitation accumulation in drought conditions.

In both 2020 and 2021, crop did not affect soil moisture across the growing seasons. However, the interaction between crop and date affected soil moisture at 10, 20, 30 and 60 cm in both years (Table 1.5). On individual dates in 2020, IWG frequently had higher soil moisture at depth compared to annual. On July 11 and August 15 and 29th, 2020, IWG had greater soil moisture at 40 cm depth than annual (Figure 1.2; Table S1.5). In contrast to 2020 trends, in June-August 2021, soil moisture trended lower in IWG compared to annual. Soil moisture was higher

in the annual system than in the IWG system at 20 and 30 cm depths on June 9 and June 25, 2021, and also at 60 cm depth on June 25, 2021(Figure 1.2; Table S1.5).

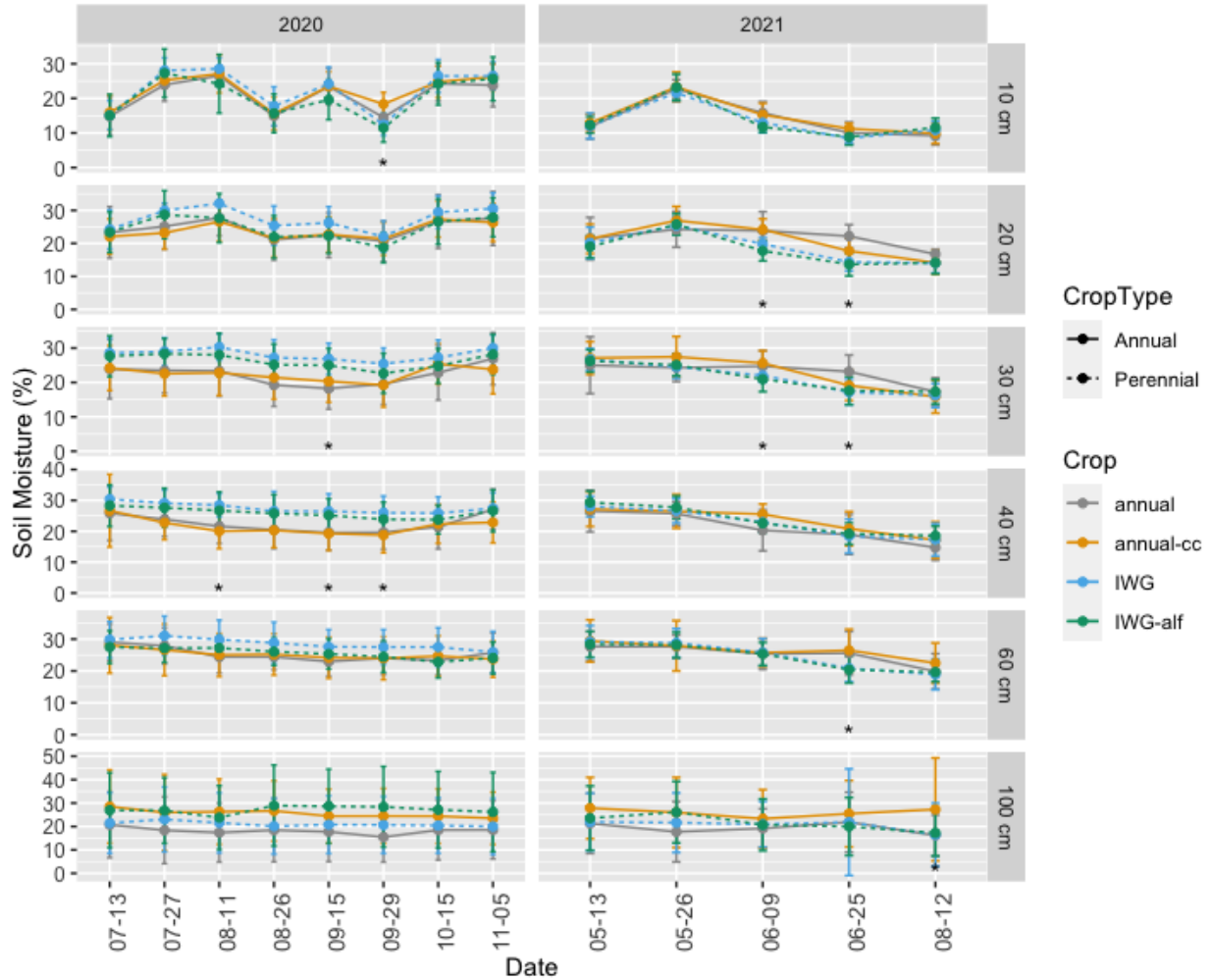


Figure 1.2: Total volumetric soil water content for each cropping system at six soil depths in 2020 and 2021. Error bars represent one standard error of the mean. * Indicates sampling dates with significantly ($p < 0.05$) or marginally significantly ($p < 0.10$) different soil moisture between cropping systems; specific cropping systems and results of comparisons of means tests are available in Supplementary Table 1.4.

Year	Predictor	10 cm		20 cm		30 cm		40 cm		60 cm		100 cm	
		F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
2020	Crop	0.11	0.951	0.19	0.904	0.97	0.419	1.02	0.397	0.26	0.853	1.37	0.266
	Date	26.05	0	12.28	0	9.57	0	6.7	0	7.16	0	0.33	0.94
	Crop:Date	2.68	0	2.34	0.001	1.9	0.012	1.3	0.176	1.5	0.077	0.91	0.576
2021	Crop	0.05	0.985	0.78	0.51	0.41	0.749	0.99	0.407	0.23	0.876	1.27	0.298
	Date	46.77	0	9.58	0	14.49	0	15.4	0	8.05	0	1.49	0.209
	Crop:Date	3.08	0	4.79	0	5.14	0	1.39	0.18	2.05	0.024	1.07	0.393

Table 1.5: Results of mixed-effects with repeated measures ANOVAs of soil moisture profile over time. Crop, date, and the interaction of crop and date were treated as fixed effects and block was treated as a random effect. Separate ANOVAs were run for each year and depth.

Depth Predictor		Total Aggregate Weight Fraction		2 mm Aggregate Weight Fraction		500 um Aggregate Weight Fraction		250 um Aggregate Weight Fraction		53 um Aggregate Weight Fraction		Mean Weight Diameter	
		F-value	p- value	F-value	p- value	F-value	p- value	F-value	p- value	F-value	p- value	F-value	p- value
0-15	Crop	1.04	0.38	1.98	0.12	1.94	0.13	2.44	0.07	1.85	0.15	1.95	0.13
	Year	3.32	0.07	15.07	0	8.47	0	24.99	0	16.66	0	13.39	0
	Crop:Year	0.71	0.55	0.86	0.47	1.76	0.16	0.33	0.81	0.56	0.64	0.76	0.52
15-30	Crop	1.23	0.31	4.04	0.01	3.46	0.03	4.07	0.01	0.01	1	3.17	0.03

Table 1.6: Water stability of soil aggregates results of mixed-effects ANOVAs. For 0-15 cm depth, Crop, Year, and the interaction of Crop and Year are treated as fixed effects and block as a random effect. Aggregate stability on 15-30 cm depth fraction was only determined on 2021 samples, so for 15-30 cm depth, Crop is the sole fixed effect and block is a random effect.

1.4.4 Soil Physical Properties

Total aggregate weight, mean weight diameter of aggregates, and the weight of each aggregate size fraction varied across the two years of the experiment at both 0-15 and 15-30 cm depth (Table 1.6). The interaction of crop and year did not affect MWD or aggregate fractions at 0-15 cm depth, suggesting that all crops responded similarly to the year-to-year changes in growing conditions. Aggregate stability was not assessed at 15-30 cm depth on 2020 samples. At 0-15 cm depth, crop had a marginal effect on the weight of the 250 um fraction aggregate fraction. In 2021, the mean annual 250 um weight fraction (0.14%) was larger than the mean IWG 250 um weight fraction (0.11%). At 15-30 cm depth in 2021, crop had an effect on the size of the 2 mm, 500 um, and 250 um fractions, as well as on total mean weight diameter. At the 15-30 cm depth, IWG had larger 2mm fractions (30%) than annual (21%) and annual-cc (22%) systems, lower 500 um fraction (28%) than annual-cc (33%) systems, and lower 250 um fraction (17%) than annual and annual-cc systems (both 21%) (Table 7). 15-30 cm soil MWD of the IWG (1.93 mm) was higher than MWD of the annual (1.55).

Mean soil sorptivity on June 22, 2021 was lower in IWG plots ($0.15 \text{ cm/sec}^{0.5}$) than in annual ($0.21 \text{ cm/sec}^{0.5}$) and annual-cc ($0.25 \text{ cm/sec}^{0.5}$) plots. While not statistically significant, soil sorptivity in the IWG-alf system ($0.18 \text{ cm/sec}^{0.5}$) also trended lower than in the annual systems.

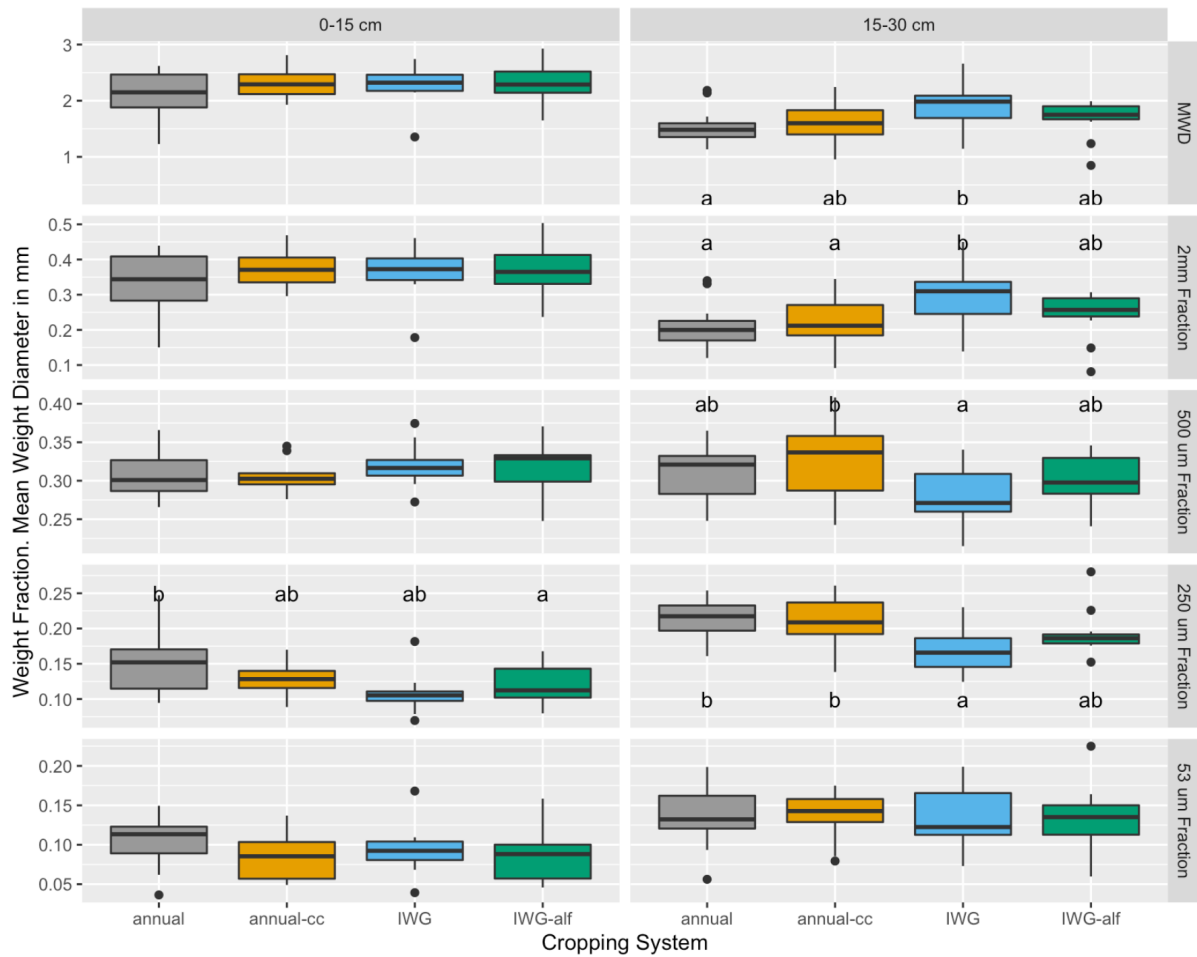


Figure 1.3: 2021 aggregate mean weight diameter and weight fractions at 0-15 and 15-30 cm depth by cropping system. Letters refer to significant differences in means, Tukey's HSD.

Depth	Year	Crop	Total Aggregate Weight Fraction			2 mm Aggregate Weight Fraction			500 um Aggregate Weight Fraction			250 um Aggregate Weight Fraction			53 um Aggregate Weight Fraction			Mean Weight Diameter		
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
0-15	2020	annual	0.96	0.11	a	0.26	0.03	a	0.28	0.03	a	0.28	0.1	a	0.14	0.02	a	1.78	0.17	a
	2020	annual-cc	0.85	0.04	a	0.28	0.02	a	0.31	0.02	a	0.16	0.02	a	0.1	0.02	a	1.84	0.09	a
	2020	IWG	0.9	0.04	a	0.33	0.02	a	0.27	0.02	a	0.14	0.01	a	0.16	0.04	a	2.02	0.12	a
	2020	IWG-alf	0.88	0.02	a	0.33	0.04	a	0.25	0.03	a	0.15	0.02	a	0.14	0.02	a	2.07	0.22	a
	2021	annual	0.89	0.01	a	0.33	0.02	a	0.31	0.01	a	0.15	0.01	b	0.1	0.01	a	2.12	0.11	a
	2021	annual-cc	0.89	0.01	a	0.37	0.02	a	0.31	0.01	a	0.13	0.01	ab	0.09	0.01	a	2.31	0.08	a
	2021	IWG	0.89	0.01	a	0.37	0.02	a	0.32	0.01	a	0.11	0.01	a	0.09	0.01	a	2.29	0.09	a
	2021	IWG-alf	0.89	0.01	a	0.36	0.02	a	0.32	0.01	a	0.12	0.01	ab	0.09	0.01	a	2.28	0.11	a
15-30	2021	annual	0.87	0.01	a	0.21	0.02	a	0.31	0.01	ab	0.21	0.01	b	0.14	0.01	a	1.55	0.09	a
	2021	annual-cc	0.89	0.01	a	0.22	0.02	a	0.33	0.01	b	0.21	0.01	b	0.14	0.01	a	1.6	0.09	ab
	2021	IWG	0.88	0.01	a	0.3	0.02	b	0.28	0.01	a	0.17	0.01	a	0.13	0.01	a	1.93	0.11	b
	2021	IWG-alf	0.87	0.01	a	0.24	0.02	ab	0.3	0.01	ab	0.19	0.01	ab	0.13	0.01	a	1.68	0.1	ab

Table 1.7: Means and standard errors of water stability of soil aggregates by cropping system, year, and depth. Letters represent results of Tukey's HSD tests comparing means of aggregate fractions by cropping system.

1.4.5 Soil Chemical Properties

Crop had marginal effects on Total C ($F= 2.42$, $p = 0.07$) at 0-15 cm depth and affected Total C ($F=2.95$, $p=0.03$) at 15-30 cm depth. Crop had marginal effects on total N ($F = 2.28$, $p =0.08$) at 15-30 cm depth (Table 1.9). There were no differences in total C at 0-15 cm depth between cropping systems in either 2020 or 2021. However, total C trended lower in both IWG systems compared to annuals in 2020 and 2021 (Table 8). At 15-30 cm depth, total soil C was higher in annual (2.27%) compared to IWG (2.08%) systems in 2020 but not 2021. In 2021 at 0-15 cm depth, total N was higher in annual systems (2.7 g/kg) than the IWG systems (2.5 g/kg).

There were no differences in mean distribution of POXC, a labile carbon fraction, across cropping systems or year. However, there was a marginal influence of crop on ACE protein at 0-15 cm depth ($F = 2.5$, $p = 0.06$), and crop and year interaction on ACE protein ($F=2.55$, $p = 0.06$) at 15-30 cm depth. ACE protein trended higher in IWG-alf systems, though there were no pairwise differences in means between cropping systems.

Crop affected soil P at 15-30 cm ($F = 4.23$, $p = 0.01$) depth, soil K at 15-30 cm depth ($F = 6.01$, $p < 0.01$), and soil pH at 0-15 ($F = 11.0$, $p <0.01$) and 15-30 ($F = 2.73$, $p = 0.05$) depths. The interaction between crop and year also affected soil K at both depths (0-15 $F = 8.48$, $P<0.01$ and 15-30 $F= 5.67$, $p <0.01$) and marginally affected soil P ($F = 2.53$, $p = 0.06$) at 15-30 cm depth and pH ($F = 2.23$, $p = 0.09$) at 0-15 cm depth. At 0-15 cm, soil K did not differ between individual systems in 2020 but in 2021 was higher in both IWG and IWG-cc systems (122 and 123 mg/kg) than annual and annual-cc systems (105 and 100 mg/kg). Conversely, soil K at 15-30 cm depth in 2020 was lower in both IWG and IWG-alf systems (71 and 75 mg/kg) than in the annual system (81 mg/kg). Soil P was lower at 15-30 cm depth in 2020 in IWG and IWG-alf (10.4 and 10.8 mg/kg) than in annual systems (12.95). In 2021 at 0-15 cm, soil pH was higher in IWG (7.34) and IWG-alf (7.2) systems than annual-cc (6.96), and higher in IWG than annual

(7.13). Differences in pH emerging in 2021 appear to solely reflect a change in the annual systems; annual and annual-cc system pH decreased between 2020 (7.28 and 7.19) and 2021 (7.13 and 6.96), while IWG-alf pH decreased slightly (7.3 to 7.20) and IWG system pH did not change (7.34). For all other soil macro and micro-nutrients, there were no differences in group mean distributions by crop or between means of individual cropping systems.

There was a significant or marginally significant difference in the mean values of almost all of the soil chemical attributes tested between years 2020 and 2021 at both 0-15 and 15-30 cm depths, with the exception of Total C, Total N, and Ca. However, there was no significant interaction between cropping system and year for most of these soil chemical attributes, with the exception of K, P, and ACE, as discussed above.

Depth	Year	Crop	Total C (Weight %)			Total N (Weight %)			CN Ratio			ACE Protein (mg/g soil)			POXC (mg/kg soil)			OM (Weight %)		
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
0-15	2020	annual	2.8	0.0	a	0.3	0.0	a	10.9	0.1	a	5.7	0.2	a	649.7	23.6	a	4.3	0.1	a
		annual-cc	2.8	0.0	a	0.3	0.0	a	10.8	0.1	a	5.6	0.1	a	692.6	13.3	a	4.4	0.1	a
		IWG	2.7	0.0	a	0.3	0.0	a	10.8	0.1	a	5.5	0.1	a	663.9	20.9	a	4.4	0.1	a
		IWG-alf	2.7	0.0	a	0.3	0.0	a	10.9	0.1	a	5.7	0.1	a	681.7	18.1	a	4.4	0.1	a
	2021	annual	2.9	0.0	a	0.3	0.0	b	10.8	0.1	a	5.2	0.1	a	758.2	5.1	a	4.3	0.0	a
		annual-cc	2.9	0.1	a	0.3	0.0	ab	10.9	0.1	a	5.4	0.1	a	754.6	4.5	a	4.3	0.0	a
		IWG	2.7	0.1	a	0.2	0.0	a	11.0	0.1	a	5.3	0.1	a	751.4	6.6	a	4.3	0.1	a
		IWG-alf	2.8	0.1	a	0.3	0.0	ab	11.1	0.1	a	5.6	0.1	a	763.8	6.6	a	4.4	0.0	a
15-30	2020	annual	2.3	0.1	b	0.2	0.0	a	10.7	0.1	a	4.2	0.1	a	525.9	32.2	a	3.8	0.1	a
		annual-cc	2.2	0.1	ab	0.2	0.0	a	10.5	0.1	a	4.1	0.1	a	522.4	29.8	a	3.7	0.1	a
		IWG	2.1	0.1	a	0.2	0.0	a	10.5	0.2	a	3.8	0.1	a	474.4	33.8	a	3.7	0.1	a
		IWG-alf	2.3	0.1	ab	0.2	0.0	a	10.8	0.1	a	4.2	0.2	a	469.2	26.7	a	3.8	0.1	a
	2021	annual	2.2	0.1	a	0.2	0.0	a	10.9	0.1	a	3.7	0.1	a	691.2	8.5	a	3.6	0.1	a
		annual-cc	2.2	0.1	a	0.2	0.0	a	10.9	0.1	a	3.9	0.2	a	688.4	9.1	a	3.6	0.1	a
		IWG	2.2	0.1	a	0.2	0.0	a	11.0	0.1	a	3.9	0.1	a	686.2	8.1	a	3.6	0.1	a
		IWG-alf	2.3	0.1	a	0.2	0.0	a	10.9	0.1	a	4.0	0.2	a	698.5	10.3	a	3.7	0.1	a

Table 1.8.1: Means and standard errors of soil chemical properties by cropping system, year, and depth. Letters represent results of

Tukey's HSD tests comparing means of aggregate fractions by cropping system.

Depth	Year	Crop	pH			CEC (meq)			P (mg/kg soil)			K (mg/kg soil)			Ca (mg/kg soil)			Mg (mg/kg soil)			Na (mg/kg soil)		
			Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
0-15	2020	annual	7.3	0.1	a	17.6	0.2	a	17.6	1.3	a	124.1	2.9	a	2756.4	49.6	a	404.0	7.3	a	18.4	0.7	a
		annual-cc	7.2	0.0	a	17.3	0.3	a	13.8	0.8	a	122.1	4.2	a	2682.8	53.8	a	404.8	4.4	a	17.2	0.5	a
		IWG	7.3	0.0	a	17.1	0.3	a	14.8	0.7	a	116.4	4.2	a	2669.9	51.1	a	396.3	8.4	a	17.7	0.8	a
		IWG-alf	7.3	0.0	a	17.1	0.2	a	15.4	1.3	a	117.6	4.3	a	2672.4	40.2	a	396.6	7.3	a	17.5	0.6	a
	2021	annual	7.1	0.1	ab	17.9	0.2	a	19.5	1.6	a	104.8	2.6	a	2753.1	47.3	a	424.5	7.6	a	12.9	0.4	a
		annual-cc	7.0	0.1	a	17.3	0.2	a	17.9	1.6	a	99.9	2.5	a	2625.7	45.7	a	417.6	6.6	a	13.6	0.6	a
		IWG	7.3	0.0	c	17.5	0.3	a	15.2	0.8	a	122.1	4.9	b	2728.2	66.6	a	415.9	8.2	a	13.9	0.7	a
		IWG-alf	7.2	0.1	bc	17.6	0.3	a	15.5	1.1	a	123.1	4.8	b	2714.4	48.2	a	420.8	6.7	a	13.7	0.5	a
15-30	2020	annual	6.7	0.1	a	15.9	0.2	a	13.0	0.5	b	81.3	3.1	b	2265.4	57.9	a	406.0	6.4	a	21.2	0.7	a
		annual-cc	6.6	0.0	a	15.9	0.2	a	11.4	0.7	ab	76.9	1.5	ab	2192.0	28.8	a	417.8	4.1	a	20.3	0.5	a
		IWG	6.7	0.1	a	15.4	0.2	a	10.4	0.5	a	71.0	1.6	a	2181.6	46.8	a	405.4	6.3	a	21.5	0.8	a
		IWG-alf	6.7	0.1	a	15.6	0.2	a	10.8	0.5	a	74.6	1.4	a	2204.7	29.0	a	408.9	5.9	a	21.1	0.8	a
	2021	annual	6.6	0.1	a	16.2	0.2	a	10.6	0.7	a	67.9	1.3	a	2215.7	35.6	a	436.0	9.0	a	17.0	0.6	a
		annual-cc	6.5	0.0	a	16.0	0.2	a	9.2	0.6	a	67.2	1.2	a	2153.4	27.6	a	443.1	6.1	a	17.2	0.7	a
		IWG	6.6	0.1	a	16.2	0.3	a	10.4	0.6	a	70.9	1.8	a	2233.8	59.8	a	447.8	7.5	a	18.5	0.7	a
		IWG-alf	6.5	0.1	a	16.1	0.2	a	10.5	0.5	a	70.5	1.7	a	2183.2	28.0	a	443.7	6.5	a	17.0	0.4	a

Table 1.8.2: Means and standard errors of soil chemical properties by cropping system, year, and depth, continued. Letters represent

results of Tukey's HSD tests comparing means of aggregate fractions by cropping system.

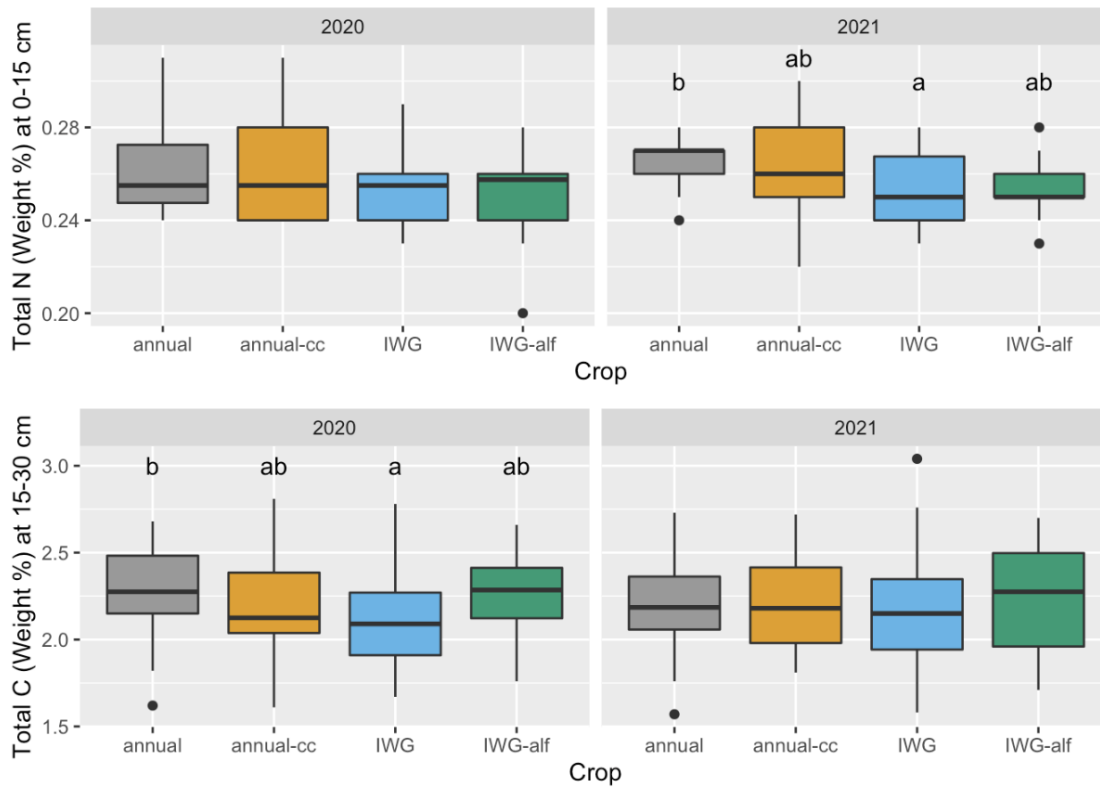


Figure 1.4: Boxplots of Total C and Total N at depths where there were differences between cropping systems. Letters refer to statistically significant differences in means, Tukey's HSD.

1.5 Discussion

Current agricultural production systems are especially vulnerable to yield decline and climate extremes because of poor soil health caused by decades of soil-degrading agricultural management practices (Oldfield et al., 2019; Gaudin et al., 2015; Williams et al., 2016; Kane et al., 2021). We explored the capacity of annual cover crops combined with reduced tillage, and the perennial grain and forage crop IWG, and IWG with legume intercropping to improve soil physical structure and soil nutrient and C pools. In line with our hypotheses, we observed increased root biomass, increased water stable aggregate size, more stable pH and K, and

differences in soil water content by depth and sampling time in IWG systems relative to maize-soybean annual systems after two years. We also observed no differences in soil quality between IWG and IWG-alf systems or annual and annual-cc systems, respectively, suggesting that within a given annual or perennial crop regime, diversification and reduced soil disturbance do little to enhance soil quality on a two year timescale.

1.5.1 Crop Yield

Modern annual cropping systems produce high per acre yields, but the intensive tillage, lack of plant functional diversity, high fertilizer requirements, seasonal bare soil, and short and shallow root systems inherent to these systems have negative impacts on soils and the environment (Van Oost et al., 2006; Tamburini et al., 2020). While perennial IWG and IWG-legume bioculture systems do not have these negative management attributes, they currently produce much lower grain yields (Law et al., 2022b). In this study, we did not assess yield differences or conduct an economic comparison of annual maize/soybean and IWG treatments. We did, however, investigate whether IWG vs. IWG-alf and annual vs. annual-cc systems produced relatively different yields and aboveground biomass, and assessed differences in belowground biomass between all four cropping systems.

Intercropping IWG with perennial legumes is a promising management strategy that increases crop system diversity, reduces fertilizer needs due to the fertilizer equivalency of the legume crop (Ryan et al., 2018), and improves the quality and quantity of forage in systems managed for dual-use grain and forage production. Previous studies have found that planting a perennial legume intercrop such as alfalfa between rows of IWG can sometimes lead to grain yield reductions of ~30% (Crews et al., 2022) to 60-80% (Tautges et al., 2018) in stand year 1, when competition from legume intercrops may outweigh the positive effects of N fixation and

transfer on grain yields (Reilly et al., 2022b; Crews et al., 2022; Hayes et al., 2017). In later years, grain yields are generally comparable or slightly higher in the intercropped systems (Crews et al., 2022; Tautges et al., 2018). However, other studies in various sites have found that grain yield is not lower in IWG-legume biculture systems even in the establishment year (ex. Reilly et al., 2022b; Dick et al., 2018; Law et al., 2022 a). We found that in Year 1, average grain yield in IWG-alf systems was only 12% of that of IWG monocultures, and in Year 2 it was 65% that of IWG monocultures. Alfalfa accounted for about 70% of the aboveground biomass of the IWG-alf system in Year 1, which likely severely limited IWG tillering and grain production. Agronomic approaches to identify and manage the relative abundance of species in a perennial grain/legume intercrop are needed. Innovative crop establishment strategies such as seeding alfalfa directly into an existing IWG stand could also promote IWG populations in an intercropping environment and potentially mitigate grain yield losses as part of a longer-term perennial crop rotation.

Despite grain yield reductions, we found that the total system aboveground biomass (i.e. forage yield) was 50% greater in IWG-alf systems compared to IWG systems in Year 1 though similar across systems in Year 2. This is similar to other findings of IWG legume intercrops managed for forage or dual-use grain and forage production (Ryan et al., 2018; Reilly et al., 2022b; Law et al., 2022a). Since ~50% of revenue in a dual-use managed system can come from forage sales (Law et al., 2022a), the economic loss of potentially lower grain yield in a biculture system could be overcome by the sale of more and higher-quality forage that we and other researchers have documented (Law et al., 2022a). For example, Law et al. (2022a) found that forage revenue increased ~28% in an IWG-red clover intercropped system compared with IWG monocrop. Additionally, legume intercrops may reduce fertilizer requirements (Hayes et al.,

2016; Crews et al., 2022; Reilly et al., 2022b). Recent research has estimated that alfalfa intercrop in IWG fixes 54-162 kg N ha⁻¹ yr⁻¹ (Crews et al, 2022) and several studies have demonstrated evidence of legume-fixed N transfer to IWG two and three years after system establishment (Reilly et al., 2022b; Crews et al., 2022). Finally, Law et al (2022b) demonstrate perennial weed suppression by legume intercrops, which may result in reductions in herbicide and other weed management costs.

Average soybean yield in 2020 was 3848 kg/ha in the annual treatment and 4251.6 kg/ha in the annual-cc treatment, which was comparable to the 2020 Dakota county average of 3,573 kg/ha (USDA, 2021). In 2021, Dakota county average maize silage yield was 60,526 kg/ha, almost twice as much as our wet yields of 31,710 kg/ha (annual) and 36646 (annual-cc) (USDA, 2022), perhaps because much of the silage corn in our area is irrigated and we harvested a bit late. We found no differences in 2020 soybean or 2021 maize silage yields between annual and annual-cc systems, suggesting that cover cropping and reduced tillage can be adopted without the expectation of positive or negative short-term yield impacts. Previous meta-analyses have similarly shown that winter cover cropping has either very little mixed effects or no effect on the yield of fertilized grain systems (Abdalla et al., 2019; Bergtold et al., 2019; Miguez and Bollero, 2005) especially when using chemical and mechanical weed control methods (Osipitan et al., 2018). Long-term conservation tillage may convey small but positive improvements in maize (~3%) and soybean (~1%) yields throughout the US corn belt (Deines et al., 2019). Any potential yield impacts of our two annual system management treatments will therefore likely take longer than two years to accumulate and will likely be minimal.

1.5.2 Root biomass

High root biomass is an important trait of perennial crops including IWG because it drives improved ecosystem services such as drastically reduced nitrate leaching relative to annual counterparts (Reilly et al., 2022a; Jungers et al., 2019; Bergquist, 2019). Increased root biomass and deeper root systems may also facilitate improvements in soil physical quality as root channels increase soil infiltration and storage capacity (Jotisankasa and Sirirattanachat, 2017; Guo et al., 2019; Liu et al., 2020) and root exudates and higher fungal biomass lead to increased soil aggregation (Rillig et al., 2015; Baumert et al., 2018). Finally, deep root biomass also allows for deep soil water use (Maeght et al., 2013). In both the first and second years of production, we find that IWG and IWG-alf systems have more than 4 times the root biomass of annual and annual-cc systems in the top 0-15 cm of soil, but that there are no significant differences in root biomass at deeper depths. These differences between annual and IWG systems are in line with previous results (Sprunger et al., 2019; Bergquist, 2019), and IWG is known to produce 60–75% of its roots in the upper 15- to 20 cm below the soil surface (Sainju et al., 2017; Bergquist, 2019; Dobbratz, 2019). In addition, we found that surface 0-15 cm soil root biomass was greater in IWG than IWG-alf systems in 2021. This is likely a result of known differences in the root architectures of IWG and alfalfa; alfalfa typically has a deep taproot while IWG has a diffuse and relatively shallower root structure (Clément et al., 2022; Rakkar et al., 2023). In 2021, we saw trending increases in root biomass in IWG systems at 15-60 cm depth, likely enabled by an additional growing season for plant establishment and driven by the IWG shifting resources deeper in the soil in response to drought stress (Maeght et al., 2013).

1.5.3 Soil Moisture Profile

We found contrasting soil water profile dynamics between annual and IWG crops throughout 2020 and 2021 which suggest an influence of crop type, plant physiological stage, and drought on evapotranspiration, soil water content, and water drainage. Similar to previous studies (Reilly et al., 2022a; McIsaac et al., 2010), we found ~20-40% greater soil water content under IWG compared to annual at 20-60 cm depths from August-September 2020 (Figure 1.2; Table S1.5), suggesting relatively higher mid-season water use by annual crops in their vegetative stage compared to IWG post-anthesis. We also found that in June 2021, soil moisture content was ~20-40% lower in IWG compared to annual systems at 10, 20, 30 and 60 cm depths, echoing previous research documenting higher water use by IWG during its vegetative stage (Reilly et al., 2022a; McIsaac et al., 2010) and potentially high water use at depth. While de Oliveira et al. (2018) report that annual evapotranspiration from IWG systems is ~ 7% higher than that of maize and ~24% higher than that of soybean, IWG has highest evapotranspiration rates (de Oliveira et al., 2020) and soil water use during its reproductive stage, which occurs mid-May to early June, and during vegetative regrowth that occurs from September to the onset of winter, when IWG can produce 3,000 kg of biomass per hectare (Hunter et al., 2020). It is fairly efficient with water use throughout July and August, when heat and drought stress tend to be more prevalent in the midwest (Mårtensson et al., 2022). In contrast, rainfed soybean and maize tend to reach maximum evapotranspiration rates about 60 days after planting, in mid-July, and maintain this high evapotranspiration for several months (Suyker and Verma, 2009), which as we saw may result in low soil moisture levels and higher water stress during this more drought-prone season. In climates with frequent water stress/drought in the summer, IWG may be more drought resilient than annual counterparts due to the timing of its water use in spring and fall when water stress is less prevalent.

Beyond physiological differences driving water use differences through time, we found some evidence of increased ability to access water deep in the soil profile in IWG systems under drought conditions. In addition to the June 2021 water content differences between IWG and annual referenced earlier, in August 2021, soil moisture content was significantly greater in annual (28%) vs. IWG systems (17%) at 100 cm depth and also trended greater at 60 cm depth. This suggests deep water use by IWG systems. In contrast, soil moisture content at depth in August 2020, a more average precipitation season, was not statistically different among annual and IWG systems, suggesting that deep soil water use by IWG may have been driven by water stress. Several studies have also reported lower soil water content under IWG compared to wheat (Culman et al., 2013), maize, and switchgrass (Jungers et al., 2019) throughout the growing season in coarse-structure soils more prone to water stress, especially at 50-100 cm depths. Increased water uptake by IWG is potentially beneficial for yield stability in drought or in conditions where nutrient leaching is of concern. It also could be detrimental in dry areas where soil water deficits persist between growing seasons and a greater total annual water use could deplete water reserves, limiting perennial crop persistence or future annual yields (Ryan et al., 2018).

Finally, in 2020 during mild drought, the IWG system generally had trending higher mean soil moisture content than the IWG-alf system (Fig. 2). Though IWG roots likely uptake more water relative to alfalfa down to 1-m depth (Clément et al., 2022), it is unsurprising that the IWG-alf system generally used more water than the IWG monoculture system because the biculture system produced ~50% more biomass than the monoculture system that year, requiring more water resources in total. Mårtensson et al., (2022) demonstrate that under drought conditions, there is indication of higher water use efficiency (WUE) by IWG in biculture IWG-

alf system than monoculture IWG system, suggesting that alfalfa intercropping may protect the system from heat stress and allow for more efficient water use and higher biomass yields (Hatfield and Dold, 2019). In 2021, during a severe drought, biomass yields were not significantly different between IWG and IWG-alf systems and there were no clear differences in soil moisture. In summary, the rooting depth and structure of IWG may be better able to access needed water and therefore be more resilient to drought than annual systems, but the severity of drought may determine whether IWG bicultures are preferable to monocultures in terms of cropping system water use.

1.5.4 Soil Physical Properties

Higher wet soil aggregate size and stability are desirable because they suggest resistance to water erosion and soil crusting (Amézketa, 1999), and are often associated with desirable increases in soil porosity, decreased greenhouse gas emissions, and increased SOM storage (Rabot et al., 2018). Aggregation is heavily tied to inherent soil properties such as texture and is generally improved by management practices that reduce soil structural disturbance, surface erodibility, and freeze-thaw consolidation (Leuther and Schlüter, 2021). It is also strongly driven by plant root traits and associated arbuscular mycorrhizal fungal traits in soil microbial communities (Rillig et al., 2015). For example, the polysaccharides excreted by roots have strong soil-binding properties (Galloway et al., 2020). We observed a 18-24% larger small macroaggregate pool (500 and 250 μm), 42% smaller large macroaggregate pool (2 mm), and overall 25% lower MWD in annual compared to IWG systems two years after establishment. Rakkar et al. (2023) similarly found that in soils across MN, after two years maize/soybean rotational systems frequently had 35-50% smaller aggregate mean weight diameter than IWG systems, driven both by smaller large aggregate fraction and a greater small aggregate fraction.

Meta-analyses have previously established that perennial and no-till management tends to improve aggregate stability relative to moldboard plow and chisel plow management throughout the soil profile and across soil types (Nunes et al., 2020). The minimal soil disturbance, dense roots and associated increased root exudates, and enhanced fungal communities of IWG systems will likely lead to more significantly improved soil aggregation over management periods longer than two years as in other perennial grass systems (Chantigny et al., 1997).

Notably, our annual treatment with strip-tillage and cover-cropping did not facilitate the same increases in soil aggregation as the IWG systems relative to the conventionally managed system. No-till management improves aggregate stability in the long run, especially in combination with cover cropping, but increases generally take at least three years to reach significant thresholds (Nunes et al., 2020). Similarly, cover cropping can improve wet aggregate stability in some soils, but effects generally take more than 2 years of management, and effects frequently do not last after cover crop termination, especially in tilled systems (Blanco-Canqui and Jasa, 2020). Therefore, it is unsurprising that we saw no significant differences between our two annual treatments. This indicates that IWG systems improve soil physical structure more quickly than cover-cropping and strip-tillage, eclipsing this positive benefit of sustainable annual management practices.

Contrary to our hypothesis, surface sorptivity was significantly lower in IWG compared to annual treatment. Sorptivity is a measure of soil's ability to rapidly absorb or desorb water by capillary action, the driving force of initial infiltration rates before gravity becomes a major factor (Smith 1999; Minasny and Cook, 2011). We measured soil sorptivity as a proxy for overall infiltration, as the two are generally correlated (Smith, 1999). Soil infiltration is known to increase in perennial management due to vegetative cover, the absence of tillage, and continuous

roots facilitating greater pore space and decreased soil crust formation (Basche and DeLonge, 2019). While soil sorptivity under annual management can temporarily increase due to tillage, conservation tillage, and even no-till seeding due to loosening of soil, it then tends to decrease through the growing season due to settling, compaction, and soil crusting (Villarreal et al., 2017; Jakab et al., 2017). Perhaps we observed lower surface soil sorptivity in IWG systems because the annual systems were plowed on 5/12/2021, a little more than a month before sorptivity measurements were taken, and there had been very little rain since annual tillage/planting to cause surface soil crusts to re-form (Figure 1.1). Indeed, Rakkar et al. (2022) found that surface soil sorptivity trended greater in IWG systems than in maize-soybean systems in Oct. two years after system establishment at multiple MN sites including Rosemount.

Generally in the long-term, infiltration is influenced more by subsurface conditions than soil surface conditions, and tillage-induced temporary increases in porosity are not as effective as natural structure-forming processes at increasing infiltration rates (Jakab et al., 2017; Bormann and Klasse, 2008). Therefore, we may expect to see greater sorptivity in IWG systems and annual-cc systems compared to annual systems later in the growing season after more compaction or in a wetter year, or see overall higher infiltration rates under full infiltration measurements. Future research on soil quality in perennial grain systems should sample infiltration throughout the growing season to capture significant intra-annual variation (Bertoni et al., 1958) and could attempt to explore the influence that drought and rainfall timing have on infiltration and soil erosion.

1.5.5 Soil Chemical Properties

Soil nutrient availability, a factor of both nutrient concentration and soil pH, controls agronomic productivity and is affected by agricultural management practices. Our data suggest

that IWG systems may maintain surface-soil neutral pH and high soil K levels better than annual maize/soybean systems. We found that in the second year of cropping system treatments, the annual systems' soil pH and K dropped significantly compared to the IWG systems' at 0-15 cm depth. These results are similar to those of Rakkar et al. (2023), who found lower pH and soil K levels in wheat systems compared to IWG systems after two years. We find no differences in K levels between IWG and IWG-alf systems after two years, suggesting that this more diverse system offers the same K stability as a monoculture system. We find this somewhat surprising given alfalfa is known to use large amounts of K relative to annual and annual forage crops, but we do not know of research quantifying K content of IWG forage tissues. Our results therefore highlight a need for future research investigating macronutrient concentrations in IWG tissues and K dynamics in IWG intercropped systems. The relative differences in pH change between annual and IWG systems are likely driven by fertilizer application; IWG monoculture systems in 2021 received almost half of the amount of urea fertilizer (80 kg/ha) as annual systems (140 kg/ha), and IWG-alf systems received none, in-line with established fertilizer recommendations (Jungers et al., 2017; Fernandez et al., 2020). Urea fertilizer application is known to be slightly acidifying to soils due to the nitrification of ammonium-N (ex. Bouman et al., 1995). In our relatively neutral and K-abundant soils, these slight decreases in pH and K availability would not be expected to impact annual system productivity. However, IWG could be a better management system to maintain neutral pH and soil K levels compared to a continuous annual maize/soybean rotation. For all other tested soil chemical attributes, the difference between years is likely due to drought conditions experienced in 2021.

We observed lower total soil C levels in IWG compared to annual in year 1 (2020) at the 15-30 cm depth, but this difference did not persist into year 2 (2021). Changes in total soil C are

not often observed when measured in consecutive years, thus these results were likely a type I error. However, Audu et al. (2022) found similar trends in reduced topsoil organic carbon stocks two years after IWG system establishment. This could be due to rhizosphere priming in the soil system, whereby increased IWG root biomass and root exudates lead to more active heterotrophic soil bacterial communities and temporarily lower total soil C (Cheng et al., 2013; Kuzyakov, 2010). Generally, total SOC is not very responsive to agricultural management practices in the short-term. Previous studies in IWG systems have seen few changes in this pool in surface soils in response to management over ~2-3 years or more (Sprunger et al., 2018; Sprunger et al., 2019; Rakkar et al., 2023), though some researchers have found increased SOC at 30-60 cm depth two years after IWG system establishment (Audu et al., 2022). On a decadal scale, conversion to unmanaged perennial vegetation or cellulosic biofuel crops increases SOC storage relative to annual crops (Paustian et al., 2019). Ledo et al. (2020) found that conversion from annual to a perennial crops increased SOC storage on average 11% over 100 cm profile in a 20-year period. However, in the case of IWG grain and other perennial crops grown for food which will likely be terminated and rotated after 3-5 years for yield maintenance and pest management, it remains to be seen whether SOC can accumulate in perennial rotations with more frequent disturbance intervals.

Several studies have found labile C fractions to be more responsive than total C fractions to perennial grain management, with early accumulation in the labile POXC (Sprunger and Robertson, 2018; Sprunger et al., 2019; van der Pol et al., 2022a), potentially mineralizable C (Culman et al., 2013), and particulate organic matter C fractions (Audu et al., 2022) in a span of several years. While not accompanied by increases in total soil C at the studied depths, increases in these fractions suggest enhanced nutrient cycling by microbial communities that may drive

increases in SOM over longer timescales (Culman et al., 2012; Hurisso et al., 2016). However, other studies in other locations have found limited positive responses in labile C fractions under 2-4 years of IWG vs. annual management (Rakkar et al., 2023; Sprunger et al., 2017; Sprunger et al., 2019b). We did not see responses in POXC or ACE protein (labile N) pools to perennial vs annual crop systems, potentially due to the initial relatively high fertility and SOC content of our experimental soils. Given the growing body of literature demonstrating few effects on total SOC and mixed effects on labile C pools in short-term 2-4 year rotations, future research seeking to investigate the C storage potential of perennial grain systems should perhaps focus on longer-term experiments with perennialized rotations, minimal-disturbance crop termination and establishment methods, and assessing SOC stocks in the whole soil profile, not just in top soils (Button et al., 2022; Ledo et al., 2020).

1.5.6 Soil property differences in IWG vs. IWG-alf treatments

We expected to see increased ecosystem and soil physical and fertility co-benefits under IWG-legume intercropping driven by increased system diversity and facultative root activity (Ryan et al., 2018; Duchene et al., 2020). Diversifying crop rotations also results in modest improvements in soil physical and chemical qualities (Beillouin et al., 2021) and increased soil organic carbon storage (Young et al., 2021), while diversifying with intercropping offers more significant soil improvements through complementary facilitation processes (Beillouin et al., 2021; Duchene et al., 2020). However, in the two years of this experiment, we found no evidence of differences or relative improvements in soil quality between IWG monoculture and IWG-alf intercrop systems. Significant benefits may accumulate on longer, decadal timescales (Ledo et al., 2020) and additional research is needed.

1.6 Conclusion

Sustainable crop management strategies that improve soil health will be essential tools for building more resource efficient, climate resilient agricultural systems with lower environmental impacts. This study examined soil moisture, soil chemical quality, soil physical quality, and agronomic performance of IWG grown in monoculture, with an alfalfa intercrop, an annual maize/soybean rotation, and maize/soybean managed with cover crops and strip-tillage for the first two years of system establishment. IWG system yields demonstrated suppressed grain yield but improved forage yield in IWG-alf compared to IWG systems, while cover cropping and reduced tillage in the annual-cc system did not affect agronomic yields compared to the annual system. Overall, soil physical and chemical attributes varied more across years than cropping systems, highlighting the strong influence of climate and drought on soil and cropping system outcomes. Perennial grain systems began to alter and improve soil aggregation compared to both conventional and sustainably managed annual maize/soybean rotations, suggesting that soil structure improvements are more rapid under IWG systems than the commonly implemented annual system best management practices of reduced-tillage and cover cropping. Relative differences between systems in soil moisture along a 1-m depth profile reinforce existing knowledge of different water use patterns through time between IWG and annual systems and highlight IWG's ability to access deep soil water in times of water stress. The IWG systems also displayed more stable, neutral soil pH and soil K concentrations than the annual systems. Nevertheless, we saw limited improvements in soil physical and chemical qualities in perennial systems two years post-establishment, echoing the large body of literature on grassland and perennial conversion which shows that significant relative improvements in soil health happen on timescales greater than 3-4 years and up to decades (Liebig et al., 2005; Cates et al., 2015; Scott et al., 2017; Nunes et al., 2020). IWG grown in short 3-4 year rotations with annuals may

offer limited soil quality improvements, especially in already fairly healthy soils, and the significant environmental co-benefits of these systems (ex. reduced nitrate leaching and inputs) may be relatively more impactful and reliable than soil health changes.

1.8 References

- Abdalla, M., Hastings, A., Cheng, K., Yue, Q., Chadwick, D., Espenberg, M., Truu, J., Rees, R.M., Smith, P., 2019. A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. *Global Change Biology* 25, 2530–2543. <https://doi.org/10.1111/gcb.14644>
- Amézqueta, E., 1999. Soil Aggregate Stability: A Review. *Journal of Sustainable Agriculture* 14, 83–151. https://doi.org/10.1300/J064v14n02_08
- Angel, J.R., Swanson, C., Boustead, B.M., Conlon, K., Hall, K.R., Jorns, J.L., Kunkel, K.E., Lemos, M.C., Lofgren, B.M., Ontl, T., Posey, J., Stone, K., Takle, E., Todey, D., 2018. Chapter 21 : Midwest. Impacts, Risks, and Adaptation in the United States: The Fourth National Climate Assessment, Volume II. U.S. Global Change Research Program. <https://doi.org/10.7930/NCA4.2018.CH21>
- Arguez, A., I. Durre, S. Applequist, R. Vose, M. Squires, X. Yin, R. Heim, and T.O., 2012. NOAA’s 1981-2010 climate normals: An overview. *Bull. Amer. Meteor.* 93, 1687–1697. <https://doi.org/10.1175/BAMS-D-11-00197.1>
- Audu, V., Rasche, F., Dimitrova Mårtensson, L.-M., Emmerling, C., 2022. Perennial cereal grain cultivation: Implication on soil organic matter and related soil microbial parameters. *Applied Soil Ecology* 174, 104414. <https://doi.org/10.1016/j.apsoil.2022.104414>
- Basche, A.D., DeLonge, M.S., 2019. Comparing infiltration rates in soils managed with conventional and alternative farming methods: A meta-analysis. *PLOS ONE* 14, e0215702. <https://doi.org/10.1371/journal.pone.0215702>
- Baumert, V.L., Vasilyeva, N.A., Vladimirov, A.A., Meier, I.C., Kögel-Knabner, I., Mueller, C.W., 2018. Root Exudates Induce Soil Macroaggregation Facilitated by Fungi in Subsoil. *Frontiers in Environmental Science* 6. <https://doi.org/10.3389/fenvs.2018.00140>

- Beillouin, D., Ben-Ari, T., Malézieux, E., Seufert, V., Makowski, D., 2021. Positive but variable effects of crop diversification on biodiversity and ecosystem services. *Global Change Biology* 27, 4697–4710. <https://doi.org/10.1111/gcb.15747>
- Bergquist, Galen., 2019. Biomass yield and soil microbial response to management of perennial intermediate wheatgrass (*Thinopyrum intermedium*) as grain crop and carbon sink. Retrieved from the University of Minnesota Digital Conservancy, <https://hdl.handle.net/11299/213040>.
- Bergtold, J.S., Ramsey, S., Maddy, L., Williams, J.R., 2019. A review of economic considerations for cover crops as a conservation practice. *Renewable Agriculture and Food Systems* 34, 62–76. <https://doi.org/10.1017/S1742170517000278>
- Bertoni, J., Larson, W. E., Shrader, W. D., 1958. Determination of infiltration rates on Marshall silt loam from runoff and rainfall records. *Soil Science Society of America Journal* 22, 571-574. <https://doi.org/10.2136/sssaj1958.03615995002200060024x>
- Black, C.K., Davis, S.C., Hudiburg, T.W., Bernacchi, C.J., DeLucia, E.H., 2017. Elevated CO₂ and temperature increase soil C losses from a soybean–maize ecosystem. *Global Change Biology* 23, 435–445. <https://doi.org/10.1111/gcb.13378>
- Blanco-Canqui, H., Jasa, P.J., 2019. Do Grass and Legume Cover Crops Improve Soil Properties in the Long Term? *Soil Science Society of America Journal* 83, 1181–1187. <https://doi.org/10.2136/sssaj2019.02.0055>
- Bormann, H., Klaassen, K., 2008. Seasonal and land use dependent variability of soil hydraulic and soil hydrological properties of two Northern German soils. *Geoderma, Modelling Pedogenesis* 145, 295–302. <https://doi.org/10.1016/j.geoderma.2008.03.017>
- Borrelli, P., Alewell, C., Alvarez, P., Anache, J.A.A., Baartman, J., Ballabio, C., Bezak, N., Biddoccu, M., Cerdà, A., Chalise, D., Chen, S., Chen, W., De Girolamo, A.M., Gessesse, G.D., Deumlich, D., Diodato, N., Efthimiou, N., Erpul, G., Fiener, P., Freppaz, M., Gentile, F., Gericke, A., Haregeweyn, N., Hu, B., Jeanneau, A., Kaffas, K., Kiani-Harchegani, M., Villuendas, I.L., Li, C., Lombardo, L., López-Vicente, M., Lucas-Borja, M.E., Märker, M., Matthews, F., Miao, C., Mikoš, M., Modugno, S., Möller, M., Naipal, V., Nearing, M., Owusu, S., Panday, D., Patault, E., Patriche, C.V., Poggio, L., Portes, R., Quijano, L., Rahdari, M.R., Renima, M., Ricci, G.F., Rodrigo-Comino, J., Saia, S., Samani, A.N., Schillaci, C., Syrris, V., Kim, H.S., Spinola, D.N., Oliveira, P.T., Teng, H., Thapa, R., Vantas, K., Vieira, D., Yang, J.E., Yin, S., Zema, D.A., Zhao, G., Panagos, P., 2021. Soil erosion modelling: A global review and statistical analysis. *Sci Total Environ* 780, 146494. <https://doi.org/10.1016/j.scitotenv.2021.146494>

- Bossio, D.A., Cook-Patton, S.C., Ellis, P.W., Fargione, J., Sanderman, J., Smith, P., Wood, S., Zomer, R.J., von Unger, M., Emmer, I.M., Griscom, B.W., 2020. The role of soil carbon in natural climate solutions. *Nat Sustain* 3, 391–398.
<https://doi.org/10.1038/s41893-020-0491-z>
- Bouman, O. T., Curtin, D., Campbell, C. A., Biederbeck, V. O., Ukrainetz, H., 1995. Soil Acidification from Long-Term Use of Anhydrous Ammonia and Urea. *Soil Science Society of America Journal* 59, 1488-1494.
<https://doi.org/10.2136/sssaj1995.03615995005900050039x>
- Bowles, T.M., Mooshammer, M., Socolar, Y., Calderón, F., Cavigelli, M.A., Culman, S.W., Deen, W., Drury, C.F., Garcia y Garcia, A., Gaudin, A.C.M., Harkcom, W.S., Lehman, R.M., Osborne, S.L., Robertson, G.P., Salerno, J., Schmer, M.R., Strock, J., Grandy, A.S., 2020. Long-Term Evidence Shows that Crop-Rotation Diversification Increases Agricultural Resilience to Adverse Growing Conditions in North America. *One Earth* 2, 284–293. <https://doi.org/10.1016/J.ONEEAR.2020.02.007>
- Burke, M., Emerick, K., 2016. Adaptation to climate change: Evidence from US agriculture. *American Economic Journal: Economic Policy* 8, 106–140.
<https://doi.org/10.1257/POL.20130025>
- Button, E.S., Pett-Ridge, J., Murphy, D.V., Kuzyakov, Y., Chadwick, D.R., Jones, D.L., 2022. Deep-C storage: Biological, chemical and physical strategies to enhance carbon stocks in agricultural subsoils. *Soil Biology and Biochemistry* 170, 108697.
<https://doi.org/10.1016/j.soilbio.2022.108697>
- Carter, P.R., Sheaffer, C.C., 1983. Alfalfa Response to Soil Water Deficits. I. Growth, Forage Quality, Yield, Water Use, and Water-Use Efficiency. *Crop Science* 23, crops1983.0011183X002300040016x.
<https://doi.org/10.2135/cropsci1983.0011183X002300040016x>
- Cates, A.M., Ruark, M.D., Grandy, A.S., Jackson, R.D., 2019. Small soil C cycle responses to three years of cover crops in maize cropping systems. *AGRICULTURE ECOSYSTEMS & ENVIRONMENT* 286. <https://doi.org/10.1016/j.agee.2019.106649>
- Cates, A.M., Ruark, M.D., Hedtcke, J.L., Posner, J.L., 2015. Long-term tillage, rotation and perennialization effects on particulate and aggregate soil organic matter. *Soil and Tillage Research* 115, 371-380. <https://doi.org/10.1016/j.still.2015.09.008>
- Chamberlain, L.A., Aguayo, T., Zerega, N.J.C., Dybzinski, R., Egerton-Warburton, L.M., 2022. Rapid improvement in soil health following the conversion of abandoned farm fields to annual or perennial agroecosystems. *Frontiers in Sustainable Food Systems* 6. <https://doi.org/10.3389/fsufs.2022.1010298>

- Chantigny, M.H., Angers, D.A., Prevost, D., Vezina, L.P., Chalifour, F.P., 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. *Soil Science Society of America Journal* 61, 262–267.
<https://doi.org/10.2136/sssaj1997.03615995006100010037x>
- Chen, S., Gong, B., 2021. Response and adaptation of agriculture to climate change: Evidence from China. *Journal of Development Economics* 148, 102557.
<https://doi.org/10.1016/j.jdeveco.2020.102557>
- Cheng, W., Parton, W.J., Gonzalez-Meler, M.A., Phillips, R., Asao, S., McNickle, G.G., Brzostek, E., Jastrow, J.D., 2014. Synthesis and modeling perspectives of rhizosphere priming. *New Phytologist* 201, 31–44. <https://doi.org/10.1111/nph.12440>
- Clément, C., Sleiderink, J., Svane, S.F., Smith, A.G., Diamantopoulos, E., Desbrøll, D.B., Thorup-Kristensen, K., 2022. Comparing the deep root growth and water uptake of intermediate wheatgrass (Kernza®) to alfalfa. *Plant and Soil*.
<https://doi.org/10.1007/s11104-021-05248-6>
- Crews, T.E., Kemp, L., Bowden, J.H., Murrell, E.G., 2022. How the Nitrogen Economy of a Perennial Cereal-Legume Intercrop Affects Productivity: Can Synchrony Be Achieved? *Frontiers in Sustainable Food Systems* 6. <https://doi.org/10.3389/fsufs.2022.755548>
- Crystal-Ornelas, R., Thapa, R., Tully, K.L., 2021. Soil organic carbon is affected by organic amendments, conservation tillage, and cover cropping in organic farming systems: A meta-analysis. *Agriculture, Ecosystems & Environment* 312, 107356.
<https://doi.org/10.1016/j.agee.2021.107356>
- Culman, S.W., DuPont, S.T., Glover, J.D., Buckley, D.H., Fick, G.W., Ferris, H., Crews, T.E., 2010. Long-term impacts of high-input annual cropping and unfertilized perennial grass production on soil properties and belowground food webs in Kansas, USA. *Agriculture, Ecosystems and Environment* 137, 13–24.
<https://doi.org/10.1016/j.agee.2009.11.008>
- Culman, S.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>
- Culman, S.W., Snapp, S.S., Ollenburger, M., Basso, B., DeHaan, L.R., 2013. Soil and water quality rapidly responds to the perennial grain Kernza wheatgrass. *Agronomy Journal* 105, 735–744. <https://doi.org/10.2134/agronj2012.0273>

- Delta-T Devices Ltd, 2016. User manual for the profile probe type PR2. Version Oct. 2016. https://delta-t.co.uk/wp-content/uploads/2017/02/PR2_user_manual_version_5.0.pdf
- de Oliveira, G., Brunzell, N.A., Crews, T.E., DeHaan, L.R., Vico, G., 2020. Carbon and water relations in perennial Kernza (*Thinopyrum intermedium*): An overview. *Plant Science* 295, 110279. <https://doi.org/10.1016/j.plantsci.2019.110279>
- de Oliveira, G., Brunzell, N.A., Sutherlin, C.E., Crews, T.E., DeHaan, L.R., 2018. Energy, water and carbon exchange over a perennial Kernza wheatgrass crop. *Agricultural and Forest Meteorology* 249, 120–137. <https://doi.org/10.1016/j.agrformet.2017.11.022>
- Deines, J.M., Wang, S., Lobell, D.B., 2019. Satellites reveal a small positive yield effect from conservation tillage across the US Corn Belt. *Environ. Res. Lett.* 14, 124038. <https://doi.org/10.1088/1748-9326/ab503b>
- Dhakal, M., West, C.P., Deb, S.K., Kharel, G., Ritchie, G.L., 2019. Field Calibration of PR2 Capacitance Probe in Pullman Clay-Loam Soil of Southern High Plains. *Agrosystems, Geosciences and Environment* 2, 1–7. <https://doi.org/10.2134/AGE2018.10.0043>
- Dick, C., Cattani, D., Entz, M.H., 2018. Kernza intermediate wheatgrass (*Thinopyrum intermedium*) grain production as influenced by legume intercropping and residue management. *Canadian Journal of Plant Science* 98, 1376–1379. <https://doi.org/10.1139/cjps-2018-0146>
- Dobbratz, M., Gutknecht, J., Wyse, D., Sheaffer, C.C., Jungers, J.M., 2022. Inconsistent effects of species diversity and N fertilization on soil microbes and carbon storage in perennial bioenergy cropping systems. *Renewable Agriculture and Food Systems* 37, 60–70. <https://doi.org/10.1017/S1742170521000302>
- Duchene, O., Bathellier, C., Dumont, B., David, C., Celette, F., 2023. Weed community shifts during the aging of perennial intermediate wheatgrass crops harvested for grain in arable fields. *European Journal of Agronomy* 143, 126721. <https://doi.org/10.1016/j.eja.2022.126721>
- Duchene, O., Celette, F., Barreiro, A., Dimitrova Mårtensson, L.-M., Freschet, G.T., David, C., 2020. Introducing Perennial Grain in Grain Crops Rotation: The Role of Rooting Pattern in Soil Quality Management. *Agronomy* 10, 1254. <https://doi.org/10.3390/agronomy10091254>
- DuPont, S.T., Beniston, J., Glover, J.D., Hodson, A., Culman, S.W., Lal, R., Ferris, H., 2014. Root traits and soil properties in harvested perennial grassland, annual wheat, and never-tilled annual wheat. *Plant and Soil* 381, 405–420. <https://doi.org/10.1007/s11104-014-2145-2>

- Ernst, O.R., Dogliotti, S., Cadenazzi, M., Kemanian, A.R., 2018. Shifting crop-pasture rotations to no-till annual cropping reduces soil quality and wheat yield. *Field Crops Research* 217, 180–187. <https://doi.org/10.1016/j.fcr.2017.11.014>
- Fagnant, L., Duchêne, O., Celette, F., David, C., Bindelle, J., Dumont, B., 2023. Learning about the growing habits and reproductive strategy of *Thinopyrum intermedium* through the establishment of its critical nitrogen dilution curve. *Field Crops Research* 291, 108802. <https://doi.org/10.1016/j.fcr.2022.108802>
- Farmaha, B.S., Sekaran, U., Franzluebbers, A.J., 2022. Cover cropping and conservation tillage improve soil health in the southeastern United States. *Agronomy Journal* 114, 296–316. <https://doi.org/10.1002/agj2.20865>
- Fernandez, C.W., Ehlke, N., Sheaffer, C.C., Jungers, J.M., 2020. Effects of nitrogen fertilization and planting density on intermediate wheatgrass yield. *Agronomy Journal* 112, 4159–4170. <https://doi.org/10.1002/agj2.20351>
- Fixen, P.E., Bruulsema, T.W., Johnston, A.M., Mikkelsen, R.L., Murrell, T.S., Snyder, C.S., Stewart, W.M., 2010. The Fertility of North American Soils. *North America* 4.
- Fox, J. and Weisberg, S., 2019. An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Galloway, A.F., Akhtar, J., Marcus, S.E., Fletcher, N., Field, K., Knox, P., 2020. Cereal root exudates contain highly structurally complex polysaccharides with soil-binding properties. *The Plant Journal* 103, 1666–1678. <https://doi.org/10.1111/tpj.14852>
- Gamble, J.D., Jungers, J.M., Wyse, D.L., Johnson, G.A., Lamb, J.A., Sheaffer, C.C., 2015. Harvest Date Effects on Biomass Yield, Moisture Content, Mineral Concentration, and Mineral Export in Switchgrass and Native Polycultures Managed for Bioenergy. *Bioenergy Research* 8, 740–749. <https://doi.org/10.1007/s12155-014-9555-0>
- Gardner, W.H., 2018. Water Content. *Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods* 493–544. <https://doi.org/10.2136/SSSABOOKSER5.1.2ED.C21>
- Gaudin, A.C.M., Tolhurst, T.N., Ker, A.P., Janovicek, K., Tortora, C., Martin, R.C., Deen, W., 2015. Increasing crop diversity mitigates weather variations and improves yield stability. *PLoS ONE* 10, 1–20. <https://doi.org/10.1371/journal.pone.0113261>
- Geisseler, D., Miller, K., Leinfelder-Miles, M., Wilson, R., 2019. Use of Soil Protein Pools as Indicators of Soil Nitrogen Mineralization Potential. *Soil Science Society of America Journal* 83, 1236–1243. <https://doi.org/10.2136/sssaj2019.01.0012>

- Glover, J.D., Culman, S.W., DuPont, S.T., Broussard, W., Young, L., Mangan, M.E., Mai, J.G., Crews, T.E., DeHaan, L.R., Buckley, D.H., Ferris, H., Turner, R.E., Reynolds, H.L., Wyse, D.L., 2010. Harvested perennial grasslands provide ecological benchmarks for agricultural sustainability. *Agriculture, Ecosystems & Environment*, Special section Harvested perennial grasslands: Ecological models for farming's perennial future 137, 3–12. <https://doi.org/10.1016/j.agee.2009.11.001>
- Guo, L., Liu, Y., Wu, G.-L., Huang, Z., Cui, Z., Cheng, Z., Zhang, R.-Q., Tian, F.-P., He, H., 2019. Preferential water flow: Influence of alfalfa (*Medicago sativa* L.) decayed root channels on soil water infiltration. *Journal of Hydrology* 578, 124019. <https://doi.org/10.1016/j.jhydrol.2019.124019>
- Gutknecht, J., Journey, A., Peterson, H., Blair, H., Cates, A., 2022.. Cover crop management practices to promote soil health and climate adaptation: Grappling with varied success from farmer and researcher observations. *Journal of Environmental Quality*, 1-17. <https://doi.org/10.1002/jeq2.20383>
- Guzmán G., Sáenz de Rodríguez M., Vanwallegem T., Vanderlinden K., Laguna A., Giráldez JV. 2015. Compatibility of Agricultural Management Practices and Types of Farming in the EU to enhance Climate Change Mitigation and Soil Health: Impacts of soil management on physical soil quality. CATCH-C, www.catch-c.eu, pp. 38.
- Halde, C., Gagné, S., Charles, A., Lawley, Y., 2017. Organic No-Till Systems in Eastern Canada: A Review. *Agriculture* 7, 36. <https://doi.org/10.3390/agriculture7040036>
- Hartemink, A.E., Zhang, Y., Bockheim, J.G., Curi, N., Silva, S.H.G., Grauer-Gray, J., Lowe, D.J., Krasilnikov, P., 2020. Chapter Three - Soil horizon variation: A review, in: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 125–185. <https://doi.org/10.1016/bs.agron.2019.10.003>
- Hatfield, J.L., Dold, C., 2019. Water-Use Efficiency: Advances and Challenges in a Changing Climate. *Frontiers in Plant Science* 10. <https://doi.org/10.3389/fpls.2019.00103>
- Hayes, R.C., Newell, M.T., Crews, T.E., Peoples, M.B., 2017. Perennial cereal crops: An initial evaluation of wheat derivatives grown in mixtures with a regenerating annual legume. *Renewable Agriculture and Food Systems* 32, 276–290. <https://doi.org/10.1017/S1742170516000260>
- Heineck, G.C., Schlautman, B., Law, E.P., Ryan, M.R., Zimbric, J.W., Picasso, V., Stoltenberg, D.E., Sheaffer, C.C., Jungers, J.M., 2022. Intermediate wheatgrass seed size and moisture dynamics inform grain harvest timing. *Crop Science* 62, 410–424. <https://doi.org/10.1002/csc2.20662>

- Huang, K., Sim, N., 2021. Adaptation May Reduce Climate Damage in Agriculture by Two Thirds. *Journal of Agricultural Economics* 72, 47–71. <https://doi.org/10.1111/1477-9552.12389>
- Huddell, A., Ernfors, M., Crews, T., Vico, G., Menge, D.N.L., 2023. Nitrate leaching losses and the fate of ¹⁵N fertilizer in perennial intermediate wheatgrass and annual wheat — A field study. *Science of The Total Environment* 857, 159255. <https://doi.org/10.1016/j.scitotenv.2022.159255>
- Hunter, M.C., Sheaffer, C.C., Culman, S.W., Lazarus, W.F., Jungers, J.M., 2020. Effects of defoliation and row spacing on intermediate wheatgrass II: Forage yield and economics. *Agronomy Journal* 1862–1880. <https://doi.org/10.1002/agj2.20124>
- 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.T., 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>
- Hurisso, T.T., Culman, S.W., Zhao, K., 2018a. Repeatability and Spatiotemporal Variability of Emerging Soil Health Indicators Relative to Routine Soil Nutrient Tests. *Soil Science Society of America Journal* 82, 939–948. <https://doi.org/10.2136/sssaj2018.03.0098>
- Hurisso, T.T., Moebius-Clune, D.J., Culman, S.W., Moebius-Clune, B.N., Thies, J.E., Es, H.M., 2018b. Soil Protein as a Rapid Soil Health Indicator of Potentially Available Organic Nitrogen. *Agricultural & Environmental Letters* 3, 180006. <https://doi.org/10.2134/ael2018.02.0006>
- Jakab, G., Madarász, B., Szabó, J.A., Tóth, A., Zacháry, D., Szalai, Z., Kertész, Á., Dyson, J., 2017. Infiltration and Soil Loss Changes during the Growing Season under Ploughing and Conservation Tillage. *Sustainability* 9, 1726. <https://doi.org/10.3390/su9101726>
- Jotisankasa, A., Sirirattanachat, T., 2017. Effects of grass roots on soil-water retention curve and permeability function. *Can. Geotech. J.* 54, 1612–1622. <https://doi.org/10.1139/cgj-2016-0281>
- Jungers, J.M., DeHaan, L.H., Mulla, D.J., Sheaffer, C.C., Wyse, D.L., 2019. Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. *Agriculture, Ecosystems & Environment* 272, 63–73. <https://doi.org/10.1016/j.agee.2018.11.007>

- Jungers, J.M., DeHaan, L.R., Betts, K.J., Sheaffer, C.C., Wyse, D.L., 2017. Intermediate wheatgrass grain and forage yield responses to nitrogen fertilization. *Agronomy Journal* 109, 462–472. <https://doi.org/10.2134/agronj2016.07.0438>
- Jungers, J.M., Frahm, C.S., Tautges, N.E., Ehlke, N.J., Wells, M.S., Wyse, D.L., Sheaffer, C.C., 2018. Growth, development, and biomass partitioning of the perennial grain crop *Thinopyrum intermedium*. *Annals of Applied Biology* 172, 346–354. <https://doi.org/10.1111/aab.12425>
- Kane, D.A., Bradford, M.A., Fuller, E., Oldfield, E.E., Wood, S.A., 2021. Soil organic matter protects US maize yields and lowers crop insurance payouts under drought. *Environmental Research Letters* 16, 044018. <https://doi.org/10.1088/1748-9326/ABE492>
- Kane, D.A., Rogé, P., Snapp, S.S., 2016. A systematic review of perennial staple crops literature using topic modeling and bibliometric analysis. *PLoS ONE* 11, 1–18. <https://doi.org/10.1371/journal.pone.0155788>
- Kaye, J.P., Quemada, M., 2017. Using cover crops to mitigate and adapt to climate change. A review. *Agron. Sustain. Dev.* 37, 4. <https://doi.org/10.1007/s13593-016-0410-x>
- Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry* 42, 1363–1371. <https://doi.org/10.1016/j.soilbio.2010.04.003>
- Lal, R., 2020. Soil organic matter content and crop yield. *Journal of Soil and Water Conservation* 75, 27A-32A. <https://doi.org/10.2489/JSWC.75.2.27A>
- Lal, R., 2016. Soil health and carbon management. *Food and Energy Security* 5, 212–222. <https://doi.org/10.1002/fes3.96>
- Law, E.P., Wayman, S., Pelzer, C.J., Culman, S.W., Gómez, M.I., DiTommaso, A., Ryan, M.R., 2022a. Multi-Criteria Assessment of the Economic and Environmental Sustainability Characteristics of Intermediate Wheatgrass Grown as a Dual-Purpose Grain and Forage Crop. *Sustainability* 14, 3548. <https://doi.org/10.3390/su14063548>
- Law, E.P., Wayman, S., Pelzer, C.J., DiTommaso, A., Ryan, M.R., 2022b. Intercropping red clover with intermediate wheatgrass suppresses weeds without reducing grain yield. *Agronomy Journal* 114, 700–716. <https://doi.org/10.1002/agi2.20914>
- Ledo, A., Smith, P., Zerihun, A., Whitaker, J., Vicente-Vicente, J.L., Qin, Z., McNamara, N.P., Zinn, Y.L., Llorente, M., Liebig, M., Kuhnert, M., Dondini, M., Don, A., Diaz-Pines, E., Datta, A., Bakka, H., Aguilera, E., Hillier, J., 2020. Changes in soil organic carbon under perennial crops. *Global Change Biology* 26, 4158–4168. <https://doi.org/10.1111/gcb.15120>

- Lenhart, C., Peterson, H., 2017.. Agricultural BMP Handbook for Minnesota 2017.
- Lenth. R.V, 2021. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.5-1. <https://CRAN.R-project.org/package=emmeans>
- Leuther, F., Schlüter, S., 2021. Impact of freeze–thaw cycles on soil structure and soil hydraulic properties. *SOIL* 7, 179–191. <https://doi.org/10.5194/soil-7-179-2021>
- Li, D.-C., Velde, B., Li, F.-M., Zhang, G.-L., Zhao, M.-S., Huang, L.-M., 2011. Impact of Long-Term Alfalfa Cropping on Soil Potassium Content and Clay Minerals in a Semi-Arid Loess Soil in China. *Pedosphere* 21, 522–531. [https://doi.org/10.1016/S1002-0160\(11\)60154-9](https://doi.org/10.1016/S1002-0160(11)60154-9)
- Liebig, M.A., Johnson, H.A., Hanson, J.D., Frank, A.B., 2005. Soil carbon under switchgrass stands and cultivated cropland. *Biomass and Bioenergy* 28, 347–354. <https://doi.org/10.1016/j.biombioe.2004.11.004>
- Liu, X., Wu, X., Liang, G., Zheng, F., Zhang, M., Li, S., 2021. A global meta-analysis of the impacts of no-tillage on soil aggregation and aggregate-associated organic carbon. *Land Degradation & Development* 32, 5292–5305. <https://doi.org/10.1002/LDR.4109>
- Liu, Y., Guo, L., Huang, Z., López-Vicente, M., Wu, G.-L., 2020. Root morphological characteristics and soil water infiltration capacity in semi-arid artificial grassland soils. *Agricultural Water Management* 235, 106153. <https://doi.org/10.1016/j.agwat.2020.106153>
- Locatelli, A., Gutierrez, L., Picasso Risso, V.D., 2022. Vernalization requirements of Kernza intermediate wheatgrass. *Crop Science* 62, 524–535. <https://doi.org/10.1002/csc2.20667>
- Lotter, D.W., Seidel, R., Liebhardt, W., 2003. The performance of organic and conventional cropping systems in an extreme climate year. *American Journal of Alternative Agriculture* 18, 146–154. <https://doi.org/10.1079/AJAA200345>
- Lubofsky, E., 2016. The promise of perennials: Working through the challenges of perennial grain crop development. *CSA News* 61, 4–7. <https://doi.org/10.2134/csa2016-61-11-1>
- Maeght, J.-L., Rewald, B., Pierret, A., 2013. How to study deep roots—and why it matters. *Front Plant Sci* 4, 299. <https://doi.org/10.3389/fpls.2013.00299>
- Mårtensson, L.-M.D., Barreiro, A., Li, S., Jensen, E.S., 2022. Agronomic performance, nitrogen acquisition and water-use efficiency of the perennial grain crop *Thinopyrum intermedium* in a monoculture and intercropped with alfalfa in Scandinavia. *Agron. Sustain. Dev.* 42, 21. <https://doi.org/10.1007/s13593-022-00752-0>

- Martin, T., Sprunger, C.D., 2022. Sensitive Measures of Soil Health Reveal Carbon Stability Across a Management Intensity and Plant Biodiversity Gradient. *Frontiers in Soil Science* 2. <https://doi.org/10.3389/fsoil.2022.917885>
- McIsaac, G.F., David, M.B., Mitchell, C.A., 2010. *Miscanthus* and Switchgrass Production in Central Illinois: Impacts on Hydrology and Inorganic Nitrogen Leaching. *J. Environ. Qual.* 39, 1790–1799. <https://doi.org/10.2134/jeq2009.0497>
- McGuire, R., Williams, P.N., Smith, P., McGrath, S.P., Curry, D., Donnison, I., Emmet, B., Scollan, N., 2022. Potential Co-benefits and trade-offs between improved soil management, climate change mitigation and agri-food productivity. *Food and Energy Security* 11, e352. <https://doi.org/10.1002/FES3.352>
- Menne, M.J., Durre, I., Vose, R.S., Gleason, B.E., Houston, T.G., 2012. An Overview of the Global Historical Climatology Network-Daily Database. *Journal of Atmospheric and Oceanic Technology* 29, 897–910. <https://doi.org/10.1175/JTECH-D-11-00103.1>
- Miguez, F.E., Bollero, G.A., 2005. Review of Corn Yield Response under Winter Cover Cropping Systems Using Meta-Analytic Methods. *Crop Science* 45, 2318–2329. <https://doi.org/10.2135/cropsci2005.0014>
- Minasny, B., Cook, F., 2011. Sorptivity of Soils, in: Gliński, J., Horabik, J., Lipiec, J. (Eds.), *Encyclopedia of Agrophysics, Encyclopedia of Earth Sciences Series*. Springer Netherlands, Dordrecht, pp. 824–826. https://doi.org/10.1007/978-90-481-3585-1_161
- Miranda-Vélez, J.F., Leuther, F., Köhne, J.M., Munkholm, L.J., Vogeler, I., 2023. Effects of freeze-thaw cycles on soil structure under different tillage and plant cover management practices. *Soil and Tillage Research* 225, 105540. <https://doi.org/10.1016/j.still.2022.105540>
- Moebius-Clune, B.N., D.J. Moebius-Clune, B.K. Gugino, O.J. Idowu, R.R. Schindelbeck, A.J. Ristow, H.M. van Es, J.E. Thies, H. A. Shayler, M. B. McBride, D.W. Wolfe, and G.S. Abawi, 2016. *Comprehensive Assessment of Soil Health – The Cornell Framework Manual, Edition 3.1*, Cornell University, Geneva, NY.
- NCERA, 2015. *Recommended Chemical Soil Test Procedures for the North Central Region*. North Central Regional Research Publication No. 221 (Revised)
- Nearing, M.A., Xie, Y., Liu, B., Ye, Y., 2017. Natural and anthropogenic rates of soil erosion. *International Soil and Water Conservation Research* 5, 77–84. <https://doi.org/10.1016/j.iswcr.2017.04.001>
- Nelson, D.W., Sommers, L.E., 2018. Total Carbon, Organic Carbon, and Organic Matter. *Methods of Soil Analysis, Part 3: Chemical Methods* 961–1010. <https://doi.org/10.2136/SSSABOOKSER5.3.C34>

- Noll, L., Zhang, S., Zheng, Q., Hu, Y., Hofhansl, F., Wanek, W., 2022. Climate and geology overwrite land use effects on soil organic nitrogen cycling on a continental scale. *Biogeosciences* 19, 5419–5433. <https://doi.org/10.5194/bg-19-5419-2022>
- Norris, C.E., Bean, G.M., Cappellazzi, S.B., Cope, M., Greub, K.L.H., Liptzin, D., Rieke, E.L., Tracy, P.W., Morgan, C.L.S., Honeycutt, C.W., 2020. Introducing the North American project to evaluate soil health measurements. *Agronomy Journal* 112, 3195–3215. <https://doi.org/10.1002/agj2.20234>
- Nunes, M.R., Karlen, D.L., Moorman, T.B., 2020. Tillage intensity effects on soil structure indicators-A US meta-analysis. *Sustainability (Switzerland)* 12. <https://doi.org/10.3390/su12052071>
- Oldfield, E.E., Bradford, M.A., Wood, S.A., 2019. Global meta-analysis of the relationship between soil organic matter and crop yields. *Soil* 5, 15–32. <https://doi.org/10.5194/soil-5-15-2019>
- Osipitan, O.A., Dille, J.A., Assefa, Y., Knezevic, S.Z., 2018. Cover Crop for Early Season Weed Suppression in Crops: Systematic Review and Meta-Analysis. *Agronomy Journal* 110, 2211–2221. <https://doi.org/10.2134/agronj2017.12.0752>
- Paustian, K., Collier, S., Baldock, J., Burgess, R., Creque, J., DeLonge, M., Dungait, J., Ellert, B., Frank, S., Goddard, T., Govaerts, B., Grundy, M., Henning, M., Izaurralde, R.C., Madaras, M., McConkey, B., Porzig, E., Rice, C., Searle, R., Seavy, N., Skalsky, R., Mulhern, W., Jahn, M., 2019. Quantifying carbon for agricultural soil management: from the current status toward a global soil information system. *Carbon Management* 10, 567–587. <https://doi.org/10.1080/17583004.2019.1633231>
- Peixoto, L., Olesen, J.E., Elsgaard, L., Enggrob, K.L., Banfield, C.C., Dippold, M.A., Nicolaisen, M.H., Bak, F., Zang, H., Dresbøll, D.B., Thorup-Kristensen, K., Rasmussen, J., 2022. Deep-rooted perennial crops differ in capacity to stabilize C inputs in deep soil layers. *Scientific Reports* 12. <https://doi.org/10.1038/S41598-022-09737-1>
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team, 2021. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-152, <https://CRAN.R-project.org/package=nlme>.
- Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. *Agriculture, Ecosystems & Environment* 200, 33–41. <https://doi.org/10.1016/j.agee.2014.10.024>
- Pugliese, J.Y., Culman, S.W., Sprunger, C.D., 2019a. Harvesting forage of the perennial grain crop kernza (*Thinopyrum intermedium*) increases root biomass and soil nitrogen. *Plant and Soil* 437, 241–254. <https://doi.org/10.1007/S11104-019-03974-6/FIGURES/4>

- Pugliese, J.Y., Culman, S.W., Sprunger, C.D., 2019b. Correction to: Harvesting forage of the perennial grain crop kernza (*Thinopyrum intermedium*) increases root biomass and soil nitrogen cycling (*Plant and Soil*, (2019), 10.1007/s11104-019-03974-6). *Plant and Soil* 241–254. <https://doi.org/10.1007/s11104-019-03974-6>
- R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rabot, E., Wiesmeier, M., Schlüter, S., Vogel, H.-J., 2018. Soil structure as an indicator of soil functions: A review. *Geoderma* 314, 122–137. <https://doi.org/10.1016/j.geoderma.2017.11.009>
- Rakkar, M., Jungers, J.M., Sheaffer, C., Bergquist, G., Grossman, J., Li, F., Gutknecht, J.L., 2023. Soil health improvements from using a novel perennial grain during the transition to organic production. *Agriculture, Ecosystems & Environment* 341, 108164. <https://doi.org/10.1016/j.agee.2022.108164>
- Reilly, E.C., Gutknecht, J.L., Sheaffer, C.C., Jungers, J.M., 2022a. Reductions in soil water nitrate beneath a perennial grain crop compared to an annual crop rotation on sandy soil. *Frontiers in Sustainable Food Systems* 6. <https://doi.org/10.3389/fsufs.2022.996586>
- Reilly, E.C., Gutknecht, J.L., Tautges, N.E., Sheaffer, C.C., Jungers, J.M., 2022b. Nitrogen transfer and yield effects of legumes intercropped with the perennial grain crop intermediate wheatgrass. *Field Crops Research* 286, 108627. <https://doi.org/10.1016/J.FCR.2022.108627>
- Renwick, L.L.R., Deen, W., Silva, L., Gilbert, M.E., Maxwell, T., Bowles, T.M., Gaudin, A.C.M., 2021. Long-term crop rotation diversification enhances maize drought resistance through soil organic matter. *Environmental Research Letters* 16. <https://doi.org/10.1088/1748-9326/AC1468>
- Rillig, M.C., Aguilar-Trigueros, C.A., Bergmann, J., Verbruggen, E., Veresoglou, S.D., Lehmann, A., 2015. Plant root and mycorrhizal fungal traits for understanding soil aggregation. *New Phytologist* 205, 1385–1388. <https://doi.org/10.1111/nph.13045>
- Rubio, V., Diaz-Rossello, R., Quincke, J.A., van Es, H.M., 2021. Quantifying soil organic carbon's critical role in cereal productivity losses under annualized crop rotations. *Agriculture, Ecosystems & Environment* 321, 107607. <https://doi.org/10.1016/j.agee.2021.107607>
- Rui, Y., Jackson, R.D., Cotrufo, M.F., Sanford, G.R., Spiesman, B.J., Deiss, L., Culman, S.W., Liang, C., Ruark, M.D., 2022. Persistent soil carbon enhanced in Mollisols by

- well- managed grasslands but not annual grain or dairy forage cropping systems 1–6.
<https://doi.org/10.1073/pnas.2118931119/-/DCSupplemental.Published>
- Ryan, M.R., Crews, T.E., Culman, S.W., Dehaan, L.R., Hayes, R.C., Jungers, J.M., Bakker, M.G., 2018. Managing for Multifunctionality in Perennial Grain Crops. *BioScience* 68, 294–304. <https://doi.org/10.1093/biosci/biy014>
- Sainju, U.M., Liptzin, D., Stevens, W.B., 2022. Autoclaved citrate-extractable protein as a soil health indicator relates to soil properties and crop production. *Nutr Cycl Agroecosyst.* <https://doi.org/10.1007/s10705-022-10230-4>
- Sainju, U.M., Allen, B.L., Lenssen, A.W., Ghimire, R.P., 2017. Root biomass, root/shoot ratio, and soil water content under perennial grasses with different nitrogen rates. *Field Crops Research* 210, 183–191. <https://doi.org/10.1016/j.fcr.2017.05.029>
- Sanford, G.R., Posner, J.L., Jackson, R.D., Kucharik, C.J., Hedtcke, J.L., Lin, T.L., 2012. Soil carbon lost from Mollisols of the North Central U.S.A. with 20 years of agricultural best management practices. *Agriculture, Ecosystems and Environment* 162, 68–76. <https://doi.org/10.1016/j.agee.2012.08.011>
- Schmidt, J., Fester, T., Schulz, E., Michalzik, B., Buscot, F., Gutknecht, J., 2017. Effects of plant-symbiotic relationships on the living soil microbial community and microbial necromass in a long-term agro-ecosystem. *Science of the Total Environment* 581–582, 756–765. <https://doi.org/10.1016/j.scitotenv.2017.01.005>
- Scott, D.A., Baer, S.G., Blair, J.M., 2017. Recovery and Relative Influence of Root, Microbial, and Structural Properties of Soil on Physically Sequestered Carbon Stocks in Restored Grassland. *Soil Science Society of America Journal* 81, 50–60. <https://doi.org/10.2136/sssaj2016.05.0158>
- Shakoor, A., Shakoor, S., Rehman, A., Ashraf, F., Abdullah, M., Shahzad, S.M., Farooq, T.H., Ashraf, M., Manzoor, M.A., Altaf, M.M., Altaf, M.A., 2021. Effect of animal manure, crop type, climate zone, and soil attributes on greenhouse gas emissions from agricultural soils—A global meta-analysis. *Journal of Cleaner Production* 278, 124019. <https://doi.org/10.1016/j.jclepro.2020.124019>
- Smith, R.E., 1999. Technical Note: Rapid Measurement of Soil Sorptivity. *Soil Science Society of America Journal* 63, 55–57. <https://doi.org/10.2136/sssaj1999.03615995006300010009x>
- Smucker, A.J.M., McBurney, S.L., Srivastava, A.K., 1982. Quantitative Separation of Roots from Compacted Soil Profiles by the Hydropneumatic Elutriation System 1. *Agronomy Journal* 74, 500–503. <https://doi.org/10.2134/AGRONJ1982.00021962007400030023X>

- Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at the following link:
<http://websoilsurvey.sc.egov.usda.gov/>. Accessed 8/13/2022.
- Sprunger, C.D., Culman, S.W., Peralta, A.L., DuPont, S.T., Lennon, J.T., Snapp, S.S., 2019. Perennial grain crop roots and nitrogen management shape soil food webs and soil carbon dynamics. *Soil Biology and Biochemistry* 137, 107573.
<https://doi.org/10.1016/j.soilbio.2019.107573>
- Sprunger, C.D., Culman, S.W., Robertson, G.P., Snapp, S.S., 2018. Perennial grain on a Midwest Alfisol shows no sign of early soil carbon gain. *Renewable Agriculture and Food Systems* 33, 360–372. <https://doi.org/10.1017/S1742170517000138>
- Sprunger, C.D., Philip Robertson, G., 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 Tvisha Martin Meredith Mann, C.D., Christine Sprunger, C.D., 2020. Systems with greater perenniality and crop diversity enhance soil biological health.
<https://doi.org/10.1002/ael2.20030>
- Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2008. Soil carbon saturation: Evaluation and corroboration by long-term incubations. *Soil Biology and Biochemistry* 40, 1741–1750. <https://doi.org/10.1016/J.SOILBIO.2008.02.014>
- Stewart, R.D., Jian, J., Gyawali, A.J., Thomason, W.E., Badgley, B.D., Reiter, M.S., Strickland, M.S., 2018. What We Talk about When We Talk about Soil Health. *Agricultural & Environmental Letters* 3, 180033.
<https://doi.org/10.2134/ael2018.06.0033>
- Stott, D.E. 2019. Recommended Soil Health Indicators and Associated Laboratory Procedures. Soil Health Technical Note No. 450-03. U.S. Department of Agriculture, Natural Resources Conservation Service.
- Strock, J.S., Johnson, J.M.F., Tollefson, D., Ranaivoson, A., 2022. Rapid change in soil properties after converting grasslands to crop production. *Agronomy Journal* 114, 1642–1654. <https://doi.org/10.1002/AGJ2.21045>
- Sutherlin, C.E., Brunsell, N.A., de Oliveira, G., Crews, T.E., DeHaan, L.R., Vico, G., 2019. Contrasting physiological and environmental controls of evapotranspiration over Kernza Perennial crop, annual crops, and C4 and mixed C3/C4 grasslands. *Sustainability (Switzerland)* 11. <https://doi.org/10.3390/su11061640>

- Suyker, A.E., Verma, S.B., 2009. Evapotranspiration of irrigated and rainfed maize–soybean cropping systems. *Agricultural and Forest Meteorology* 149, 443–452.
<https://doi.org/10.1016/j.agrformet.2008.09.010>
- Tamburini, G., Bommarco, R., Wanger, T.C., Kremen, C., van der Heijden, M.G.A., Liebman, M., Hallin, S., 2020. Agricultural diversification promotes multiple ecosystem services without compromising yield. *Science Advances* 6, eaba1715.
<https://doi.org/10.1126/sciadv.aba1715>
- Tautges, N.E., Jungers, J.M., Dehaan, L.R., Wyse, D.L., Sheaffer, C.C., 2018. Maintaining grain yields of the perennial cereal intermediate wheatgrass in monoculture v. bi-culture with alfalfa in the Upper Midwestern USA. *Journal of Agricultural Science* 156, 758–773. <https://doi.org/10.1017/S0021859618000680>
- Thaler, E.A., Kwang, J.S., Quirk, B.J., Quarrier, C.L., Larsen, I.J., 2022. Rates of Historical Anthropogenic Soil Erosion in the Midwestern United States. *Earth’s Future* 10, e2021EF002396. <https://doi.org/10.1029/2021EF002396>
- United States Department of Agriculture National Agricultural Statistics Service, 2021. Minnesota Ag News 2020 Soybean County Estimates. <https://quickstats.nass.usda.gov/>
- United States Department of Agriculture National Agricultural Statistics Service, 2022. Minnesota Ag News 2021 Corn for Silage County Estimates.
<https://quickstats.nass.usda.gov/>
- van Es, H.M., 1993. Evaluation of temporal, spatial, and tillage-induced variability for parameterization of soil infiltration. *Geoderma* 60, 187–199.
[https://doi.org/10.1016/0016-7061\(93\)90026-H](https://doi.org/10.1016/0016-7061(93)90026-H)
- Van Oost, K., Govers, G., De Alba, S., Quine, T.A., 2006. Tillage erosion: a review of controlling factors and implications for soil quality. *Progress in Physical Geography: Earth and Environment* 30, 443–466. <https://doi.org/10.1191/0309133306pp487ra>
- Villarreal, R., Soracco, C.G., Lozano, L.A., Melani, E.M., Sarli, G.O., 2017. Temporal variation of soil sorptivity under conventional and no-till systems determined by a simple laboratory method. *Soil and Tillage Research* 168, 92–98.
<https://doi.org/10.1016/j.still.2016.12.013>
- Wallander, S., Smith, D., Bowman, M., Claassen, R. (Eds.), 2021. Cover Crop Trends, Programs, and Practices in the United States, EIB-222.
<https://doi.org/10.22004/ag.econ.309562>
- Wander, M.M., Cihacek, L.J., Coyne, M., Drijber, R.A., Grossman, J.M., Gutknecht, J.L.M., Horwath, W.R., Jagadamma, S., Olk, D.C., Ruark, M., Snapp, S.S., Tiemann, L.K., Weil, R., Turco, R.F., 2019. Developments in Agricultural Soil Quality and Health:

- Reflections by the Research Committee on Soil Organic Matter Management. *Frontiers in Environmental Science* 7. <https://doi.org/10.3389/fenvs.2019.00109>
- Waring, B.G., Smith, K.R., Grote, E.E., Howell, A., Reibold, R., Tucker, C.L., Reed, S.C., 2021. Climatic Controls on Soil Carbon Accumulation and Loss in a Dryland Ecosystems. *Journal of Geophysical Research: Biogeosciences* 126. <https://doi.org/10.1029/2021jg006492>
- Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *American Journal of Alternative Agriculture* 18, 3–17. <https://doi.org/10.1079/AJAA2003003>
- Wickham, H, 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- Williams, A., Hunter, M.C., Kammerer, M., Kane, D.A., Jordan, N.R., Mortensen, D.A., Smith, R.G., Snapp, S., Davis, A.S., 2016. Soil water holding capacity mitigates downside risk and volatility in US rainfed maize: Time to invest in soil organic matter? *PLoS ONE* 11, 1–11. <https://doi.org/10.1371/journal.pone.0160974>
- Wright, S.F., Upadhyaya, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science* 161, 575-586. 10.1097/00010694-199609000-00003
- Young, M.D., Ros, G.H., de Vries, W., 2021. Impacts of agronomic measures on crop, soil, and environmental indicators: A review and synthesis of meta-analysis. *Agriculture, Ecosystems & Environment* 319, 107551. <https://doi.org/10.1016/j.agee.2021.107551>
- Zhang, Q., Shao, M., Jia, X., Wei, X., 2019. Changes in soil physical and chemical properties after short drought stress in semi-humid forests. *Geoderma* 338, 170–177. <https://doi.org/10.1016/j.geoderma.2018.11.051>

1.7 Supplementary Tables and Figures

System(s)	Crop	Variety	Seeding Rate	Row Spacing	Planting Date	Harvest Date
IWG, IWG-alf	IWG	MN Clearwater	13.5 kg/ha	30.5 cm	9/05/2019	8/4/2020, 7/21/2021
IWG-alf	Alfalfa	Bluejay 4hr (Blue River Seed)	9 kg/ha	Two alfalfa rows spaced 20.32 cm apart between each row of IWG. Spacing between IWG and alfalfa is 5.09 cm.	9/05/2019	8/4/2020, 7/21/2021
Annual, annual-cc	Soybean		395,000 plants/ha	30.5 cm	5/12/2020	9/22/2020
annual-cc	Winter Rye		92 kg/ha	20.23 cm	9/05/2019	5/4/2020 (terminated 5/7/2020)
Annual, Annual-cc	Maize	Anderson 611R 100 Day CRM Roundup Ready corn	83,950 plants/ha	76.2 cm	5/14/2021 - 5/17/2021	8/12/2021 (sillage)
Annual-cc	Vetch		39.2 kg/ha	20.23 cm	9/22/2020	5/4/2021 (terminated 5/12/2021)

Supplementary Table 1.1: Agronomic information for 2020 and 2021 growing seasons.

Date	Cropping System	Target Plant	Active Ingredient	Concentration (kg/ha)	Purpose
5/7/2020	Annual-cc	Rye cover crop	glyphosate	1.6 kg/ha	Terminate cover crop
5/12/2021	Annual-cc	Vetch cover crop	glyphosate	1.6 kg/ha	Terminate cover crop
6/15/2021 and 6/16/2021	Annual and Annual-cc	Weeds	Glyphosate	1.6 kg/ha	Weed Management between rows
6/16/2021	Annual and Annual-cc	Weeds	2, 4-D	1.1 kg/ha	Weed management between rows

Supplementary Table 1.2a: Herbicide Treatments and Timing in 2020 and 2021 growing seasons.

Date	System(s)	Fertilizer	Concentration	Purpose
4/30/2020	IWG	Urea	90 kg N/ha	Spring N application prior to stem elongation
4/20/2021	IWG	Urea	90 kg N/ha	Spring N application prior to stem elongation
5/17/2021	Annual, annual-cc	Urea	70 kg N/ha	Spring N application at planting
6/25/2021	Annual, annual-cc	Urea	70 kg N/ha	N application at V6 maize growth stage

Supplementary Table 1.2b: Urea fertilizer amounts and timing in 2020 and 2021 growing seasons.

Variable	Year	Crop	emmean	SE	Group
IWG Grain Yield (log transformed kg/ha)	2021	IWG-alf	5.43	0.23	a
		IWG	6.03	0.23	b
	2020	IWG-alf	3.58	0.81	a
		IWG	4.47	0.81	b
IWG System Total Biomass (kg/ha)	2021	IWG-alf	8.32	0.08	a
		IWG	8.4	0.08	a
	2020	IWG	7.7	0.14	a
		IWG-alf	8.35	0.14	b
IWG Straw Yield (kg/ha)	2021	IWG-alf	7.98	0.1	a
		IWG	8.4	0.1	b
	2020	IWG-alf	7.18	0.22	a
		IWG	7.7	0.22	b
Harvest Index	2021	IWG-alf	9.01	1.09	a
		IWG	10.15	1.09	a
	2020	IWG-alf	4.25	2.12	a
		IWG	8.92	2.12	b
Soybean Yield (kg/ha)	2020	annual	4027.5	167.55	a
		annual-cc	4306.68	167.55	a
Silage Yield (dry kg/ha)	2021	annual	35503.27	3617.72	a
		annual-cc	36008.17	3617.72	a
Silage Moisture (%)	2021	annual-cc	0.24	0.01	a
		annual	0.24	0.01	a

Supplementary Table 1.4: Results of post-hoc tests for annual and IWG system agronomic yields.

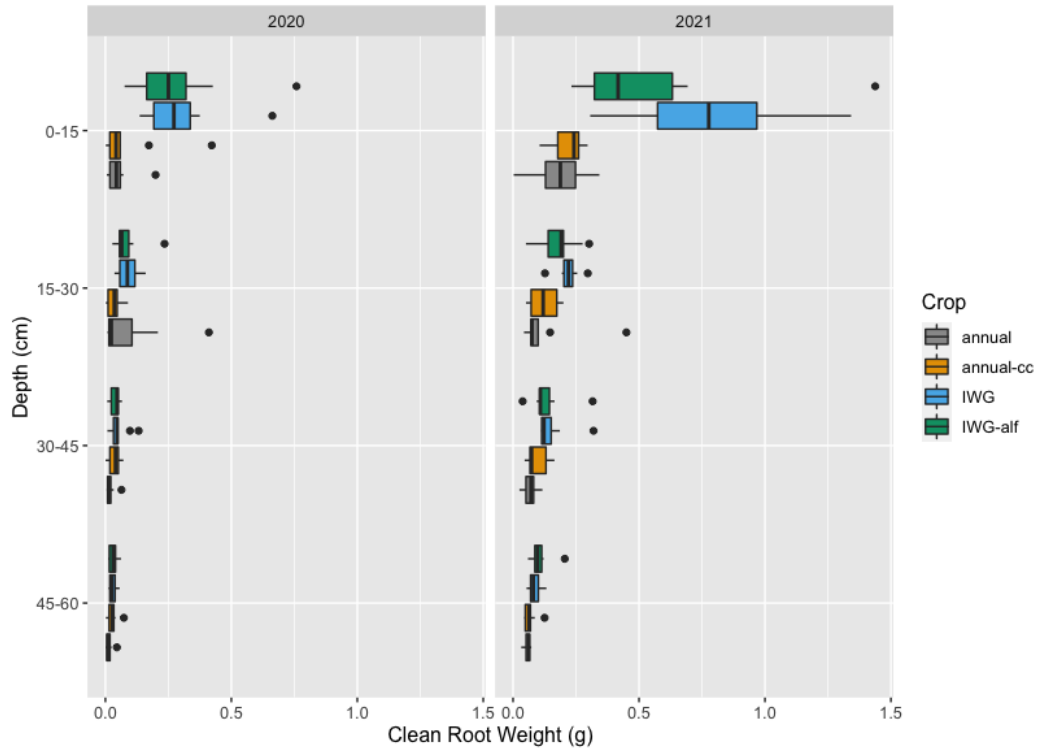


Figure S1.1: Boxplots of root biomass by depth and cropping system. Note: if keeping this, will add Tukey HSD significance indicators.

Year	Date	Depth	Crop	emmean	SE	Group
2020	8/11/20	40	annual-cc	0.200	0.022	a
			annual	0.209	0.023	ab
			IWG-alf	0.260	0.022	ab
			IWG	0.267	0.022	b
	9/15/20	30	annual	0.189	0.020	a
			annual-cc	0.200	0.020	ab
			IWG-alf	0.244	0.020	ab
			IWG	0.264	0.020	b
		40	annual-cc	0.190	0.022	a
			annual	0.195	0.022	ab
			IWG-alf	0.246	0.022	ab
			IWG	0.257	0.022	b
	9/29/20	10	IWG-alf	0.118	0.017	a
			IWG	0.126	0.017	a
			annual	0.155	0.018	ab
			annual-cc	0.186	0.017	b
		40	annual-cc	0.185	0.022	a
			annual	0.203	0.022	ab
			IWG-alf	0.232	0.022	ab
			IWG	0.252	0.022	b
2021	6/9/21	10	IWG-alf	0.111	0.009	a
			IWG	0.135	0.009	ab
			annual-cc	0.154	0.009	b
			annual	0.157	0.009	b
		20	IWG-alf	0.169	0.012	a
			IWG	0.204	0.012	ab
			annual-cc	0.238	0.012	b
			annual	0.244	0.012	b
		30	IWG-alf	0.201	0.014	a
			IWG	0.225	0.015	ab
			annual-cc	0.251	0.014	ab
			annual	0.256	0.015	b
	6/25/21	20	IWG-alf	0.134	0.012	a

			IWG	0.148	0.012	a
			annual-cc	0.177	0.012	a
			annual	0.225	0.012	b
		30	IWG-alf	0.174	0.014	a
			IWG	0.175	0.014	a
			annual-cc	0.192	0.014	ab
			annual	0.242	0.014	b
		60	IWG-alf	0.202	0.018	a
			IWG	0.203	0.018	a
			annual	0.254	0.018	ab
			annual-cc	0.259	0.018	b
		8/12/21	100	annual	0.167	0.058
	IWG			0.170	0.058	ab
	IWG-alf			0.180	0.058	ab
	annual-cc			0.283	0.058	b

Supplementary Table 1.5: Results of Tukey's HSD test on soil moisture values along a depth profile. Only significant differences in means are reported due to large sample size.

Chapter 2: Surface soil bacterial and fungal community composition and potential function after two years under annual and perennial grain crop management

2.1 Abstract

Soil microbial communities are key regulators of soil nutrient cycling, soil physical structure, and plant-soil interactions. In order to better understand the processes driving agricultural soil health under management changes, we must empirically connect soil microbial community composition and activity to specific soil qualities and ecosystem functions. In this study, we explore soil microbial community and soil physical and chemical quality responses to contrasting cropping systems representing a range of perenniality, crop diversity, and disturbance over a two year period. The four cropping systems we assess are perennial intermediate wheatgrass (IWG, *Thinopyrum intermedium*, Host Buckworth and Dewey), IWG intercropped with alfalfa (*Medicago sativa*), an annual rotation of soybean and corn (*Glycine max*, *Zea mays*) with strip tillage and winter cover crops (winter cereal rye, *Triticum aestivum*, and hairy vetch, *Vicia villosa*), and an annual rotation of soybean and corn with conventional tillage. We assessed soil microbial community diversity, composition, and function one and two years after system establishment. We observed no significant differences in microbial community function as measured by extracellular enzyme activity and litter decomposition rates between the four cropping systems two years after establishment. We find no statistically significant differences in fungal and bacterial community richness indices between cropping systems in 2020 and 2021. We find that crop explains 4% of variation in bacterial community composition in 2020, and 5% and 10% of variation in fungal community composition in 2020 and 2021, respectively. Both 2020 NLFA indicators and 2021 fungal taxonomic abundance show a significant increase in arbuscular mycorrhizal fungi (AMF) in IWG systems compared to annual systems. Extracellular enzyme activity varies by cropping

system and trends higher in systems with continuous living cover (IWG and cover-cropped), but there are no significant differences in mean activity by cropping system. Finally, bacterial community diversity varied significantly with soil pH in 2021, but there were no other significant relationships between soil properties or active and total nutrient pools and microbial community structure, diversity, and potential function.

2.2 Introduction

Globally, annual and biennial herbaceous crops account for about 11% of global land use (FAO, 2021). Annual management practices including frequent soil disturbance through tillage, seasonally bare soils, shallow annual crop roots, and lack of organic inputs have resulted in widespread soil degradation and nutrient loss because of disruptions to natural soil structure and nutrient cycling systems (DeHann et al., 2007; Bai and Cortrufo, 2022). Perennial grain crops, in contrast, provide continuous living cover of the soil, reduce the need for tillage and other soil disturbances, and allocate significantly more biomass to their deep root systems than annual crops (Sprunger et al., 2019; Duchene et al., 2020), and are therefore seen as a promising method for building more sustainable agroecosystems (Crews and Rumsey, 2017; Duchene et al., 2019; Zhang et al., 2022). In natural ecosystems, larger, perennial root systems and increased root exudation are associated with distinct and more active soil microbial communities, which together with root inputs drive improvements in soil nutrient pools and fluxes (Sokol and Bradford, 2019; Zatta et al., 2014) and physical structure (Rui et al., 2022; Culman et al., 2010; DuPont et al., 2014; Pérès et al., 2013; Stockmann et al., 2013). Similarly, practices which increase living cover on soils and reduce soil disturbance in annual crop systems are associated with changes in soil microbial community composition and function which drive improvements in soil function (ex. Liu et al.,

2021; Poeplau and Don, 2015; Stewart et al., 2018). Perennial grains therefore have significant potential to improve agricultural soil nutrient cycling, nutrient storage functions, and physical quality through processes driven by soil microbial communities.

Soil microbial communities are key regulators of soil nutrient cycling and organic matter storage, soil physical structure (ex. mycorrhizae) (Rillig and Mummey, 2006), and soil-plant interactions (Jacoby et al., 2017; Nadarajah et al., 2021). For example, under the current model of SOM formation and persistence, microbial communities drive the formation and stability of plant and microbially-derived SOM within the context of plant inputs and abiotic factors, which regulate their activity (Lehman and Kleber, 2015; Whalen et al., 2022; Baldock and Skjemstad, 2000; Birkhofer et al., 2021). Within agricultural soils, bacterial and fungal community composition and function are sensitive to management practices (Chang et al., 2021; Paungfoo-Lonhienne et al., 2015; Schmidt et al., 2017), and plant type (Jacoby et al., 2017; Haichar et al., 2008; Pérès et al., 2013), which should drive changes in soil physical and chemical properties. However, we need to increase our mechanistic understanding of how crop type, management, and environmental factors affect microbial communities, and in turn to improve our understanding of how these microbial communities actually regulate soil functions such as stabilization and turnover of SOC, in order to more accurately predict and model the effect of management strategies on soil nutrient cycling (Button et al., 2022; Bailey et al., 2019). Studying the responses of soil microbial community composition and function to management practices is therefore key to improving our understanding of the mechanisms behind soil quality responses to management.

Previous research provides evidence of significant and swift relative differences in microbial community biomass, diversity, and function under perennial vs. annual grain crops that may lead to desirable changes in soil function over time. After only a few years, perennial

management systems can result in increased fungal biomass and mycorrhizal abundance (Mann et al., 2019), and a fungal community structure more similar to native ecosystems than annual agricultural soils (McKenna et al., 2020). Audu et al. (2022) found increased SOC, microbial biomass, and potential enzyme activities at 30-60 cm depth in IWG systems compared to conventionally managed annual winter wheat crop two years after system establishment, indicating an increase in desired microbial community function and potential soil carbon storage outcomes in IWG systems (Beniston et al., 2014; Gill and Jackson, 2000). Bak et al. (2022) found that IWG and alfalfa recruit N-fixing bacteria to depth 3m and found evidence of decreased C:N ratios which may facilitate long-term carbon storage (Kopittke et al., 2020). There are still rapid developments in perennial crop management techniques and the potential for significant changes to plant biomass allocations due to breeding for increased yield, which could affect the plant's biomass allocations and interactions with microbial communities. Additional studies of the responsiveness of soil microbial communities to IWG cropping systems, especially directly compared to annual grain cropping systems, are necessary as the germplasm and management strategies evolve.

Many studies have explored changes in microbial community composition in IWG vs. annual systems (Bak et al., 2022; Piexoto et al., 2022; Taylor et al., 2022; McKenna et al., 2020; Sprunger et al., 2019), but few have concurrently measured changes in potential microbial community function (Rakkar et al., 2023; Audu et al., 2022). However, changes in potential microbial community activity under perennial grains may provide insights into the mechanisms underlying microbially-induced soil quality improvements such as improved soil structure and significant SOM accumulation. For example, there is a growing body of research exploring potential SOM accrual in IWG systems because of significant excitement about the potential environmental (Oldfield et al., 2022; Bossio et al., 2020), economic, and fertility (Lal, 2020)

benefits of SOM accumulation under perennial grains (Gill and Jackson, 2000; Audu et al., 2022). These studies show little consistent evidence of improvements in total SOC stocks or operationally-defined labile carbon pools in surface soils over the 2-3 year periods when the crop produces significant grain yields (Culman et al., 2013; Sprunger et al., 2018; Sprunger et al., 2019; Rakkar et al., 2023), but significant accrual of particulate organic matter (POM) carbon (Audu et al., 2022; Van de Pol et al., 2022) and microbial stabilization of rhizodeposited carbon (Peixoto et al., 2020) at depth over the same short time periods. Since it is well established that IWG has greater shallow root biomass than annual counterparts (Sprunger et al., 2019; Sainju et al., 2017; Bergquist, 2019; Dobbratz, 2019; Link et al., in preparation) but also twice the C:N ratio (Sprunger et al., 2019), lack of corresponding SOM increases in surface soils is likely due to microbial community priming, or the increased rate of consumption of carbon inputs (Kuzyakov, 2010; Bailey et al., 2019; Kopittke et al., 2020). This hypothesis could be investigated using measures of potential activity such as extracellular enzyme activity and litter decomposition. Improved understanding of the structure and especially activity of microbial communities under IWG systems could provide insights into the decomposition rates and microbial priming dynamics that mediate soil qualities at different depths and timescales.

In this study, we assess the linkages between agricultural management systems, soil microbial community characteristics, and potential microbial community function one and two years after the implementation of annual and perennial grain management systems. We also examined how these measurements relate to trends in total active soil C and N pools and soil physical and chemical properties. These systems represent gradients of perenniality/continuous cover, cropping system diversity, and soil disturbance intervals. Our research questions were 1) How are soil microbial community diversity, composition, and function affected by two years of

crop management systems representing a range of perenniality, crop diversity, and soil disturbance intensity?; and 2) Is there co-variability of direct soil microbial community composition and function, total active soil C and N pools, and soil physical and chemical properties? We hypothesized that after two years, perennial and annual cropping systems would have distinct bacterial and especially fungal community composition and that perennial systems would display increased microbial growth and enhanced microbial function.

2.3 Methods

2.3.1 Experimental Design

The experiment was conducted at the University of Minnesota's Rosemount Research and Outreach Center in Rosemount, MN, USA (44°42'42.3"N 93°06'51.1"W). Before the experiment was established, the field was under cultivation in alfalfa for two growing seasons. Alfalfa was terminated and experiment was fallowed in fall 2018, and planted to oats (*Avena sativa*) for the 2019 growing season. The experimental design was a split-plot, randomized complete block design with five blocks. The split-plot design allowed for climate treatments to be imposed on main plots in 2022 and 2023, after establishment of the cropping system treatments that were imposed on the sub plots. However, the 2020 and 2021 data reported here were collected from sub plots prior to main plot treatment application. Therefore, the experiment as described for this study was conducted as a randomized block design. Each block contained four replicates of each cropping system grown in 6.1 by 6.1 m plots, for a total of 20 replicates per cropping system. Analyses in this study were performed on all replicates (n=80). Detailed agronomic methods are available in Link et al., in preparation.

The soil type is Waukegan silt loam with 0 to 1 percent slopes (Fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludoll) (Soil Survey Staff, accessed June 8, 2022). From 1991-2020, the mean annual precipitation at the site was 87 cm and mean annual temperature was 6.7 °C (Arguez et al., 2010, accessed 8/18/2022). Historical average monthly precipitation and growing degree days (GDD) and annual cumulative precipitation and GDD over the course of the experiment are shown in Link et al., in preparation (Figure 1.1). Of note, the field site experienced below average precipitation during the first growing season (2020; total annual accumulation 21% below 30-year average), and a historic drought during the second growing season (2021; total annual accumulation 31% below 30-year average).

We assessed fungal and bacterial community composition using a metabarcoding approach of the 16S and ITS regions, total microbial biomass and functional group biomass using PLFA/NLFA, and potential microbial function via both hydrolytic enzyme activity and litter decomposition rates. We also assessed total and active C and N pools, soil fertility, soil physical structure, and agronomic and belowground biomass (presented in Chapter 1), and investigated relationships between soil microbiological communities and these nutrient statuses and soil qualities.

2.3.2 Soil sampling and storage

In 2020, soil samples were taken from each plot at 0-15 cm and 15-30 cm depths using a hand soil push probe with an inner diameter of 2.5 cm. 10 subsamples were taken from throughout the crop aisles and inter row and row spaces of each plot on August 20 and 25th. Probes were wiped of visible soil between plots. In 2021, a hydraulic soil probe (Giddings Machine Co, Windsor, CO) with an internal diameter of 5 cm was used to collect bulk soil samples and root

biomass samples on August 12 and 13th. Three subsamples from 0-15 and 15-30 cm increments were collected for each plot, one from in-row, row shoulder, and crop aisle.

In both 2020 and 2021, bulk soils were homogenized by hand and subsampled in the field for extracellular enzymes (carried on ice until frozen at -20°C), PLFA/NLFA (carried on ice, frozen to -20°C ; freeze dried within 4 weeks), DNA extraction (carried on dry ice until frozen to -80°C). Subsampling equipment was cleaned with 70% ethanol between samples. The remaining field wet bulk sample was refrigerated until subsampled for soil gravimetric moisture; 20-25g subsamples were oven dried at 105°C for at least 24 hours, and weight was used to calculate gravimetric water content (Gardner, 2018). See Link et al. (in preparation) for soil subsampling and laboratory methods related to soil physical and chemical property analysis.

2.3.3 Phospholipid Fatty Acid /Neutral Lipid Fatty Acid

PLFAs and NLFAs were extracted and quantified using a modified Bligh and Dyer (1959) protocol (Firestone lab, 2021). This is a 3 step process of lipid extraction, separation, and methylation. Fatty acids were extracted from 8 g whole soil by adding 5:6:12 ml citrate buffer:chloroform:methanol, sonicating for 15 minutes, centrifuging, and removing the supernate. The extraction process was repeated 3 times, followed by adjustment of the ratios of solvents to 0.9:1:1 in the supernate and then leaving it overnight for phases to separate. Then, the lipid-containing CHCl_3 layer of extraction replicates was combined and liquids evaporated using a Rapid Vac. To separate lipids, silica acid chromatography was performed; dried lipids were resuspended in chloroform, eluted through a silica column with CHCl_3 (saved as neutral lipid fraction) followed by acetone (waste/glycolipids) and methanol (saved as phospholipid fraction). PLFA and NLFA samples were again dried in the Rapid Vac, topped with nitrogen, and stored frozen at -20°C . Methylation was performed no more than two weeks before analysis. The

methylation process involved first forming fatty acid methyl esters (FAMES) by resuspending samples in 0.5:0.5:1 ml chloroform:methanol:0.2 M methanolic potassium hydroxide and incubating at 37°C for 30 minutes. Then, FAMES were extracted by adding 2:0.2:2 ml hexane:acetic acid:water, centrifuging, and removing the top FAME-containing layer. Extraction was repeated three times, and extracts were dried in the Rapid Vac. Samples were resuspended in 50 µl of a C 13:0 (250 ng/µl) and transferred to GC vials. Samples were analyzed using an Agilent 7890 gas chromatograph. Chromatogram peaks were named as lipid markers by visual inspection and assignment. A total of 17 PLFAs were identified in all samples, as well as 16:1 ω5c NLFA (Table S2.1). Peak lipid biomass (nmol g soil⁻¹) was defined as Equation 1:

$$\text{Lipid Biomass: nmol/g soil} = \frac{(\text{Peak area/K value}) * (50\text{ul}/2)}{\text{molecular weight of peak} * \text{soil mass (g)}}$$

Where K is the peak area of the 13:0 standard from the batch divided by the 500 ng present in the 13:0 FAME standard. Total biomass was calculated as the sum of peaks less than or equal to 20 carbons in length identified below. Microbial group assignments were made based on existing literature (see Table S2.1). NLFA 16:1 ω5c rather than PLFA 16:1 ω5c was assigned to arbuscular mycorrhizal fungi (Lekberg et al., 2022; Olsson and Lekberg, 2022). Fungal:Bacterial ratio was defined as the ratio of identified fungal group biomass (AMF, actinomycetes, saprotrophic fungi) to bacterial group biomass (Actinobacteria, Gram +, Gram -).

2.3.4 Litter Decomposition

We performed a litter decomposition experiment in June-July 2021, 22 months after IWG system implementation. To minimize cost and time and increase data comparability, we used the Tea Bag Index method (Keuskamp et al., 2006; Blume-Werry et al., 2021) rather than creating litter bags with native litters. This method has previously been adopted to assess organic matter

decomposition in soils in a variety of ecological contexts, including agricultural soils (Toth et al., 2017; Toleikiene et al., 2020), as tea leaf decomposition is representative of the decomposition of other leaf litters (Duddigan et al., 2020). This method utilizes the contrast between highly labile green tea and more recalcitrant rooibos tea to explore differences in decomposition of the two represented fractions.

We used commercially available green tea and rooibos tea from Riishi tea company packaged in tetrahedron-shaped synthetic tea bags with sides of 5-cm made of 250 um mesh. Tea bag weight was obtained prior to burial. Two separate sets of green and Rooibos tea bags were buried pairwise at a depth of 5 cm on a crop row shoulder in each plot on 6/2/2021. One set was retrieved on 6/30/2021 (28 days) and the other on 7/30/2021(58 days); removal was necessary prior to plot maintenance in August. Bags were cleaned using the following process: soil was gently brushed off, bags were gently rinsed in cool water until they ran clear, and bags were air-dried at room temperature for several days (Gluseen et al., accessed 5/2021). We report tea mass loss (weight %) at both retrieval dates (Houben et al., 2018).

2.3.5 Extracellular Enzyme Activity

We performed a microplate fluorometric assay based on German et al., 2011 to assess activity of hydrolytic enzymes in response to four substrates representing forms of C, N, and P found in soil; β -glucosidase (BG) and cellobiohydrolase (Cello) for cellulose and polysaccharide degradation, phosphatase (P) for polyphosphate degradation, and N-acetylglucosaminidase (NAG) for chitin degradation. All samples were circumneutral pH, so EEA was performed with a buffer adjusted to pH = 7. Briefly, 50 mM Tris buffer was added to 0.5 g soil samples and sonicated in a bath sonicator for 5 minutes. One clear 96-well plate was prepared for each sample with 10 replicate assay wells (soil slurry + substrates) for each substrate and well as control wells of

substrate blanks (substrates + Tris buffer), buffer blanks, homogenate blanks (soil slurry + Tris buffer), and MUB standards (4-Methylumbelliferone + soil slurry). For each batch of no more than 12 samples, a plate with Tris buffer + 4-Methylumbelliferone was also prepared. Plates were incubated at room temperature for 1 hour starting when soil slurry was added. After 1 hour, 10 μ L of 1 M NaOH were added to each well and plate absorbance was read exactly 1 minute after this addition. Calculations were performed as outlined in German et al, 2011, Equation 2:

$$\text{Activity (nmol/hr/g soil)} = (\text{net absorbance}/\epsilon) \times (1/\text{hrs incubation}) \times (\text{Buffer volume} / \text{homogenate volume}) \times (1/\text{soil weight})$$

Where:

Net absorbance = net absorbance units = assay absorbance-homogenate control-substrate control

ϵ = Extinction coefficient, 2 absorbance/nmol

Buffer volume= the total buffer added to soil at the beginning of the assay, in mL

homogenate volume= 0.2 mLs

soil wt. = oven dry equivalent, g.

C:N assay ratio was calculated as the sum of BG and Cello activities divided by NAGase activities (Tiemann et al., 2015), though we are aware of problems with this method due to NAGase correlating well with both C and N and approach interpretation of this ratio with caution. Unfortunately, 2020 data had to be excluded due to experimental error and insufficient sample to re-run the assay, and we report EEA from only 2021.

2.3.6 DNA Extraction

In 2020, DNA was extracted from soil using Qiagen's DNEasy PowerSoil extraction kit following the suggested protocol. Samples were lysized for 20 minutes. This kit was subsequently taken off market, and in 2021 samples were extracted using Qiagen's DNEasy PowerSoil Pro

extraction kit, which uses a different chemistry. Again, kit instructions were followed exactly; samples were lysized for 20 minutes each, and DNA suspended in 100 μ l 10 mM Tris and stored in -80 °C. Ten 2021 samples were also run using the old DNEasy PowerSoil kit to assess comparability of DNA extraction quantity, quality, and potential effects on diversity metrics (See Appendix 1).

2.3.7 Amplicon sequencing

We used a metabarcoding approach to characterize the soil bacterial and fungal communities via the 16S and ITS regions, respectively. Samples were sent to the University of Minnesota Genomics Center (UMGC) for library preparation and amplicon sequencing using 2x300 bp Illumina MiSeq. Amplification of the V4 16S rRNA region was carried out using primers 515F-806R (Caporaso et al. 2011) and amplification of the ITS2 region was carried out using primers 5.8S-Fun and ITS4-Fun (Taylor et al., 2016). Reads were received from UMGCC demultiplexed. Reads were processed using DADA2 version 1.16.0 (Callahan et al., 2016) following standard pipeline procedures with the following modifications on Minnesota Supercomputing Institute (MSI) resources. Primers were removed from 16S reads by forward trimming (F-19 bp, R-20 bp). Forward and reverse reads were truncated to 250 bp based on quality profile plots using trim-paired; quality trimming and filtering included the additional parameters maxEE=2 and trunQ=2. Primers were removed from ITS2 reads using cutadapt (Martin, 2011). ITS2 reads were not truncated; reads less than 50 bp were discarded and reads were quality filtered on the same parameters as 16S. We performed dereplication using DADA2's 'derep' function and denoising using the 'dada' function based on errors calculated with the 'learnErrors' function on paired sequences. Reads were paired using 'mergePairs'; poor quality of ITS2 reverse reads resulted in significant loss of reads during pairing, so we re-ran the above pipeline with single-end

reads and proceeded. 16S reads not within target amplicon length of 251-257 base pairs were removed. Chimeras were removed using the ‘removeBimeraDenovo’ function with the “consensus” method. We then used pre-trained classifiers on the SILVA [Version 138.1] (Quast et al., 2013; McLauren and Callahan, 2021; Callahan et al., 2016) and UNITE [Version 10.05.2021](Nilsson et al., 2019; Abarenkov et al., 2021) databases to assign taxonomy to 16S and ITS sequences, respectively. 16S rRNA sequences not assigned to kingdom Bacteria or Archaea were removed before proceeding. A total of 23,966 amplicon sequence variants (ASVs) for 16S and 12,691 ASV’s for ITS2 were obtained. See Appendix 2 for access to raw sequences.

2.3.8 Data Analysis: Extracellular Enzymes, Litter Decomposition, PLFA/NLFA

Analyses were conducted with R [Version 4.0.2] (R Core Team, 2022). R code available in Appendix 2. Datasets were assessed for adherence to the assumptions of ANOVA and post-hoc tests including homogeneity of variance and normal distribution. Analysis of variance (ANOVA) was performed using the lme command of the nlme package followed by a type III/marginal sum of squares ANOVA using nlme’s anova command (Pineiro et al., 2022). Crop system was treated as a fixed effect and block as a random effect. For all datasets, Tukey’s post-hoc analysis was performed to determine differences in distributions between cropping systems nested by year using estimated marginal means via the emmeans package (Lenth, 2021). Significant differences for all analyses were determined at $p < 0.05$, and marginally significant differences at $p < 0.10$.

2.3.8 Bioinformatic data analysis

Data analyses were performed in R [Version 4.1.0] (R Core Team, 2021) using the packages phyloseq (McMurdie & Holmes, 2013) and vegan (Oksanen et al., 2022). Data were analyzed separately by year after method testing and extensive analysis to determine the effect of

kit on DNA sequence results (see Appendix 1). For soil microbial community diversity and composition estimates (alpha and beta diversity), data were rarefied using sampling without replacement (phyloseq 'rarefy_even_depth') to 90% of the size of the smallest sample. After this step, 16,198 ASVs for bacteria and 6,279 ASVs for fungi remained. We assessed for differences in alpha diversity estimated by the Hill-Simpson and Hill-Shannon diversity indices (phyloseq 'estimate_richness'). Since the Hill-Shannon index was consistently normally distributed, we used ANOVA and Tukey's HSD to test for significant differences. Hill-Simpson index was not normally distributed and therefore pairwise differences between crop types were investigated using a Kruskal-Wallis test ('kruskal.test'). We also report species richness (total number of ASVs identified). Beta diversity was calculated using a Bray-Curtis index using phyloseq's 'distance' function, again separately by year. Differences between cropping systems were assessed via PERMANOVA using vegan's 'adonis' function with crop and block as factors. Homogeneity of group dispersions were assessed using vegan's 'betadisper' function. Beta diversity was visualized using a NMDS ordination (function 'ordinate') on bray-curtis index. To determine taxonomic relative abundance, unique ASVs were defined for each crop using indicpecies' 'multipatt' function. We also report the most abundant genera in each crop group, defined as genera making up greater than 1% of reads, and their relative abundances.

2.3.9 Covariability of microbial community and environmental variables

We individually assessed covariability of 2021 fungal and bacterial community composition, 2021 extracellular enzyme activities, and 2020 PLFA/NLFAs with soil physical and chemical properties. These properties were Total C, Total N, POX-C, ACE Protein, pH, OM, Total water stable aggregate fraction, water stable aggregate mean weight diameter, and aggregate size fractions 2mm-53 um (Link et al., in preparation). Data analyses were conducted in R [Version

4.0.2] using the vegan package (Oksanen et al., 2022). We calculated distance matrices of the environmental and microbial community datasets using Bray-Curtis dissimilarity with the ‘vegdist’ function. We then performed Mantel tests comparing scaled distance matrices of the datasets with the ‘mantel’ function using the Spearman method. We also performed ordinations of the microbial community datasets with the ‘MetaMDS’ function on Bray-Curtis dissimilarity index. Finally, we performed multiple regression of environmental variables with the first two axes of the microbial dataset ordinations using the envfit function followed by a false-discovery rate (FDR) correction.

2.4 Results

2.4.1 Litter decomposition - microbial community function

In the litter decomposition experiment, crop did not significantly affect mass loss from either green tea/labile or rooibos tea/recalcitrant litter over either the 28 day or the 58 time periods ($p > 0.05$). Litter type ($p < 0.0001$) and the interaction of the retrieval date and litter type ($p < 0.0001$) significantly affected mass loss. Green tea had higher mass loss than rooibos tea at both sampling dates (Figure S2.1). Fractional mass loss of green tea after 30 days was the same as mass loss after 58 days (means of 0.598 ± 0.013 se and 0.610 ± 0.0125 se, respectively). Fractional mass loss of rooibos tea after 30 days (emmean) was significantly lower than after 58 days (means of 0.429 ± 0.0125 se and 0.477 ± 0.0128 se, respectively).

2.4.2 Extracellular enzyme activity - microbial community function

Crop significantly affected Cello activity and NAG activity in samples taken in August 2021, two years after system implementation. There were no significant differences in the means of individual cropping systems. Cellobiohydrolase, N-acetylglucosaminidase, and Phosphatase

activities trended lower in annual systems (Cello = 54.85, NAGase = 54.57, $P = 1087.93$), compared to annual-cc (Cello = 71.81, NAGase = 68.1, $P = 1219.68$), IWG (Cello = 57.55, NAGase = 68.69, $P = 1265.4$), and IWG-alf systems (Cello = 69.81, NAGase = 89.41, $P = 1287.58$) (Figure 2.1, Table S2.2). As extracellular enzyme activities became more dissimilar, soil physical and chemical properties did not become more dissimilar ($r = -0.001$, significance = 0.50). We found no relationships between EEA 2D ordination (Stress = 0.04) and variance in soil physical and chemical properties or root biomass.

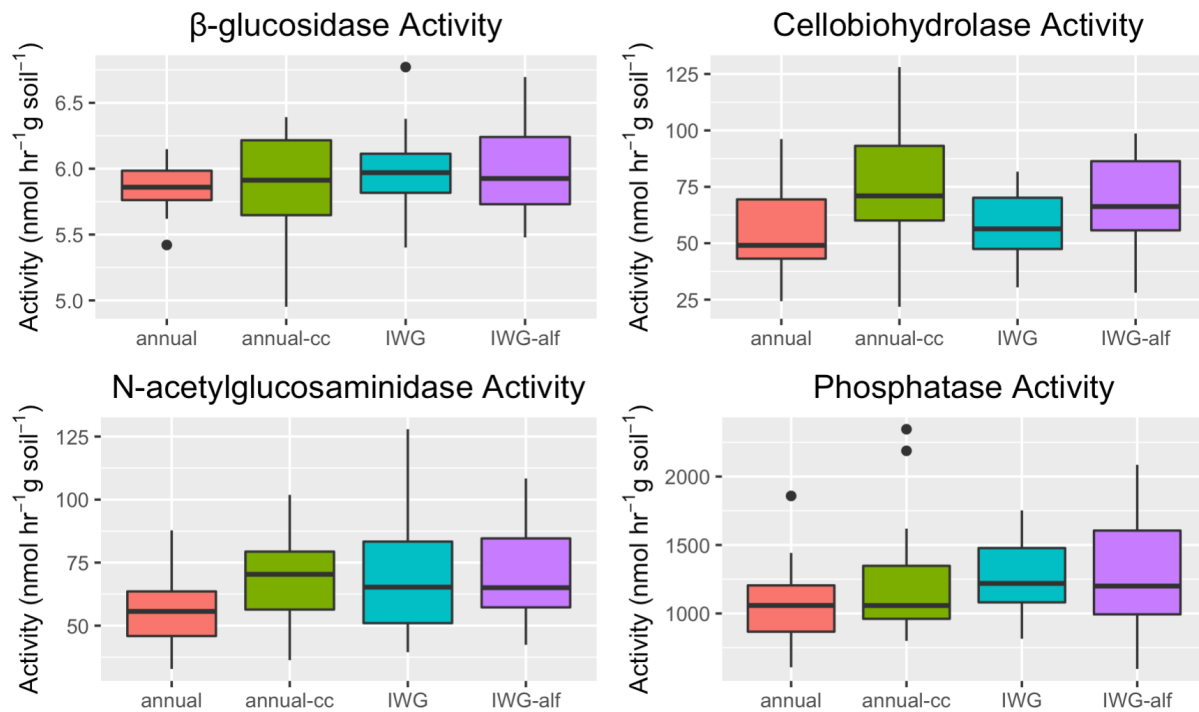


Figure 2.1: Boxplots of enzyme activity across cropping systems in 2021. ANOVA reveals that Cellobiohydrolase and N-Acetylglucosaminidase activity vary by cropping system. There are no significant differences in activity estimated marginal means as shown by Tukey's HSD post-hoc testing.

2.4.3 PLFA/NLFA - Microbial biomass and community composition

In 2020, log-transformed AMF biomass varied significantly by cropping system (ANOVA: $F=5.38$, $P = 0.002$). There was more AMF biomass in IWG systems (mean = 22.1 nmol/gsoil , se

= 3.06) than annual (mean = 9.13, se = 0.95), annual-cc (mean = 12.7, se = 2.23) and IWG-alf (mean = 8.19, se = 0.92) (Figure 2.2). There were no differences in total microbial lipid biomass or biomass of actinomycetes, gram negative bacteria, or gram positive bacteria by cropping systems (Table S2.2, S2.3). The 2021 PLFA/NLFA dataset is still in progress.

There was no covariation in soil physical and chemical properties and PLFA+NLFA lipid class biomasses (ANOVA: $r = 0.022$, significance = 0.304). There were no linear relationships between any soil physical and chemical properties or root biomass and a 2D ordination of PLFA+NLFA lipid classes (stress = 0.077).

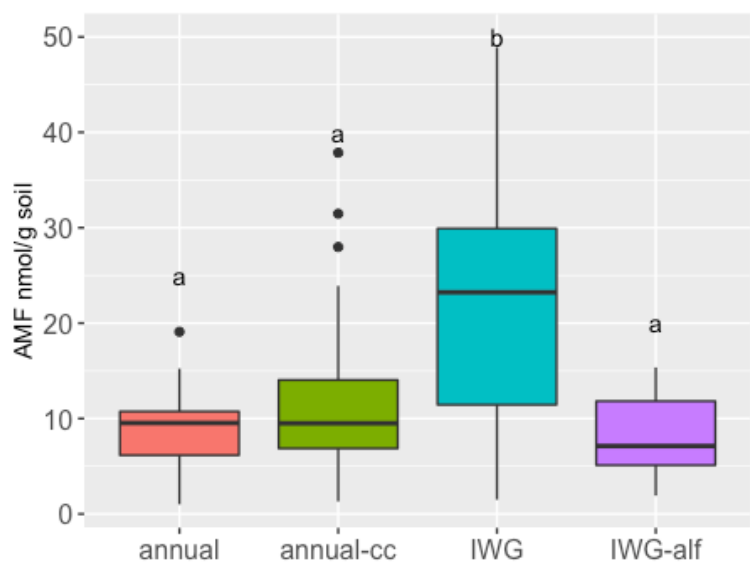


Figure 2.2: Boxplot of NLFA AMF fungi lipid biomass by cropping system. Letters indicate significant differences, Tukey's HSD.

2.4.4 Bacterial and Fungal diversity and community composition

We found no significant variation in bacterial Hill-Shannon and Hill-Simpson indices by crop in either 2020 or 2021, and no significant pairwise differences between crop systems (Table S2.4). In 2020, fungal Hill-Shannon and Hill-Simpson indices were marginally and significantly influenced by crop ($F = 2.26$ and $P = 0.09$, Kruskal-Wallis chi-squared = 8.3 and $p = 0.039$). There

were no significant pairwise differences between systems, though Wilcoxon rank-sum exact test revealed marginally significant differences between Hill-Simpson indices of annual-cc and IWG-alf systems ($p = 0.08$) and annual and annual-cc systems ($p=0.08$) (Table S2.5). In 2021, fungal diversity indices did not vary by crop and there were no statistical differences between cropping systems (Table 2.1).

We assessed differences in species diversity by cropping system. We found that bacterial community composition varied by cropping system in 2020, and the model explained 4% of the total variance in the pool of samples (PERMANOVA: $p = 0.03$, $R^2 = 0.04$) (Table 2.1; Ordination visualizations Figure S2.2). However, bacterial community composition did not vary by cropping system in 2021. Fungal community composition varied significantly by cropping system in both 2020 and 2021, with the model explaining 6% and 10% of the total variation in distances each year, respectively (Table 2.1). Betadisper, a multivariate test for homogeneity of variances, revealed significant differences in the distribution of community composition only in the bacterial 2021 dataset, where there were no significant differences in community composition centroids. Therefore, we accept the significance of the PERMANOVA results.

We assessed taxonomic relative abundance and guild relative abundance for 2021 sample data. Relative abundances of the most abundant phylum are shown in Figure 2.3, and relative abundances of genera making up >1% of reads in each cropping system are shown in Tables 2.2 and 2.3. ASVs identified as indicator genera for cropping systems are reported in Tables S2.6 and S2.7. Fungal indicator taxa for IWG and IWG-alf systems include several members of the orders *Diversisporales* and *Glomerales*, which are fungi forming arbuscular mycorrhizae.

Finally, we investigated covariability of 2021 fungal and bacterial community composition with measured soil physical and chemical properties, surface soil (top 0-15 cm) root biomass, and

EEA. As bacterial and fungal communities become more dissimilar, environmental variables do not become more dissimilar (Mantel tests: ITS: $r = 0.06527$, significance = 0.17; 16S $r = 0.005$, significance = 0.45). pH significantly related to bacterial community composition 2D ordination (stress = 0.15) ($R^2 = 0.1658$, $p = 0.008$) (Figure S2.3). We found no linear relationships between fungal community composition 2D ordination and measured soil physical and chemical properties, root biomass, or EEA.

Year	Test	16S				ITS			
		Sum of Sqs.	R^2	F-stat	P-value	Sum of Sqs.	R^2	F-stat	P-value
2020	Adonis	0.45	0.04	1.17	0.03	0.71	0.06	1.45	0.001
	Betadisper	0.0002	-	0.48	0.70	0.006	-	1.13	0.34
2021	Adonis	0.34	0.04	1.04	0.169	1.2	0.10	2.73	0.001
	Betadisper	0.02	-	2.92	0.04	0.007	-	0.38	0.82

Table 2.1: Results of Adonis and betadisper tests assessing differences between community composition of fungal and bacterial communities in 2020 and 2021.

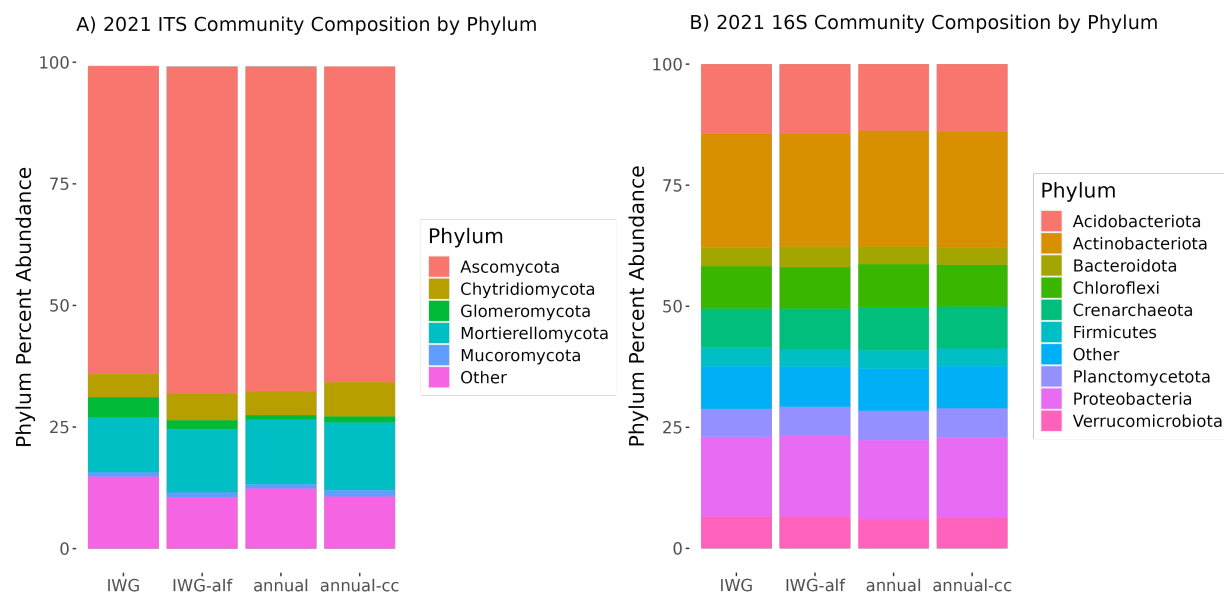


Figure 2.3: The most abundant A) fungal and B) bacterial phyla across cropping systems in 2021 in topsoils 0-15 cm.

Genus	Annual	Annual-cc	IWG	IWG-alf
<i>Mortierella</i>	16.2	17.1	14.4	16.1
<i>Fusarium</i>	11.2	11.5	9.1	8.9
<i>Gibellulopsis</i>	10.1	4.8	4.6	4.8
<i>Plectosphaerella</i>	5.1	2.9	2.6	4.4
<i>Tetracladium</i>	4.7	5.3	4.2	4
<i>Alternaria</i>	3.5	5.4	7.2	7.1
<i>Bolbitius</i>	2.3	0	0	0
<i>Ascobolus</i>	1.7	1.1	0	1.1
<i>Penicillium</i>	1.6	1.3	0	1.7
<i>Solicoccozyma</i>	1.6	2.3	1.5	1.6
<i>Pseudogymnoascus</i>	1.5	1.7	1.5	1.4
<i>Mrakia</i>	1.5	0	0	0
<i>Nectria</i>	1.5	2	0	0
<i>Gibberella</i>	1.4	0	1.1	0
<i>Coniochaeta</i>	1.3	1.3	1	0

<i>Clonostachys</i>	1.3	1.7	0	0
<i>Trichoderma</i>	1.3	1.5	1.5	1.2
<i>Myrmecridium</i>	1.2	1.3	1.6	1.6
<i>Bipolaris</i>	1.1	1	1.7	1.9
<i>Humicola</i>	1	1.3	1.1	0
<i>Staphylotrichum</i>	1	1.4	1.9	1.1
<i>Tausonia</i>	0	1.4	0	0
<i>Talaromyces</i>	0	1.2	0	0
<i>Pseudopithomyces</i>	0	1.1	2.5	1.4
<i>Cladosporium</i>	0	1	1	1.6
<i>Acremonium</i>	0	1	1.1	0
<i>Glomus</i>	0	0	2.2	0
<i>Phaeosphaeria</i>	0	0	1.2	1.2
<i>Conocybe</i>	0	0	1.1	0
<i>Oidiodendron</i>	0	0	1.1	0
<i>Pyrenochaetopsis</i>	0	0	1	1.2
<i>Rhizophlyctis</i>	0	0	1	2.1
<i>Neosetophoma</i>	0	0	0	1.9
<i>Epicoccum</i>	0	0	0	1.3
<i>Pseudorobillarda</i>	0	0	0	1.2
Sum all	72.1	70.6	67.2	68.8
Sum Common	58.5	56.2	51.8	54.1

Table 2.2: Relative abundance of fungal genera making up >1% of all reads in each cropping system, 2021.

Genus	Annual	Annual- cc	IWG	IWG-alf
<i>Candidatus Udaeobacter</i>	8.1	9	9.8	8.9
<i>Candidatus Nitrocosmicus</i>	5.9	5.9	5.1	5.3
<i>Bacillus</i>	5.5	5.2	5.6	5.3
<i>Gaiella</i>	5.3	5.1	5.6	5
<i>Arthrobacter</i>	2.9	3.3	2.7	2.7
<i>Nocardioides</i>	2.3	2.3	2.2	2.4
<i>RB41</i>	2.1	2.2	2.4	2.3
<i>Conexibacter</i>	2.1	2	2.1	2
<i>Sphingomonas</i>	2	2	2	2
<i>Solirubrobacter</i>	2	2	1.9	1.9
<i>Nitrospira</i>	1.8	1.8	1.7	1.5
<i>Pirellula</i>	1.7	1.6	1.8	1.7
<i>Bradyrhizobium</i>	1.6	1.6	1.7	1.7
<i>Haliangium</i>	1.6	1.6	1.6	1.5
<i>MNDI</i>	1.5	1.4	1.4	1.1
<i>Streptomyces</i>	1.4	1.4	1.1	1.3
<i>Pseudolabrys</i>	1.3	1.4	1.3	1.2
<i>Hyphomicrobium</i>	1.3	1.2	1.3	1.2
<i>Microclunatus</i>	1.2	1.4	1.4	1.4
<i>Subgroup 10</i>	1.2	1.1	1.1	1.2
<i>Steroidobacter</i>	1.2	1.1	1.3	1.2
<i>Chthoniobacter</i>	1	0	1	1.1
<i>Bryobacter</i>	1	1	0	0
<i>Mycobacterium</i>	1	0	0	0
<i>Acidibacter</i>	0	1	1.1	0
Sum all	57	56.6	57.2	53.9
Sum Common	54	54.6	55.1	52.8

Table 2.3: Relative abundance of bacterial genera making up >1% of all reads in each cropping system, 2021.

2.5 Discussion

This study investigates microbial community changes in annual and perennial IWG grain systems representing a gradient in continuous living cover, system diversity, and disturbance intervals after two growing seasons. We found evidence of increased AMF total biomass and indicator AMF species in IWG systems relative to annual systems and higher extracellular enzyme activity indicating potentially enhanced nutrient cycling in IWG and cover cropped annual systems relative to the conventional annual system. We found no significant relationships between measured soil microbial community composition and function metrics and soil physical and chemical properties despite significant differences in aggregate stability, pH, and K availability between annual and IWG systems after two growing seasons (Link et al., in preparation). Previous studies have investigated the short-term impacts of IWG systems on soil food webs, carbon stabilization and storage and, recently, linkages between SOC, microbial structure, and potential microbial function (Sprunger et al., 2019; Peixoto et al., 2020; Bak et al., 2022; Peixoto et al., 2022; Audu et al., 2022). However, none that we know of have thoroughly assessed microbial community structure and function concurrently with soil chemical/physical quality in a field setting with a high degree of replication. This work directly contributes to increased understanding of how perennial vs. annual management practices affect soil microbial community composition and function and the relationships between microbial communities and desired soil health outcomes.

2.5.1 Enhanced microbial activity under continuous living cover systems after two years.

Extracellular enzyme activities are generally responsive to agricultural and land management practices on longer implementation timescales (Tiemann and Grandy, 2015; Dose et al., 2015; Veres et al., 2015). After only two years of system implementation, we begin to see

enhanced potential microbial processing of cellulose, chitin, and phosphorous containing compounds in systems providing continuous living cover of the soil (Huang et al., 2011; Veres et al., 2015). Generally, our observations of slightly enhanced EEA in soils with continuous living cover are in line with previous understanding of the responsiveness of potential EEA to management; several IWG studies with less replication than our experiment have found that surface soil enzyme activities are not responsive to two years of IWG management (Rakkar et al., 2023), though C-obtaining enzyme activities are enhanced at 30-60 cm depth even within that short timeframe (Audu et al., 2022). With the higher degree of replication in our experiment, we are perhaps more able to observe small, initial changes in surface EEA which we expect will increase over time and with depth. Increasing enzyme activity indicates a functional response of the microbial community to litter supply and a shift in effort by microorganisms towards obtaining C, N, and P from these sources (Veres et al., 2015). Root biomass, turnover, and exudation are significant sources of C and other nutrients to soils (Brzostek et al., 2013; Freschet et al., 2017). Here, it is likely that increased root biomass and exudates from perennials and cover crops are increasing labile C, N, and P pools and driving increased microbial activity and enzyme excretion (Hargreaves et al., 2013). For example, Cello is active in later phases of degradation of cellulose, a main component of plant roots (Turner et al., 2002; Galloway et al., 2020). While we might therefore expect that increased EEA may be associated with increases in labile or total C and N pools, we observed no such relationships, nor did we observe increases in total or labile C or N pools (Chapter 1). This could reflect increased microbial decomposition rates (priming) of increased labile inputs, which may be indicated by increases in total microbial biomass when we have that dataset. It could also reflect little actual labile carbon and other nutrient pool accrual in surface soils under two years of IWG/cover cropping management (Sprunger et al., 2019).

Previous research has also reported a decoupling of EEA and SOC pool sizes (Sinsabaugh et al., 2007), though they are often positively associated (Audu et al., 2022, Hargreaves et al., 2013).

Contrary to our hypothesis and to our results indicating enhanced potential soil EEA under crop systems with continuous living cover, we observed no differences in litter decomposition rates by cropping system. This is likely because we are just beginning to see differences in soil biological community activity two years after cropping system implementation. It could also be a function of the historic drought that occurred during the litter decomposition experiment, as soil moisture limitations may have been more limiting to microbial community function than microbial community differences between cropping systems (Xie et al., 2020; Glass et al., 2022). Litter decomposition is a function of climate, litter quality, and decomposer type and abundance (Coûteaux et al., 1995) and is sensitive to agricultural management practices including soil disturbance interval and annual vs. perennial crop types (Wardle et al., 1999; Glass et al., 2022; Carlesso et al., 2019). As the first step in the decomposition of litter inputs, it represents a primary control on SOC POM and MAOM formation. While the majority of carbon/litter inputs in annual soil systems come from surface litter inputs and its incorporation via tillage, perennial bioenergy crops provide fewer surface litter inputs and the majority of inputs come from root biomass (Anderson-Teixeira et al., 2013). This leads to more active root-associated C cycling in perennials vs. active surface soil C cycling in annual systems. Previous literature has found that after 4 and 7 years of annual crop vs. perennial crop management, decomposition rates of litter buried at 5 cm depth are higher in annual vs. perennial crops and higher in mulched vs. unmulched perennial crops, especially in the first few months of decomposition (Wardle et al., 1999). We expect, therefore, that if this experiment were replicated under longer-term management, surface litter

decomposition would be enhanced in annual systems compared to perennial systems, especially under adequate moisture conditions.

2.5.2 AMF biomass greater than annual systems' in IWG but not IWG-alf systems

We observed increased AMF biomass under IWG vs. annual, annual-cc, and IWG-alf systems after just one year of cropping system implementation, when the annual systems were planted in soybean. Similarly, Duchene et al. (2020) found significant increases in fungal lipid markers indicating AMF in IWG vs annual systems in the topsoil layer (10 cm) after only one growing season. In contrast, two previous studies in Rosemount soils have shown no responses in AMF biomass even after two years (Rakkar et al., 2023; Bergquist, 2019). The differences between our results and these previous Rosemount studies could be due to the fact that these previous studies utilized 16:1 ω 5 PLFA to estimate AMF biomass while in this study we utilized 16:1 ω 5 NLFA, which has higher specificity and better indicates AMF spore density, biomass, and colonization (Sharma and Buyer, 2015; Lekberg et al., 2022; Duchene et al., 2020). Our experiment also contained a greater number of replicates (n=20 for each cropping system), increasing power and our ability to observe small but significant differences. Regardless, the increase in AMF biomass in IWG systems seen in our experiment points to a host-specific AMF growth response relative to annual and IWG systems containing alfalfa intercrop (Boerner, 1992; Vestberg et al., 2015). In 2020, our IWG-alf plots were dominated by alfalfa, which also forms relationships with AMF and, as a legume, needs significant P for P-intensive N acquisition. We only assessed microbial biomass and community composition in 0-15 cm surface soils, but perhaps alfalfa, with its deep taproot, maintains more AMF relationships and draws more P from deeper in the soil profile. This lack of AMF growth response could have implications for the soil quality improvement potential in IWG-alf biculture systems, since increased AMF abundance generally

confers enhanced soil aggregation and enhance tolerance to climate extremes through enhanced nutrient acquisition (Begum et al., 2019; Rillig, 2004). One year after system establishment, we find no statistically significant relationships between AMF microbial biomass and soil physical and chemical qualities, and no differences in soil aggregate stability by cropping system (Link et al., in preparation). However, in 2021 we observed increased soil aggregate stability in IWG but not IWG-alf systems compared to annual systems. 2021 NLFA/PLFA 16:105 will indicate whether this trend is statistically associated with differences in AMF biomass indicators.

We observed no responses in PLFA indicator total microbial biomass, total bacterial biomass, or total fungal biomass by cropping system one year after implementation, reflecting previous studies which have found no change in soil bacterial biomass and sometimes limited increases in fungal biomass in IWG systems 1-3 years after implementation (Duchene et al., 2020; Rakkar et al., 2023; Bergquist et al., 2019). We expect that PLFA/NLFA in 2021 will also reflect increases in AMF and perhaps also general fungal biomass in IWG vs annual systems due to increased root inputs of recalcitrant material (Six et al., 2016; Audu et al., 2022; Bai and Cortrufo, 2022). Generally, we may expect that total microbial biomass and bacterial microbial biomass may increase eventually in IWG systems under the longer-term cultivation due to increased plant carbon inputs, as seen in some sites of previous studies (Rakkar et al., 2023).

2.5.3 Soil bacterial and fungal diversity and community composition responses to annual vs perennial management

Our results demonstrate that after two years of management, annual vs. IWG systems begin to influence fungal community composition but not bacterial community composition or overall bacterial and fungal diversity. Other studies have similarly found that fungal community composition often responds to 2-3 years of IWG vs. annual system management (Taylor et al., in

preparation; Audu et al., 2022; Bergquist, 2019), though this is not always the case in surface soils (Rakkar et al., 2023; Audu et al., 2022). Land use intensity change is generally associated with responses in fungal community composition and especially AMF community composition, perhaps due to the fact that this taxonomic group has similar lifestyle strategies and low host specificity (Balami et al., 2020; Birkhofer et al., 2021). Echoing our observation that AMF biomass increased in 2020 in IWG systems, we see evidence that differences in AMF taxonomic abundance drive the fungal community compositional differences between cropping systems in 2021. While we find no statistically significant relationships between fungal community composition indicators and soil physical and chemical qualities, increased abundance of these specific taxonomic and functional groups is generally associated with desirable improvements in soil structure, yield, and yield resilience to climate extremes (McKenna et al., 2020). Future experiments exploring microbial community responses at multiple depths may reveal that increases in AMF biomass and changes in community composition are associated with improvements in soil physical structure seen after 2-3 years in IWG vs. annual systems (Link et al., in preparation; Rakkar et al., 2023). Previous research has found that the soil fungal saprotroph and symbiotroph community composition under 13-year IWG monoculture systems are more similar to that of native grassland vegetation than annual agricultural systems, indicating that removing soil disturbance alters microbial community composition even without increases in plant species functional diversity (McKenna et al., 2020; Birkhofer et al., 2021). Even in our short-term experiment, we found many indicator taxa in common in monoculture/biculture IWG systems that were distinct from annual/annual-cc systems. Changes in these particular fungal functional groups may, in longer term experiments, be associated with desirable improvements in soil C storage and nutrient pools and soil physical structure.

We observed significant differences in bacterial community composition between cropping systems in 2020 but not in 2021. Unfortunately, it is impossible for us to disentangle the effect of kit extraction type and year on our observed results in order to directly compare changes across years (Appendix 1). If there truly is a difference in community composition rather than kit-defined community composition in 2020 vs. 2021, perhaps it is due to increased root inputs of labile C into IWG systems driving temporary increased diversity in copiotrophic bacterial communities in IWG systems (Liao et al., 2023). Our results showing no differences in bacterial community composition two years after system establishment echo previous research which has also found limited responses in surface soil bacterial community beta diversity or microbial biomass in 2-3 year IWG rotations (Bak et al., 2022; Peixoto et al., 2022; Taylor et al., in preparation), though some after four years (Sprunger et al., 2019).

2.5.4 Covariability of soil microbial community characteristics and function and desired soil quality outcomes

We find no evidence of covariability of soil microbial community structure and potential function with soil physical and chemical parameters and root biomass in IWG vs. annual cropping systems. This is perhaps surprising that in these soils, we began to see some differences in soil aggregate stability and pH, and very stark differences in 0-15 cm root biomass, and these attributes are closely related to bacterial and fungal communities (Link et al., in preparation). Perhaps methods such as measuring gene expression through qPCR (eg. Su et al., 2021) and rhizospheric rather than bulk soil sampling (eg. Peixoto et al., 2022) should be used to better capture active microbial community composition. Research has shown significant improvements in soil aggregation (Link et al., in preparation) and POM and other labile carbon pools (Sprunger et al., 2018; Peixoto et al., 2020; Audu et al., 2022) in deeper soil horizons under IWG cultivation. This

evidence points to the need to look deeper into the soil profile for carbon sequestration potential in IWG systems and changes in microbial community dynamics that will regulate these carbon pools and fluxes. Agroecosystem subsoils may have a large potential for sequestering C due to decreased microbial abundance and activity and increased opportunity for MAOM C storage soil, but there is limited knowledge about storage potential across landscapes and time, subsoil processes including microbial dynamics that govern this potential SOM accumulation, and even current global subsoil carbon stocks (Button et al., 2022). Soil carbon sequestration is also not the only benefit of increasing SOM, and increasing surface SOM which has been degraded by annual crop management is a primary goal of many practices including perennial crops. Higher surface soil SOM is associated with overall higher agronomic yields (e.g. Oldfield et al., 2019) and higher yield resilience in the face of climate extremes like drought or extreme heat (e.g. Gaudin et al., 2015; Williams et al., 2016; Kane et al., 2021). It is therefore still important to assess surface soil responses to perennial vs. annual grain management, especially in longer term rotations, and to hopefully develop perennial grain management strategies that improve soil quality and SOC storage throughout the soil profile both for C offset and sequestration and improved soil agronomic function.

2.6 Conclusion

In this study, we assessed bacterial and fungal total biomass, community composition, and potential microbial function, as well as potential covariance of microbial community diversity and function metrics, total and active soil nutrient pools, and soil physical structure. After two years, we observed significant shifts in fungal community composition, including AMF indicator taxa in IWG and IWG-alf systems, and trending increases in extracellular enzyme activity between annual and IWG cropping systems. After only one year, we saw no changes in total microbial biomass

but a significant increase in AMF biomass in IWG but not IWG-alf systems. These observed changes in microbial community structure and function were generally not statistically associated with differences in soil physical and chemical quality and belowground biomass observed between cropping systems. Future research should continue to investigate relationships between microbial community composition + function and soil quality at different soil depths and under longer term management systems to better understand the mechanisms behind desired improvements.

2.7 References

- Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2021. UNITE general FASTA release for Fungi. Version 10.05.2021. UNITE Community. <https://doi.org/10.15156/BIO/1280049>
- Abdalla, M., Hastings, A., Cheng, K., Yue, Q., Chadwick, D., Espenberg, M., Truu, J., Rees, R.M., Smith, P., 2019. A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. *Global Change Biology* 25, 2530–2543. <https://doi.org/10.1111/gcb.14644>
- Amézketa, E., 1999. Soil Aggregate Stability: A Review. *Journal of Sustainable Agriculture* 14, 83–151. https://doi.org/10.1300/J064v14n02_08
- Angel, J.R., Swanson, C., Boustead, B.M., Conlon, K., Hall, K.R., Jorns, J.L., Kunkel, K.E., Lemos, M.C., Lofgren, B.M., Ontl, T., Posey, J., Stone, K., Takle, E., Today, D., 2018. Chapter 21 : Midwest. Impacts, Risks, and Adaptation in the United States: The Fourth National Climate Assessment, Volume II. U.S. Global Change Research Program. <https://doi.org/10.7930/NCA4.2018.CH21>
- Arguez, A., I. Durre, S. Applequist, R. Vose, M. Squires, X. Yin, R. Heim, and T.O., 2012. NOAA’s 1981-2010 climate normals: An overview. *Bull. Amer. Meteor.* 93, 1687–1697. <https://doi.org/10.1175/BAMS-D-11-00197.1>
- Audu, V., Rasche, F., Dimitrova Mårtensson, L.-M., Emmerling, C., 2022. Perennial cereal grain cultivation: Implication on soil organic matter and related soil microbial parameters. *Applied Soil Ecology* 174, 104414. <https://doi.org/10.1016/j.apsoil.2022.104414>

- Basche, A.D., DeLonge, M.S., 2019. Comparing infiltration rates in soils managed with conventional and alternative farming methods: A meta-analysis. *PLOS ONE* 14, e0215702. <https://doi.org/10.1371/journal.pone.0215702>
- Baumert, V.L., Vasilyeva, N.A., Vladimirov, A.A., Meier, I.C., Kögel-Knabner, I., Mueller, C.W., 2018. Root Exudates Induce Soil Macroaggregation Facilitated by Fungi in Subsoil. *Frontiers in Environmental Science* 6. <https://doi.org/10.3389/fenvs.2018.00140>
- Beillouin, D., Ben-Ari, T., Malézieux, E., Seufert, V., Makowski, D., 2021. Positive but variable effects of crop diversification on biodiversity and ecosystem services. *Global Change Biology* 27, 4697–4710. <https://doi.org/10.1111/gcb.15747>
- Bergquist, Galen., 2019. Biomass yield and soil microbial response to management of perennial intermediate wheatgrass (*Thinopyrum intermedium*) as grain crop and carbon sink. Retrieved from the University of Minnesota Digital Conservancy, <https://hdl.handle.net/11299/213040>.
- Bergtold, J.S., Ramsey, S., Maddy, L., Williams, J.R., 2019. A review of economic considerations for cover crops as a conservation practice. *Renewable Agriculture and Food Systems* 34, 62–76. <https://doi.org/10.1017/S1742170517000278>
- Bertoni, J., Larson, W. E., Shrader, W. D., 1958. Determination of infiltration rates on Marshall silt loam from runoff and rainfall records. *Soil Science Society of America Journal* 22, 571-574. <https://doi.org/10.2136/sssaj1958.03615995002200060024x>
- Black, C.K., Davis, S.C., Hudiburg, T.W., Bernacchi, C.J., DeLucia, E.H., 2017. Elevated CO₂ and temperature increase soil C losses from a soybean–maize ecosystem. *Global Change Biology* 23, 435–445. <https://doi.org/10.1111/gcb.13378>
- Blanco-Canqui, H., Jasa, P.J., 2019. Do Grass and Legume Cover Crops Improve Soil Properties in the Long Term? *Soil Science Society of America Journal* 83, 1181–1187. <https://doi.org/10.2136/sssaj2019.02.0055>
- Bormann, H., Klaassen, K., 2008. Seasonal and land use dependent variability of soil hydraulic and soil hydrological properties of two Northern German soils. *Geoderma, Modelling Pedogenesis* 145, 295–302. <https://doi.org/10.1016/j.geoderma.2008.03.017>
- Borrelli, P., Alewell, C., Alvarez, P., Anache, J.A.A., Baartman, J., Ballabio, C., Bezak, N., Biddoccu, M., Cerdà, A., Chalise, D., Chen, S., Chen, W., De Girolamo, A.M., Gessesse, G.D., Deumlich, D., Diodato, N., Efthimiou, N., Erpul, G., Fiener, P., Freppaz, M., Gentile, F., Gericke, A., Haregeweyn, N., Hu, B., Jeanneau, A., Kaffas, K., Kiani-Harchegani, M., Villuendas, I.L., Li, C., Lombardo, L., López-Vicente, M.,

- Lucas-Borja, M.E., Märker, M., Matthews, F., Miao, C., Mikoš, M., Modugno, S., Möller, M., Naipal, V., Nearing, M., Owusu, S., Panday, D., Patault, E., Patriche, C.V., Poggio, L., Portes, R., Quijano, L., Rahdari, M.R., Renima, M., Ricci, G.F., Rodrigo-Comino, J., Saia, S., Samani, A.N., Schillaci, C., Syrris, V., Kim, H.S., Spinola, D.N., Oliveira, P.T., Teng, H., Thapa, R., Vantas, K., Vieira, D., Yang, J.E., Yin, S., Zema, D.A., Zhao, G., Panagos, P., 2021. Soil erosion modelling: A global review and statistical analysis. *Sci Total Environ* 780, 146494.
<https://doi.org/10.1016/j.scitotenv.2021.146494>
- Bossio, D.A., Cook-Patton, S.C., Ellis, P.W., Fargione, J., Sanderman, J., Smith, P., Wood, S., Zomer, R.J., von Unger, M., Emmer, I.M., Griscom, B.W., 2020. The role of soil carbon in natural climate solutions. *Nat Sustain* 3, 391–398.
<https://doi.org/10.1038/s41893-020-0491-z>
- Bouman, O. T., Curtin, D., Campbell, C. A., Biederbeck, V. O., Ukrainetz, H., 1995. Soil Acidification from Long-Term Use of Anhydrous Ammonia and Urea. *Soil Science Society of America Journal* 59, 1488-1494.
<https://doi.org/10.2136/sssaj1995.03615995005900050039x>
- Bowles, T.M., Mooshammer, M., Socolar, Y., Calderón, F., Cavigelli, M.A., Culman, S.W., Deen, W., Drury, C.F., Garcia y Garcia, A., Gaudin, A.C.M., Harkcom, W.S., Lehman, R.M., Osborne, S.L., Robertson, G.P., Salerno, J., Schmer, M.R., Strock, J., Grandy, A.S., 2020. Long-Term Evidence Shows that Crop-Rotation Diversification Increases Agricultural Resilience to Adverse Growing Conditions in North America. *One Earth* 2, 284–293. <https://doi.org/10.1016/J.ONEEAR.2020.02.007>
- Burke, M., Emerick, K., 2016. Adaptation to climate change: Evidence from US agriculture. *American Economic Journal: Economic Policy* 8, 106–140.
<https://doi.org/10.1257/POL.20130025>
- Button, E.S., Pett-Ridge, J., Murphy, D.V., Kuzyakov, Y., Chadwick, D.R., Jones, D.L., 2022. Deep-C storage: Biological, chemical and physical strategies to enhance carbon stocks in agricultural subsoils. *Soil Biology and Biochemistry* 170, 108697.
<https://doi.org/10.1016/j.soilbio.2022.108697>
- Callahan, B.J., McMurdie, P.J., Rosen, MJ, Han AW, Johnson AJA, Holmes SP, 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13, 581-583. doi: 10.1038/nmeth.3869.

- Carter, P.R., Sheaffer, C.C., 1983. Alfalfa Response to Soil Water Deficits. I. Growth, Forage Quality, Yield, Water Use, and Water-Use Efficiency. *Crop Science* 23, <https://doi.org/10.2135/cropsci1983.0011183X002300040016x>
- Cates, A.M., Ruark, M.D., Grandy, A.S., Jackson, R.D., 2019. Small soil C cycle responses to three years of cover crops in maize cropping systems. *AGRICULTURE ECOSYSTEMS & ENVIRONMENT* 286. <https://doi.org/10.1016/j.agee.2019.106649>
- Cates, A.M., Ruark, M.D., Hedtcke, J.L., Posner, J.L., 2015. Long-term tillage, rotation and perennialization effects on particulate and aggregate soil organic matter. *Soil and Tillage Research* 115, 371-380. <https://doi.org/10.1016/j.still.2015.09.008>
- Chamberlain, L.A., Aguayo, T., Zerega, N.J.C., Dybzinski, R., Egerton-Warburton, L.M., 2022. Rapid improvement in soil health following the conversion of abandoned farm fields to annual or perennial agroecosystems. *Frontiers in Sustainable Food Systems* 6. <https://doi.org/10.3389/fsufs.2022.1010298>
- Chantigny, M.H., Angers, D.A., Prevost, D., Vezina, L.P., Chalifour, F.P., 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. *Soil Science Society of America Journal* 61, 262–267. <https://doi.org/10.2136/sssaj1997.03615995006100010037x>
- Chen, S., Gong, B., 2021. Response and adaptation of agriculture to climate change: Evidence from China. *Journal of Development Economics* 148, 102557. <https://doi.org/10.1016/j.jdeveco.2020.102557>
- Cheng, W., Parton, W.J., Gonzalez-Meler, M.A., Phillips, R., Asao, S., McNickle, G.G., Brzostek, E., Jastrow, J.D., 2014. Synthesis and modeling perspectives of rhizosphere priming. *New Phytologist* 201, 31–44. <https://doi.org/10.1111/nph.12440>
- Clément, C., Sleiderink, J., Svane, S.F., Smith, A.G., Diamantopoulos, E., Desbrøll, D.B., Thorup-Kristensen, K., 2022. Comparing the deep root growth and water uptake of intermediate wheatgrass (Kernza®) to alfalfa. *Plant and Soil*. <https://doi.org/10.1007/s11104-021-05248-6>
- Crews, T.E., Kemp, L., Bowden, J.H., Murrell, E.G., 2022. How the Nitrogen Economy of a Perennial Cereal-Legume Intercrop Affects Productivity: Can Synchrony Be Achieved? *Frontiers in Sustainable Food Systems* 6. <https://doi.org/10.3389/fsufs.2022.755548>
- Crystal-Ornelas, R., Thapa, R., Tully, K.L., 2021. Soil organic carbon is affected by organic amendments, conservation tillage, and cover cropping in organic farming systems: A meta-analysis. *Agriculture, Ecosystems & Environment* 312, 107356. <https://doi.org/10.1016/j.agee.2021.107356>

- Culman, S.W., DuPont, S.T., Glover, J.D., Buckley, D.H., Fick, G.W., Ferris, H., Crews, T.E., 2010. Long-term impacts of high-input annual cropping and unfertilized perennial grass production on soil properties and belowground food webs in Kansas, USA. *Agriculture, Ecosystems and Environment* 137, 13–24.
<https://doi.org/10.1016/j.agee.2009.11.008>
- Culman, S.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>
- Culman, S.W., Snapp, S.S., Ollenburger, M., Basso, B., DeHaan, L.R., 2013. Soil and water quality rapidly responds to the perennial grain Kernza wheatgrass. *Agronomy Journal* 105, 735–744. <https://doi.org/10.2134/agronj2012.0273>
- Delta-T Devices Ltd, 2016. User manual for the profile probe type PR2. Version Oct. 2016.
https://delta-t.co.uk/wp-content/uploads/2017/02/PR2_user_manual_version_5.0.pdf
- de Oliveira, G., Brunzell, N.A., Crews, T.E., DeHaan, L.R., Vico, G., 2020. Carbon and water relations in perennial Kernza (*Thinopyrum intermedium*): An overview. *Plant Science* 295, 110279. <https://doi.org/10.1016/j.plantsci.2019.110279>
- de Oliveira, G., Brunzell, N.A., Sutherlin, C.E., Crews, T.E., DeHaan, L.R., 2018. Energy, water and carbon exchange over a perennial Kernza wheatgrass crop. *Agricultural and Forest Meteorology* 249, 120–137. <https://doi.org/10.1016/j.agrformet.2017.11.022>
- Deines, J.M., Wang, S., Lobell, D.B., 2019. Satellites reveal a small positive yield effect from conservation tillage across the US Corn Belt. *Environ. Res. Lett.* 14, 124038.
<https://doi.org/10.1088/1748-9326/ab503b>
- Dhakar, M., West, C.P., Deb, S.K., Kharel, G., Ritchie, G.L., 2019. Field Calibration of PR2 Capacitance Probe in Pullman Clay-Loam Soil of Southern High Plains. *Agrosystems, Geosciences and Environment* 2, 1–7. <https://doi.org/10.2134/AGE2018.10.0043>
- Dick, C., Cattani, D., Entz, M.H., 2018. Kernza intermediate wheatgrass (*Thinopyrum intermedium*) grain production as influenced by legume intercropping and residue management. *Canadian Journal of Plant Science* 98, 1376–1379.
<https://doi.org/10.1139/cjps-2018-0146>
- Dobbratz, M., Gutknecht, J., Wyse, D., Sheaffer, C.C., Jungers, J.M., 2022. Inconsistent effects of species diversity and N fertilization on soil microbes and carbon storage in

- perennial bioenergy cropping systems. *Renewable Agriculture and Food Systems* 37, 60–70. <https://doi.org/10.1017/S1742170521000302>
- Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2020. PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 38, 685–688. <https://doi.org/10.1038/s41587-020-0548-6>
- Duchene, O., Bathellier, C., Dumont, B., David, C., Celette, F., 2023. Weed community shifts during the aging of perennial intermediate wheatgrass crops harvested for grain in arable fields. *European Journal of Agronomy* 143, 126721. <https://doi.org/10.1016/j.eja.2022.126721>
- Duchene, O., Celette, F., Barreiro, A., Dimitrova Mårtensson, L.-M., Freschet, G.T., David, C., 2020. Introducing Perennial Grain in Grain Crops Rotation: The Role of Rooting Pattern in Soil Quality Management. *Agronomy* 10, 1254. <https://doi.org/10.3390/agronomy10091254>
- DuPont, S.T., Beniston, J., Glover, J.D., Hodson, A., Culman, S.W., Lal, R., Ferris, H., 2014. Root traits and soil properties in harvested perennial grassland, annual wheat, and never-tilled annual wheat. *Plant and Soil* 381, 405–420. <https://doi.org/10.1007/s11104-014-2145-2>
- Ernst, O.R., Dogliotti, S., Cadenazzi, M., Kemanian, A.R., 2018. Shifting crop-pasture rotations to no-till annual cropping reduces soil quality and wheat yield. *Field Crops Research* 217, 180–187. <https://doi.org/10.1016/j.fcr.2017.11.014>
- Fagnant, L., Duchêne, O., Celette, F., David, C., Bindelle, J., Dumont, B., 2023. Learning about the growing habits and reproductive strategy of *Thinopyrum intermedium* through the establishment of its critical nitrogen dilution curve. *Field Crops Research* 291, 108802. <https://doi.org/10.1016/j.fcr.2022.108802>
- Farmaha, B.S., Sekaran, U., Franzluebbers, A.J., 2022. Cover cropping and conservation tillage improve soil health in the southeastern United States. *Agronomy Journal* 114, 296–316. <https://doi.org/10.1002/agj2.20865>
- Fernandez, C.W., Ehlke, N., Sheaffer, C.C., Jungers, J.M., 2020. Effects of nitrogen fertilization and planting density on intermediate wheatgrass yield. *Agronomy Journal* 112, 4159–4170. <https://doi.org/10.1002/agj2.20351>
- Fixen, P.E., Bruulsema, T.W., Johnston, A.M., Mikkelsen, R.L., Murrell, T.S., Snyder, C.S., Stewart, W.M., 2010. The Fertility of North American Soils. *North America* 4.
- Fox, J. and Weisberg, S., 2019. An {R} Companion to Applied Regression,

- Third Edition. Thousand Oaks CA: Sage.
<https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Galloway, A.F., Akhtar, J., Marcus, S.E., Fletcher, N., Field, K., Knox, P., 2020. Cereal root exudates contain highly structurally complex polysaccharides with soil-binding properties. *The Plant Journal* 103, 1666–1678. <https://doi.org/10.1111/tpj.14852>
- Gamble, J.D., Jungers, J.M., Wyse, D.L., Johnson, G.A., Lamb, J.A., Sheaffer, C.C., 2015. Harvest Date Effects on Biomass Yield, Moisture Content, Mineral Concentration, and Mineral Export in Switchgrass and Native Polycultures Managed for Bioenergy. *Bioenergy Research* 8, 740–749. <https://doi.org/10.1007/s12155-014-9555-0>
- Gardner, W.H., 2018. Water Content. *Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods* 493–544.
<https://doi.org/10.2136/SSSABOOKSER5.1.2ED.C21>
- Gaudin, A.C.M., Tolhurst, T.N., Ker, A.P., Janovicek, K., Tortora, C., Martin, R.C., Deen, W., 2015. Increasing crop diversity mitigates weather variations and improves yield stability. *PLoS ONE* 10, 1–20. <https://doi.org/10.1371/journal.pone.0113261>
- Geisseler, D., Miller, K., Leinfelder-Miles, M., Wilson, R., 2019. Use of Soil Protein Pools as Indicators of Soil Nitrogen Mineralization Potential. *Soil Science Society of America Journal* 83, 1236–1243. <https://doi.org/10.2136/sssaj2019.01.0012>
- Glover, J.D., Culman, S.W., DuPont, S.T., Broussard, W., Young, L., Mangan, M.E., Mai, J.G., Crews, T.E., DeHaan, L.R., Buckley, D.H., Ferris, H., Turner, R.E., Reynolds, H.L., Wyse, D.L., 2010. Harvested perennial grasslands provide ecological benchmarks for agricultural sustainability. *Agriculture, Ecosystems & Environment, Special section Harvested perennial grasslands: Ecological models for farming’s perennial future* 137, 3–12. <https://doi.org/10.1016/j.agee.2009.11.001>
- Guo, L., Liu, Y., Wu, G.-L., Huang, Z., Cui, Z., Cheng, Z., Zhang, R.-Q., Tian, F.-P., He, H., 2019. Preferential water flow: Influence of alfalfa (*Medicago sativa* L.) decayed root channels on soil water infiltration. *Journal of Hydrology* 578, 124019.
<https://doi.org/10.1016/j.jhydrol.2019.124019>
- Gutknecht, J., Journey, A., Peterson, H., Blair, H., Cates, A., 2022.. Cover crop management practices to promote soil health and climate adaptation: Grappling with varied success from farmer and researcher observations. *Journal of Environmental Quality*, 1-17.
<https://doi.org/10.1002/jeq2.20383>
- Guzmán G., Sáenz de Rodríguez M., Vanwallegem T., Vanderlinden K., Laguna A., Giráldez JV. 2015. Compatibility of Agricultural Management Practices and Types of

- Farming in the EU to enhance Climate Change Mitigation and Soil Health: Impacts of soil management on physical soil quality. CATCH-C, www.catch-c.eu, pp. 38.
- Halde, C., Gagné, S., Charles, A., Lawley, Y., 2017. Organic No-Till Systems in Eastern Canada: A Review. *Agriculture* 7, 36. <https://doi.org/10.3390/agriculture7040036>
- Hartemink, A.E., Zhang, Y., Bockheim, J.G., Curi, N., Silva, S.H.G., Grauer-Gray, J., Lowe, D.J., Krasilnikov, P., 2020. Chapter Three - Soil horizon variation: A review, in: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 125–185. <https://doi.org/10.1016/bs.agron.2019.10.003>
- Hatfield, J.L., Dold, C., 2019. Water-Use Efficiency: Advances and Challenges in a Changing Climate. *Frontiers in Plant Science* 10. <https://doi.org/10.3389/fpls.2019.00103>
- Hayes, R.C., Newell, M.T., Crews, T.E., Peoples, M.B., 2017. Perennial cereal crops: An initial evaluation of wheat derivatives grown in mixtures with a regenerating annual legume. *Renewable Agriculture and Food Systems* 32, 276–290. <https://doi.org/10.1017/S1742170516000260>
- Heineck, G.C., Schlautman, B., Law, E.P., Ryan, M.R., Zimbric, J.W., Picasso, V., Stoltenberg, D.E., Sheaffer, C.C., Jungers, J.M., 2022. Intermediate wheatgrass seed size and moisture dynamics inform grain harvest timing. *Crop Science* 62, 410–424. <https://doi.org/10.1002/csc2.20662>
- Huang, K., Sim, N., 2021. Adaptation May Reduce Climate Damage in Agriculture by Two Thirds. *Journal of Agricultural Economics* 72, 47–71. <https://doi.org/10.1111/1477-9552.12389>
- Huddell, A., Ernfors, M., Crews, T., Vico, G., Menge, D.N.L., 2023. Nitrate leaching losses and the fate of 15N fertilizer in perennial intermediate wheatgrass and annual wheat — A field study. *Science of The Total Environment* 857, 159255. <https://doi.org/10.1016/j.scitotenv.2022.159255>
- Hunter, M.C., Sheaffer, C.C., Culman, S.W., Lazarus, W.F., Jungers, J.M., 2020. Effects of defoliation and row spacing on intermediate wheatgrass II: Forage yield and economics. *Agronomy Journal* 1862–1880. <https://doi.org/10.1002/agj2.20124>
- 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.T., 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>

- Hurisso, T.T., Culman, S.W., Zhao, K., 2018a. Repeatability and Spatiotemporal Variability of Emerging Soil Health Indicators Relative to Routine Soil Nutrient Tests. *Soil Science Society of America Journal* 82, 939–948.
<https://doi.org/10.2136/sssaj2018.03.0098>
- Hurisso, T.T., Moebius-Clune, D.J., Culman, S.W., Moebius-Clune, B.N., Thies, J.E., Es, H.M., 2018b. Soil Protein as a Rapid Soil Health Indicator of Potentially Available Organic Nitrogen. *Agricultural & Environmental Letters* 3, 180006.
<https://doi.org/10.2134/ael2018.02.0006>
- Jakab, G., Madarász, B., Szabó, J.A., Tóth, A., Zacháry, D., Szalai, Z., Kertész, Á., Dyson, J., 2017. Infiltration and Soil Loss Changes during the Growing Season under Ploughing and Conservation Tillage. *Sustainability* 9, 1726.
<https://doi.org/10.3390/su9101726>
- Jotiskansa, A., Sirirattanachit, T., 2017. Effects of grass roots on soil-water retention curve and permeability function. *Can. Geotech. J.* 54, 1612–1622. <https://doi.org/10.1139/cgj-2016-0281>
- Jungers, J.M., DeHaan, L.H., Mulla, D.J., Sheaffer, C.C., Wyse, D.L., 2019. Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. *Agriculture, Ecosystems & Environment* 272, 63–73.
<https://doi.org/10.1016/j.agee.2018.11.007>
- Jungers, J.M., DeHaan, L.R., Betts, K.J., Sheaffer, C.C., Wyse, D.L., 2017. Intermediate wheatgrass grain and forage yield responses to nitrogen fertilization. *Agronomy Journal* 109, 462–472. <https://doi.org/10.2134/agronj2016.07.0438>
- Jungers, J.M., Frahm, C.S., Tautges, N.E., Ehlke, N.J., Wells, M.S., Wyse, D.L., Sheaffer, C.C., 2018. Growth, development, and biomass partitioning of the perennial grain crop *Thinopyrum intermedium*. *Annals of Applied Biology* 172, 346–354.
<https://doi.org/10.1111/aab.12425>
- Kane, D.A., Bradford, M.A., Fuller, E., Oldfield, E.E., Wood, S.A., 2021. Soil organic matter protects US maize yields and lowers crop insurance payouts under drought. *Environmental Research Letters* 16, 044018. <https://doi.org/10.1088/1748-9326/ABE492>
- Kane, D.A., Rogé, P., Snapp, S.S., 2016. A systematic review of perennial staple crops literature using topic modeling and bibliometric analysis. *PLoS ONE* 11, 1–18.
<https://doi.org/10.1371/journal.pone.0155788>

- Kaye, J.P., Quemada, M., 2017. Using cover crops to mitigate and adapt to climate change. A review. *Agron. Sustain. Dev.* 37, 4. <https://doi.org/10.1007/s13593-016-0410-x>
- Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry* 42, 1363–1371. <https://doi.org/10.1016/j.soilbio.2010.04.003>
- Lal, R., 2020. Soil organic matter content and crop yield. *Journal of Soil and Water Conservation* 75, 27A-32A. <https://doi.org/10.2489/JSWC.75.2.27A>
- Lal, R., 2016. Soil health and carbon management. *Food and Energy Security* 5, 212–222. <https://doi.org/10.1002/fes3.96>
- Law, E.P., Wayman, S., Pelzer, C.J., Culman, S.W., Gómez, M.I., DiTommaso, A., Ryan, M.R., 2022a. Multi-Criteria Assessment of the Economic and Environmental Sustainability Characteristics of Intermediate Wheatgrass Grown as a Dual-Purpose Grain and Forage Crop. *Sustainability* 14, 3548. <https://doi.org/10.3390/su14063548>
- Law, E.P., Wayman, S., Pelzer, C.J., DiTommaso, A., Ryan, M.R., 2022b. Intercropping red clover with intermediate wheatgrass suppresses weeds without reducing grain yield. *Agronomy Journal* 114, 700–716. <https://doi.org/10.1002/agi2.20914>
- Ledo, A., Smith, P., Zerihun, A., Whitaker, J., Vicente-Vicente, J.L., Qin, Z., McNamara, N.P., Zinn, Y.L., Llorente, M., Liebigh, M., Kuhnert, M., Dondini, M., Don, A., Diaz-Pines, E., Datta, A., Bakka, H., Aguilera, E., Hillier, J., 2020. Changes in soil organic carbon under perennial crops. *Global Change Biology* 26, 4158–4168. <https://doi.org/10.1111/gcb.15120>
- Lenhart, C., Peterson, H., 2017. *Agricultural BMP Handbook for Minnesota 2017*.
- Lenth, R.V., 2021. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.5-1. <https://CRAN.R-project.org/package=emmeans>
- Leuther, F., Schlüter, S., 2021. Impact of freeze–thaw cycles on soil structure and soil hydraulic properties. *SOIL* 7, 179–191. <https://doi.org/10.5194/soil-7-179-2021>
- Li, D.-C., Velde, B., Li, F.-M., Zhang, G.-L., Zhao, M.-S., Huang, L.-M., 2011. Impact of Long-Term Alfalfa Cropping on Soil Potassium Content and Clay Minerals in a Semi-Arid Loess Soil in China. *Pedosphere* 21, 522–531. [https://doi.org/10.1016/S1002-0160\(11\)60154-9](https://doi.org/10.1016/S1002-0160(11)60154-9)
- Liebigh, M.A., Johnson, H.A., Hanson, J.D., Frank, A.B., 2005. Soil carbon under switchgrass stands and cultivated cropland. *Biomass and Bioenergy* 28, 347–354. <https://doi.org/10.1016/j.biombioe.2004.11.004>

- Liu, X., Wu, X., Liang, G., Zheng, F., Zhang, M., Li, S., 2021. A global meta-analysis of the impacts of no-tillage on soil aggregation and aggregate-associated organic carbon. *Land Degradation & Development* 32, 5292–5305. <https://doi.org/10.1002/LDR.4109>
- Liu, Y., Guo, L., Huang, Z., López-Vicente, M., Wu, G.-L., 2020. Root morphological characteristics and soil water infiltration capacity in semi-arid artificial grassland soils. *Agricultural Water Management* 235, 106153. <https://doi.org/10.1016/j.agwat.2020.106153>
- Locatelli, A., Gutierrez, L., Picasso Risso, V.D., 2022. Vernalization requirements of *Kernza* intermediate wheatgrass. *Crop Science* 62, 524–535. <https://doi.org/10.1002/csc2.20667>
- Lotter, D.W., Seidel, R., Liebhardt, W., 2003. The performance of organic and conventional cropping systems in an extreme climate year. *American Journal of Alternative Agriculture* 18, 146–154. <https://doi.org/10.1079/AJAA200345>
- Lubofsky, E., 2016. The promise of perennials: Working through the challenges of perennial grain crop development. *CSA News* 61, 4–7. <https://doi.org/10.2134/csa2016-61-11-1>
- Maeght, J.-L., Rewald, B., Pierret, A., 2013. How to study deep roots—and why it matters. *Front Plant Sci* 4, 299. <https://doi.org/10.3389/fpls.2013.00299>
- Mårtensson, L.-M.D., Barreiro, A., Li, S., Jensen, E.S., 2022. Agronomic performance, nitrogen acquisition and water-use efficiency of the perennial grain crop *Thinopyrum intermedium* in a monoculture and intercropped with alfalfa in Scandinavia. *Agron. Sustain. Dev.* 42, 21. <https://doi.org/10.1007/s13593-022-00752-0>
- Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17, 10–12. <https://doi.org/10.14806/ej.17.1.200>
- Martin, T., Sprunger, C.D., 2022. Sensitive Measures of Soil Health Reveal Carbon Stability Across a Management Intensity and Plant Biodiversity Gradient. *Frontiers in Soil Science* 2. <https://doi.org/10.3389/fsoil.2022.917885>
- McIsaac, G.F., David, M.B., Mitchell, C.A., 2010. *Miscanthus* and Switchgrass Production in Central Illinois: Impacts on Hydrology and Inorganic Nitrogen Leaching. *J. Environ. Qual.* 39, 1790–1799. <https://doi.org/10.2134/jeq2009.0497>
- McGuire, R., Williams, P.N., Smith, P., McGrath, S.P., Curry, D., Donnison, I., Emmet, B., Scollan, N., 2022. Potential Co-benefits and trade-offs between improved soil management, climate change mitigation and agri-food productivity. *Food and Energy Security* 11, e352. <https://doi.org/10.1002/FES3.352>

- McLaren, Michael R., & Callahan, Benjamin J. (2021). Silva 138.1 prokaryotic SSU taxonomic training data formatted for DADA2 [Data set]. Zenodo.
<https://doi.org/10.5281/zenodo.4587955>
- 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.
- Menne, M.J., Durre, I., Vose, R.S., Gleason, B.E., Houston, T.G., 2012. An Overview of the Global Historical Climatology Network-Daily Database. Journal of Atmospheric and Oceanic Technology 29, 897–910. <https://doi.org/10.1175/JTECH-D-11-00103.1>
- Miguez, F.E., Bollero, G.A., 2005. Review of Corn Yield Response under Winter Cover Cropping Systems Using Meta-Analytic Methods. Crop Science 45, 2318–2329.
<https://doi.org/10.2135/cropsci2005.0014>
- Minasny, B., Cook, F., 2011. Sorptivity of Soils, in: Gliński, J., Horabik, J., Lipiec, J. (Eds.), Encyclopedia of Agrophysics, Encyclopedia of Earth Sciences Series. Springer Netherlands, Dordrecht, pp. 824–826. https://doi.org/10.1007/978-90-481-3585-1_161
- Miranda-Vélez, J.F., Leuther, F., Köhne, J.M., Munkholm, L.J., Vogeler, I., 2023. Effects of freeze-thaw cycles on soil structure under different tillage and plant cover management practices. Soil and Tillage Research 225, 105540.
<https://doi.org/10.1016/j.still.2022.105540>
- Moebius-Clune, B.N., D.J. Moebius-Clune, B.K. Gugino, O.J. Idowu, R.R. Schindelbeck, A.J. Ristow, H.M. van Es, J.E. Thies, H. A. Shayler, M. B. McBride, D.W. Wolfe, and G.S. Abawi, 2016. Comprehensive Assessment of Soil Health – The Cornell Framework Manual, Edition 3.1, Cornell University, Geneva, NY.
- NCERA, 2015. Recommended Chemical Soil Test Procedures for the North Central Region. North Central Regional Research Publication No. 221 (Revised)
- Nearing, M.A., Xie, Y., Liu, B., Ye, Y., 2017. Natural and anthropogenic rates of soil erosion. International Soil and Water Conservation Research 5, 77–84.
<https://doi.org/10.1016/j.iswcr.2017.04.001>
- Nelson, D.W., Sommers, L.E., 2018. Total Carbon, Organic Carbon, and Organic Matter. Methods of Soil Analysis, Part 3: Chemical Methods 961–1010.
<https://doi.org/10.2136/SSSABOOKSER5.3.C34>
- Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20, 241–248.
<https://doi.org/10.1016/j.funeco.2015.06.006>

- Noll, L., Zhang, S., Zheng, Q., Hu, Y., Hofhansl, F., Wanek, W., 2022. Climate and geology overwrite land use effects on soil organic nitrogen cycling on a continental scale. *Biogeosciences* 19, 5419–5433. <https://doi.org/10.5194/bg-19-5419-2022>
- Norris, C.E., Bean, G.M., Cappellazzi, S.B., Cope, M., Greub, K.L.H., Liptzin, D., Rieke, E.L., Tracy, P.W., Morgan, C.L.S., Honeycutt, C.W., 2020. Introducing the North American project to evaluate soil health measurements. *Agronomy Journal* 112, 3195–3215. <https://doi.org/10.1002/agj2.20234>
- Nunes, M.R., Karlen, D.L., Moorman, T.B., 2020. Tillage intensity effects on soil structure indicators-A US meta-analysis. *Sustainability (Switzerland)* 12. <https://doi.org/10.3390/su12052071>
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, H.M., Szoecs, E., and Wagner, H., 2019. *vegan: Community Ecology Package*. R package version 2.5-6. <https://CRAN.R-project.org/package=vegan>
- Oldfield, E.E., Bradford, M.A., Wood, S.A., 2019. Global meta-analysis of the relationship between soil organic matter and crop yields. *Soil* 5, 15–32. <https://doi.org/10.5194/soil-5-15-2019>
- Osipitan, O.A., Dille, J.A., Assefa, Y., Knezevic, S.Z., 2018. Cover Crop for Early Season Weed Suppression in Crops: Systematic Review and Meta-Analysis. *Agronomy Journal* 110, 2211–2221. <https://doi.org/10.2134/agronj2017.12.0752>
- Paustian, K., Collier, S., Baldock, J., Burgess, R., Creque, J., DeLonge, M., Dungait, J., Ellert, B., Frank, S., Goddard, T., Govaerts, B., Grundy, M., Henning, M., Izaurralde, R.C., Madaras, M., McConkey, B., Porzig, E., Rice, C., Searle, R., Seavy, N., Skalsky, R., Mulhern, W., Jahn, M., 2019. Quantifying carbon for agricultural soil management: from the current status toward a global soil information system. *Carbon Management* 10, 567–587. <https://doi.org/10.1080/17583004.2019.1633231>
- Peixoto, L., Olesen, J.E., Elsgaard, L., Enggrob, K.L., Banfield, C.C., Dippold, M.A., Nicolaisen, M.H., Bak, F., Zang, H., Dresbøll, D.B., Thorup-Kristensen, K., Rasmussen, J., 2022. Deep-rooted perennial crops differ in capacity to stabilize C inputs in deep soil layers. *Scientific Reports* 12. <https://doi.org/10.1038/S41598-022-09737-1>
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team, 2021. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-152, <https://CRAN.R-project.org/package=nlme>.

- Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. *Agriculture, Ecosystems & Environment* 200, 33–41. <https://doi.org/10.1016/j.agee.2014.10.024>
- Pugliese, J.Y., Culman, S.W., Sprunger, C.D., 2019a. Harvesting forage of the perennial grain crop kernza (*Thinopyrum intermedium*) increases root biomass and soil nitrogen. *Plant and Soil* 437, 241–254. <https://doi.org/10.1007/S11104-019-03974-6/FIGURES/4>
- Pugliese, J.Y., Culman, S.W., Sprunger, C.D., 2019b. Correction to: Harvesting forage of the perennial grain crop kernza (*Thinopyrum intermedium*) increases root biomass and soil nitrogen cycling (*Plant and Soil*, (2019), 10.1007/s11104-019-03974-6). *Plant and Soil* 241–254. <https://doi.org/10.1007/s11104-019-03974-6>
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucl. Acids Res.* 41 (D1): D590-D596.
- R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rabot, E., Wiesmeier, M., Schlüter, S., Vogel, H.-J., 2018. Soil structure as an indicator of soil functions: A review. *Geoderma* 314, 122–137. <https://doi.org/10.1016/j.geoderma.2017.11.009>
- Rakkar, M., Jungers, J.M., Sheaffer, C., Bergquist, G., Grossman, J., Li, F., Gutknecht, J.L., 2023. Soil health improvements from using a novel perennial grain during the transition to organic production. *Agriculture, Ecosystems & Environment* 341, 108164. <https://doi.org/10.1016/j.agee.2022.108164>
- Reilly, E.C., Gutknecht, J.L., Sheaffer, C.C., Jungers, J.M., 2022a. Reductions in soil water nitrate beneath a perennial grain crop compared to an annual crop rotation on sandy soil. *Frontiers in Sustainable Food Systems* 6. <https://doi.org/10.3389/fsufs.2022.996586>
- Reilly, E.C., Gutknecht, J.L., Tautges, N.E., Sheaffer, C.C., Jungers, J.M., 2022b. Nitrogen transfer and yield effects of legumes intercropped with the perennial grain crop intermediate wheatgrass. *Field Crops Research* 286, 108627. <https://doi.org/10.1016/J.FCR.2022.108627>
- Renwick, L.L.R., Deen, W., Silva, L., Gilbert, M.E., Maxwell, T., Bowles, T.M., Gaudin, A.C.M., 2021. Long-term crop rotation diversification enhances maize drought resistance through soil organic matter. *Environmental Research Letters* 16. <https://doi.org/10.1088/1748-9326/AC1468>

- Rillig, M.C., Aguilar-Trigueros, C.A., Bergmann, J., Verbruggen, E., Veresoglou, S.D., Lehmann, A., 2015. Plant root and mycorrhizal fungal traits for understanding soil aggregation. *New Phytologist* 205, 1385–1388. <https://doi.org/10.1111/nph.13045>
- Rubio, V., Diaz-Rossello, R., Quincke, J.A., van Es, H.M., 2021. Quantifying soil organic carbon's critical role in cereal productivity losses under annualized crop rotations. *Agriculture, Ecosystems & Environment* 321, 107607. <https://doi.org/10.1016/j.agee.2021.107607>
- Rui, Y., Jackson, R.D., Cotrufo, M.F., Sanford, G.R., Spiesman, B.J., Deiss, L., Culman, S.W., Liang, C., Ruark, M.D., 2022. Persistent soil carbon enhanced in Mollisols by well- managed grasslands but not annual grain or dairy forage cropping systems 1–6. <https://doi.org/10.1073/pnas.2118931119/-/DCSupplemental.Published>
- Ryan, M.R., Crews, T.E., Culman, S.W., Dehaan, L.R., Hayes, R.C., Jungers, J.M., Bakker, M.G., 2018. Managing for Multifunctionality in Perennial Grain Crops. *BioScience* 68, 294–304. <https://doi.org/10.1093/biosci/biy014>
- Sainju, U.M., Liptzin, D., Stevens, W.B., 2022. Autoclaved citrate-extractable protein as a soil health indicator relates to soil properties and crop production. *Nutr Cycl Agroecosyst.* <https://doi.org/10.1007/s10705-022-10230-4>
- Sainju, U.M., Allen, B.L., Lenssen, A.W., Ghimire, R.P., 2017. Root biomass, root/shoot ratio, and soil water content under perennial grasses with different nitrogen rates. *Field Crops Research* 210, 183–191. <https://doi.org/10.1016/j.fcr.2017.05.029>
- Sanford, G.R., Posner, J.L., Jackson, R.D., Kucharik, C.J., Hedtcke, J.L., Lin, T.L., 2012. Soil carbon lost from Mollisols of the North Central U.S.A. with 20 years of agricultural best management practices. *Agriculture, Ecosystems and Environment* 162, 68–76. <https://doi.org/10.1016/j.agee.2012.08.011>
- Schmidt, J., Fester, T., Schulz, E., Michalzik, B., Buscot, F., Gutknecht, J., 2017. Effects of plant-symbiotic relationships on the living soil microbial community and microbial necromass in a long-term agro-ecosystem. *Science of the Total Environment* 581–582, 756–765. <https://doi.org/10.1016/j.scitotenv.2017.01.005>
- Scott, D.A., Baer, S.G., Blair, J.M., 2017. Recovery and Relative Influence of Root, Microbial, and Structural Properties of Soil on Physically Sequestered Carbon Stocks in Restored Grassland. *Soil Science Society of America Journal* 81, 50–60. <https://doi.org/10.2136/sssaj2016.05.0158>
- Shakoor, A., Shakoor, S., Rehman, A., Ashraf, F., Abdullah, M., Shahzad, S.M., Farooq, T.H., Ashraf, M., Manzoor, M.A., Altaf, M.M., Altaf, M.A., 2021. Effect of animal

- manure, crop type, climate zone, and soil attributes on greenhouse gas emissions from agricultural soils—A global meta-analysis. *Journal of Cleaner Production* 278, 124019. <https://doi.org/10.1016/j.jclepro.2020.124019>
- Smith, R.E., 1999. Technical Note: Rapid Measurement of Soil Sorptivity. *Soil Science Society of America Journal* 63, 55–57. <https://doi.org/10.2136/sssaj1999.03615995006300010009x>
- Smucker, A.J.M., McBurney, S.L., Srivastava, A.K., 1982. Quantitative Separation of Roots from Compacted Soil Profiles by the Hydropneumatic Elutriation System 1. *Agronomy Journal* 74, 500–503. <https://doi.org/10.2134/AGRONJ1982.00021962007400030023X>
- Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at the following link: <http://websoilsurvey.sc.egov.usda.gov/>. Accessed 8/13/2022.
- Sprunger, C.D., Culman, S.W., Peralta, A.L., DuPont, S.T., Lennon, J.T., Snapp, S.S., 2019. Perennial grain crop roots and nitrogen management shape soil food webs and soil carbon dynamics. *Soil Biology and Biochemistry* 137, 107573. <https://doi.org/10.1016/j.soilbio.2019.107573>
- Sprunger, C.D., Culman, S.W., Robertson, G.P., Snapp, S.S., 2018. Perennial grain on a Midwest Alfisol shows no sign of early soil carbon gain. *Renewable Agriculture and Food Systems* 33, 360–372. <https://doi.org/10.1017/S1742170517000138>
- Sprunger, C.D., Philip Robertson, G., 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 Tvisha Martin Meredith Mann, C.D., Christine Sprunger, C.D., 2020. Systems with greater perenniality and crop diversity enhance soil biological health. <https://doi.org/10.1002/ael2.20030>
- Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2008. Soil carbon saturation: Evaluation and corroboration by long-term incubations. *Soil Biology and Biochemistry* 40, 1741–1750. <https://doi.org/10.1016/J.SOILBIO.2008.02.014>
- Stewart, R.D., Jian, J., Gyawali, A.J., Thomason, W.E., Badgley, B.D., Reiter, M.S., Strickland, M.S., 2018. What We Talk about When We Talk about Soil Health. *Agricultural & Environmental Letters* 3, 180033. <https://doi.org/10.2134/ael2018.06.0033>
- Stott, D.E. 2019. Recommended Soil Health Indicators and Associated Laboratory Procedures. Soil Health Technical Note No. 450-03. U.S. Department of Agriculture,

- Natural Resources Conservation Service.
- Strock, J.S., Johnson, J.M.F., Tollefson, D., Ranaivoson, A., 2022. Rapid change in soil properties after converting grasslands to crop production. *Agronomy Journal* 114, 1642–1654. <https://doi.org/10.1002/AGJ2.21045>
- Sutherlin, C.E., Brunsell, N.A., de Oliveira, G., Crews, T.E., DeHaan, L.R., Vico, G., 2019. Contrasting physiological and environmental controls of evapotranspiration over Kernza Perennial crop, annual crops, and C4 and mixed C3/C4 grasslands. *Sustainability (Switzerland)* 11. <https://doi.org/10.3390/su11061640>
- Suyker, A.E., Verma, S.B., 2009. Evapotranspiration of irrigated and rainfed maize–soybean cropping systems. *Agricultural and Forest Meteorology* 149, 443–452. <https://doi.org/10.1016/j.agrformet.2008.09.010>
- Tamburini, G., Bommarco, R., Wanger, T.C., Kremen, C., van der Heijden, M.G.A., Liebman, M., Hallin, S., 2020. Agricultural diversification promotes multiple ecosystem services without compromising yield. *Science Advances* 6, eaba1715. <https://doi.org/10.1126/sciadv.aba1715>
- Tautges, N.E., Jungers, J.M., Dehaan, L.R., Wyse, D.L., Sheaffer, C.C., 2018. Maintaining grain yields of the perennial cereal intermediate wheatgrass in monoculture v. bi-culture with alfalfa in the Upper Midwestern USA. *Journal of Agricultural Science* 156, 758–773. <https://doi.org/10.1017/S0021859618000680>
- Thaler, E.A., Kwang, J.S., Quirk, B.J., Quarrier, C.L., Larsen, I.J., 2022. Rates of Historical Anthropogenic Soil Erosion in the Midwestern United States. *Earth’s Future* 10, e2021EF002396. <https://doi.org/10.1029/2021EF002396>
- United States Department of Agriculture National Agricultural Statistics Service, 2021. Minnesota Ag News 2020 Soybean County Estimates. <https://quickstats.nass.usda.gov/>
- United States Department of Agriculture National Agricultural Statistics Service, 2022. Minnesota Ag News 2021 Corn for Silage County Estimates. <https://quickstats.nass.usda.gov/>
- van Es, H.M., 1993. Evaluation of temporal, spatial, and tillage-induced variability for parameterization of soil infiltration. *Geoderma* 60, 187–199. [https://doi.org/10.1016/0016-7061\(93\)90026-H](https://doi.org/10.1016/0016-7061(93)90026-H)
- Van Oost, K., Govers, G., De Alba, S., Quine, T.A., 2006. Tillage erosion: a review of controlling factors and implications for soil quality. *Progress in Physical Geography: Earth and Environment* 30, 443–466. <https://doi.org/10.1191/0309133306pp487ra>

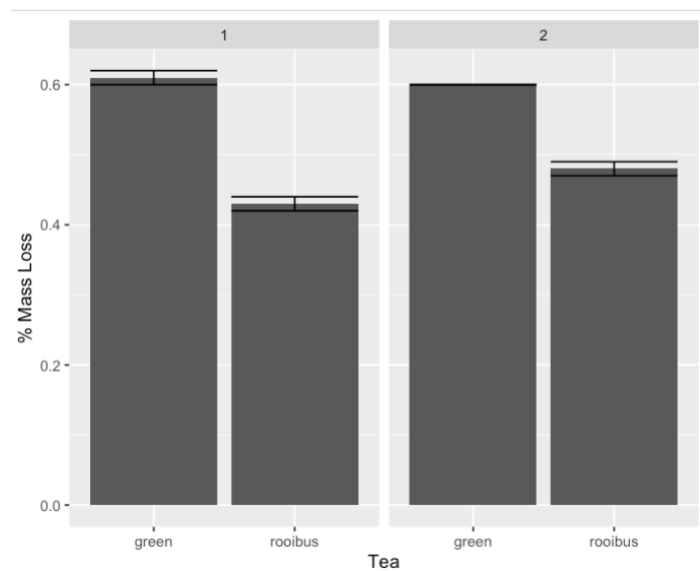
- Villarreal, R., Soracco, C.G., Lozano, L.A., Melani, E.M., Sarli, G.O., 2017. Temporal variation of soil sorptivity under conventional and no-till systems determined by a simple laboratory method. *Soil and Tillage Research* 168, 92–98.
<https://doi.org/10.1016/j.still.2016.12.013>
- Wallander, S., Smith, D., Bowman, M., Claassen, R. (Eds.), 2021. *Cover Crop Trends, Programs, and Practices in the United States*, EIB-222.
<https://doi.org/10.22004/ag.econ.309562>
- Wander, M.M., Cihacek, L.J., Coyne, M., Drijber, R.A., Grossman, J.M., Gutknecht, J.L.M., Horwath, W.R., Jagadamma, S., Olk, D.C., Ruark, M., Snapp, S.S., Tiemann, L.K., Weil, R., Turco, R.F., 2019. Developments in Agricultural Soil Quality and Health: Reflections by the Research Committee on Soil Organic Matter Management. *Frontiers in Environmental Science* 7. <https://doi.org/10.3389/fenvs.2019.00109>
- Waring, B.G., Smith, K.R., Grote, E.E., Howell, A., Reibold, R., Tucker, C.L., Reed, S.C., 2021. Climatic Controls on Soil Carbon Accumulation and Loss in a Dryland Ecosystems. *Journal of Geophysical Research: Biogeosciences* 126.
<https://doi.org/10.1029/2021jg006492>
- Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *American Journal of Alternative Agriculture* 18, 3–17.
<https://doi.org/10.1079/AJAA2003003>
- Wickham, H, 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- Williams, A., Hunter, M.C., Kammerer, M., Kane, D.A., Jordan, N.R., Mortensen, D.A., Smith, R.G., Snapp, S., Davis, A.S., 2016. Soil water holding capacity mitigates downside risk and volatility in US rainfed maize: Time to invest in soil organic matter? *PLoS ONE* 11, 1–11. <https://doi.org/10.1371/journal.pone.0160974>
- Wright, S.F., Upadhyaya, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science* 161, 575-586. 10.1097/00010694-199609000-00003
- Young, M.D., Ros, G.H., de Vries, W., 2021. Impacts of agronomic measures on crop, soil, and environmental indicators: A review and synthesis of meta-analysis. *Agriculture, Ecosystems & Environment* 319, 107551. <https://doi.org/10.1016/j.agee.2021.107551>

Zhang, Q., Shao, M., Jia, X., Wei, X., 2019. Changes in soil physical and chemical properties after short drought stress in semi-humid forests. *Geoderma* 338, 170–177. <https://doi.org/10.1016/j.geoderma.2018.11.051>

2.8 Supplementary Tables and Figures

Identified Marker	Microbial Group Assignment
16:0	Total Biomass
16:0 10-methyl	Actinobacteria
16:1 ω 5c (NLFA and PLFA)	Arbuscular Mycorrhizal Fungi (NLFA)
16:1 ω 7c	Gram-negative
16:1 ω 9c	Gram-negative
17:0	Total Biomass
17:0 anteiso	Gram-positive
17:0 iso	Gram-positive
17:0 cyclo	Gram-negative
18:0	Total Biomass
18:0 10-methyl	Actinomycetes
18:1 ω 7c	Gram-negative
18:1 ω 9t	Gram-negative
18:1 ω 9c	Saprotrophic Fungi
18:2 ω 6,9c	Saprotrophic Fungi
19:0	Total Biomass
19:0 cyclo	Gram-negative

Supplementary Table 2.1: Identified lipids, their molecular weights used to calculate total lipid biomass, and microbial group assignments (Frostegård et al., 1993; Balsler et al., 2005; Veum et al., 2021; Lekberg et al., 2022; Olsson and Lekberg, 2022).



Supplementary Figure 2.1: % Mass loss from green and rooibos teas from collection day 1 (28 day incubation) and 2 (58 day incubation). Error bars represent one standard deviation.

		Annual	Annual-cc	IWG	IWG-alf
Total Lipid Biomass (nmol/g soil)	Mean	36.2	39.4	34.6	33.7
	SD	14.6	17.0	8.5	17.7
AMF Lipid Biomass (nmol/g soil)	Mean	9.1	13.5	22.1	8.2
	SD	4.3	10.3	13.7	4.1
Gram Positive Lipid Biomass(nmol/g soil)	Mean	2.3	2.0	2.1	1.8
	SD	0.5	1.1	0.6	1.0
Gram Negative Lipid Biomass (nmol/g soil)	Mean	12.5	11.8	10.5	10.2
	SD	5.6	8.7	5.5	6.7
Saprotrophic Lipid Biomass (nmol/g soil)	Mean	3.6	3.4	3.5	3.7
	SD	1.5	2.1	1.5	2.0
Actinobacteria Lipid Biomass (nmol/g soil)	Mean	3.2	2.9	2.7	2.6
	SD	0.9	1.7	1.1	1.6
Actinomycetes Lipid Biomass (nmol/g soil)	Mean	0.7	0.6	0.5	0.5
	SD	0.5	0.4	0.2	0.3
Fungal/Bacterial Lipid Biomass Ratio	Mean	0.4	0.2	0.3	0.3
	SD	0.1	0.1	0.2	0.2
Cellobiohydrolase Activity (nmol/hr/g soi)	Mean	54.85	71.81	57.55	69.81
	SE	19.33	28.1	14.82	20.34
N-acetylglucosaminidase Activity (nmol/hr/g soi)	Mean	54.57	68.1	68.69	89.41
	SE	14.64	16.61	22.8	19.9
Phosphatase Activity (nmol/hr/g soi)	Mean	1087.93	1219.68	1265.3	1287.58
	SE	273.71	421.25	273.88	412.92
Beta Glucosaminidase Activity(nmol/hr/g soi)	Mean	354.55	377.44	407.12	429.28
	SE	64.1	132.44	147.49	170.32
CN Activity Ratio	Mean	8.15	6.85	7.12	7.78
	SE	2.36	2.82	2.23	2.79

Supplementary Table 2.2: Mean and standard deviation of EEA and PLFA/NLFA lipid biomass fractions by cropping system. CN Ratio here refers to the ratio of the sum of Cello and BG activities to NAG activity. Tukey's HSD on estimated marginal means revealed that there was significantly more AMF biomass in IWG systems (mean = 22.1 nmol/gsoil , se = 3.06) than annual (mean =9.13, se =0.95) , annual-cc (mean = 12.7, se = 2.23) and IWG-alf (mean = 8.19, se = 0.92). There were no significant differences in mean EEA or other PLFA lipid fractions between cropping systems.

Response Variable	Explanatory Variable	F-value	p-value
Total Lipid Biomass (nmol/g soil)	Crop	0.40172	0.75
AMF Lipid Biomass (nmol/g soil)	Crop	5.38008	0.002
Gram Positive Lipid Biomass(nmol/g soil)	Crop	0.87765	0.46
Gram Negative Lipid Biomass (nmol/g soil)	Crop	2.1240	0.11
Saprotrophic Lipid Biomass (nmol/g soil)	Crop	0.0991	0.96
Actinobacteria Lipid Biomass (nmol/g soil)	Crop	0.69440	0.56
Actinomycetes Lipid Biomass (nmol/g soil)	Crop	1.06032	0.37
Fungal/Bacterial Lipid Biomass Ratio	Crop	2.45517	0.071
Cellobiohydrolase Activity (nmol/hr/g soi)	Crop	3.021	0.036
N-acetylglucosaminidase Activity (nmol/hr/g soi)	Crop	0.835	0.479
Phosphatase Activity (nmol/hr/g soi)	Crop	2.944	0.038
Beta Glucosaminidase Activity (nmol/hr/g soi)	Crop	1.284	0.286
CN Activity Ratio	Crop	9.75	0.410

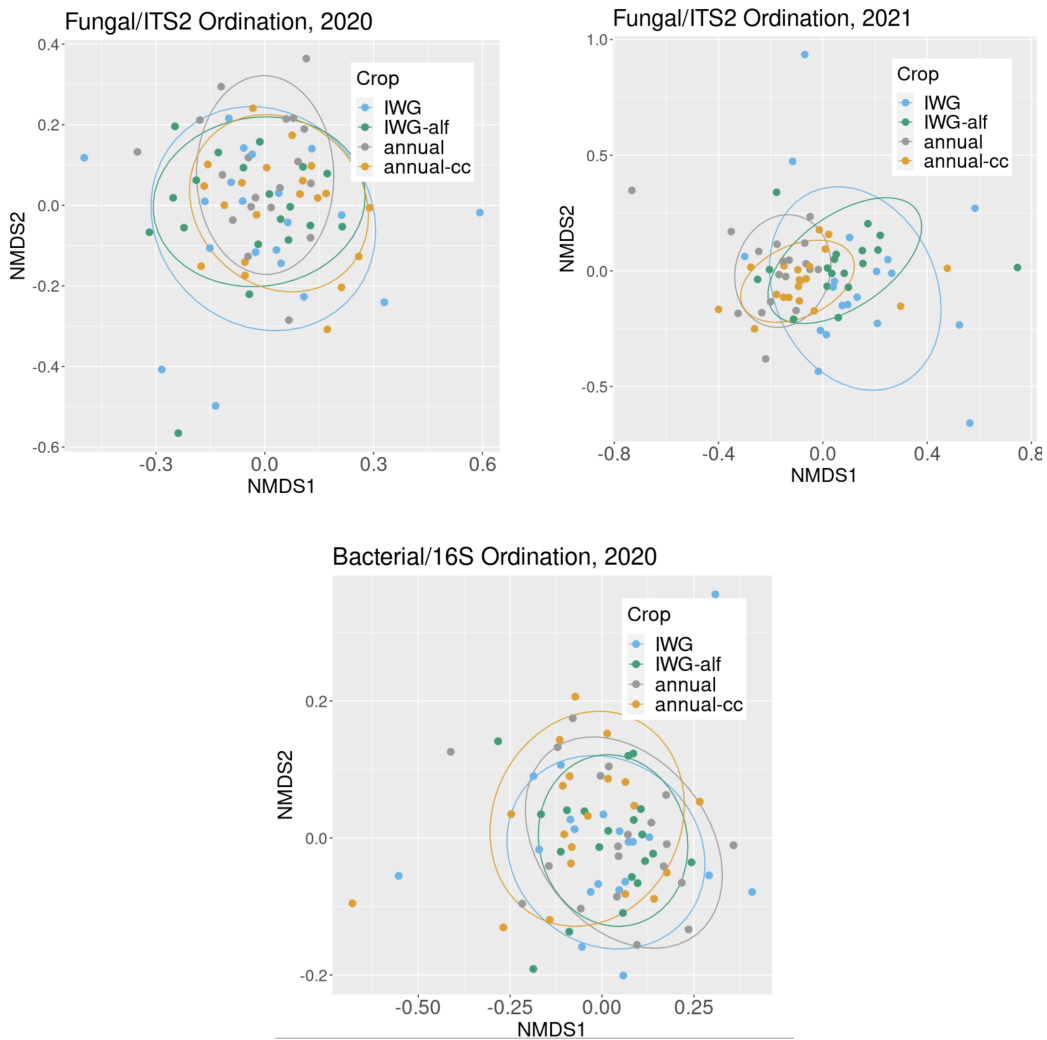
Supplementary Table 2.3: Results of ANOVA assessing differences EEA (2021) and PLFA/NLFA Lipid fractions (2020), with Crop modeled as a fixed effect and Block as a random effect. Degrees of freedom = 3 for all tests.

Year	Index	16S		ITS	
		Chi-sq.	p-value	Chi-sq.	p-value
2020	Hill-Shannon	1.75	0.62	7.332	0.06
	Hill-Simpson	0.96	0.81	8.35	0.04
2021	Hill-Shannon	5.05	0.17	5.39	0.15
	Hill-Simpson	3.65	0.302	5.53	0.14

Supplementary Table 2.4: Results of Kruskal-Wallis rank sum tests on Hill-Shannon and Hill-Simpson diversity indices by cropping system of bacterial and fungal metagenomic datasets in 2020 and 2021.

Year	Index	16S				ITS			
		IWG	IWG-alf	annual	annual-cc	IWG	IWG-alf	annual	annual-cc
2020	Richness	1503	1467	1491	1541	398	391	390	409
	Hill-Shannon	955.73	946.47	955.57	988.69	164.92	151.13	146.13	169.5
	Hill-Simpson	1.00	1.00	1.00	1.00	1.02	1.02	1.03	1.02
2021	Richness	833	921	967	916	306	298	288	291
	Hill-Shannon	517.46	575.56	594.98	568.36	108.51	108.24	93.94	105.04
	Hill-Simpson	1.00	1.00	1.00	1.00	1.03	1.02	1.04	1.02

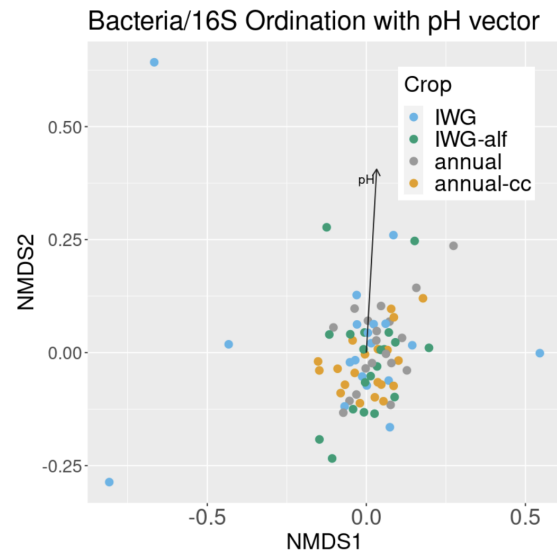
Supplementary Table 2.5: Mean Richness, Hill-Shannon Index, and Hill-Simpson Index by cropping system for bacterial and fungal communities, 2020 and 2021. There were no significant differences between individual cropping systems in any metric, though Wilcoxon rank-sum exact test revealed marginally significant differences in 2020 fungal community Hill-Simpson indices of annual-cc and IWG-alf systems ($p = 0.08$) and annual and annual-cc systems ($p=0.08$).



Supplementary Figure 2.2: A) 2020 fungal data ordination grouped by cropping system. Crop significantly explains 5% of distance matrix variation ($p = 0.001$). Stress = 0.25. Ordination goodness of fit, non-metric $R^2 = 0.939$, linear fit $R^2 = 0.76$. B) 2021 fungal data ordination grouped by cropping system. Crop significantly explains 10% of distance matrix variation ($p = 0.001$). Stress = 0.2. Ordination goodness of fit, non-metric $R^2 = 0.965$, linear fit $R^2 = 0.887$. C) 2020 bacterial data ordination grouped by cropping system. Crop significantly explains 5% of distance matrix variation ($p = 0.03$). Stress = 0.17. Ordination goodness of fit, non-metric $R^2 = 0.974$, linear fit $R^2 = 0.915$.

Supplementary Table 2.6: [Fungal Indicator Taxa](#)

Supplementary Table 2.7: [Bacteria indicator taxa](#)



Supplemental Figure 2.3: 2021 Bacterial community ordination with pH vector. [Result]. Stress = 0.15

Appendix 1 - Differing microbial communities from Qiagen PowerSoil and PowerSoil Pro extraction kits

Due to the unanticipated removal of the qiagen PowerSoil DNA extraction kit from the market in 2021, we were forced to use two different extraction kits in 2020 and 2021. It is well established that different extraction kits can capture widely different microbial communities (ex. Pearman et al., 2020), and the qiagen PowerSoil DNA extraction kit and PowerSoil Pro DNA extraction kits proved no exception to this rule. In a subset of 10 2021 samples extracted with both the old and the new PowerSoil Pro kits, we found significant differences in species richness and species diversity with no significant differences in homogeneity of group dispersions by cropping system (Figures below). In an attempt to normalize differences between kits, we trimmed this subset of samples to only ASVs identified using both kits. We implemented a custom R function and rarefied the data. This reduced the number of ASVs in ITS datasets from 2244 to 577 and in 16S from 6391 to 2404. We found that significant differences in species richness and diversity metrics persisted between kits, with the exception of bacterial community richness (Figures below). These differences could be due to inherent differences in DNA/RNA extracted by the kits, older and degraded extraction reagents from the PowerSoil extraction kit, and/or inherent high variability of microbial community characteristics targeted by these methods even within a very small sample. Regardless, this demonstrated that it is impossible to confidently disentangle the effects of kit type and year in the multi-year, multi-kit dataset by subsetting to commonly found asvs. While the effect of year is clearly stronger, there remains some variation in community composition due to kit. Therefore, we chose to analyze the full datasets separately by year and compare presence/absence of relative differences in diversity metrics over time.

A1.1 Differences seen in diversity indices by extraction kit (subset 10 samples)

To assess differences in taxa and abundance captured by extraction kits used in 2020 and 2021, 10 samples from 2021 were extracted with both kits and sequenced. I assessed differences in alpha diversity, beta diversity, and taxonomic relative abundance between these OldKit 2020 samples, OldKit2021 samples, and NewKit2021 samples. In this subset there were 2244 taxa found in ITS and 6391 16S.

A1.1.1 Fungal/ITS alpha and beta diversity

Year and extraction kit both significantly affect alpha-diversity of the ITS amplicon data. The simpson index is significantly different among groups (OldKit, NewKit, OldKit2020) (Kruskal-Wallis chi-squared = 16.27, df = 2, p-value = 0.0002932). Wilcoxon rank sum exact test reveals that the Simpson index of group is different from that of each other group (Table A1). Year and kit also both significantly affect beta-diversity. The centroid of the bray-curtis dissimilarity matrix is different by extraction kit, while dispersion of the centroid is not (betadisper) (Fig. A2 and Table A2). The differences between years are greater than the differences between kits in 2021, but when only 2021 data is considered, there is still a significant kit effect (Table A2).

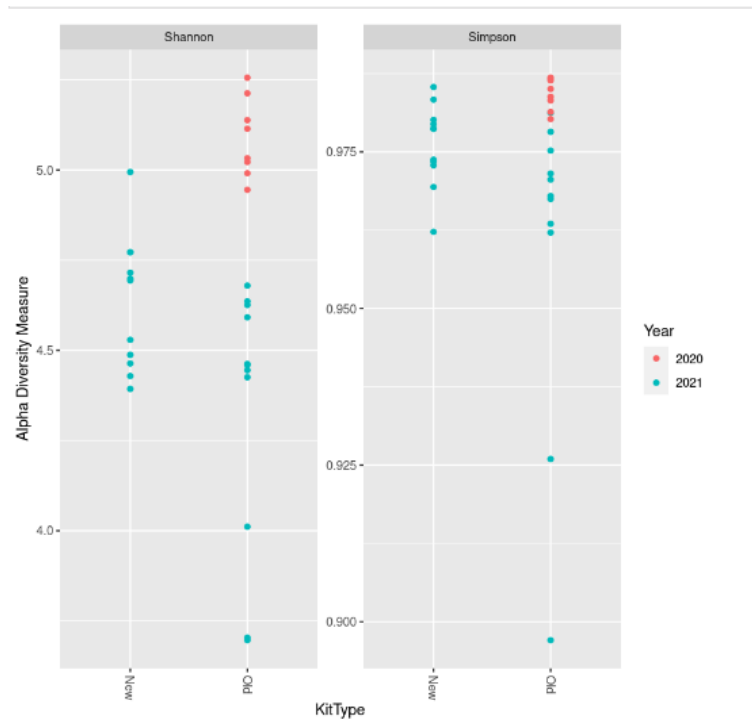


Figure A1: ITS Shannon and Simpson diversity indices of old and new extraction kits.

	New	Old
Old 2020	0.01207	-
Old 2021	0.01047	0.00056

Table A1: Results of Wilcoxon rank sum exact test assessing differences in ITS Simpson Diversity index of old and new extraction kits.

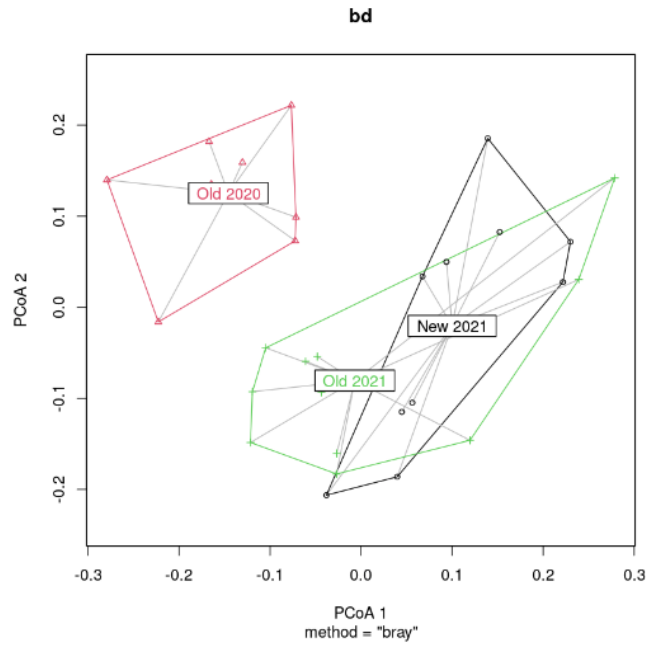


Figure A2: Bray-Curtis dissimilarity matrix of ITS beta diversity of new and old extraction kits.

	Df	SumOfSqs	R2	F	Pr(>F)
	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
KitYear	2	0.6432108	0.1378481	2.158494	0.001
Residual	27	4.0228718	0.8621519	NA	NA
Total	29	4.6660827	1.0000000	NA	NA

	Df	SumOfSqs	R2	F	Pr(>F)
	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
Year	1	0.4826873	0.1034459	3.230688	0.001
Residual	28	4.1833954	0.8965541	NA	NA
Total	29	4.6660827	1.0000000	NA	NA

	Df	SumOfSqs	R2	F	Pr(>F)
	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
Kit	1	0.4193452	0.1348332	3.116929	0.001
Residual	20	2.6907590	0.8651668	NA	NA
Total	21	3.1101042	1.0000000	NA	NA

Table A2: Results of adonis PERMANOVA of Bray-Curtis dissimilarity matrix of ITS beta diversity of a) new and old extraction kits, both years of data included; b) year, both kits included; and c) new and old extraction kits, only 2021 samples included.

A1.1.2 16S/bacterial alpha and beta diversity

Year and extraction kit both significantly affected alpha-diversity of the 16S amplicon data. The Simpson index is significantly different among groups (OldKit, NewKit, OldKit2020) (Kruskal-Wallis chi-squared = 14.677, df = 2, p-value = 0.0006501). Wilcoxon rank sum exact test reveals that the Simpson index of each group is different or marginally from that of each other group (Table A3). Year and kit also both significantly affect beta-diversity. The centroid of the bray-curtis dissimilarity matrix is different by extraction kit even when only 2021 data is considered, while dispersion of the centroid is not (betadispr) (Table A4).

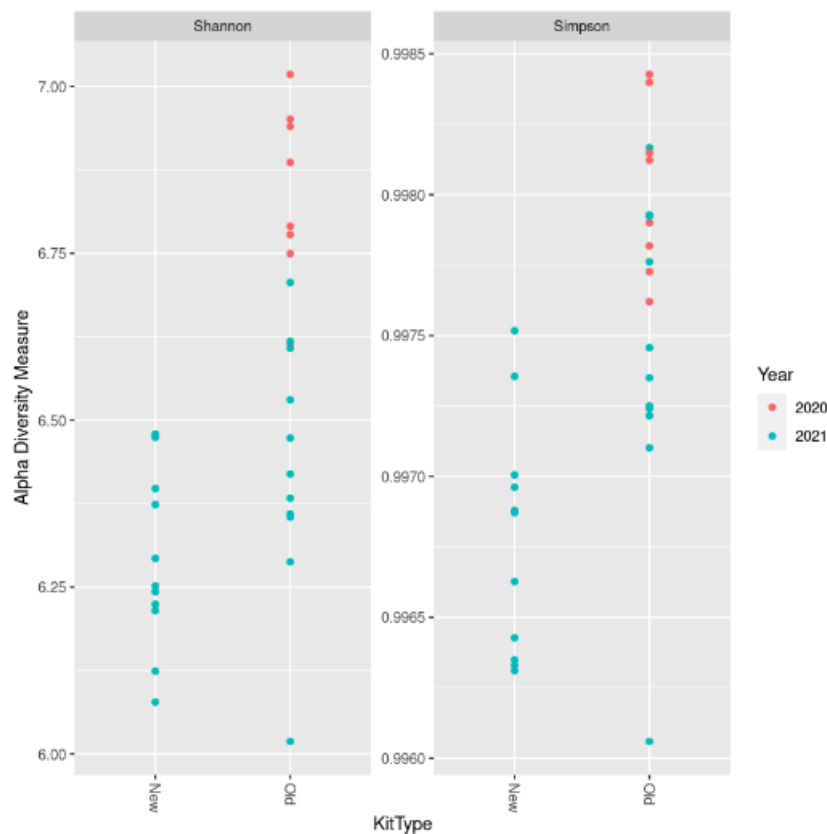


Figure A3: 16S Shannon and Simpson diversity indices of old and new extraction kits.

	New 2021	Old 2020
Old 2020	0.00032	-
Old 2021	0.01327	0.10865

P value adjustment method: holm

Table A3: Results of Wilcoxon rank sum exact test assessing differences in 16S Simpson Diversity index of old and new extraction kits.

	Df	SumOfSqs	R2	F	Pr(>F)
	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
Kit	1	0.4263296	0.1734181	4.19603	0.001
Residual	20	2.0320618	0.8265819	NA	NA
Total	21	2.4583915	1.0000000	NA	NA

Table A4: Results of adonis PERMANOVA of Bray-Curtis dissimilarity matrix of 16S beta diversity of new and old extraction kits, only 2021 data considered.

A1.2 Common taxa between both extraction kits

I subset the dataset to only the ASVs seen in both kits in the 2021 and 2020 samples. This reduced the number of taxa in ITS from 2244 to 577 and in 16S from 6391 to 2404. Lists of all taxa present and taxa present only from both extraction kits are available [here](#).

A1.2.1 ITS/fungal alpha and beta diversity

Year and extraction kit both significantly affected alpha-diversity of the ITS amplicon data. The simpson index is significantly different among groups (OldKit, NewKit, OldKit2020) (Kruskal-Wallis chi-squared = 12.28, df = 2, p-value = 0.0006501). Wilcoxon rank sum exact test reveals that new and old kit 2021 samples and old kit 2020 and 2021 samples were significantly different (Table A5). Year and kit also both significantly affect beta-diversity. The centroid of the bray-curtis dissimilarity matrix is different by extraction kit even when only 2021 data is considered, while dispersion of the centroid is not (betadisper) (Table A6). Figure A4 shows that taxonomic differences in the most abundant phylum and classes persist in this subset dataset.

	New 2021	Old 2020
Old 2020	0.2375	-
Old 2021	0.0167	0.0024

Table A5: Results of Kruskal-Wallis and Wilcoxon rank sum tests showing that ITS Simpson indices are different by extraction kit when asvs are pruned to only those found in common by both kits.

	Df	SumOfSqs	R2	F	Pr(>F)
	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
KitYear	1	0.3949429	0.1452137	3.397661	0.001
Residual	20	2.3247925	0.8547863	NA	NA
Total	21	2.7197354	1.0000000	NA	NA

Table A6: Results of PERMANOVA showing that ITS beta diversity is different by extraction kit when asvs are pruned to only those found in common by both kits.

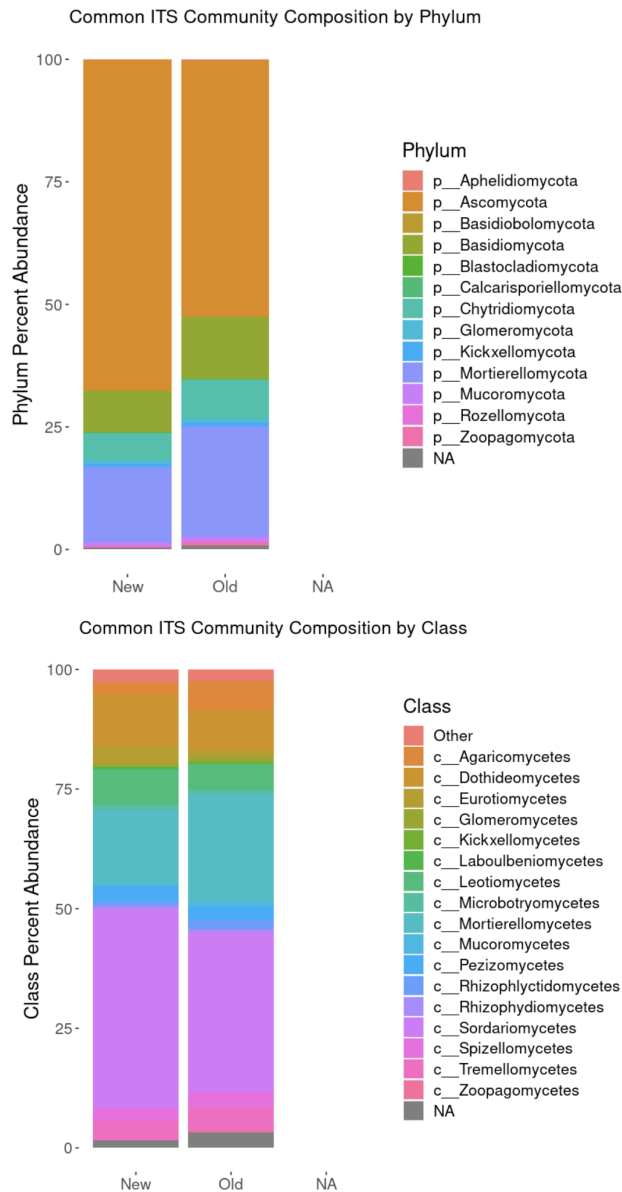


Figure A4: Abundance of phylum and classes of identified ITS taxa found in both old and new extraction kits.

A1.2.2 16S/bacterial alpha and beta diversity

Extraction kit did not affect alpha-diversity of the subset 16S amplicon data. The simpson index is not significantly different among groups (OldKit, NewKit) (Kruskal-Wallis chi-squared = 1.1635, df = 1, p-value = 0.2807), and there were no differences between groups (p = 0.3). However, kit significantly affects beta-diversity. The centroid of the bray-curtis dissimilarity matrix is different by extraction kit even when only 2021 data is considered, while dispersion of the centroid is not (betadisper) (Table A7). Figure A4 shows that taxonomic differences in the most abundant phylum and classes persist in this subset dataset.

	Df	SumOfSqs	R2	F	Pr(>F)
	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
KitType	1	0.3673965	0.2055689	5.175247	0.001
Residual	20	1.4198220	0.7944311	NA	NA
Total	21	1.7872185	1.0000000	NA	NA

Table A7: Results of PERMANOVA showing that 16S beta diversity is different by extraction kit when asvs are pruned to only those found in common by both kits. Beta diversity is significantly different by kit type. No difference in centroid dispersion.

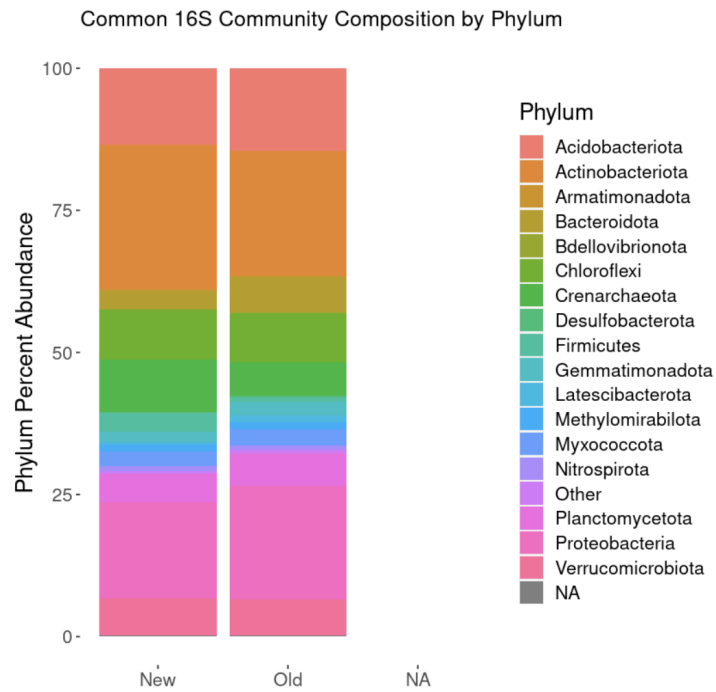
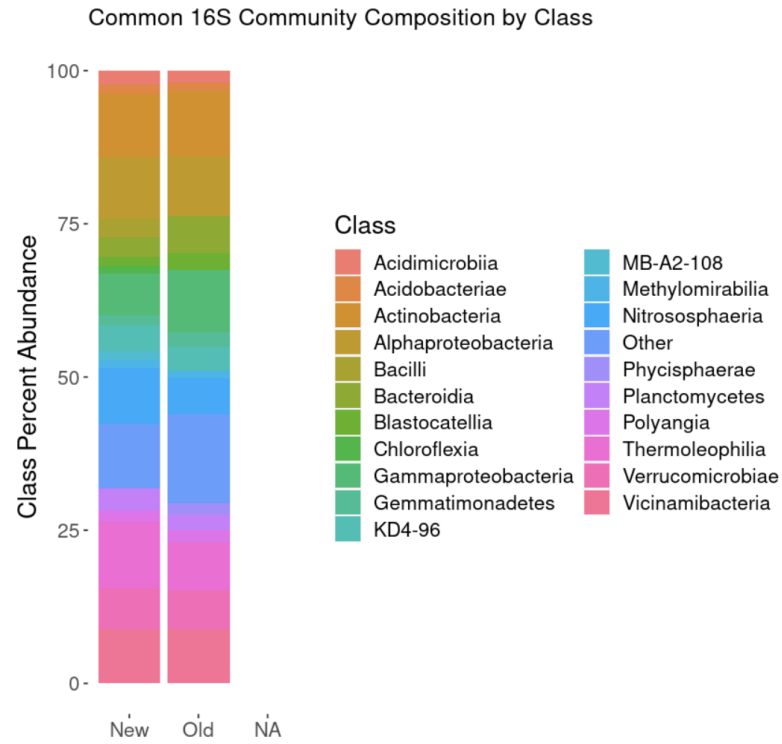


Figure A6: Abundance of phylum and classes of identified 16S taxa found in both old and new extraction kits.

Appendix 2: Data and code

Complete R code and raw datasets for Chapter 1 can be [found on github](#).

R code and raw datasets for Chapter 2, PLFA, EEA, and litter decomposition can be [found on github](#).

R code for amplicon sequencing data analysis can be [found on github](#). Due to size constraints, only a subset dataset is available in this github repository. Please contact Emma (link0126@umn.edu) or look to forthcoming publication for dataset repository.