From microbes to farmers perspectives: Enhancing soil health through on-farm sustainable agricultural practices

by

Carlos Augusto Bonini Pires

B.S., Federal University of Santa Maria, 2016
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Abstract

One of the pivotal challenges for ensuring the sustainability and resiliency of agroecosystems is the selection of agricultural practices that can effectively nurture soil health and maintain productivity levels. In this scenario, regenerative agriculture practices, such as cover crops, can offer multiple benefits to soils by improving soil health and sustaining the soil microbial community structure and activity. The overall goal of this research was to explore on-farm sustainable agricultural practices to design farming systems for sustaining soil health management in Kansas. The objective was to quantify the impact of cover crops and nutrient management on biological, chemical, and physical soil health indicators at the farm level. Besides soil nutrients, selected indicators included phospholipid fatty acids, enzyme activity, soil organic carbon, potentially mineralizable nitrogen, and water-stable aggregates. Additionally, farmers' perspectives were assessed regarding collaborative on-farm research and incorporate farmer input and collaboration to enhance the effectiveness of these practices in "real-world" agricultural settings.

Overall, cover crops improved soil health by increasing soil microbial biomass, bacteria, fungi, β-glucosidase activity, soil organic carbon and other biological soil health indicators. The extent of these changes occurred in response to the precipitation patterns across sites. Our findings support the hypothesis that increasing soil organic carbon levels and stimulating soil microbial growth and activity under water-limited environments requires time. Besides increasing several biological soil health indicators, cover crops improved aggregate stability. The continuous presence of living roots from cover crops significantly impacted the development and maintenance of macroaggregates as affected by increased fungi and bacterial communities. Maintaining and increasing soil organic carbon (e.g., soil organic matter) is considered an

essential strategy to improve N management. Our study indicated that after 25 years of regenerative agriculture practices, in the field conditions of the study, decreasing the N rate by 48% for winter wheat production is feasible without compromising yields and soil organic carbon levels in the short term. However, reducing the N rate would not be advisable for corn production as it may lead to economic losses. This approach can empower farmers to take advantage of the mineralized N from the soil they built over time and use external inputs more efficiently, ultimately promoting soil health.

Finally, our research survey demonstrated that farmers' high trust in on-farm research outcomes can influence the adoption of sustainable practices. Limitations like time management and plot setup were identified in on-farm research, while strengths included practical knowledge gained and unbiased insights. Notably, a shift towards conservation practices has been made in the last ten years, with cover crops and no-till at the forefront of these changes. Farmers were 40% more likely to adopt practices supported by findings of on-farm research compared to off-farm ones, and farmers' primary motivators for doing on-farm research were productivity and profitability. Insights of this nature have implications for fostering collaborations, addressing constraints, and maximizing the impact of on-farm research, offering guidance for sustainable agriculture progress in the U.S. and beyond. Results from this research survey can drive further policy and initiatives promoting on-farm research.

Ultimately, the diverse scientific methods employed in this work, from laboratory-based analysis to on-farm research and nationwide survey, significantly contributed to advancing the scientific understanding of how agricultural practices impact soil-crop outcomes and how farmers' perspectives about on-farm research impact their decision-making. Overall, this research offered a strong foundation for practical applications that can contribute to developing more

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Major Professor
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Table of Contents

List of Figures xiii
List of Tables xviii
Acknowledgements xxii
Dedication xxiv
Chapter 1 - Introduction 1
Regenerative agriculture 2
Soil health 2
Soil health assessment and indicators 4
Cover crops 9
Soil microbial communities and enzyme activity 10
On-farm research and the Kansas Soil Health Partnership 12
Study Objectives 13
References 15
Chapter 2 - Cover crop-mediated changes in soil microbial community and enzyme activity: implications for sustainable on-farm cropping systems 30
Abstract 30
Introduction 31
Materials and Methods 33
Study site and field characteristics 33
Experimental design and treatment descriptions 34
Soil and biomass sampling 35
Soil physical and chemical properties analysis 36
Soil microbiological properties analysis 37
Statistical analysis 38
Results 39
Soil microbial community composition 39
Extracellular enzyme activity 46
Soil organic carbon and total nitrogen 53

Influence of soil chemical properties on the soil microbial community and enzyme activity 55
Cropping system biomass and cash crop yield 57
Discussion 59
Conclusion 64
References 65
Supplementary material 78
Chapter 3 - Amplifying the bond: cover crops strengthening the soil aggregates-microbial community relationship 104
Abstract 104
Introduction 105
Materials and Methods 107
Study site and field characteristics 107
Experimental design and treatments description 107
Soil sampling 109
Sand-free water stable aggregates analysis 109
Phospholipid fatty acids analysis (PLFA) 110
Statistical analysis 111
Results 112
Aggregates size distribution 112
Soil microbial community 116
Soil microbial community and macroaggregates relationship 118
Discussion 121
Conclusion 125
References 125
Supplementary material 136
Chapter 4 - Short-term impact of nitrogen rates on potentially mineralizable nitrogen, soil organic matter, and crop yield after 25 years of on-farm regenerative agriculture 141
Abstract 141
Introduction 142
Materials and Methods 145

Study site and field characteristics 145
Experimental design and treatment descriptions 147
Soil and yield sampling 148
Soil measurements 149
Data analysis 150
Results 151
Discussion 156
Conclusion 160
References 161
Chapter 5 - Perspective of U.S. farmers on collaborative on-farm agronomic research 172
Abstract 172
Introduction 173
Materials and Methods 175
Results 178
Discussion 187
References 196
Supplementary material 202
Chapter 6 - Conclusion 203
Future Research and Considerations 205
Appendix A – Chapter 2 207

List of Figures

Figure 1. 1. Relevant spatial scales, functions, ecosystem services, and stakeholders of soil security, health, quality, and fertility. Adapted from Lehman et al. (2020). 3
Figure 1. 2. Soil health and conservation agriculture principles..... 4
Figure 1. 3. Soil health indicators. *PLFA: phospholipid fatty acid analysis. 5
Figure 1. 4. Main soil organism groups and their soil functions and involved mechanisms..... 6
Figure 1. 5. Soil health biological indicators and related soil functions..... 7
Figure 1. 6. Soil physical functions. 8
Figure 1. 7. Commonly used cover crop species separated by purpose and type..... 10
Figure 1. 8. Enzymes and biogeochemical cycles. 11
Figure 2.1. Microbial biomass by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level..... 40
Figure 2. 2. Microbial biomass by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level..... 42
Figure 2. 3. β-glucosidase activity by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level..... 47
Figure 2. 4. β-glucosidase activity by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level..... 49
Figure 2. 5. β-glucosidase activity by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level..... 52
Figure 2. 6. Soil organic carbon by treatment and year at 0-5 cm at Bucyrus (A), Solomon (B), and Beloit (C). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level..... 54
Figure 2. 7. Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activity constrained by soil chemical properties at 0-5 cm in Bucyrus (A), Solomon (B), and

Beloit (C). Microbial biomass (TMB), gram-positive bacteria (Gram+), gram-negative bacteria (Gram-), actinomycetes (Actin), arbuscular mycorrhizal fungi (AMF), saprophytic fungi (Fungi), β-glucosidase (βG), acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS), soil organic carbon (SOC), and total nitrogen (TN)..... 56
Figure S2. 1. Daily rainfall, cumulative rainfall, and maximum, average, and minimum temperatures recorded during the study years in Bucyrus, KS. 93
Figure S2. 2. Daily rainfall, cumulative rainfall, and maximum, average, and minimum temperatures recorded during the study years in Solomon, KS. 94
Figure S2. 3. Daily rainfall, cumulative rainfall, and maximum, average, and minimum temperatures recorded during the study years in Beloit, KS..... 95
Figure S2. 4. Study experimental design. Bucyrus, Kansas. 96
Figure S2. 5. Study experimental design. Solomon, Kansas. 97
Figure S2. 6. Study experimental design. Beloit, Kansas. 98
Figure S2. 7. Crop biomass by treatment and year at Bucyrus, KS..... 99
Figure S2. 8. Crop biomass by treatment and year at Solomon, KS..... 99
Figure S2. 9. Crop biomass by treatment and year at Beloit, KS. 100
Figure S2. 10. Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activity constrained by soil chemical properties at 0-30 cm in Bucyrus. Microbial biomass (TMB), gram-positive bacteria (Gram+), gram-negative bacteria (Gram-), actinomycetes (Actin), arbuscular mycorrhizal fungi (AMF), saprophytic fungi (Fungi), β-glucosidase (βG), acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS), soil organic carbon (SOC), and total nitrogen (TN). 101
Figure S2. 11. Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activity constrained by soil chemical properties at 0-30 cm in Solomon. Microbial biomass (TMB), gram-positive bacteria (Gram+), gram-negative bacteria (Gram-), actinomycetes (Actin), arbuscular mycorrhizal fungi (AMF), saprophytic fungi (Fungi), β-glucosidase (βG), acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS), soil organic carbon (SOC), and total nitrogen (TN). 102
Figure S2. 12. Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activity constrained by soil chemical properties at 0-30 cm in Beloit. Microbial biomass (TMB), gram-positive bacteria (Gram+), gram-negative bacteria (Gram-),

actinomycetes (Actin), arbuscular mycorrhizal fungi (AMF), saprophytic fungi (Fungi), β-glucosidase (βG), acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS), soil organic carbon (SOC), and total nitrogen (TN). 103
Figure 3. 1. Sand-free water stable aggregate (WSA) distribution by treatment and year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Lowercase letters compare the interaction or year effect, while capitalized letters compare the treatment effect: *No cover, **Cover crop. 113
Figure 3. 2. Sand-free water stable aggregate (WSA) distribution by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. 114
Figure 3. 3. Sand-free water stable aggregate (WSA) distribution by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. 115
Figure 3. 4. Macroaggregates by treatment and year at 0-5 cm depth at Bucyrus (A), Solomon (B), and Beloit (C). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. 116
Figure 3. 5. Total fungi by treatment and year at 0-5 cm depth at Bucyrus (A), Solomon (B), and Beloit (C). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. 117
Figure 3. 6. Total bacteria by treatment and year at 0-5 cm depth at Bucyrus (A), Solomon (B), and Beloit (C). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. 118
Figure 3. 7. Relationship between total fungi and macroaggregates as affected by treatment and year at 0-5 cm depth at Bucyrus (A), Solomon (B), and Beloit (C). 119
Figure 3. 8. Relationship between total bacteria and macroaggregates as affected by treatment and year at 0-5 cm depth in Bucyrus (A), Solomon (B), and Beloit (C). 120
Figure 3. 9. Pearson correlation matrix between total fungi, total bacteria, soil organic carbon (SOC), macroaggregates, and mean weight diameter (MWD). 121
Figure S3. 1. Mean weight diameter (mm) by treatment and year at 0-5 cm depth in Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. 140

Figure S3. 2. Mean weight diameter (mm) by treatment and year at 0-5 cm depth in Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. 140
Figure 4. 1. Daily rainfall, cumulative rainfall, and maximum, average, and minimum temperatures for 2019 (corn) and 2021 (wheat). 146
Figure 4. 2. Study experimental design. Glen Elder, Kansas, U.S. 148
Figure 4. 3. Corn grain yield, agronomic optimum nitrogen rate (AONR), and economic optimum nitrogen rate (EONR) response to nitrogen management. Yellow dots indicate grain yield at each N rate. The AONR is represented by horizontal lines and EONR by vertical dashed lines. 152
Figure 4. 4. Winter wheat yield, agronomic optimum nitrogen rate (AONR), and economic optimum nitrogen rate (EONR) response to nitrogen management. Yellow dots indicate grain yield at each N rate. The AONR is represented by horizontal lines and EONR by vertical dashed lines. 153
Figure 4. 5. Cumulative potentially mineralizable nitrogen (PMN) for soil samples taken in 2019 and 2022. 154
Figure 4. 6. Soil organic carbon (SOC) by year and depth. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. 155
Figure 4. 7. Total nitrogen (TN) by year (A) and depth (B). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. 156
Figure 5.1. Number of participants by U.S. states that completed the on-farm research survey. 176
Figure 5. 2**.** Farmers response to: Is on-farm research a new concept for you? (a). Have you participated in on-farm research? (b). In your opinion, what best describes on-farm research? (c). 179
Figure 5. 3**.** Farmers response to: How likely are you to collaborate with a university (e.g., researchers and extension educators) for conducting on-farm research? (a). How likely are you to adopt practices recommended based on an on-farm study? (b). How likely are you to adopt practices recommended based on a research study other than on-farm study? (c). I consider on-farm research/experimentation an important way to strengthen relationships between producers and universities (d). 181

Figure 5. 4**.** Farmers response to: In your opinion, what are the top 3 on-farm research limitations? (a). In your opinion, what are the top 3 on-farm research strengths? (b)..... 182
Figure 5. 5**.** Farmers response to: What is your primary source for field information? (a). How often do you consult field information sources? (b). 186
Figure 5. 6. Farmers response to: Did you have any major changes to your farming in the last 10 years? Minimum and maximum frequency: 1, 106. 187
Figure S5. 1. Do you use available precision agriculture technologies? 202
Figure S5. 2. Do you have yield monitor in your combine?..... 202
Figure 6. 1. Time scale changes and sensitiveness of biological soil health indicators to cover crops (CCs). 205

List of Tables

Table 2.1. Site name, location, elevation, soil classification, average annual precipitation, and average mean annual temperature. 34
Table 2. 2. Changes in soil microbial community (nmol PLFA g^{-1} soil) by treatment, year, and depth in Bucyrus, KS. 41
Table 2. 3. Changes in soil microbial community (nmol PLFA g^{-1} soil) by treatment, year, and depth in Solomon, KS. 43
Table 2. 4. Changes in soil microbial community (nmol PLFA g^{-1} soil) by treatment, year, and depth in Beloit, KS. 46
Table 2. 5. Changes in enzyme activity (µg PNP $soil^{-1}$ h^{-1}) by treatment, year, and depth in Bucyrus, KS. 48
Table 2. 6. Changes in enzyme activity (µg PNP $soil^{-1}$ h^{-1}) by treatment, year, and depth in Solomon, KS. 50
Table 2. 7. Changes in enzyme activity (µg PNP $soil^{-1}$ h^{-1}) by treatment, year, and depth in Beloit, KS. 52
Table 2. 8. Cash crop yield (kg ha^{-1}) by treatment and year at Bucyrus, KS. 58
Table 2. 9. Cash crop yield (kg ha^{-1}) by treatment and year at Solomon, KS. 58
Table 2. 10. Cash crop yield (kg ha^{-1}) by treatment and year at Beloit, KS. 58
Table S2. 1. Soil physicochemical properties by treatment, year, and depth at Bucyrus, KS (n = 4). 78
Table S2. 2. Soil physicochemical properties by treatment, year, and depth at Solomon, KS (n = 4). 79
Table S2. 3. Soil physicochemical properties by treatment, year, and depth at Beloit, KS (n = 4). 80
Table S2. 4. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on microbial biomass (TMB), gram-positive bacteria, gram-negative bacteria, actinomycetes, arbuscular mycorrhizal fungi (AMF), and saprophytic fungi at 0-5, 5-15, and 15-30 cm soil depth at Bucyrus, KS. 81

Table S2. 5. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on β-glucosidase, acid phosphatase, N-acetyl-β-glucosaminidase, and arylsulfatase at 0-5, 5-15, and 15-30 cm soil depth at Bucyrus, KS. 82
Table S2. 6. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on soil organic carbon, total nitrogen, and C: N ratio at 0-5, 5-15, and 15-30 cm soil depth atBucyrus, KS. 83
Table S2. 7. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on microbial biomass (TMB), gram-positive bacteria, gram-negative bacteria, actinomycetes, arbuscular mycorrhizal fungi (AMF), and saprophytic fungi at 0-5, 5-15, and 15-30 cm soil depth at Solomon, KS. 84
Table S2. 8. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on β-glucosidase, acid phosphatase, N-acetyl-β-glucosaminidase, and arylsulfatase at 0-5, 5-15, and 15-30 cm soil depth at Solomon, KS. 85
Table S2. 9. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on soil organic carbon, total nitrogen, and C: N ratio at 0-5, 5-15, and 15-30 cm soil depth at Solomon, KS. 86
Table S2. 10. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on microbial biomass (TMB), gram-positive bacteria, gram-negative bacteria, actinomycetes, arbuscular mycorrhizal fungi (AMF), and saprophytic fungi at 0-5, 5-15, and 15-30 cm soil depth at Beloit, KS. 87
Table S2. 11. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on β-glucosidase, acid phosphatase, N-acetyl-β-glucosaminidase, and arylsulfatase at 0-5, 5-15, and 15-30 cm soil depth at Beloit, KS. 88
Table S2. 12. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on soil organic carbon, total nitrogen, and C: N ratio at 0-5, 5-15, and 15-30 cm soil depth at Beloit, KS. 89
Table S2. 13. Changes in soil organic carbon (g C kg $soil^{-1}$), total nitrogen (g N kg $soil^{-1}$), and C: N ratio by treatment, year, and depth at Bucyrus, KS. 90
Table S2. 14. Changes in soil organic carbon (g C kg $soil^{-1}$), total nitrogen (g N kg $soil^{-1}$), and C: N ratio by treatment, year, and depth at Solomon, KS. 91

Table S2. 15. Changes in soil organic carbon (g C kg soil^{-1}), total nitrogen (g N kg soil^{-1}), and C: N ratio by treatment, year, and depth at Beloit, KS. 92
Table 3. 1. Site name, location, elevation, soil classification, average annual precipitation, and average mean annual temperature. 107
Table S3. 1. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on water stable aggregates (WSA) >2 mm, 2 – 0.250 mm, 0.250 – 0.053 mm, 0.053 – 0.02 mm, macroaggregates, mean weight diameter (MWD), total fungi, and total bacteria at 0-5 cm soil depth in Bucyrus. 136
Table S3. 2. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on water stable aggregates (WSA) >2 mm, 2 – 0.250 mm, 0.250 – 0.053 mm, 0.053 – 0.02 mm, macroaggregates, mean weight diameter (MWD), total fungi, and total bacteria at 0-5 cm soil depth in Solomon. 137
Table S3. 3. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on water stable aggregates (WSA) >2 mm, 2 – 0.250 mm, 0.250 – 0.053 mm, 0.053 – 0.02 mm, macroaggregates, mean weight diameter (MWD), total fungi, and total bacteria at 0-5 cm soil depth in Beloit. 138
Table S3. 4. Model parameters of the bivariate regression between macroaggregates and total fungi. 139
Table S3. 5. Model parameters of the bivariate regression between macroaggregates and total bacteria. 139
Table 4. 1. Soil texture by nitrogen management and depth (n = 4). 145
Table 4. 2. Soil chemical properties by nitrogen management and depth (n = 4). 146
Table 4. 3. Analysis of variance (ANOVA) of the effect of nitrogen management, depth, year, and their respective interactions on soil organic carbon and total nitrogen. Depth effect was not tested for potentially mineralizable nitrogen. 154
Table 5.1. Questions description, identification number, and number of responses recorded for all 24 questions. 177
Table 5. 2**.** Surveyed farmer profiles related to age, years in production, and cropland area expressed in acres. 178
Table 5. 3. Farmers response to: How do you compare on-farm research to other education opportunities (e.g., greenhouse research, small plots research) available to you? On-farm

research can foster innovation (cover crops, nutrient management, precision agriculture, etc.) to my farm. 183
Table 5. 4**.** Farmers response to: What has been and/or would be your primary motivator for doing on-farm research? 184
Table 5. 5. Farmers response to: I am satisfied with the investment universities make in outreach and extension. What type of extension events do you usually attend? How many field days to you attend annually. 185

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Dedication

I dedicate this dissertation to my family – To my grandparents Arduino Bonini, Candida Bonini, Jose Carlos Pires, and Maria Eugenia Pires. To my parents and brothers Sergio Renato Pires, Neiva Fatima Bonini Pires, Luis F. Bonini Pires, and Marcos A. Bonini Pires. To my wife, Jessica Demarco.

Chapter 1 - Introduction

The concerns surrounding soil degradation and its impacts on global food security and agricultural sustainability have become increasingly relevant over the past decades (Amundson et al., 2015; Karlen & Rice, 2015). To withstand soil degradation and climate variability, including extreme precipitation events and frequent droughts, agricultural systems must be resource-efficient, profitable, resilient, and healthy (Bowles et al., 2020; Reimer et al., 2022). One of the pivotal challenges for ensuring the sustainability and resiliency of agroecosystems lies in selecting agricultural practices that can effectively nurture soil health and maintain productivity levels (Foley et al., 2011; Pittelkow et al., 2015). Agricultural production in the U.S. Midwest frequently relies on external inputs due to degraded soil health and nutrient cycling (Sanchez et al., 2019; Schipanski et al., 2016). Practices involving continued tillage and monoculture have disrupted the soil ecosystem equilibrium, resulting in decreased soil health and degradation through soil erosion and organic matter depletion (Hatfield, 2012; Lal, 2010). Although the interest in soil health and sustainable agriculture has increased in recent decades, farms in the U.S. Midwest continue to exhibit vulnerability to extreme weather events and market fluctuations, indicating a lack of resilience (Prokopy et al., 2020). Currently, soil heath promoting practices have been adopted on less than 14% of the current U.S. land capacity (USDA NASS, 2019). In this scenario, regenerative agriculture (RA) practices, such as no-tillage, cover cropping, crop rotation, and nutrient management, offer multiple benefits to soils by improving soil health (Giller et al., 2021; Newton et al., 2020), sustaining the soil microbial community structure and activity (Lin et al., 2023; Pires et al., 2020), and making agriculture resource-efficient, profitable and resilient (Lal et al., 2011; Reimer et al., 2022; Schreefel et al., 2020).

Regenerative agriculture

Regenerative agriculture is gaining momentum worldwide among farmers, scientists, and policy makers (Tittonell et al., 2022). Currently, RA does not have a comprehensively described scientific definition, and various definitions of RA can be found in the literature (Schreefel et al., 2020). For instance, Malik & Verma (2014) define RA as a technique that uses organic farming methods, whereas Elevitch et al. (2018) define RA as a farming approach that has the ability for self-renewal and resiliency, promote soil health, and support biodiversity while sequestering carbon. In this dissertation, RA is defined as a farming strategy that uses natural processes and minimal external inputs to increase biological activity, enhance soil health, improve nutrient cycling, restore landscape function, and produce food and fiber (Elevitch et al., 2018; Khangura et al., 2023; Newton et al., 2020; Schreefel et al., 2020). The goal of RA is not the restoration of the native, pre-agricultural ecology and its biological functions. Instead, it aims to harness ecological processes inherent in nature and integrate them into an agricultural system, thereby enhancing the overall health of the farming system through reliance on biological interactions and ecosystem services (Gordon et al., 2022; Khangura et al., 2023). To achieve this, researchers have proposed the use of farming practices such as no-tillage, cover cropping, crop rotation, and nutrient management (Giller et al., 2021; Newton et al., 2020)

Soil health

Soil health is commonly defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA–NRCS, 2018). Soil health encompasses scales, stakeholders, functions, and assessment tools relevant to soil quality and fertility and shares some of the policy dimensions of soil security (Lehmann et al., 2020). Soil

health (i.e., soil quality) can be assessed to understand the sustainability of different types of land management, specifically ecosystem services for environmental health and agricultural productivity (Doran & Parkin, 1994; Karlen et al., 1997). There is a broad understanding that soils are not just a support for crops but also underpin other essential ecosystem services, such as climate regulation, nutrient cycling, flood regulation, carbon sequestration, water purification, and habitats for microorganisms (Karlen et al., 1997). This understanding manifests the importance of proper soil management to be sustainable for future generations. Lehmann et al. (2020) state that several other related concepts exist, including soil fertility, quality, and security (Figure 1.1).

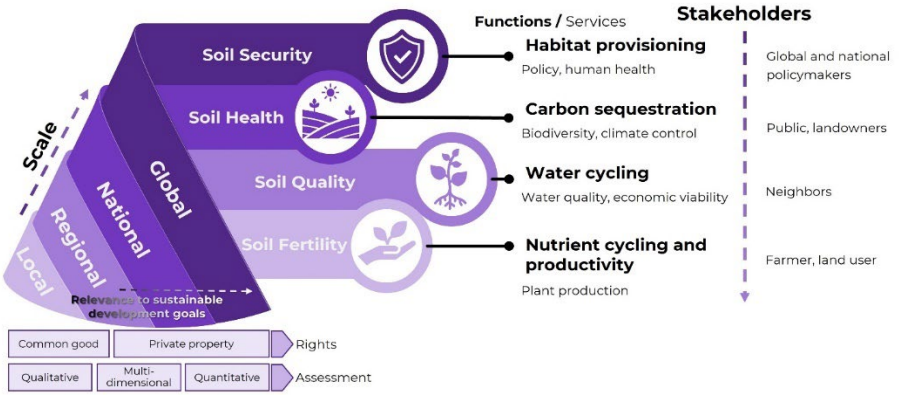


Figure 1. 1. Relevant spatial scales, functions, ecosystem services, and stakeholders of soil security, health, quality, and fertility. Adapted from Lehman et al. (2020).

Farming practices that minimize soil disturbance, maximize soil cover, biodiversity, and continuous living roots, and make the most efficient use of external inputs are practices that follow soil health and conservation agriculture principles (Doran & Zeiss, 2000; Kibblewhite et al., 2008; Pittelkow et al., 2015; USDA–NRCS, 2018) (Figure 1.2). Moreover, considering the context of the geographic location is as important as the other principles since climate patterns,

soil type, landscape, and land-use change across space. Contemplating local markets, supply chains, and agricultural suitability are also essential aspects to contextualize when implementing farming practices that pursue soil health principles (Bowman et al., 2022; da Luz et al., 2019; Krupek et al., 2022; Lehmann et al., 2020).

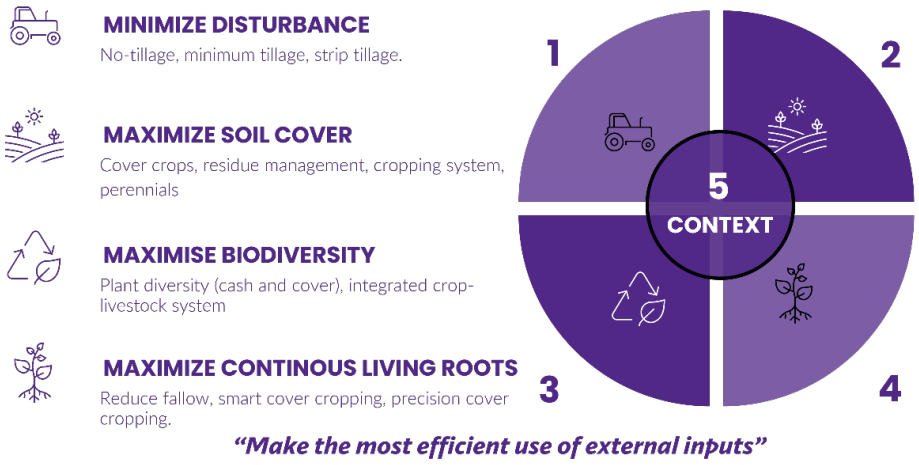


Figure 1. 2. Soil health and conservation agriculture principles.

Soil health assessment and indicators

A soil health assessment determines how well soil performs its functions and how those functions are being preserved for the future (Rinot et al., 2019). While crop yield is one outcome of soil health, it incorporates other soil functions that affect nutrient efficiency, water quality, and climate resilience. Soil health indicators include biological, physical, or chemical properties (Figure 1.3), and for plant production, often include plant-available nutrients, soil organic carbon (SOC), pH, cation exchange capacity (CEC), penetration resistance, potentially mineralizable nitrogen (PMN), aggregation, bulk density, and water storage (Bagnall et al., 2020; Doran & Parkin, 1997; Lehmann et al., 2020; USDA–NRCS, 2015). Overall, soil health indicators are

measurable properties that allow interpretation on a relative scale by monitoring changes over time in relation to standard reference values. Standard values can be soils from native ecosystems or non-degraded soils. However, using reference values from a standard reference soil may not represent the optimal value a managed system can achieve after undergoing management. Soil health indicators should be management-sensitive, time-sensitive, interpretable, and valuable (Stott, 2019).

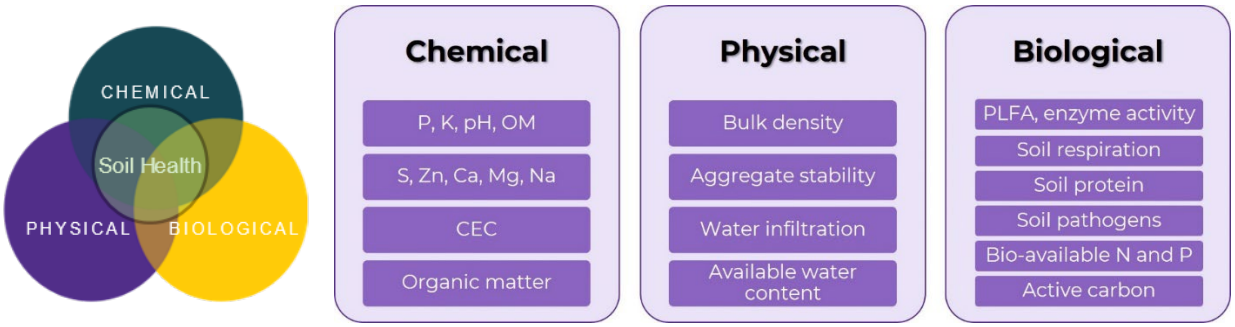


Figure 1. 3. Soil health indicators. *PLFA: phospholipid fatty acid analysis.

Assessing soil biological indicators of soil health is key to understanding the soil's living component (Fierer et al., 2021; Nunes et al., 2020). Soil microbes play crucial roles in the decomposition of soil organic matter, nutrient cycling, soil pollutant degradation, and the formation and stability of soil aggregates (Das et al., 2019; Fabrizzi et al., 2009; White & Rice, 2009). Soil organisms also respond rapidly and are very sensitive to changes in tillage and cropping systems (Pires et al., 2020). Most of the indicators will reflect the quantity and diversity of living organisms, such as fungi, bacteria, and earthworms, as well as their bioproducts (Figure 1.4).

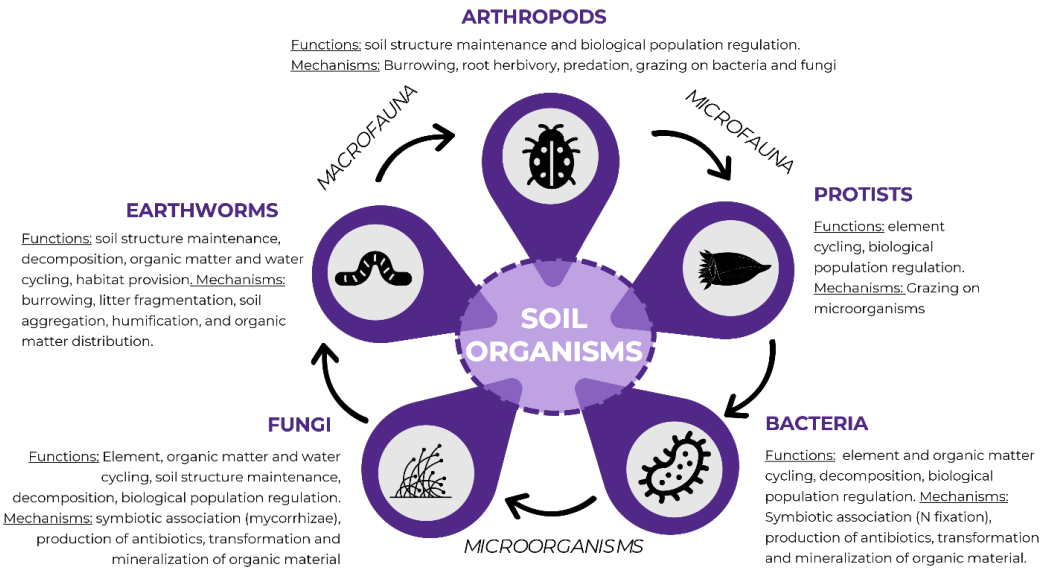


Figure 1. 4. Main soil organism groups and their soil functions and involved mechanisms.

Soil biological indicators can relate to soil properties at 1) Individual, population, and community level; and 2) Ecosystem level. Most individual, population, and community-level indicators are associated with soil organisms' presence, richness or abundance, microbial biomass and ratios, and community composition (Bünemann et al., 2018). At the ecosystem level, the indicators are linked to soil respiration, nitrogen mineralization, denitrification, nitrification, protein synthesis, enzymatic activity, and SOC (Bünemann et al., 2018). Generally, biological indicators that tell us about soil processes mediated by soil organisms are the most revealing about soil functions (Figure 1.5). It is important to state that there are limitations in directly measuring soil organisms as indicators of soil health (Fierer et al., 2021). Thus, biological dynamics properties are often measured as alternatives to processes mediated by soil organisms (Creamer et al., 2022; Ndiaye et al., 2000). Besides the soil microbial community composition measured by phospholipid fatty acids (PLFA), the most frequently used biological

soil health indicators proposed by the Soil Microbial Agroecology Lab at Kansas State University are enzymatic activity, SOC, soil respiration, and PMN).

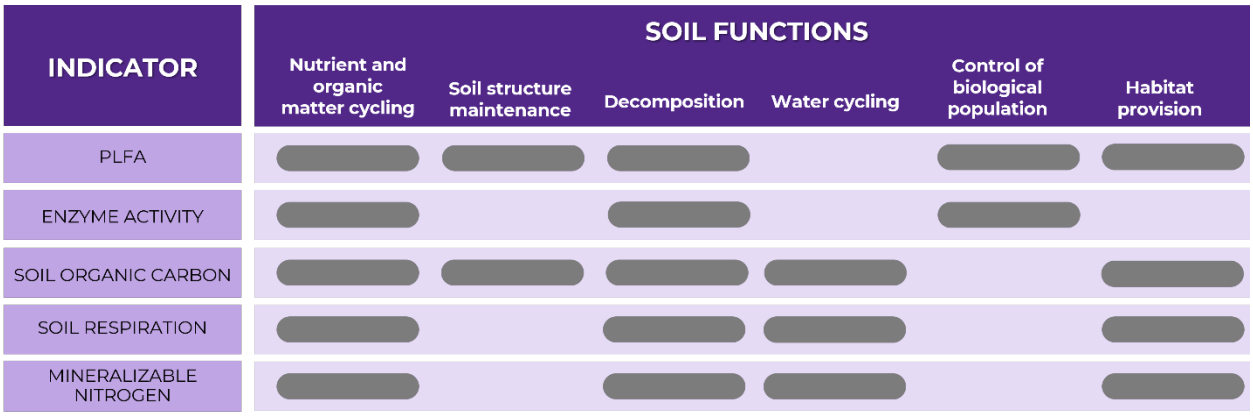


Figure 1. 5. Soil health biological indicators and related soil functions.

Physical soil health indicators are soil properties that can be measured and observed to assess the soil's physical condition. Optimal soil physical conditions reduce limitations to root growth, improve water infiltration and aeration, decrease soil compaction, and increase water storage capacity (Cherubin et al., 2016; Moraes et al., 2016). Optimal conditions and physical indicators are also linked to soil functions (Figure 1.6). Overall, soil with good physical health has a good structure that allows for water and airflow, resists erosion and compaction, permits root growth, and provides a habitat for soil microbes (Cardoso et al., 2006; Cavalcante et al., 2021). Soil structure is the composition of silt, sand, and clay that build soil blocks. The arrangement of these soil particles, as well as the combination of particles, roots, fungi hyphae, and other compounds (e.g., sugars, proteins, etc.) released by microbes, builds soil aggregates, which correspond to the soil structure (Mikha & Rice, 2004; Six et al., 2006; Tisdall & Oades, 1982). Poor soil physical health can reduce crop yield and increase soil erosion and water runoff

(Bonini Da Luz et al., 2020). Minimal disturbance (i.e., no-tillage) and plants with robust root systems are key for building aggregates and improving soil structure (Lin et al., 2023; A. Liu et al., 2005). There are several indicators for evaluating soil physical health. Among the most suggested are aggregate stability, water infiltration rate, bulk density, and penetration resistance (Bünemann et al., 2018; Cherubin et al., 2016; Moraes et al., 2014).

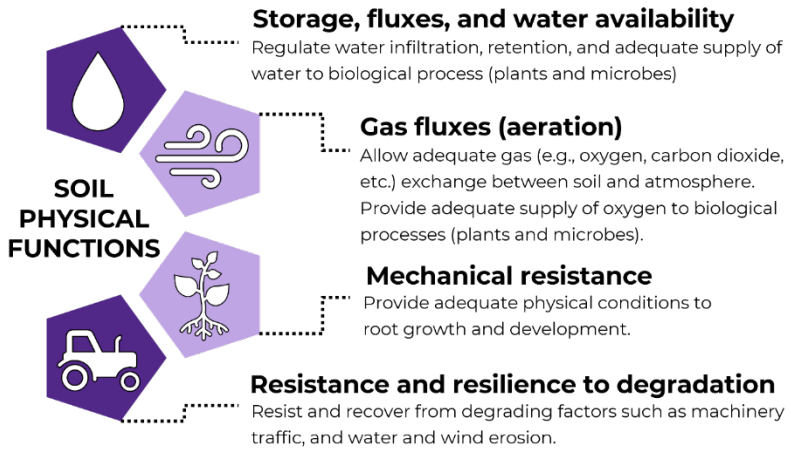


Figure 1. 6. Soil physical functions.

Chemical indicators of soil health are correlated with the capacity of the soil to provide nutrients for plants and retain chemical elements or compounds harmful to the environment and plant growth (Cardoso et al., 2013). The availability and quantity of macro- and micronutrients in agricultural soils can significantly influence the occurrence of deficiencies or toxicities, which, in turn, impact crop productivity (Osman, 2013). Chemical indicators of soil health are related to measurable soil nutrients dependent on soil pH, CEC, and percent base saturation (Schoenholtz et al., 2000). Measured nutrients often include extractable P, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Fe, Zn, Cu, and Mn (Alloway, 2008; Graham, 2008; Idowu et al., 2008; O'Neill et al., 2021).

Cover crops

Cover cropping involves growing a crop between harvest and planting of the main crop (i.e., cash crop) (Chamberlain et al., 2020; Chapagain et al., 2020), and it is expected to reduce soil erosion, protect the soil surface from raindrop energy, improve nutrient cycling, suppress weeds, increase carbon sequestration, and promote microbial growth (Blanco-Canqui, 2022; Hobbs et al., 2008; Kim et al., 2020). In 2017, Kansas led the nation with 48% of farmed acres under no-till (NT) (LaRose & Myers, 2017). However, only 3.2% of the available acres were in winter cover crops (excludes acres in winter wheat) (LaRose & Myers, 2017). Cover crops are an important component of cropping systems and a vital companion practice to NT and crop rotation to improve soil health and increase SOC (Bowman et al., 2022; Pires et al., 2020). Cover crop species distinctly affect the soil due to different root properties, exudates, and functions (Bronick & Lal, 2005). Moreover, changes in soil properties vary among species due to differences in the quality and quantity of biomass (Blanco-Canqui et al., 2011; Koudahe et al., 2022). Grasses (e.g., cereal rye, sorghum sudan, sudangrass, triticale) are expected to have a greater positive effect on decreasing soil erosion and improving aggregation than legumes (e.g., hairy vetch, winter peas, sunn hemp) and brassicas (e.g., radish, turnip) due to their extensive and dense fibrous root system and aboveground biomass production (Blanco-Canqui & Jasa, 2019; Chan & Heenan, 1999; Haynes & Beare, 1997). On the other hand, legumes and brassicas are recommended to decrease soil compaction, balance substrate C: N ratio, and fix atmospheric N that can be used by the following crop (Koudahe et al., 2022; Snapp et al., 2005). Moreover, legumes and brassicas can decompose faster than grasses due to their lower C: N ratio. Ultimately, cover crop selection should be based on the services each species provides (Figure 1.7).

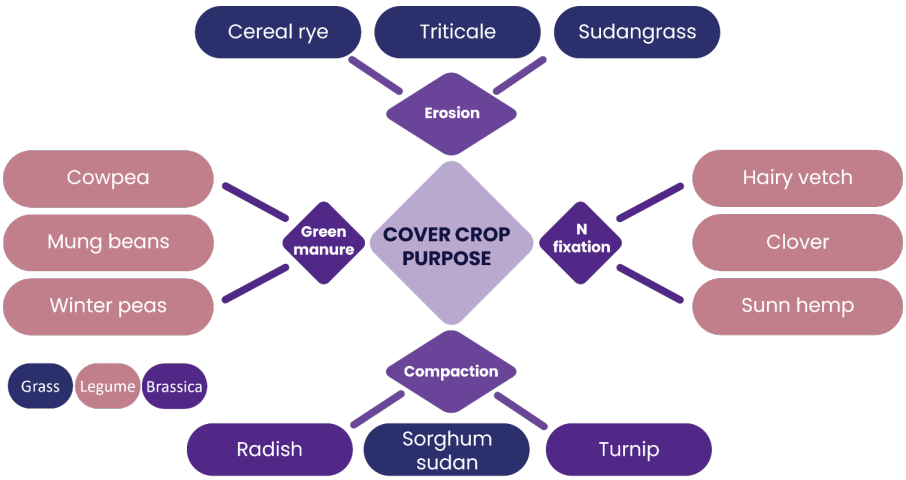


Figure 1. 7. Commonly used cover crop species separated by purpose and type.

Soil microbial communities and enzyme activity

Soils hold a wide variety of biodiversity with complex interactions (Crecchio et al., 2018; Hartmann & Six, 2022; Roy Chowdhury et al., 2019). Their interactions of soil microorganisms with plants can be either beneficial, neutral, or detrimental. Recognizing the dynamics of the plant-soil microbiome interaction is essential in evaluating overall soil and plant health (Turner et al., 2013). Soil microbes are key organic matter decomposers. While soil fungi are an important decomposer of recalcitrant material (e.g., lignin, cellulose), soil bacteria are better adapted to metabolizing easily decomposed organic matter (Liu et al., 2023). Soil microbial diversity and activity are essential in regulating biogeochemical cycles (Acosta-Martínez et al., 2003; Chavarría et al., 2016; Hsiao et al., 2018). Soil bacteria and fungi communities provide essential services to soil agroecosystems, including soil aggregate formation, organic carbon protection, decomposition of recalcitrant components, and nutrient cycling (Das et al., 2019; Glick, 2018). Likewise, it is well known that enzymes catalyze all biochemical reactions and are

essential for cycling nutrients more efficiently (Nannipieri et al., 2017; Tabatabai, 1994). Specifically, β-glucosidase (βG), N-acetyl-β-glucosaminidase (NAG), acid phosphatase (AcidP), and arylsulfatase (ArylS) are hydrolytic enzymes responsible for facilitating C, N, P, and S cycling in the soil, respectively (Figure 1.8) (Bowles et al., 2014; Hsiao et al., 2018).

Soil microbial diversity is a key aspect of healthy soils. Microbial diversity refers to the relative abundance of specific microbial species within a microbial community (Dey et al., 2012). Within terrestrial ecosystems, this diversity contributes to the multifunctionality of the soil, enabling it to simultaneously carry out a variety of ecosystem functions and services (Delgado-baquerizo et al., 2018).

A diverse microbial community comprises both dominant and rare taxa, with the dominant groups primarily driving microbial activity (Escalas et al., 2019; Jia et al., 2018). Despite being less abundant, the rare microbial groups contribute to genetic and functional diversity (Torsvik & Øvreås, 2002). Such microbial diversity enables the soil to adapt to shifting environmental conditions, a critical factor in a changing climate (Bent & Forney, 2008; García-García et al., 2019).

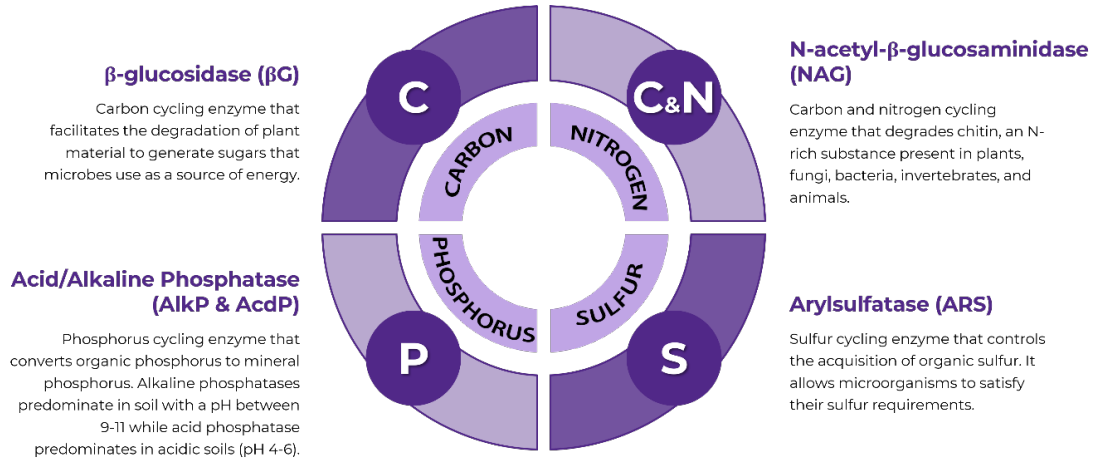


Figure 1. 8. Enzymes and biogeochemical cycles.

On-farm research and the Kansas Soil Health Partnership

On-farm research has emerged in recent years as a unique approach to involve farmers and other agricultural stakeholders as active participants in knowledge development and as an effective method of technology and innovation transfer across farms (Wood et al., 2014). Additionally, farmers worldwide are increasingly emphasizing agricultural practices to enhance sustainability and build resilience in the face of various environmental and production challenges (Jayaraman et al., 2021). On-farm research is broadly defined, according to the Sustainable Agriculture Research and Education (SARE) program, as a study with straightforward experimental design, replications, and statistical analysis conducted as part of a farm using plots large enough to allow the use of farmer's machinery (Chaney, 2017). The core idea of on-farm research is a partnership between researchers and the farmer throughout the project. This process brings agricultural stakeholders together around mutually beneficial experimentation to support farmers' management decisions (Wood et al., 2014). Chaney (2017) and Lacoste et al. (2022) acknowledged that on-farm research is generally supported by three mechanisms that build on the complex and interconnected histories of formal and farmer participatory research: (1) farm fields at meaningful scales (real-scale/field-scale) rather than in small experimental plots that are designed in a small part of a field or externally (e.g., industry or university plots); (2) the interests of farmers and/or surrounding community are explicitly acknowledged to allow for building productive relationships; and (3) on-farm research is understood as a process of partnership between researchers and other stakeholders with farmers that pursue shared goals.

In this context, Bagnall et al. (2020) reported that mentoring and partnerships were critical in getting non-adopters to start a new practice. Thus, there is a need for science-based information on soil health metrics and integrated research, extension, and education efforts to

identify and implement management practices that sustainably increase agricultural productivity and protect against yield losses from environmental stresses while improving soil health. Without such interventions, sustainability of agricultural systems is in jeopardy, and ultimately, this system is likely to stagnate or decline.

In 2018, recognizing the necessity for decentralized, inclusive, diverse, and networked approaches to agricultural research and extension to support farmers in their decision-making processes, Kansas State University, with financial support from the Kansas Corn Commission and in collaboration with the Soil Health Partnership, launched the Kansas Soil Health Partnership (KSHP) and established four soil health research farms across the state. The KSHP's goal was to measure and communicate the environmental benefits of different farming practices. This foundational network laid the groundwork for the development of the Kansas Soil Health Network.

Study Objectives

The overall goal of this research was to explore on-farm sustainable agricultural practices to design farming systems for sustaining soil health management in Kansas. The objective was to quantify the impact of cover crops and nutrient management on biological, chemical, and physical soil health indicators at the farm level. Additionally, farmers' perspectives regarding collaborative on-farm research were assessed. The objectives of the individual chapters were:

Chapter 2: Cover crop-mediated changes in soil microbial community and enzyme activity: implications for sustainable on-farm cropping systems. The objective of this study was to investigate the effect of cover crops on soil microbial community composition, enzyme activity, and soil organic carbon in different cropping systems.

Chapter 3: Amplifying the bond: cover crops strengthening the soil aggregates-microbial community relationship. The objectives of this study were to assess the effect of cover crops on (i) soil aggregation, (ii) fungal and bacterial biomass, and (iii) the relationship between soil microbes, cover crops, and macroaggregates.

Chapter 4: Short-term impact of nitrogen rates on potentially mineralizable nitrogen, soil organic matter, and crop yield after 25 years of on-farm regenerative agriculture. The objectives of this study were to (i) evaluate the effect of N rates on crop yield, and (ii) evaluate the short-term effect of N rates on SOC, total nitrogen (TN), and PMN.

Chapter 5: Perspective of U.S. farmers on collaborative on-farm agronomic research. The objective of this study was to assess and understand the perspective and knowledge of U.S. farmers regarding on-farm research via the implementation of a survey instrument distributed across most of the U.S. Midwest and South-Central regions.

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Chapter 2 - Cover crop-mediated changes in soil microbial community and enzyme activity: implications for sustainable on-farm cropping systems

Abstract

One of the pivotal challenges for ensuring the sustainability and resiliency of agroecosystems is the selection of agricultural practices that can effectively nurture soil health and maintain productivity levels. In this scenario, regenerative agriculture practices, such as cover crops, can offer multiple benefits to soils by improving soil health and sustaining the soil microbial community structure and activity. Three on-farm research were conducted for five years in Kansas, U.S., to investigate the effect of cover crops on the soil microbial community composition, enzyme activity, and soil organic carbon in different cropping systems. We hypothesized that cover crops are an important tool for managing the soil microbial community and enzyme activity while increasing soil organic carbon. Cover crops improved soil health as demonstrated by a notable increase in various biological soil health indicators, including soil microbial biomass, bacteria, fungi, soil organic carbon, and enzyme activity. Importantly, the magnitude of these improvements was strongly influenced by regional precipitation patterns, reinforcing the hypothesis that increasing soil organic carbon levels and stimulating soil microbial growth in water-limited environments is a time-dependent process. Cover crops emerge as indispensable tools to augment aboveground plant biomass production in water-limited regions. Conversely, in non-water-limited environments, such as eastern Kansas, the substantial productivity of cash crops may limit the additional impact of cover crops on total

biomass production. Nevertheless, adopting diverse cropping systems that incorporate cover crops proved to be a compelling and sustainable strategy for improving soil health.

Introduction

Agricultural systems must be resource-efficient, profitable, resilient, and healthy to withstand climate variability, including extreme precipitation events and frequent droughts (Bowles et al., 2020; Karlen & Rice, 2015; Reimer et al., 2022). One of the pivotal challenges for ensuring the sustainability and resiliency of agroecosystems in the face of a changing climate and growing food demand lies in the selection of agricultural practices that can effectively nurture soil health and maintain productivity levels (Foley et al., 2011; Pittelkow et al., 2015). Healthy soils provide ecosystem services such as fresh water, food production, and carbon sequestration (McBratney et al., 2014)

Agricultural production in the U.S. Midwest has frequently relied on external inputs due to degraded soil health and nutrient cycling (Hatfield, 2012; Sanchez et al., 2019). In this scenario, regenerative agriculture (RA) practices, such as no-tillage, cover cropping, and crop rotation, offer multiple benefits to soils by improving soil health (Giller et al., 2021; Newton et al., 2020) and sustaining the soil microbial community structure and activity (Lin et al., 2023; Pires et al., 2020). No-tillage promotes soil and water conservation by reducing soil erosion, increasing water infiltration, and improving soil aggregation by encouraging fungal networks (Blanco-Canqui et al., 2017; Calegari et al., 2008; Fabrizzi et al., 2009; Pires et al., 2020). Cover crops reduce soil erosion, improve nutrient cycling and soil aggregation, suppress weeds, and increase carbon sequestration (Blanco-Canqui, 2022; Jian et al., 2020; Kim et al., 2020)

Regenerative agriculture can enhance microbial diversity and activity, which plays an important role in regulating biogeochemical cycling (Acosta-Martínez et al., 2003; Ashworth et al., 2017; Chavarría et al., 2016; Hsiao et al., 2018; White & Rice, 2009). Soil bacteria and fungi communities provide essential services to soil agroecosystems, including soil aggregate formation, organic carbon protection, decomposition of recalcitrant components, and nutrient cycling (Das et al., 2019; Fabrizzi et al., 2009; Glick, 2018). Likewise, it is well known that enzymes catalyze all biochemical reactions and are essential for cycling nutrients more efficiently (Nannipieri et al., 2017; Tabatabai, 1994). Specifically, β-glucosidase (βG), N-acetyl-β-glucosaminidase (NAG), acid phosphatase (AcidP), and arylsulfatase (ArylS) are hydrolytic enzymes responsible for facilitating C, N, P, and S cycling in the soil, respectively (Bowles et al., 2014; Hsiao et al., 2018). Thus, implementing cropping systems that promote the growth and development of the soil microbiome and biological activity may be key for agricultural resiliency and sustainability.

Although there is evidence of the benefits of crop rotation and diverse cropping systems to the overall soil health (Chamberlain et al., 2020; Lin et al., 2023; Q. Liu et al., 2023; Simão et al., 2023), there is still a lack of understanding on how cover crops (CCs) interplay with the soil microbial community and enzyme activity in different cropping systems. Both crop rotation and CCs increase cropping system plant biodiversity on a temporal basis, which is expected to positively interact with the soil microbiome (Kim et al., 2020). However, it is unclear whether the benefits primarily come from the diverse plant biomass produced by rotating cash crops or if CCs play a significant role in modifying the soil microbial community and its biological activity. In water-limited environments such as the U.S. Central Great Plains (CGP), the role of CCs in dryland cropping systems becomes even more uncertain due to the potential negative impacts of

CCs on subsequent cash crops grain yield and biomass production (Rosa et al., 2021). Additionally, due to water limitations, time constraints, and a short planting window, CCs may represent only a small percentage of the total plant biomass produced during a growing season. Consequently, a pressing question from producers to scientists nowadays is whether investing in planting CCs to manage the soil microbial community is worthwhile given these challenges.

Therefore, it is essential to understand these relationships thoroughly to assist producers in making informed decisions about land management on their farms (Chamberlain et al., 2020). To address these issues, this study investigated the effect of CCs on the soil microbial community composition, enzyme activity, and soil organic carbon in different cropping systems. We hypothesized that CCs are an important tool for managing the soil microbial community and enzyme activity while increasing soil organic carbon (SOC).

Materials and Methods

Study site and field characteristics

On-farm research studies were conducted in three farm fields in Kansas, U.S., as part of the Kansas Soil Health Partnership. Research was conducted in the Bucyrus site from 2018 to 2022, whereas the Solomon and Beloit sites were between 2019 and 2023. The climate for all three sites was classified as Hot-summer humid continental (Dfa) according to (Peel et al., 2007). Soil classification (Soil Survey Staff, 2014), coordinates, elevation, average annual precipitation, and average mean annual temperature for all three sites are presented in Table 2.1. Soil physicochemical characterization by treatment, depth, and year is presented in Tables S2.1 (Bucyrus), S2.2 (Solomon), and S2.3 (Beloit). For all sites, the rainfall was concentrated from March to November, with higher intensity between May and July (Figure S2.1; S2.2; S2.3).

Table 2.1. Site name, location, elevation, soil classification, average annual precipitation, and average mean annual temperature.

Site	Location	Elevation	Soil taxonomy	Average annual precipitation	Average annual temperature
Bucyrus	38.7395N -94.7068W	341 m	Fine, smectitic, mesic Aquertic Argiudoll	998 mm	13°C
Solomon	38.8406N -97.3928W	381 m	Fine, mixed, superactive, mesic Pachic Argiustoll	848 mm	13°C
Beloit	39.3226N -981545W	460 m	Fine, smectitic, mesic Typic Argiustoll	700 mm	12°C

Experimental design and treatment descriptions

The experiments were established as a randomized complete block design, with two treatments [cover crop (CC) and no cover crop (NC)] and four replications (strips) (Figure S2.4; S2.5; S2.6). The strip size matched the field size and farming equipment. At Bucyrus, the field size was 21.6 ha, with a strip size of 2.7 ha and a sampling grid of 0.45 ha. At Solomon, the field size was 13.6 ha, with a strip size of 1.7 ha and a sampling grid of 0.43 ha. At Beloit, the field had 8.8 ha, with a strip size of 1.1 ha and a sampling grid of 0.37 ha. All fields were under no-tillage management.

Throughout the five years of study, the cropping system at Bucyrus (Figure S2.7) was corn (2018), cereal rye as CC (2018/2019), soybean (2019), oat, cereal rye, radish, and hairy vetch as a mix of CCs (2019/2020), corn (2020), cereal rye as CC (2020/2021), soybean (2021), cereal rye, rapeseed, winter pea, and hairy vetch as a mix of CCs (2021/2022), corn (2022), cereal rye as CC (2022/2023). Additionally, poultry litter was applied across the entire field at a rate of 600 kg ha^{-1}. At Solomon (Figure S2.8), the cropping system was winter wheat (2019/2020), pearl millet, sudangrass, sunflower, radish, turnip, and flax as a mix of CCs (2020),

triticale as CC (2020/2021), soybean (2021), winter wheat (2021/2022), pearl millet, sudangrass, sunflower, radish, turnip, and flax as a mix of CCs (2022) hairy vetch (2022/2023), and grain sorghum (2023). At Beloit (Figure S2.9), the cropping system was winter wheat (2018/2019), winter pea, oat, barley, and radish as CC (2019), grain sorghum (2020), triticale as CC (2020/2021), soybean (2021), winter wheat (2021/2022), winter pea, oat, barley, and radish as CC (2022), and grain sorghum (2023). The distinguishing factor among all the sites treatments was the inclusion or absence of CCs. All implemented crops can be defined as corn (*Zea mays)*, cereal rye (*Secale cereale*), soybean (*Glycine max*), oat (*Avena sativa*), radish (*Raphanus sativus*), hairy vetch (*Vicia sativa*), rapeseed (*Brassica napus*), winter pea (*Pisum sativum*), pearl millet (*Pennisetum glaucum*), sudangrass (*Sorghum bicolor × S. sudanense), sunflower (Helianthus annus), turnip (Brassica rapa), flex (Linum usitatissimum), barley (Hordeum vulgare), grain sorghum (Sorghum bicolor), triticale (×Triticosecale Wittmack), and winter wheat (Triticum aestivum).*

Soil and biomass sampling

Soil samples were taken at 0-5, 5-15, and 15-30 cm soil depth in a GPS coordinate sampling grid at the first, third, and fifth year at each site. At Bucyrus, soil sampling was done during the spring after CCs termination and before planting the cash crop. At Solomon, soil sampling was conducted in the fall prior to planting winter wheat in 2019, and in the spring after CCs termination and before planting soybeans and sorghum in 2021 and 2023, respectively. At Beloit, 2019 soil sampling was conducted in the spring when winter wheat was at Feekes 4, and in 2021 and 2023 during the spring following the termination of the CC and preceding the planting of the cash crop. The first-year sampling was considered as the baseline. Soil samples

for microbial properties were kept in a cooler (4°C) and frozen (-20°C) within 2 hrs after sampling and stored until analysis. Samples for physical and chemical analysis were cleaned of roots, air-dried, ground, and passed through a 2-mm sieve.

Cover crop aboveground biomass was collected after CC termination at all sites. One 0.98 m^2 aboveground biomass sample was collected at each GPS point. Samples were dried in a forced-air oven at 50°C for a minimum of 7 days and weighed when constant weight was achieved. Cash crop aboveground biomass was estimated using a harvest index (Prince et al., 2001; Schapaugh & Wilcox, 1980; Sharma & Smith, 1987) of 0.54 for corn (Johnson et al., 2006; Ruiz et al., 2023; West et al., 2010), 0.46 for winter wheat (Johnson et al., 2006; Prince et al., 2001; West et al., 2010), 0.45 for grain sorghum (Johnson et al., 2006; Prince et al., 2001; West et al., 2010), and 0.43 for soybean (Johnson et al., 2006; Prince et al., 2001; West et al., 2010).

Soil physical and chemical properties analysis

The sand, silt, and clay fraction for each site was determined by the hydrometer method using a solution of 50 g/L of sodium hexametaphosphate as a dispersing solution (Gavlak et al., 2005). Soil chemical properties were analyzed using the standard Mehlich-3 method (NCR221, 2015) for extractable P and the standard ammonium acetate method for extractable K, Ca, Mg, and Na (NCR221, 2015) at the Soil Testing Lab at Kansas State University, Manhattan, KS. Soil pH was determined with a 1:10 soil: water slurry method. Soil organic carbon (SOC) and total nitrogen (TN) were determined by dry combustion using a LECO® CN 828 analyzer (Leco Corp; USA).

Soil microbiological properties analysis

Phospholipid fatty acid (PLFA) analysis was performed with modifications to the original procedure (D. C. White & Ringelberg, 1998) to assess the soil microbial community composition. Total lipids were extracted with 10 mL of methanol, 5 mL of chloroform, and 4 mL of phosphate buffer (pH 7.4) on 5 g freeze-dried soil. Water and chloroform were added after 3 hours to separate the mixture into polar and non-polar fractions. Total lipids remained in the non-polar phase. Phospholipids were isolated from neutral lipids and glycolipids by using silicic acid chromatography columns (Disposable BAKERBOND® SPE Columns, J.T. Baker®, Avantor, Radnor, Pennsylvania, USA) and eluted with methanol. The phospholipids were then saponified with KOH, methylated to fatty acid methyl esters (FAME) and analyzed by Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Helium was used as carrier gas. FAME peaks were recognized by different retention times in comparison with the bacterial acid methyl esters mix (BAME; Matreya 1,114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). Internal standard 19:0 FAME was used to determine concentrations. A total of 30 biomarkers were identified for all samples. Microbial groups were assigned based on characteristics of the biomarkers: iso and ante-iso branches often belong to Gram-positive bacteria (Gram+); monosaturated and cyclopropyl lipids often belong to Gram-negative bacteria (Gram-); actinobacteria (Actino) have more methyl-branched fatty acids; and methyl linoleate typically belong to fungi, as describe by Pires et al. (2020). Phospholipid fatty acid abundance was reported as nmol PLFA g^{-1} soil. Microbial biomass (TMB) was estimated as the sum of all PLFA biomarkers.

The potential activity of four extracellular and hydrolytic soil enzymes indicative of C-cycling (β-glucosidase, βG, EC 3.2.1.21), C/N-cycling (*N*-acetyl-β-glucosaminidase, NAG, EC

3.2.1), P-cycling (acid phosphatase, AcidP, EC 3.1.3.2), and S-cycling (arylsulfatase, ArylS, EC 3.1.6.1) was evaluated. These enzyme activities were assayed using 0.5g of air-dried soil with the appropriate p-nitrophenyl substrate and incubated for 1 hour at 37°C at the optimal pH as described by Tabatabai (1994). Following incubation, 0.5 mL of 0.5 M $CaCl_2$ and 2 mL of stop buffer were added to the samples. Enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation and subtracted from the sample value. Samples were filtered using Whatman 2V 9.0-cm diameter filter paper and absorbance measured using a Genesys 20 Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at a wavelength of 400 nm.

Statistical analysis

The R Programming software (R Core Team, 2023) was used for all the analyses and figure production. Prior to statistical analysis, the dataset was spatially transformed to a sf object using x (longitude) and y (latitude) coordinates from the GPS sampling points (Pebesma & Bivand, 2023). The effect of treatment (CCs) and year, and their respective interactions (fixed effects) on the response variables was analyzed by ANOVA after fitting a linear mixed effects model using the nlme R package (Pinheiro et al., 2017) for each depth. Blocks were set as random effects. The normality of the residuals was tested using the Shapiro–Wilk test, and square-root transformation was applied when necessary. Models were tested with exponential, spherical, gaussian, and rational spatial correlation structures without nugget effect using a maximized log-likelihood method (Pinheiro et al., 2017). Model selection for correlation structure was performed following the Akaike information criterion (AIC). Model means were extracted using the emmeans R package (Lenth, 2023) and a pairwise comparison was used to

determine significant differences among treatments for all the fixed effects presenting a significance equal to or lower than 0.05 using Fisher's LSD (multcomp R package) (Hothorn et al., 2008).

Distance-based redundancy analysis (dbRDA) (Legendre & Andersson, 1999) was used to explore linear relationships between key chemical properties and axes of the ordination of sites based upon Bray-Curtis dissimilarities using the function capscale of the *vegan* R package (Oksanen et al., 2017). Their significance was tested by a Monte Carlos permutation test using 999 permutations. Prior to analysis, microbiological data were log-transformed. Likewise, the analysis of variance, dbRDA was performed separately for each site at 0-5 and 0-30 cm. The direction and magnitude of the relationship between chemical and microbiological variables were presented visually in the dbRDA triplot (sites as points and soil microbiological and chemical variables as vectors).

Results

Soil microbial community composition

The soil microbial community composition was assessed by the abundance of PLFA biomarkers according to treatment and year at each depth and site location. All other microbial groups at Bucyrus, excluding saprophytic fungi, had a significant two-way interaction for treatment and year at 0-5 cm (Table S2.4). Soil microbial biomass was significantly higher in the CC treatment in 2022 than in all other treatment and year combinations, showing an increase of 5x compared to the 2018 CC treatment (Figure 2.1A). Likewise, NC 2022 had a microbial biomass 4x higher than NC 2018.

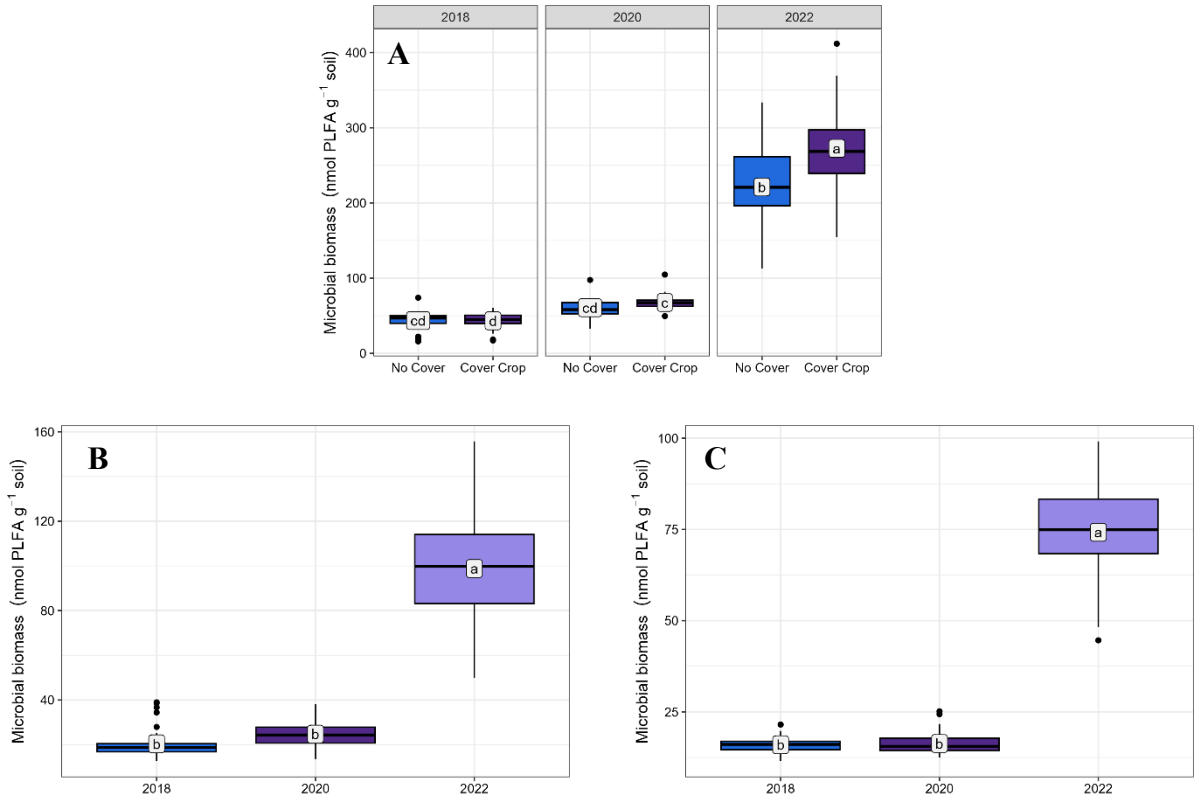


Figure 2.1. Microbial biomass by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

The same trend was observed for Gram+, Gram -, actinomycetes, and AMF (Table 2.2). Saprophytic fungi were significant for year only (Table S2.4), with an increase of 3.1 nmol PLFA g^{-1} soil from 2018 to 2022 (Table 2.2). At 5-15 cm, all microbial groups were significantly affected by year, with 2022 having the highest values, excluding AMF, which had higher biomass in 2020 (Figure 2.1B; Table 2.2). At 15-30 cm, microbial biomass, Gram-, actinomycetes, and fungi were significant for year (Table S2.4). Excluding saprophytic fungi, which had higher biomass in 2020 (same as 5-15 cm), microbial biomass (Figure 2.1C), Gram-, and actinomycetes (Table 2.2) had higher biomass in 2022. This increase was 3.6x for microbial

biomass, 4.4x for Gram-, and 2.6x for actinomycetes. Gram+ and AMF had a two-way interaction between treatment and year (Table S2.4). Cover crop 2022 had the highest Gram+ biomass, while NC and CC 2020 had the highest AMF biomass (Table 2.2).

Table 2. 2. Changes in soil microbial community (nmol PLFA g^{-1} soil) by treatment, year, and depth in Bucyrus, KS.

Treatment	Year	Gram+	Gram-	Actino	AMF	Fungi
0-5 cm						
No cover	2018	12.0 cd	7.0 c	2.3 d	2.7 c	0.6 b
Cover crop		11.8 d	7.3 c	2.6 cd	2.6 c	
No cover	2020	16.5 cd	9.2 c	3.6 c	7.7 b	0.8 b
Cover crop		17.0 c	11.0 c	3.5 cd	8.6 b	
No cover	2022	58.4 b	40.0 b	13.2 b	8.5 b	3.7 a
Cover crop		71.1 a	51.4 a	16.0 a	12.9 a	
5-15 cm						
No cover	2018	7.0 b	2.5 b	1.3 b	0.7 c	0.3 b
Cover crop						
No cover	2020	8.5 b	3.2 b	1.3 b	3.3 a	0.2 b
Cover crop						
No cover	2022	22.7 a	13.2 a	6.0 a	2.2 b	0.5 a
Cover crop						
15-30 cm						
No cover	2018	6.9 c	1.4 b	1.0 b	0.4 d	0.1 b
Cover crop		6.7 c			0.4 d	
No cover	2020	6.8 c	1.6 b	1.2 b	1.8 a	0.1 b
Cover crop		6.6 c			1.8 a	
No cover	2022	13.6 b	7.6 a	3.6 a	0.8 c	0.2 a
Cover crop		15.2 a			1.0 b	

Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Gram-positive bacteria (Gram+), gram-negative bacteria (Gram+), actinomycetes (Actino), arbuscular mycorrhizal fungi (AMF), and saprophytic fungi (Fungi).

At Solomon, a significant two-way interaction between treatment and year occurred for all microbial groups at 0-5 cm (Table S2.6). Microbial biomass (Figure 2.2A), Gram+, Gram-, Actino, and AMF (Table 2.3) were significantly higher for CC 2023 than all other treatment and

year combinations (Table 2.3). Microbial biomass for CC 2023 was 1x higher than CC 2019 (baseline).

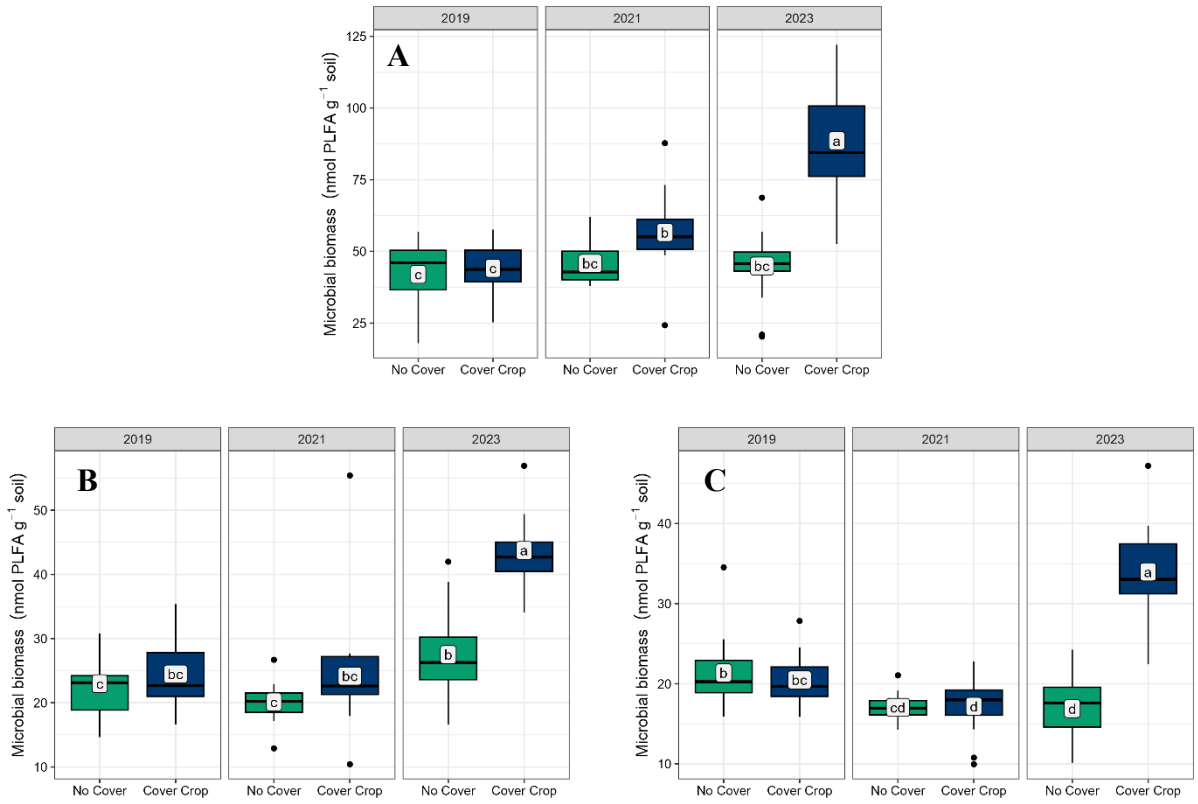


Figure 2. 2. Microbial biomass by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Similar increments were observed for Gram+, Gram-, actinomycetes, and AMF. For saprophytic fungi, CC 2023 was not different from CC 2021 and 2019. However, saprophytic fungi were higher for CC 2023 (1.7 nmol PLFA g^{-1} soil) compared to NC 2023 (0.6 nmol PLFA g^{-1} soil) (Table 2.3). Similar trends were observed at 5-15 cm, where microbial biomass (Figure 2.2B), Gram+, and Gram- were significantly higher for CC 2023 than all other treatment and

year combinations (Table 2.3). For AMF, there was no significant difference between CC 2023, CC 2019, and NC 2019. Year significantly affected actinomycetes, while year and treatment, separately, affected saprophytic fungi (Table S2.7). Actinomycete biomass decreased by 50% from 2019 to 2023, while saprophytic fungi decreased by 54%. On the other hand, CC increased saprophytic fungi by 100% (Table 2.3). At 15-30 cm, there was a two-way interaction between year and treatment for microbial biomass, Gram+, Gram-, actinomycetes, and AMF (Table S2.7). Microbial biomass decreased by 21% from 2019 to 2023 with NCs (Figure 2.2C). In contrast, there was an increase of 66% in microbial biomass during the same period for CC. Gram-positive bacteria decreased by 47% from 2019 to 2023, whereas there was no significant difference between 2019 and 2023 for the CC treatment. Gram-negative population significantly increased for both CC and NC treatments, with a significantly higher Gram- population under the CC treatment in 2023 (Table 2.3). Arbuscular mycorrhizal fungi slightly decreased from 2019 to 2023 for the CC treatment. However, AMF significantly reduced (64%) for the NC treatment. Similarly to the 5-15 cm depth, saprophytic fungi experienced a one-way effect from both year and treatment. Saprophytic fungi were increased by CC and decreased by year (Table 2.3).

Table 2. 3. Changes in soil microbial community (nmol PLFA g^{-1} soil) by treatment, year, and depth in Solomon, KS.

Treatment	Year	Gram+	Gram-	Actino	AMF	Fungi
			0-5 cm			
No cover	2018	13.0 bc	4.3 b	1.3 abc	6.6 cd	1.2 b
Cover crop		12.9 c	5.4 b	1.2 bc	6.7 cd	1.3 b
No cover	2020	12.1 bc	4.6 b	1.4 ab	7.2 bc	1.3 b
Cover crop		15.5 b	6.5 b	1.7 ab	8.8 b	2.0 a
No cover	2022	13.1 bc	5.9 b	0.8 c	5.3 d	0.6 c
Cover crop		25.8 a	11.8 a	1.8 a	11.3 a	1.7 ab
			5-15 cm			

No cover	2018	8.7 b	2.2 c	1.0 a	3.3 ab	0.2 B*	0.42 a
Cover crop		9.1 b	2.3 c		3.6 a		
No cover	2020	8.0 bc	2.0 c	0.8 a	2.7 c		0.35 a
Cover crop		8.6 bc	2.4 c		3.3 bc		
No cover	2022	7.1 c	4.1 b	0.5 b	1.8 d	0.4 A**	0.19 b
Cover crop		11.9 a	6.9 a		3.5 ab		
			15-30 cm				
No cover	2018	6.9 c	1.4 b	1.0 b	0.4 d	0.2 B*	0.4 a
Cover crop		6.7 c			0.4 d		
No cover	2020	6.8 c	1.6 b	1.2 b	1.8 a		0.2 b
Cover crop		6.6 c			1.8 a		
No cover	2022	13.6 b	7.6 a	3.6 a	0.8 c	0.3 A**	0.1 c
Cover crop		15.2 a			1.0 b		

*No cover, **Cover crop. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Gram-positive bacteria (Gram+), gram-negative bacteria (Gram+), actinomycetes (Actino), arbuscular mycorrhizal fungi (AMF), and saprophytic fungi (Fungi).

At Beloit, there was a two-way interaction between year and treatment for microbial biomass, Gram-, and AMF (Table S2.10). Microbial biomass (81.3 nmol PLFA g^{-1} soil) and Gram- (26.3 nmol PLFA g^{-1} soil) were higher for CC 2023 than any other treatment and year combination (Figure 2.3; Table 2.4), with a difference of 51% from CC 2019 (baseline) to CC 2023. Arbuscular mycorrhizal fungi were higher for CC 2023 than CC 2019 and NC 2023 but not different from CC and NC 2021. Actinomycete and Gram+ were significant for year, increasing from 2019 to 2023 (Table S2.10; 2.4).

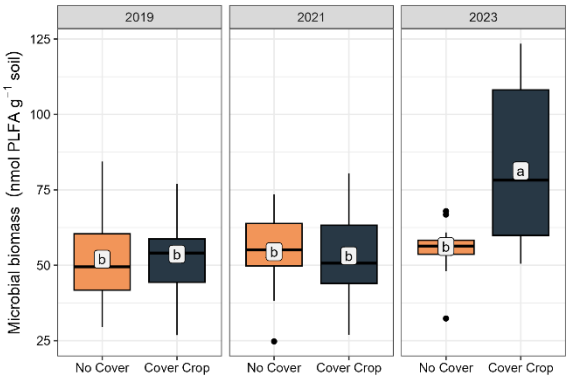


Figure 3. Microbial biomass by treatment and year at 0-5 cm at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

For 5-15 cm, there were no statistical differences for microbial biomass and Gram- (Table S2.10). Still, there was a two-way interaction between treatment and year for Gram+, AMF, and saprophytic fungi (Table S2.10). In 2023, the CC treatment presented a 30% increase in Gram+ compared to the CC treatment in 2019. However, this difference was not statistically significant when compared to the other treatments, except for NC 2021 (Table 2.4). Arbuscular mycorrhizal fungi increased by 25% for CC 2023 compared to CC 2019. Although CC 2021 had a higher AMF than CC 2023, it was not statistically different. Moreover, AMF significantly decreased by 26% from NC 2019 to NC 2023 (Table 2.4). There were no differences between CC 2019, NC 2019, and CC 2021 for saprophytic fungi. The lowest quantities of saprophytic fungi were found in NC 2021 and 2023 (Table 2.4). In contrast to 0-5 cm, actinomycetes significantly decreased (25%) from 2019 to 2023 (Table 2.4). Only actinomycetes (one-way) and AMF (two-way) had statistical differences at 15-30 cm (Table S2.10). Actinomycetes decreased by 75% from 2019 to 2023 (Table 2.4). Arbuscular mycorrhizal fungi increased from 2019 to 2021 with CC. However, there was a subsequent decrease in 2023 for the same treatment (Table 2.4).

Table 2. 4. Changes in soil microbial community (nmol PLFA g^{-1} soil) by treatment, year, and depth in Beloit, KS.

Treatment	Year	Gram+	Gram-	Actino	AMF	Fungi
0-5 cm						
No cover	2018	16.28 c	13.5 b	0.9 a	2.7 b	1.6 b
Cover crop			16.3 b		2.6 b	
No cover	2020	19.26 b	12.8 b	0.4 b	3.1 ab	2.0 ab
Cover crop			12.3 b		3.3 ab	
No cover	2022	22.4 a	14.0 b	0.5 b	2.6 b	2.1 a
Cover crop			26.3 a		3.9 a	
5-15 cm						
No cover	2018	9.5 ab	6.3 ns	0.47 a	0.70 bc	0.4 ab
Cover crop		8.1 b	7.0 ns		0.68 cd	0.4 ab
No cover	2020	7.7 b	10.5 ns	0.28 b	0.60 d	0.2 c
Cover crop		10.1 ab	10.1 ns		0.96 a	0.5 a
No cover	2022	9.67 ab	12.0 ns	0.35 b	0.58 d	0.3 bc
Cover crop		10.5 a	12.5 ns		0.85 ab	0.5 ab
15-30 cm						
No cover	2018	6.0 ns	3.0 ns	0.4 a	0.36 ab	0.2 ns
Cover crop		4.6 ns	2.3 ns		0.28 b	0.2 ns
No cover	2020	5.4 ns	8.3 ns	0.2 b	0.35 b	0.1 ns
Cover crop		6.2 ns	5.8 ns		0.49 a	0.2 ns
No cover	2022	5.8 ns	6.5 ns	0.1 b	0.33 b	0.1 ns
Cover crop		5.8 ns	5.3 ns		0.39 ab	0.3 ns

Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Gram-positive bacteria (Gram+), gram-negative bacteria (Gram+), actinomycetes (Actino), arbuscular mycorrhizal fungi (AMF), and saprophytic fungi (Fungi).

Extracellular enzyme activity

At Bucyrus, all tested enzymes had a significant two-way interaction for treatment and year at 0-5 cm (Table S2.5). For the 0-5 cm soil depth, βG activity increased from 360 µg PNP $soil^{-1}$ h^{-1} (2018) to 568 µg PNP $soil^{-1}$ h^{-1} (2022) with CCs, representing an increase of 58% (Figure 2.4A). Acid phosphatase activity also increased with CCs (Table 2.5). For the CC treatment, there was a difference of 24% between 2018 and 2022. On the other hand, NC

decreased AcidP activity from 2018 to 2022 by 17% (Table 2.5). *N*-acetyl-β-glucosaminidase activity had a similar trend, with a temporal increase of 39% from 2018 to 2020 with CCs and no significant changes with NC (Table 2.5). For ArylS, there was a significant increase from 2018 to 2020 and a decrease from 2020 to 2022. Nonetheless, ArylS activity was 30% higher for the CC treatment in 2022 than NC (Table 2.5).

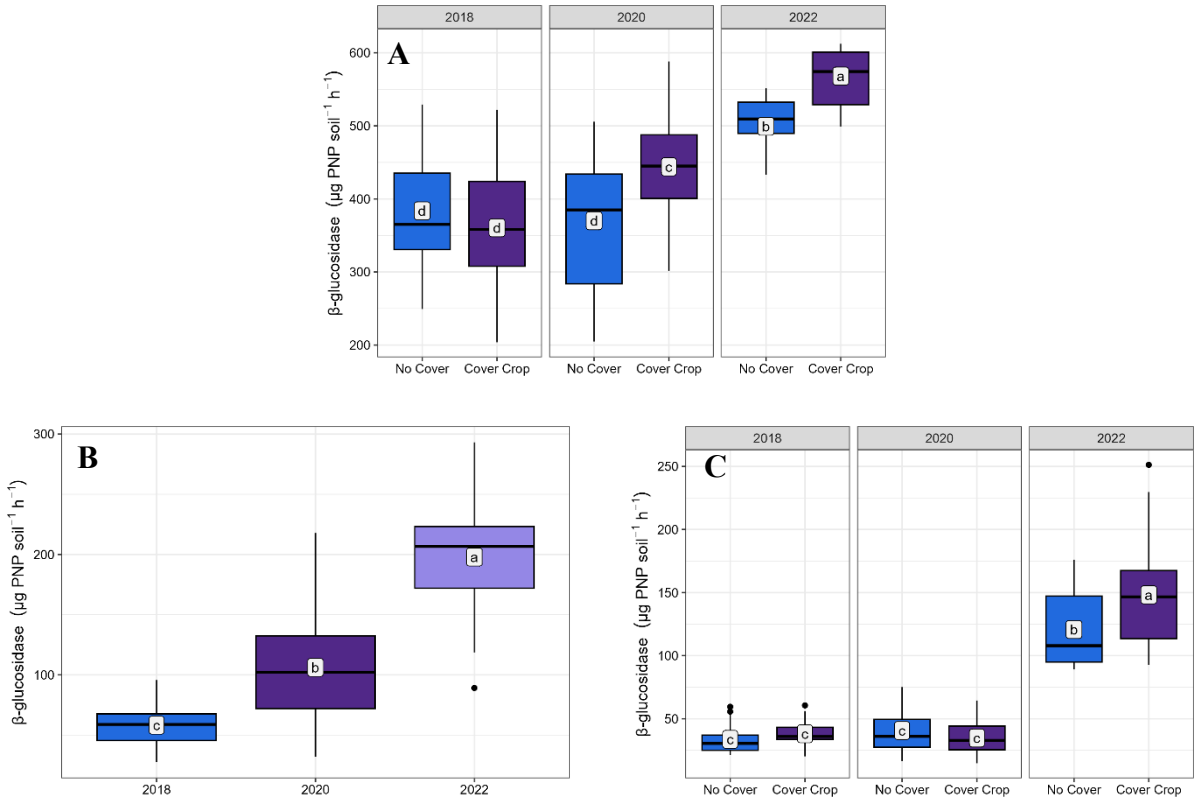


Figure 2. 3. β-glucosidase activity by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Table 2. 5. Changes in enzyme activity (µg PNP $soil^{-1}$ h^{-1}) by treatment, year, and depth in Bucyrus, KS.

Treatment	Year	AcidP	NAG	ArylS
		0-5 cm		
No cover	2018	585 ab	59.4 b	213 cd
Cover crop		521 bc	58.2 b	199 de
No cover	2020	594 ab	60.5 b	221 bc
Cover crop		628 a	81.2 a	256 a
No cover	2022	484 c	60.0 b	181 e
Cover crop		649 a	77.7 a	235 b
		5-15 cm		
No cover	2018	285 b	24.7 b	54.7 c
Cover crop		259 bc		52.7 c
No cover	2020	295 ab	28.7 a	83.9 b
Cover crop		334 a		105 a
No cover	2022	246 c	29.2 a	73.1 b
Cover crop		282 bc		85.2 b
		15-30 cm		
No cover	2018	302 a	43.8 a	63.2 a
Cover crop		266 b		
No cover	2020	249 bc	28.0 b	66.0 a
Cover crop		255 bc		
No cover	2022	187 d	25.5 b	56.0 b
Cover crop		232 c		

Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS).

For 5-15 cm, there was a two-way interaction between treatment and year for AcidP and ArylS, and a one-way effect of year for βG and NAG (Table S2.5). For AcidP and ArylS we observed the same trend as 0-5 cm, with an increase from 2018 to 2020 and a decrease from 2020 to 2022 for the CC treatment. However, CC and NC 2023 were not statistically different. Both βG (240%) and NAG (18%) activity increased from 2018 to 2022 (Figure 2.4B; Table 2.5). At 15-30 cm, there was a two-way interaction between treatment and year for βG and AcidP, and a one-way effect of year on NAG and ArylS (Table S2.5). Independent of treatment, βG

significantly increased from 2019 to 2023 (Figure 2.4C). However, greater βG was found for CC (148 µg PNP soil^{-1} h^{-1}) than NC (121 µg PNP soil^{-1} h^{-1}) in 2023. Contrastingly, AcidP activity continuously decreased with time for both treatments (Table 2.5). A greater decrease was found for NC (38%) than for CC (13%). *N*-acetyl-β-glucosaminidase (42%) and ArylS (11%) significantly decreased with time (Table 2.5).

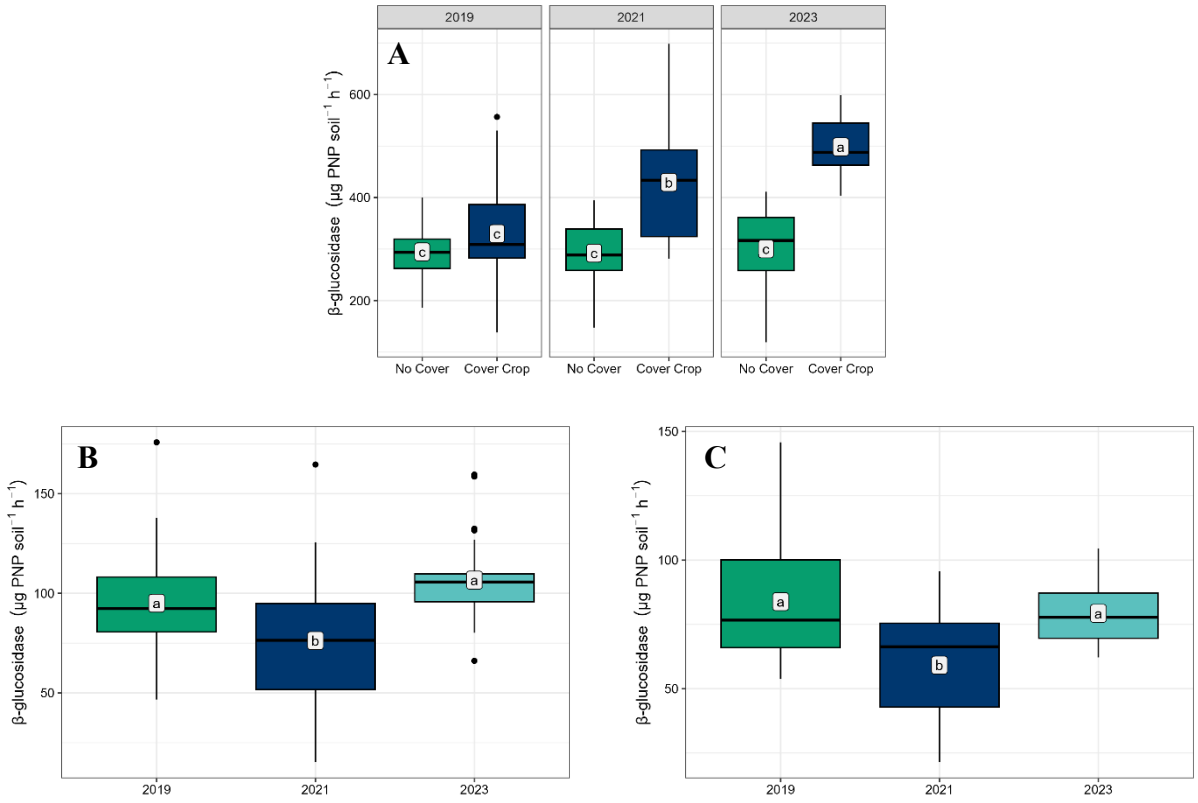


Figure 2. 4. β-glucosidase activity by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

At Solomon, there was a two-way interaction between treatment and year for all tested enzymes at 0-5 cm (Table S2.8). β-glucosidase activity temporally (2019-2023) increased by

51% with CCs (Figure 2.5A). A similar increase was observed for AcidP activity (20%) (Table 2.6). While there was no decrease in βG activity with NC, AcidP activity decreased by 21%. A comparable result was found for NAG, which decreased by 19% with NC (Table 2.6). Arylsulfatase significantly increased with CCs from 2019-2023 (Table 2.6). At 5-15 cm, there was a one-way effect of year for βG and AcidP, and year and treatment for NAG (Table S2.8). β-glucosidase activity increased from 2019 to 2021 and decreased from 2021 to 2023, with no statistical difference between the first and fifth years (Figure 2.5B). Acid phosphatase and NAG activity decreased by 17% and 20% with time, respectively (Table 2.6). Nonetheless, CCs significantly increased NAG by 14%. At 15-30 cm, a one-way effect of year was found for βG, AcidP, and NAG (Table S2.8), where the activity of all three enzymes decreased with time by 5%, 16%, and 43%, respectively (Figure 2.5C; Table 2.6).

Table 2. 6. Changes in enzyme activity (μg PNP $soil^{-1}$ h^{-1}) by treatment, year, and depth in Solomon, KS.

Treatment	Year	AcidP	NAG	ArylS
		0-5 cm		
No cover	2018	1135 ab	102 a	55.9 c
Cover crop		1112 b	120 a	58.3 bc
No cover	2020	977 bc	75.5 b	69.2 b
Cover crop		1309 a	119 a	110 a
No cover	2022	896 c	82.3 b	64.0 bc
Cover crop		1337 a	109 a	101 a
		5-15 cm		
No cover	2018	591 a	28.5 B*	33.7 a	43.4 *ns*
Cover crop					48.1 *ns*
No cover	2020	501 b		31.2 a	38.0 *ns*
Cover crop					47.4 *ns*
No cover	2022	493 b	32.6 A**	26.8 b	42.1 *ns*
Cover crop					49.0 *ns*
		15-30 cm		

No cover	2018	453 a	37.4 a	41.6 *ns*
Cover crop				42.8 *ns*
No cover	2020	409 b	28.4 b	35.0 *ns*
Cover crop				38.8 *ns*
No cover	2022	379 b	21.3 c	31.5 *ns*
Cover crop				41.7 *ns*

Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS).

At Beloit, there was a two-way interaction between treatment and year for all tested enzymes at 0-5 cm (Table S2.11). β-glucosidase, AcidP, NAG, and ArylS activity increased from 2019 to 2023 with CCs by 157%, 32%, 38%, and 156%, respectively (Figure 2.5A; Table 2.7). For βG, AcidP, and NAG, there was no difference over time with NC (Table 2.7). Arylsulfatase increased by 33% even in the absence of CCs. The same two-way interaction observed at the 0-5 cm depth was also found at 5-15 cm (Table S2.11). While there was an increase in activity for AcidP, NAG, and ArylS, no temporal differences were found for βG with CCs (Figure 2.6B; Table 2.7). At 15-30 cm, there was a two-way interaction between treatment and year for NAG and ArylS, and a one-way effect of year for βG (Table S2.11). β-glucosidase activity increased by 22% over time (Figure 2.6C). Arylsulfatase activity increased over time in both the CC and NC treatments, with a significant 21% difference observed between the treatments in 2023 (Table 2.7). *N*-acetyl-β-glucosaminidase activity initially decreased from 2019 to 2021, followed by an increase from 2019 to 2023 for both CC and NC. The treatment without CC had the lowest statistically significant NAG activity in 2021 (Table 2.7).

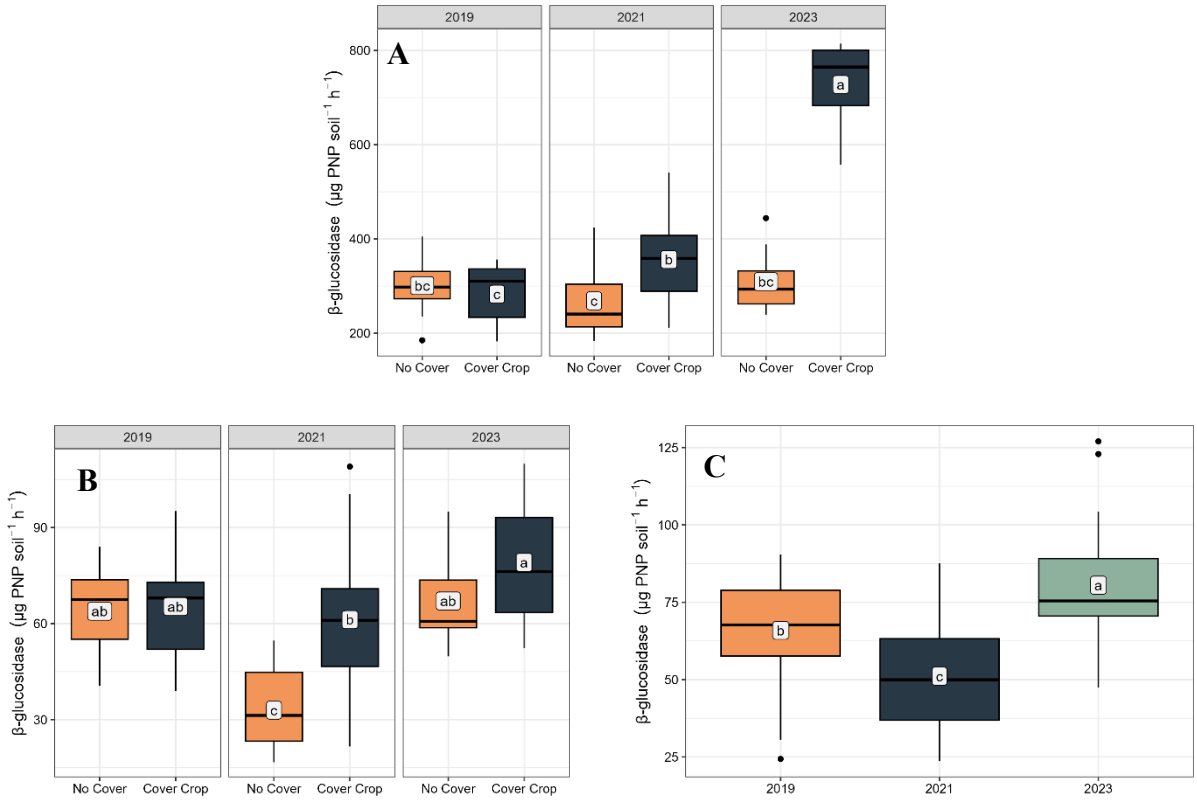


Figure 2. 5. β-glucosidase activity by treatment and year at 0-5 (A), 5-15 (B), and 15-30 (C) cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Table 2. 7. Changes in enzyme activity (µg PNP soil^{-1} h^{-1}) by treatment, year, and depth in Beloit, KS.

Treatment	Year	AcidP	NAG	ArylS
		0-5 cm		
No cover	2018	841 b	76.4 b	65.1 de
Cover crop		804 b	72.9 b	62.0 e
No cover	2020	869 b	76.5 b	80.3 cd
Cover crop		979 a	110 a	109 b
No cover	2022	773 b	68.6 b	82.9 c
Cover crop		1069 a	101 a	159 a
		5-15 cm		
No cover	2018	355 b	21.9 c	21.6 d

Cover crop		356 b	23.0 c	24.9 d
No cover	2020	402 b	24.0 c	25.0 d
Cover crop		483 a	34.3 a	40.0 b
No cover	2022	379 b	22.3 c	32.7 bc
Cover crop		472 a	29.0 b	51.5 a
		15-30 cm		
No cover	2018	336 ns	43.7 ab	39.0 c
Cover crop		345 ns	42.1 abc	35.5 c
No cover	2020	398 ns	32.7 c	37.8 c
Cover crop		351 ns	33.8 bc	37.2 c
No cover	2022	385 ns	35.2 abc	47.9 b
Cover crop		371 ns	51.5 a	60.4 a

Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS).

Soil organic carbon and total nitrogen

At Bucyrus, there was a significant two-way interaction between treatment and year for SOC at 0-5 cm (Table 2.S6). A one-way effect of year was found for 5-15 and 15-30 cm soil depth (Table 2.S6). At 0-5 cm, CC increased soil organic carbon by 21% from 2018 to 2022 (Figure 2.7A). Soil organic carbon was also higher for NC 2023 than CC 2018 (baseline) but not different from NC 2018 (Figure 2.7A). For both 5-15 and 15-30 cm there was an increase in SOC of 19% and 13% with time, respectively (Table S2.13). Total nitrogen was significant for treatment and year for 0-5 cm and year for 5-15 and 15-30 cm (Table S2.6). Inversely to SOC, there was no increase in TN with time with CCs (Table S2.13). Moreover, TN temporally decreased by 36% without CCs.

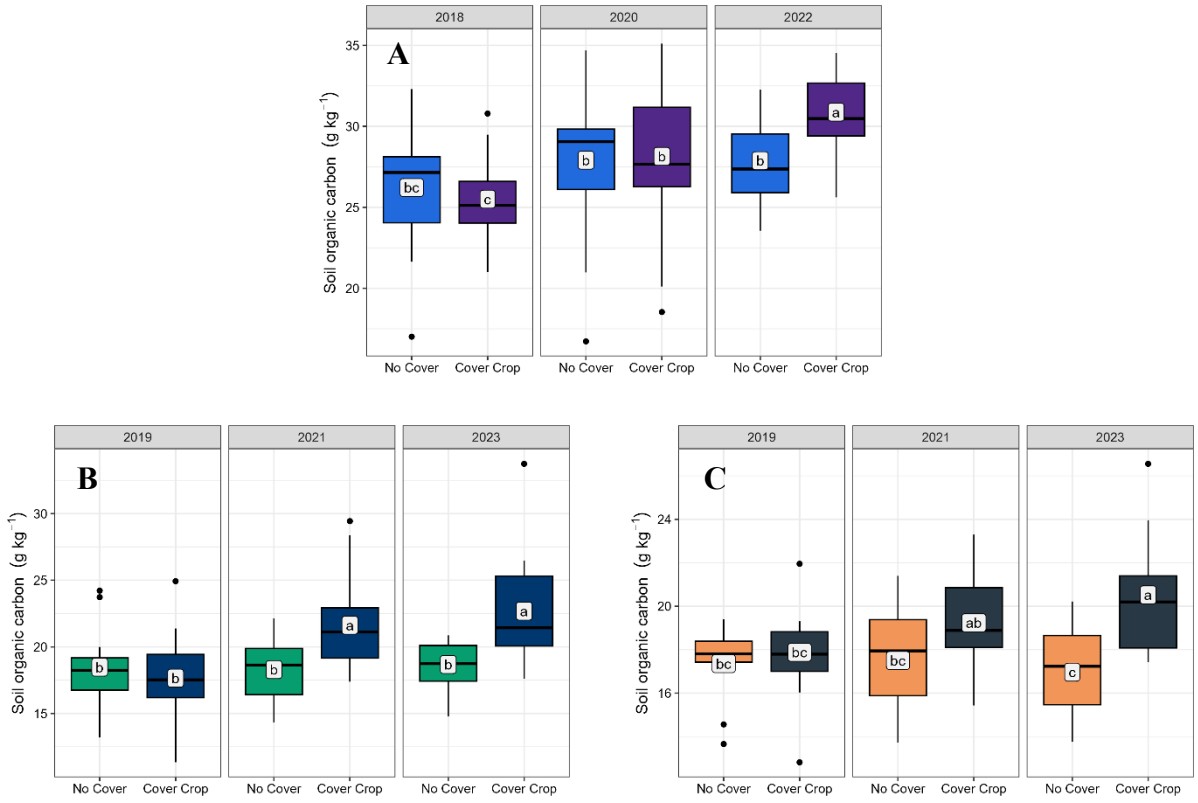


Figure 2. 6. Soil organic carbon by treatment and year at 0-5 cm at Bucyrus (A), Solomon (B), and Beloit (C). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Similar to the findings at Bucyrus, Solomon observed a two-way interaction between treatment and year for SOC at the 0-5 cm depth, while a one-way effect of year was identified at both the 5-15 cm and 15-30 cm depths (Table S2.9). While there were no changes in SOC over time in the absence of CCs at 0-5 cm, there was a significant increase of 29% when CCs were employed (Figure 2.7B). At 5-15 cm, SOC increased from 2019 to 2021 and decreased from 2021 to 2023. Nonetheless, the difference in SOC between 2019 and 2023 was not statistically significant (Table S2.9; S2.14). At 15-30 cm, a significant 5% decrease in SOC was observed from 2019 to 2023 (Table S2.9; S2.14). Total nitrogen was significant for treatment and year at

0-5 cm and year at 5-15 and 15-30 cm (Table S2.9). Total nitrogen increased with CCs by 22% from 2019 to 2023 at 0-5 cm (Table S2.14). However, TN 2023 was not different between CC and NC treatments (Table S2.14).

For the Beloit site, a two-way interaction between treatment and year was identified for SOC at 0-5 cm (Table S2.12). While there were no statistical differences for the 5-15 cm soil depth, a single effect of year was identified at 15-30 cm (Table S2.12). The use of CCs led to a 14% increase in SOC at the 0-5 cm depth, resulting in 21% more SOC than the treatment without CCs (Figure 2.7C). At 15-30 cm, there was an increase of 13% over time (Table S2.15). Regarding TN, a two-way interaction between treatment and year occurred at 0-5 cm (Table S2.12). Total nitrogen fluctuated from 2019 to 2023 at 0-5 cm soil depth, with a TN loss from 2019 to 2021 and a gain from 2021 to 2023 (Table S2.15).

Influence of soil chemical properties on the soil microbial community and enzyme activity

A dbRDA was performed to examine the relative influence of soil chemical properties and CCs on the soil microbial community and enzyme activity at 0-5 cm in Bucyrus (Figure 2.8A), Solomon (Figure 2.8B), and Beloit (Figure 2.8C). The soil chemical properties used in the model (SOC, TN, P, K^+, and pH) explained 40% in Bucyrus (999 permutations, $p = 0.001$), 30% in Solomon (999 permutations, $p = 0.03$), and 37.4% in Beloit (999 permutations, $p = 0.014$) of the total variation by the first two axis as influenced by CCs.

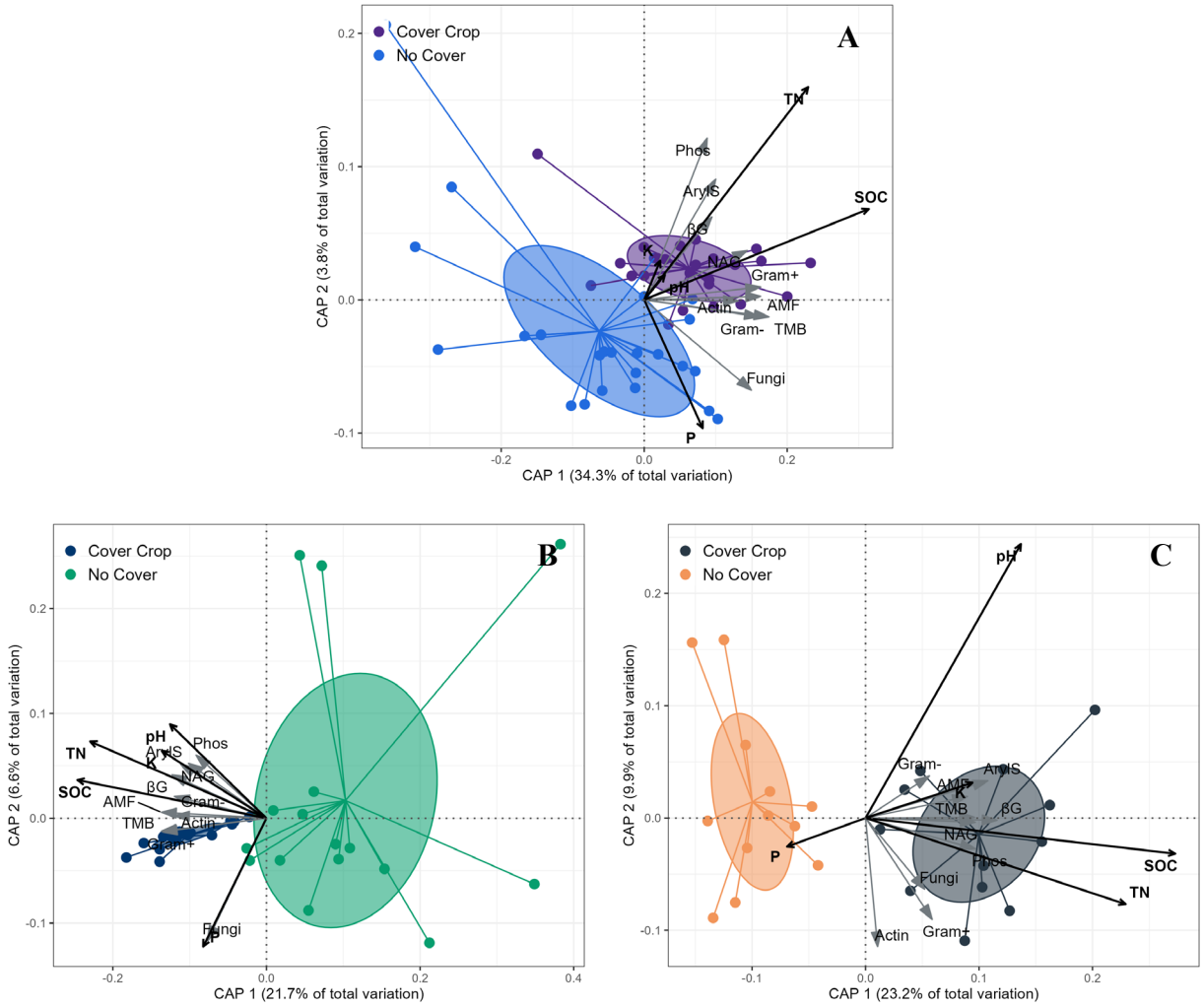


Figure 2. 7. Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activity constrained by soil chemical properties at 0-5 cm in Bucyrus (A), Solomon (B), and Beloit (C). Microbial biomass (TMB), gram-positive bacteria (Gram+), gram-negative bacteria (Gram-), actinomycetes (Actin), arbuscular mycorrhizal fungi (AMF), saprophytic fungi (Fungi), β-glucosidase (βG), acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS), soil organic carbon (SOC), and total nitrogen (TN).

All soil microbes and enzymes were strongly correlated with SOC (Bucyrus $p = 0.001$; Solomon $p = 0.006$; Beloit $p = 0.002$) as a result of the Monte Carlo permutation test ($p \leq 0.05$).

For all sites, only the first axis (CAP 1, horizontal) was significant and explained most of the variance. Likewise, the ordination of treatments was primarily clustered by CAP 1, which separated the CC from NC treatment for all locations. Overall, there was a positive correlation between the soil microbial community, enzymes, and CCs. When analyzed for 0-30 cm soil depth, the positive correlation between the soil microbial community, enzymes, and CCs was maintained for all locations, with SOC being the main driver of the relationship (Figure S2.10; S11; S12). However, while there was a clear separation between CC and NC at Solomon and Beloit, there was no clear clustering in Bucyrus as the poultry litter masked the CC effect.

Cropping system biomass and cash crop yield

At the Bucyrus site, the base cropping system yielded 82.7 Mg ha^{-1} of shoot biomass (Figure S2.7). The CC treatment added 5.3 Mg ha^{-1} for 88 Mg ha^{-1} (Figure S2.7). The fundamental idea behind incorporating CCs into the cropping system was to introduce a layer of residue cover ahead of soybean planting and a mix of legumes and grasses to supply nitrogen (N) for corn. At Solomon, the base cropping system yielded 18.3 Mg ha^{-1} of shoot biomass (Figure S2.8). The addition of CCs yielded an additional 10.6 Mg ha^{-1} of shoot biomass, for a total of 28.9 Mg ha^{-1} (Figure S2.8). The farmer's goal at Solomon was to add a high biomass mix of summer CCs for potential grazing and carbon accrual after winter wheat. Similarly to Bucyrus, the goal of adding a fall/winter CC was to add residue before soybean and provide N to grain sorghum. While at Bucyrus, the CCs represented only 6% of the total biomass, at Solomon CCs represented 36.7%. At Beloit, the total biomass produced by the base cropping system was 32.2 Mg ha^{-1} (Figure S2.9). The CCs added 3.2 Mg ha^{-1} of biomass to the cropping system, for a total of 35.4 Mg ha^{-1}. The CCs represented 9% of the total biomass. The farmer’s goal at Beloit was to

add a mix of legume, brassica, and grasses to provide N for grain sorghum, alleviate soil compaction, and cover the soil to reduce wind erosion. Cash crop yield by treatment and year for location is presented on tables 2.8, 2.9, and 2.10.

Table 2. 8. Cash crop yield (kg ha^{-1}) by treatment and year at Bucyrus, KS.

Crop	Year	Treatment	
		No cover	Cover crop
Corn	2018	12948 ± 220	13050 ± 284
Soybean	2019	3802 ± 91	3791 ± 75
Corn	2020	10343 ± 122	10002 ± 121
Soybean	2021	4254 ± 120	3958 ± 114
Corn	2022	11279 ± 88	11403 ± 84

Means for crop yield with the standard error of the mean.

Table 2. 9. Cash crop yield (kg ha^{-1}) by treatment and year at Solomon, KS.

Crop	Year	Treatment	
		No cover	Cover crop
Wheat	2020	3972 ± 61	3917 ± 56
Soybean	2021	2931 ± 86	3034 ± 71
Wheat	2022	1335 ± 93	1521 ± 79

Means for crop yield with the standard error of the mean.

Table 2. 10. Cash crop yield (kg ha^{-1}) by treatment and year at Beloit, KS.

Crop	Year	Treatment	
		No cover	Cover crop
Wheat	2019	1137 ± 25	1145 ± 27
Sorghum	2020	7445 ± 112	7552 ± 133
Soybean	2021	2846 ± 153	2843 ± 211
Wheat	2022	3038 ± 139	2911 ± 78

Means for crop yield with the standard error of the mean.

Discussion

Soil microorganisms play an essential role in agroecosystems and have been recognized as a critical component of soil health, often serving as indicators for assessing soil health (Ashworth et al., 2017; Fierer et al., 2021; Kim et al., 2020). In general, cover cropping led to an overall increase in all microbial parameters at the 0-5 cm soil depth, with the extent of this increase varying depending on the site location due to environmental properties such as SOC and cropping system intensity and diversity. Muhammad et al. (2021) also found increased soil microbial community abundance with CCs compared to no CCs. At Bucyrus, the increase in microbial biomass, Gram+, Gram-, and actinomycetes was increased by the poultry litter application. In a study from (Acosta-Martínez & Harmel, 2006), higher microbial biomass and peak area of fatty acid methyl ester (FAMEs) was attributed to poultry litter application and its nutrient richness. Moreover, CCs enhanced this effect, resulting in higher microbial abundance and enzyme activity than NC. This effect was most likely provided by the additional plant C provided by CCs (Campos et al., 2011; Kim et al., 2020). A similar increase was observed in Solomon and Beloit, albeit with a lower degree of magnitude. A plausible explanation for these results is that both sites had lower levels of SOC and produced less crop biomass over time. The presence of living roots from CCs during most of the intercrop period supplies root exudates (Chavarría et al., 2016), contributing to increased microbial growth and population (Loveland & Webb, 2003). A good example of this process is the release of high molecular weight compounds like polysaccharides and proteins, which have been shown to stimulate soil microbial biomass (Haichar et al., 2014; Kamilova et al., 2006). Therefore, maintaining a continuous and diverse presence of live roots in the system is a crucial factor in promoting the development of the soil microbial community. Crop residue is another important factor for the preservation and

development of the microbial community as it affects soil water relations. The residue provided by CCs maintains soil moisture (Rosa et al., 2021) and regulates temperature during summer (Blanco-Canqui & Ruis, 2020), creating a more favorable and stable environment for microbes (Wang et al., 2011). Additionally, CCs can potentially reduce the frequency of wetting-drying cycles by enhancing soil structure and increasing water retention. This, in turn, may mitigate the adverse effects of these cycles in the soil microbial community (Tivet et al., 2013; Vezzani & Mielniczuk, 2009).

A response similar to that of SOC was observed for microbial biomass, Gram+, Gram-, and Actinomycetes at deeper layers in Bucyrus, showing a positive correlation between SOC and microbes, as described by the dbRDA. Adding high-carbon materials, such as poultry litter, is known for increasing bacteria populations (Acosta-Martínez & Harmel, 2006). Although CCs favored saprophytic fungi, they decreased with depth as the availability of more recalcitrant material decreased (Pires et al., 2020). It has been reported that soil cover allows fungi to establish bridges at the soil-litter interface, facilitating residue decomposition and soil organic matter formation (Fabrizzi et al., 2009). At Solomon, there was a decrease in saprophytic fungi abundance for both 5-15 and 15-30 cm. This result may be associated with high-quality residue from CCs (hairy vetch) and soybean. Residue with high N content or low C/N ratio, favors bacterial dominance, whereas low-quality residue favors fungal growth (Bossuyt et al., 2001; Liu et al., 2023). For instance, Muhammad et al. (2021) found that legume CCs increased bacteria, but decreased fungi compared to nonlegume CCs.

β-glucosidase, NAG, AcidP, and ArylS activity increased with time and CCs at 0-5 cm at all sites, corroborating with the findings of other studies (Mbuthia et al., 2015; Pires et al., 2020). These findings are likely associated with the quantity and diversity of crop residues and the

proportion of readily decomposable organic materials reintroduced into the soil in response to the inclusion of CCs (Chavarría et al., 2016). β-glucosidase catalyzes the decomposition of cellulose; hence its higher activity may be explained by the SOC content (H. Liu et al., 2016). Furthermore, our study agrees with the study of Pires et al. (2020) that βG may be considered a good biological soil health indicator due to its quick response to changes in soil management.

Enzyme activity can pose challenges as a soil health indicator when it is not adequately interpreted within its proper context. According to (Margenot & Wade, 2023), there is a reductionist assumption that fertilization always attenuates or inhibits soil enzyme activity, mainly phosphomonoesterase (PME). For instance, P fertilizer can increase overall crop growth and thus soil organic matter, which may increase the activity of PME (Margenot et al., 2017). In this context, higher enzyme activity can be interpreted as nutrient deficiency (feedback inhibition) or greater microbial activity and, thus increased rates of nutrient cycling (Margenot & Wade, 2023). In our study, overall enzyme activity followed SOC and microbial biomass increases as affected by CCs, demonstrating a linkage with possibly higher nutrient cycling rates (Six et al., 2000). Nonetheless, we also observed a decrease in AcidP activity at the 5-15 cm and 15-30 cm depths over time where poultry litter was applied (Bucyrus), indicating a potential P feedback inhibition despite the concurrent increase in SOC and microbial biomass.

Arylsulfatase is responsible for the release of plant-available SO42- from sulfate esters (Hai-Ming et al., 2014). Supporting our results, Bandick & Dick (1999 and Sanchez et al. (2019) found increased ArylS activity after CCs. Increased plant diversity from the cropping system with CCs coupled with an increased root density may have also contributed to increased ArylS in our study. N-acetyl-β-glucosaminidase, which is linked to N cycling through chitin degradation in the soil, increased over time with CCs, responding to increased SOC rather than specific plant

biomass inputs (Sanchez et al., 2019). Although CCs positively affected NAG at 5-15 cm at Solomon, the activity decreased from 2019 to 2023. On the other hand, at Beloit NAG activity increased over time for all depths when CCs were implemented. The decline in NAG activity over time at Bucyrus could be linked to a decrease in TN, which was decoupled from SOC. A recent study from (Rosinger et al., 2019) showed that adding high C/N ratio plant material shifted fungal growth into N limitation.

The observation that enzyme activity changes in response to soil microbial biomass and SOC offers compelling evidence that the composition of the microbial community significantly impacted the potential for enzyme-mediated substrate catalysis in soil (Acosta-Martínez & Harmel, 2006; Kandeler et al., 1996; Ndiaye et al., 2000; Sun et al., 2004). This theory is supported by several authors that also found a correlation between microbial biomass and enzyme activity (Acosta-Martínez & Harmel, 2006; Bandick & Dick, 1999; Pires et al., 2020). Furthermore, greater enzyme activity with the implementation of CCs can be partially explained because enzymes can also be plant-produced (Margenot & Wade, 2023; Mori et al., 2023).

The results from this study demonstrated that SOC accrual is not singularly governed by shoot crop biomass (i.e., aboveground plant residue) and precipitation, although these two variables were positively correlated with SOC (Jian et al., 2020; Kuo et al., 1997). At Bucyrus and Beloit, CCs represented only 6 and 9% of the total biomass produced. At Solomon, a double sequence of summer and winter CCs in 2020 and 2022 represented 36.7% of the total biomass produced. Even though the CC biomass represented a small amount of the total biomass, it significantly increased SOC at 0-5 cm at all sites. The higher increase in SOC at Bucyrus might be attributed to the poultry litter application, which is a carbon-rich organic material (Acosta-Martínez & Harmel, 2006; Ashworth et al., 2017; Franzluebbers, 2005; Sharpley et al., 1993).

The utilization of poultry litter is a common practice in southeast Kansas, which besides providing a good source of many other nutrients (P, N, K^+), is generally less expensive than mineral fertilizers (Franzluebbers, 2005). Solomon and Beloit had no external sources of organic carbon besides the carbon derived from plants. In a global meta-analysis with more than 131 publications and 1195 comparisons between SOC concentrations when CCs were included in rotations versus SOC in NC controls, Jian et al. (2020) found that incorporating CCs into cropping systems increased SOC by 15.5% to 30%. The SOC increase in our study was within the range of SOC previously reported in the literature. At Solomon, planting CCs after crop harvest in summer and terminating late to increase biomass production has been reported to increase SOC (Blanco-Canqui, 2022). Moreover, some studies have reported that SOC increases after CC introduction due to a significant decrease in runoff and erosion (Jian et al., 2020; Kaspar & Singer, 2015). Reduced runoff and erosion rates can help minimize SOC losses from the field, creating a positive feedback loop (Berhe et al., 2007; Kaye & Quemada, 2017). Likewise, root biomass, rhizo-deposits, and microbial biomass C represent significant sources of SOC that CCs supply or contribute to during their growth and development (Balakrishna et al., 2017; Kutsch et al., 2010). In contrast to the 0-5 cm depth, there was no effect of CCs in deeper layers. At Bucyrus, poultry litter could have played a pivotal role in the increase of SOC from 2018 to 2022. Soluble C from the surface applied poultry litter may translocate in the soil and promote SOC accumulation in underlying soil layers (Nicoloso et al., 2018). Although there is still a debate regarding the capacity of CCs to sequester SOC (Blanco-Canqui, 2022), our study demonstrated that CCs could increase SOC over time.

The precipitation gradient across the three sites demonstrated to be a governing factor in the biomass production of the cropping system (Jian et al., 2020; Souza et al., 2023). The

difference in crop biomass production between eastern and central Kansas sites was substantial. While the 5-year accumulated precipitation in Bucyrus was 6010 mm, it was 3349 mm in Solomon and 2709 mm in Beloit. Notably, the total biomass produced in the CC treatment in Bucyrus was 204% higher than in Solomon and 149% higher than in Beloit. Besides precipitation, the choice of cover and cash crops employed in the cropping system affected total crop biomass production. At Solomon, the cropping system incorporated two consecutive CCs (one in summer and one in winter) in two out of five years, contributing significantly to the diversification of crops within the system and the total biomass produced in that location. At Beloit, where CCs were exclusively used before cash crops and not preceding another CC, the cropping system had higher biomass production. This was primarily attributed to the inclusion of full-season grain sorghum with high biomass production in 2020. Even though the 2020 total precipitation was below average, the rain was concentrated during the grain sorghum growing season, and water availability did not appear to be a significant limiting factor.

Conclusion

Our study underscores the significance of CCs in improving soil health, as evidenced by the increase in soil microbial biomass, bacteria, fungi, SOC, βG, and many other biological soil health indicators. Overall, the extent of these changes occurred in response to the precipitation patterns across sites. Our findings support the hypothesis that increasing SOC levels and stimulating soil microbial growth and activity under water-limited environments requires a longer time. This also implies that in water-limited environments, CCs are crucial to augment aboveground plant biomass production. In contrast, in non-water-limited environments like eastern Kansas, CCs may not substantially impact total biomass production due to the already

high-yielding nature of cash crops. Overall, diverse cropping systems incorporating CCs are a compelling and sustainable solution for improving soil health. While addressing issues related to soil erosion, compaction, and nutrient availability, it promotes the accumulation of SOC, leading to higher levels of microbial biomass and enzyme activity.

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Supplementary material

Table S2. 1. Soil physicochemical properties by treatment, year, and depth at Bucyrus, KS (n = 4).

Treatment	Year	Clay	Silt	Sand	K^+	P	Ca^{2+}	Mg^{2+}	Na^+	pH
		-------- g kg^{-1} --------			---------- mg kg^{-1} ----------					
0-5 cm										
No cover	2018	234 ± 10.6	633 ± 10.5	133 ± 2.5	198 ± 11.9	45.3 ± 2.6	2565 ± 65	185 ± 5.6	13.3 ± 0.6	6.6 ± 0.1
Cover crop	2018	245 ± 9.6	630 ± 9.9	125 ± 4.3	173 ± 8	39.9 ± 2.4	2622 ± 55.1	188 ± 5.5	12.3 ± 0.7	6.8 ± 0.1
No cover	2020	-----	-----	-----	218 ± 11.7	63 ± 5.4	2610 ± 74	197 ± 8.4	29 ± 0.9	6.8 ± 0.1
Cover crop	2020	-----	-----	-----	207 ± 13.1	51.4 ± 3.9	2539 ± 61.2	197 ± 7.1	25.8 ± 0.7	6.9 ± 0
No cover	2022	-----	-----	-----	179 ± 11.1	50 ± 5	2305 ± 54.6	170 ± 5.8	24.4 ± 0.7	6.9 ± 0.1
Cover crop	2022	-----	-----	-----	186 ± 6.4	46.7 ± 4.1	2373 ± 55	182 ± 6.9	24.8 ± 0.9	7 ± 0
5-15 cm										
No cover	2018	282 ± 8.1	594 ± 9.1	124 ± 2.1	102 ± 6.3	17.3 ± 1.9	2216 ± 64.8	162 ± 8.3	34.1 ± 1	5.6 ± 0.1
Cover crop	2018	290 ± 9.1	590 ± 10.7	120 ± 3.1	103 ± 6.9	15.3 ± 2.1	2312 ± 56.1	172 ± 10.2	35.6 ± 1.3	5.7 ± 0.1
No cover	2020	-----	-----	-----	145 ± 9	26.4 ± 3.6	2379 ± 82.5	166 ± 8.4	31.2 ± 1	6.2 ± 0.1
Cover crop	2020	-----	-----	-----	136 ± 8	23.2 ± 2.6	2446 ± 88.1	176 ± 11.6	29.3 ± 1.1	6.3 ± 0.1
No cover	2022	-----	-----	-----	120 ± 8	17.3 ± 2.6	2230 ± 62.5	150 ± 8.2	24 ± 1.3	6.3 ± 0.1
Cover crop	2022	-----	-----	-----	117 ± 7.6	13.1 ± 2.6	2417 ± 77.6	163 ± 13.1	25.5 ± 1.3	6.6 ± 0.1
15-30 cm										
No cover	2018	301 ± 9.7	582 ± 10.5	117 ± 2.7	113 ± 9.4	7.3 ± 1.7	2203 ± 95.8	194 ± 14.6	41 ± 2	5.7 ± 0.1
Cover crop	2018	308 ± 9.7	576 ± 10.2	116 ± 2.6	101 ± 7.1	4.1 ± 0.4	2166 ± 64.6	194 ± 14.9	39.9 ± 1.6	5.8 ± 0.1
No cover	2020	-----	-----	-----	130 ± 7.9	11.1 ± 2.3	2259 ± 98.9	183 ± 14.3	33 ± 0.9	5.8 ± 0.1
Cover crop	2020	-----	-----	-----	129 ± 9.9	11.9 ± 2.4	2245 ± 102.3	183 ± 19	31.3 ± 1.9	5.9 ± 0.1
No cover	2022	-----	-----	-----	115 ± 7.1	8.6 ± 2.1	2133 ± 65.7	155 ± 9.9	27.2 ± 1.9	5.9 ± 0.1
Cover crop	2022	-----	-----	-----	120 ± 8.9	8.7 ± 1.9	2423 ± 84.9	176 ± 16.2	29.1 ± 1.6	6.2 ± 0.1

Means for soil texture with the standard error of the mean.

Table S2. 2. Soil physicochemical properties by treatment, year, and depth at Solomon, KS (n = 4).

Treatment	Year	Clay	Silt	Sand	K^+	P	Ca^{2+}	Mg^{2+}	Na^+	pH
		-------- g kg^{-1} --------			---------- mg kg^{-1} ----------					
0-5 cm										
No cover	2019	265 ± 6	600 ± 6.6	135 ± 3.4	331 ± 12	53 ± 5.6	1586 ± 55.6	448 ± 42.6	12.7 ± 0.7	5.3 ± 0.1
Cover crop	2019	285 ± 7.3	578 ± 8	136 ± 6.9	322 ± 13	55.9 ± 5.2	1676 ± 79.3	476 ± 32.5	15.3 ± 1.4	5.2 ± 0.2
No cover	2021	-----	-----	-----	345 ± 7.6	63.5 ± 4.4	1742 ± 118	508 ± 58.5	18.9 ± 1.1	5.4 ± 0.2
Cover crop	2021	-----	-----	-----	386 ± 12	57.4 ± 4.8	1801 ± 77	527 ± 34.7	21.7 ± 1.4	5.5 ± 0.1
No cover	2023	-----	-----	-----	414 ± 20.3	67.6 ± 5.2	1710 ± 95.9	474 ± 38.1	15.2 ± 0.9	5.5 ± 0.2
Cover crop	2023	-----	-----	-----	434 ± 16.9	66.3 ± 4.3	1748 ± 100.6	473 ± 22.3	17 ± 1.1	5.5 ± 0.2
5-15 cm										
No cover	2019	280 ± 6.8	589 ± 8	130 ± 5.9	198 ± 7.9	19.7 ± 3	1393 ± 33.4	534 ± 57.7	17.1 ± 1.4	5.2 ± 0.1
Cover crop	2019	300 ± 8.2	568 ± 11.3	130 ± 8.6	204 ± 5.6	16.8 ± 2.2	1557 ± 73.2	554 ± 37.7	21.1 ± 2.5	5.3 ± 0.2
No cover	2021	-----	-----	-----	193 ± 6.1	15.6 ± 1.9	1527 ± 38.3	627 ± 74.4	26.5 ± 2.4	5.4 ± 0.1
Cover crop	2021	-----	-----	-----	194 ± 9.2	13.6 ± 1.9	1710 ± 101.4	624 ± 39.4	30.7 ± 2.4	5.6 ± 0.2
No cover	2023	-----	-----	-----	202 ± 11.1	25.9 ± 5.8	1523 ± 58.8	524 ± 45.5	21.6 ± 2.2	5.2 ± 0.1
Cover crop	2023	-----	-----	-----	199 ± 10.4	17 ± 2.7	1675 ± 112.3	523 ± 27.2	24.2 ± 1.8	5.4 ± 0.2
15-30 cm										
No cover	2019	287 ± 9.3	585 ± 7.5	127 ± 6.4	179 ± 7.3	11 ± 1.6	1452 ± 43.8	577 ± 60.1	24.5 ± 2.5	5.4 ± 0.1
Cover crop	2019	307 ± 8	569 ± 9.2	123 ± 6.4	184 ± 5.4	9.2 ± 1.4	1740 ± 162.3	603 ± 42.6	31.7 ± 4	5.5 ± 0.2
No cover	2021	-----	-----	-----	166 ± 6.4	8.1 ± 1.1	1565 ± 48.2	692 ± 78.6	36.9 ± 4.1	5.7 ± 0.2
Cover crop	2021	-----	-----	-----	168 ± 5.9	6.9 ± 0.7	1711 ± 88.1	656 ± 37.4	38.2 ± 4.1	5.6 ± 0.1
No cover	2023	-----	-----	-----	168 ± 9.6	6.6 ± 0.8	1567 ± 67.5	624 ± 58.7	36 ± 4.7	5.4 ± 0.1
Cover crop	2023	-----	-----	-----	169 ± 5.9	5.5 ± 0.6	1786 ± 137.7	603 ± 35.7	39 ± 4.5	5.6 ± 0.2

Means for soil texture with the standard error of the mean.

Table S2. 3. Soil physicochemical properties by treatment, year, and depth at Beloit, KS (n = 4).

Treatment	Year	Clay	Silt	Sand	K^+	P	Ca^{2+}	Mg^{2+}	Na^+	pH
		-------- g kg^{-1} --------			---------- mg kg^{-1} ----------					
0-5 cm										
No cover	2019	224 ± 10.8	574 ± 15.3	201 ± 14.6	493 ± 12.7	80.5 ± 3.6	1897 ± 80.9	183 ± 11.3	13.9 ± 0.7	5.8 ± 0.2
Cover crop	2019	255 ± 18.2	565 ± 15.9	179 ± 14.8	502 ± 13.5	82.3 ± 5.2	1907 ± 121.6	208 ± 19.7	14.6 ± 0.7	5.7 ± 0.2
No cover	2021	-----	-----	-----	483 ± 11.2	109.8 ± 4.6	2069 ± 60	197 ± 14.6	15.9 ± 0.5	5.7 ± 0.1
Cover crop	2021	-----	-----	-----	561 ± 17.4	108.2 ± 5.2	2417 ± 152.1	244 ± 23.9	21 ± 1.5	6 ± 0.2
No cover	2023	-----	-----	-----	600 ± 19.2	97 ± 5.4	2253 ± 169.5	193 ± 15.1	9.4 ± 0.6	6 ± 0.2
Cover crop	2023	-----	-----	-----	626 ± 24.7	93.5 ± 5.2	2404 ± 178.5	219 ± 23.5	12.8 ± 0.9	6.1 ± 0.2
5-15 cm										
No cover	2019	265 ± 10.1	478 ± 18.4	255 ± 19.1	339 ± 8.2	39.7 ± 2.5	1341 ± 76.3	210 ± 17.5	15.1 ± 0.9	4.9 ± 0
Cover crop	2019	279 ± 17	504 ± 26.6	216 ± 25.7	371 ± 17.3	38.2 ± 3.3	1620 ± 192	268 ± 37.5	18.3 ± 1.7	5 ± 0.1
No cover	2021	-----	-----	-----	345 ± 11.3	40.6 ± 4.7	1763 ± 86.6	259 ± 19.8	19 ± 1.3	5.1 ± 0
Cover crop	2021	-----	-----	-----	388 ± 12.3	50.5 ± 6.8	1942 ± 145.9	286 ± 32	22.5 ± 1.7	5.2 ± 0.1
No cover	2023	-----	-----	-----	369 ± 11.7	45.6 ± 4.6	1740 ± 103.9	209 ± 16.9	10.4 ± 0.6	5 ± 0.1
Cover crop	2023	-----	-----	-----	382 ± 19.2	38.6 ± 6.2	2028 ± 180.6	271 ± 33.1	13.9 ± 1.4	5.2 ± 0.1
15-30 cm										
No cover	2019	313 ± 11.8	494 ± 12.2	192 ± 14.4	397 ± 19.8	3.7 ± 0.5	2941 ± 123.6	534 ± 30.1	34.7 ± 2	6.3 ± 0.1
Cover crop	2019	341 ± 23.7	465 ± 12	193 ± 24.5	437 ± 13	4.8 ± 1.7	3171 ± 124.7	592 ± 23.5	39.3 ± 2.2	6.4 ± 0.1
No cover	2021	-----	-----	-----	353 ± 16.6	9.5 ± 0.9	2417 ± 128.2	402 ± 26.1	27.2 ± 1.1	5.5 ± 0.1
Cover crop	2021	-----	-----	-----	390 ± 15.7	16.2 ± 3.5	2586 ± 194.2	431 ± 40.1	32.6 ± 2.6	5.7 ± 0.1
No cover	2023	-----	-----	-----	329 ± 12.7	9.9 ± 1.3	2497 ± 119.8	372 ± 27.5	15.9 ± 1.4	5.5 ± 0.1
Cover crop	2023	-----	-----	-----	371 ± 14.3	8.9 ± 1.7	2935 ± 191.5	463 ± 37.2	21 ± 2	5.8 ± 0.1

Means for soil texture with the standard error of the mean.

Table S2. 4. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on microbial biomass (TMB), gram-positive bacteria, gram-negative bacteria, actinomycetes, arbuscular mycorrhizal fungi (AMF), and saprophytic fungi at 0-5, 5-15, and 15-30 cm soil depth at Bucyrus, KS.

Type III Tests of Fixed Effects								
0-5 cm								
TMB			Gram-positive			Gram-negative		
Sf	χ^2	p	Sf	χ^2	p	Sf	χ^2	P
Cover crop (CC)	3.71	0.05	Cover crop (CC)	3.18	0.75	Cover crop (CC)	2.55	0.11
Year (Y)	1176	0.00	Year (Y)	1291	0.00	Year (Y)	453	0.00
CC*Y	18.33	0.00	CC*Y	20.08	0.00	CC*Y	9.20	0.01
Actinomycetes			AMF			Saprophytic fungi		
Cover crop (CC)	3.25	0.07	Cover crop (CC)	12.67	0.00	Cover crop (CC)	0.82	0.37
Year (Y)	1316	0.00	Year (Y)	417	0.00	Year (Y)	421	0.00
CC*Y	17.90	0.00	CC*Y	35.22	0.00	CC*Y	1.61	0.45
5-15 cm								
TMB			Gram-positive			Gram-negative		
Cover crop (CC)	0.60	0.44	Cover crop (CC)	0.97	0.32	Cover crop (CC)	0.01	0.91
Year (Y)	1052	0.00	Year (Y)	353	0.00	Year (Y)	249	0.00
CC*Y	2.37	0.31	CC*Y	2.30	0.32	CC*Y	0.75	0.69
Actinomycetes			AMF			Saprophytic fungi		
Cover crop (CC)	0.76	0.38	Cover crop (CC)	2.26	0.13	Cover crop (CC)	0.09	0.76
Year (Y)	463	0.00	Year (Y)	315	0.00	Year (Y)	46.59	0.00
CC*Y	4.09	0.13	CC*Y	2.61	0.27	CC*Y	1.74	0.42
15-30 cm								
TMB			Gram-positive			Gram-negative		
Cover crop (CC)	0.02	0.89	Cover crop (CC)	0.92	0.34	Cover crop (CC)	1.10	0.29
Year (Y)	2452	0.00	Year (Y)	448	0.00	Year (Y)	791	0.00
CC*Y	0.40	0.82	CC*Y	6.19	0.05	CC*Y	1.89	0.39
Actinomycetes			AMF			Saprophytic fungi		
Cover crop (CC)	0.19	0.66	Cover crop (CC)	4.16	0.04	Cover crop (CC)	1.15	0.28
Year (Y)	468	0.00	Year (Y)	974	0.00	Year (Y)	35.63	0.00
CC*Y	1.21	0.55	CC*Y	7.24	0.03	CC*Y	0.55	0.76

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S2. 5. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on β-glucosidase, acid phosphatase, N-acetyl-β-glucosaminidase, and arylsulfatase at 0-5, 5-15, and 15-30 cm soil depth at Bucyrus, KS.

Type III Tests of Fixed Effects					
0-5 cm					
β-glucosidase			Acid phosphatase		
Sf	χ^2	p	Sf	χ^2	p
Cover crop (CC)	10.99	0.00	Cover crop (CC)	2.64	0.10
Year (Y)	179	0.00	Year (Y)	11.34	0.00
CC*Y	18.97	0.00	CC*Y	38.44	0.00
N-acetyl-β-glucosaminidase			Arylsulfatase		
Cover crop (CC)	14.21	0.00	Cover crop (CC)	25.27	0.00
Year (Y)	22.11	0.00	Year (Y)	35.46	0.00
CC*Y	27.59	0.00	CC*Y	33.81	0.00
5-15 cm					
β-glucosidase			Acid phosphatase		
Cover crop (CC)	13.45	0.00	Cover crop (CC)	3.21	0.07
Year (Y)	413	0.00	Year (Y)	23.98	0.00
CC*Y	5.32	0.07	CC*Y	11.19	0.00
N-acetyl-β-glucosaminidase			Arylsulfatase		
Cover crop (CC)	1.93	0.16	Cover crop (CC)	5.30	0.02
Year (Y)	8.91	0.01	Year (Y)	69.88	0.00
CC*Y	4.75	0.09	CC*Y	5.70	0.05
15-30 cm					
β-glucosidase			Acid phosphatase		
Cover crop (CC)	1.65	0.20	Cover crop (CC)	0.22	0.64
Year (Y)	644	0.00	Year (Y)	64.50	0.00
CC*Y	14.84	0.00	CC*Y	21.22	0.00
N-acetyl-β-glucosaminidase			Arylsulfatase		
Cover crop (CC)	1.55	0.21	Cover crop (CC)	2.94	0.09
Year (Y)	76.70	0.00	Year (Y)	21.05	0.00
CC*Y	2.31	0.32	CC*Y	2.66	0.26

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S2. 6. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on soil organic carbon, total nitrogen, and C: N ratio at 0-5, 5-15, and 15-30 cm soil depth at Bucyrus, KS.

Type III Tests of Fixed Effects								
0-5 cm								
Soil organic carbon			Total nitrogen			C: N ratio		
Sf	χ^2	p	Sf	χ^2	p	Sf	χ^2	P
Cover crop (CC)	2.50	0.11	Cover crop (CC)	0.07	0.80	Cover crop (CC)	0.35	0.55
Year (Y)	30.32	0.00	Year (Y)	29.95	0.00	Year (Y)	141.50	0.00
CC*Y	8.93	0.01	CC*Y	7.00	0.03	CC*Y	0.77	0.68
5-15 cm								
Soil organic carbon			Total nitrogen			C: N ratio		
Cover crop (CC)	0.06	0.80	Cover crop (CC)	1.31	0.25	Cover crop (CC)	0.35	0.55
Year (Y)	49.87	0.00	Year (Y)	37.04	0.00	Year (Y)	133.75	0.00
CC*Y	1.36	0.51	CC*Y	4.93	0.08	CC*Y	2.68	0.26
15-30 cm								
Soil organic carbon			Total nitrogen			C: N ratio		
Cover crop (CC)	0.12	0.73	Cover crop (CC)	1.03	0.31	Cover crop (CC)	0.31	0.58
Year (Y)	73.03	0.00	Year (Y)	43.94	0.00	Year (Y)	108.09	0.00
CC*Y	2.21	0.33	CC*Y	5.47	0.06	CC*Y	2.43	0.30

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S2. 7. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on microbial biomass (TMB), gram-positive bacteria, gram-negative bacteria, actinomycetes, arbuscular mycorrhizal fungi (AMF), and saprophytic fungi at 0-5, 5-15, and 15-30 cm soil depth at Solomon, KS.

Type III Tests of Fixed Effects								
0-5 cm								
TMB			Gram-positive			Gram-negative		
Sf	χ^2	p	Sf	χ^2	p	Sf	χ^2	P
Cover crop (CC)	25.78	0.00	Cover crop (CC)	36.09	0.00	Cover crop (CC)	18.64	0.00
Year (Y)	66.56	0.00	Year (Y)	66.93	0.00	Year (Y)	25.62	0.00
CC*Y	55.36	0.00	CC*Y	58.47	0.00	CC*Y	9.46	0.01
Actinomycetes			AMF			Saprophytic fungi		
Cover crop (CC)	7.45	0.06	Cover crop (CC)	13.18	0.00	Cover crop (CC)	18.38	0.00
Year (Y)	2.83	0.24	Year (Y)	8.97	0.01	Year (Y)	11.43	0.00
CC*Y	8.57	0.01	CC*Y	27.58	0.00	CC*Y	10.20	0.01
5-15 cm								
TMB			Gram-positive			Gram-negative		
Cover crop (CC)	19.89	0.00	Cover crop (CC)	14.46	0.00	Cover crop (CC)	20.23	0.00
Year (Y)	143.08	0.00	Year (Y)	8.70	0.01	Year (Y)	162.31	0.00
CC*Y	39.89	0.00	CC*Y	35.58	0.00	CC*Y	24.34	0.00
Actinomycetes			AMF			Saprophytic fungi		
Cover crop (CC)	0.68	0.11	Cover crop (CC)	16.00	0.00	Cover crop (CC)		0.00
Year (Y)	10.30	0.01	Year (Y)	37.01	0.00	Year (Y)		0.00
CC*Y	3.28	0.19	CC*Y	18.55	0.00	CC*Y		0.24
15-30 cm								
TMB			Gram-positive			Gram-negative		
Cover crop (CC)	39.01	0.00	Cover crop (CC)	7.03	0.01	Cover crop (CC)	12.42	0.00
Year (Y)	81.11	0.00	Year (Y)	55.80	0.00	Year (Y)	320.05	0.00
CC*Y	119.09	0.00	CC*Y	46.53	0.00	CC*Y	55.11	0.00
Actinomycetes			AMF			Saprophytic fungi		
Cover crop (CC)	0.75	0.39	Cover crop (CC)	17.52	0.00	Cover crop (CC)	9.28	0.00
Year (Y)	23.05	0.00	Year (Y)	71.36	0.00	Year (Y)	70.02	0.00
CC*Y	8.40	0.01	CC*Y	36.94	0.00	CC*Y	2.76	0.25

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S2. 8. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on β-glucosidase, acid phosphatase, N-acetyl-β-glucosaminidase, and arylsulfatase at 0-5, 5-15, and 15-30 cm soil depth at Solomon, KS.

Type III Tests of Fixed Effects					
0-5 cm					
β-glucosidase			Acid phosphatase		
Sf	χ^2	p	Sf	χ^2	p
Cover crop (CC)	20.78	0.00	Cover crop (CC)	26.58	0.00
Year (Y)	9.78	0.01	Year (Y)	0.22	0.89
CC*Y	19.39	0.00	CC*Y	16.66	0.00
N-acetyl-β-glucosaminidase			Arylsulfatase		
Cover crop (CC)	34.77	0.00	Cover crop (CC)	33.18	0.00
Year (Y)	9.28	0.01	Year (Y)	19.13	0.00
CC*Y	6.80	0.03	CC*Y	16.11	0.00
5-15 cm					
β-glucosidase			Acid phosphatase		
Cover crop (CC)	1.22	0.27	Cover crop (CC)	0.00	0.99
Year (Y)	23.91	0.00	Year (Y)	20.14	0.00
CC*Y	0.28	0.87	CC*Y	1.12	0.57
N-acetyl-β-glucosaminidase			Arylsulfatase		
Cover crop (CC)	9.40	0.00	Cover crop (CC)	3.18	0.07
Year (Y)	17.44	0.00	Year (Y)	2.05	0.36
CC*Y	2.52	0.28	CC*Y	2.15	0.34
15-30 cm					
β-glucosidase			Acid phosphatase		
Cover crop (CC)	0.71	0.40	Cover crop (CC)	0.27	0.61
Year (Y)	53.34	0.00	Year (Y)	12.99	0.00
CC*Y	1.56	0.46	CC*Y	0.51	0.77
N-acetyl-β-glucosaminidase			Arylsulfatase		
Cover crop (CC)	2.29	0.13	Cover crop (CC)	3.04	0.08
Year (Y)	46.78	0.00	Year (Y)	4.95	0.08
CC*Y	5.01	0.08	CC*Y	2.72	0.26

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S2. 9. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on soil organic carbon, total nitrogen, and C: N ratio at 0-5, 5-15, and 15-30 cm soil depth at Solomon, KS.

Type III Tests of Fixed Effects								
0-5 cm								
Soil organic carbon			Total nitrogen			C: N ratio		
Sf	χ^2	p	Sf	χ^2	p	Sf	χ^2	P
Cover crop (CC)	7.31	0.01	Cover crop (CC)	0.80	0.37	Cover crop (CC)	1.59	0.21
Year (Y)	14.80	0.00	Year (Y)	3.24	0.20	Year (Y)	13.27	0.00
CC*Y	13.90	0.00	CC*Y	12.57	0.00	CC*Y	0.70	0.71
5-15 cm								
Soil organic carbon			Total nitrogen			C: N ratio		
Cover crop (CC)	0.63	0.80	Cover crop (CC)	0.29	0.59	Cover crop (CC)	0.48	0.49
Year (Y)	11.16	0.00	Year (Y)	14.32	0.00	Year (Y)	46.38	0.00
CC*Y	5.87	0.05	CC*Y	2.72	0.26	CC*Y	0.71	0.70
15-30 cm								
Soil organic carbon			Total nitrogen			C: N ratio		
Cover crop (CC)	0.02	0.89	Cover crop (CC)	0.00	0.99	Cover crop (CC)	0.05	0.81
Year (Y)	9.25	0.01	Year (Y)	17.00	0.00	Year (Y)	44.08	0.00
CC*Y	3.51	0.17	CC*Y	2.20	0.33	CC*Y	1.80	0.41

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S2. 10. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on microbial biomass (TMB), gram-positive bacteria, gram-negative bacteria, actinomycetes, arbuscular mycorrhizal fungi (AMF), and saprophytic fungi at 0-5, 5-15, and 15-30 cm soil depth at Beloit, KS.

Type III Tests of Fixed Effects								
0-5 cm								
TMB			Gram-positive			Gram-negative		
Sf	χ^2	p	Sf	χ^2	p	Sf	χ^2	P
Cover crop (CC)	5.18	0.02	Cover crop (CC)	3.07	0.08	Cover crop (CC)	2.84	0.09
Year (Y)	19.01	0.00	Year (Y)	25.48	0.00	Year (Y)	8.26	0.02
CC*Y	11.95	0.00	CC*Y	4.17	0.12	CC*Y	6.02	0.05
Actinomycetes			AMF			Saprophytic fungi		
Cover crop (CC)	0.53	0.47	Cover crop (CC)	3.78	0.52	Cover crop (CC)	0.93	0.34
Year (Y)	47.81	0.00	Year (Y)	5.24	0.07	Year (Y)	7.45	0.02
CC*Y	0.92	0.63	CC*Y	6.06	0.05	CC*Y	2.38	0.30
5-15 cm								
TMB			Gram-positive			Gram-negative		
Cover crop (CC)	1.58	0.21	Cover crop (CC)	1.39	0.24	Cover crop (CC)	0.02	0.89
Year (Y)	5.31	0.07	Year (Y)	4.51	0.10	Year (Y)	4.56	0.10
CC*Y	0.65	0.72	CC*Y	6.10	0.05	CC*Y	0.06	0.97
Actinomycetes			AMF			Saprophytic fungi		
Cover crop (CC)	0.03	0.87	Cover crop (CC)	5.10	0.02	Cover crop (CC)	9.30	0.00
Year (Y)	17.04	0.00	Year (Y)	0.81	0.67	Year (Y)	2.98	0.23
CC*Y	5.04	0.08	CC*Y	21.66	0.00	CC*Y	9.11	0.01
15-30 cm								
TMB			Gram-positive			Gram-negative		
Cover crop (CC)	0.57	0.45	Cover crop (CC)	0.26	0.61	Cover crop (CC)	0.69	0.41
Year (Y)	2.80	0.25	Year (Y)	1.44	0.49	Year (Y)	4.24	0.12
CC*Y	0.19	0.91	CC*Y	6.10	0.06	CC*Y	0.17	0.92
Actinomycetes			AMF			Saprophytic fungi		
Cover crop (CC)	0.01	0.97	Cover crop (CC)	1.83	0.18	Cover crop (CC)	2.37	0.12
Year (Y)	53.08	0.00	Year (Y)	6.29	0.04	Year (Y)	0.99	0.61
CC*Y	0.64	0.73	CC*Y	8.66	0.01	CC*Y	1.90	0.39

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S2. 11. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on β-glucosidase, acid phosphatase, N-acetyl-β-glucosaminidase, and arylsulfatase at 0-5, 5-15, and 15-30 cm soil depth at Beloit, KS.

Type III Tests of Fixed Effects					
0-5 cm					
β-glucosidase			Acid phosphatase		
Sf	χ^2	p	Sf	χ^2	p
Cover crop (CC)	59.97	0.00	Cover crop (CC)	16.27	0.00
Year (Y)	160.47	0.00	Year (Y)	11.11	0.00
CC*Y	131.96	0.00	CC*Y	22.97	0.00
N-acetyl-β-glucosaminidase			Arylsulfatase		
Cover crop (CC)	22.20	0.00	Cover crop (CC)	30,50	0.00
Year (Y)	26.91	0.00	Year (Y)	1877	0.00
CC*Y	33.33	0.00	CC*Y	108.1	0.00
5-15 cm					
β-glucosidase			Acid phosphatase		
Cover crop (CC)	12.05	0.00	Cover crop (CC)	16.08	0.00
Year (Y)	29.02	0.00	Year (Y)	26.70	0.00
CC*Y	7.53	0.02	CC*Y	7.86	0.02
N-acetyl-β-glucosaminidase			Arylsulfatase		
Cover crop (CC)	25.55	0.00	Cover crop (CC)	16.04	0.00
Year (Y)	26.25	0.00	Year (Y)	104.22	0.00
CC*Y	12.88	0.00	CC*Y	16.15	0.00
15-30 cm					
β-glucosidase			Acid phosphatase		
Cover crop (CC)	0.15	0.70	Cover crop (CC)	0.92	0.34
Year (Y)	33.18	0.00	Year (Y)	4.34	0.11
CC*Y	3.45	0.18	CC*Y	2.10	0.35
N-acetyl-β-glucosaminidase			Arylsulfatase		
Cover crop (CC)	1.04	0.31	Cover crop (CC)	1.89	0.17
Year (Y)	10.09	0.01	Year (Y)	68.93	0.00
CC*Y	7.23	0.03	CC*Y	13.51	0.00

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S2. 12. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on soil organic carbon, total nitrogen, and C: N ratio at 0-5, 5-15, and 15-30 cm soil depth at Beloit, KS.

Type III Tests of Fixed Effects								
0-5 cm								
Soil organic carbon			Total nitrogen			C: N ratio		
Sf	χ^2	p	Sf	χ^2	p	Sf	χ^2	P
Cover crop (CC)	6.33	0.01	Cover crop (CC)	6.33	0.01	Cover crop (CC)	6.08	0.01
Year (Y)	4.43	0.11	Year (Y)	4.43	0.11	Year (Y)	367.67	0.00
CC*Y	7.79	0.02	CC*Y	7.79	0.02	CC*Y	0.14	0.93
5-15 cm								
Soil organic carbon			Total nitrogen			C: N ratio		
Cover crop (CC)	0.01	0.91	Cover crop (CC)	0.30	0.59	Cover crop (CC)	0.33	0.57
Year (Y)	3.93	0.14	Year (Y)	468.08	0.00	Year (Y)	720.31	0.00
CC*Y	2.52	0.28	CC*Y	2.12	0.35	CC*Y	1.49	0.48
15-30 cm								
Soil organic carbon			Total nitrogen			C: N ratio		
Cover crop (CC)	0.41	0.52	Cover crop (CC)	2.57	0.11	Cover crop (CC)	1.80	0.18
Year (Y)	11.05	0.00	Year (Y)	195.21	0.00	Year (Y)	91.81	0.00
CC*Y	1.28	0.53	CC*Y	7.77	0.02	CC*Y	3.19	0.20

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S2. 13. Changes in soil organic carbon (g C kg soil^{-1}), total nitrogen (g N kg soil^{-1}), and C: N ratio by treatment, year, and depth at Bucyrus, KS.

Treatment	Year	SOC	TN	C:N ratio
		0-5 cm		
No cover	2018	26.2 bc	3.0 a	9.5 c
Cover crop		25.5 c	2.7 ab	
No cover	2020	28.0 b	2.4 bc	11.5 b
Cover crop		28.1 b	2.4 bc	
No cover	2022	28.0 b	2.2 c	12.5 a
Cover crop		30.9 a	2.4 bc	
		5-15 cm		
No cover	2018	17.1 c	2.1 a	9.2 c
Cover crop				
No cover	2020	18.3 b	1.6 b	11.5 b
Cover crop				
No cover	2022	20.3 b	1.5 b	13.5 a
Cover crop				
		15-30 cm		
No cover	2018	15.7 b	2.0 a	9.2 c
Cover crop				
No cover	2020	17.4 a	1.4 b	11.8 b
Cover crop				
No cover	2022	17.8 a	1.3 b	13.7 a
Cover crop				

Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Soil organic carbon (SOC), total nitrogen (TN), soil organic carbon: total nitrogen ratio (C: N ratio).

Table S2. 14. Changes in soil organic carbon (g C kg soil^{-1}), total nitrogen (g N kg soil^{-1}), and C: N ratio by treatment, year, and depth at Solomon, KS.

Treatment	Year	SOC	TN	C:N ratio
0-5 cm				
No cover	2018	18.5 b	1.8 ab	10.8 b
Cover crop		17.6 b	1.6 b	
No cover	2020	18.3 b	1.6 b	11.9 a
Cover crop		21.6 a	1.8 ab	
No cover	2022	18.7 b	1.7 ab	11.4 ab
Cover crop		22.7 a	1.95 a	
5-15 cm				
No cover	2018	13.4 b	1.1 a	12.3 b
Cover crop				
No cover	2020	14.3 a	0.9 b	15.9 a
Cover crop				
No cover	2022	13.3 b	1.1 a	11.6 b
Cover crop				
15-30 cm				
No cover	2018	13.0 ab	1.1 a	11.9 b
Cover crop				
No cover	2020	13.3 a	0.9 b	16.9 a
Cover crop				
No cover	2022	12.33 b	1.0 a	11.9 b
Cover crop				

Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Soil organic carbon (SOC), total nitrogen (TN), soil organic carbon: total nitrogen ratio (C: N ratio).

Table S2. 15. Changes in soil organic carbon (g C kg soil^{-1}), total nitrogen (g N kg soil^{-1}), and C: N ratio by treatment, year, and depth at Beloit, KS.

Treatment	Year	SOC	TN	C:N ratio	
		0-5 cm			
No cover	2018	17.4 bc	1.9 a	11.2 B*	9.5 c
Cover crop		17.9 bc	1.8 a		
No cover	2020	17.5 bc	1.34 c		13.2 a
Cover crop		19.2 ab	1.4 bc		
No cover	2022	17.0 c	1.6 b	11.6 **	11.3 b
Cover crop		20.5 a	1.8 a		
		5-15 cm			
No cover	2018	10.8 ns	1.1 a	9.4 c	
Cover crop		10.6 ns			
No cover	2020	10.9 ns	0.73 c	15.1 a	
Cover crop		11.2 ns			
No cover	2022	11.0 ns	1.0 b	10.7 b	
Cover crop		11.2 ns			
		15-30 cm			
No cover	2018	8.5 b	1.0 a	9.6 a	
Cover crop			0.9 a		
No cover	2020	9.6 a	0.7 b	14.9 a	
Cover crop			0.6 b		
No cover	2022	9.6 a	0.9 a	10.3 b	
Cover crop			1.0 a		

*No cover, **Cover crop. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Soil organic carbon (SOC), total nitrogen (TN), soil organic carbon: total nitrogen ratio (C: N ratio).

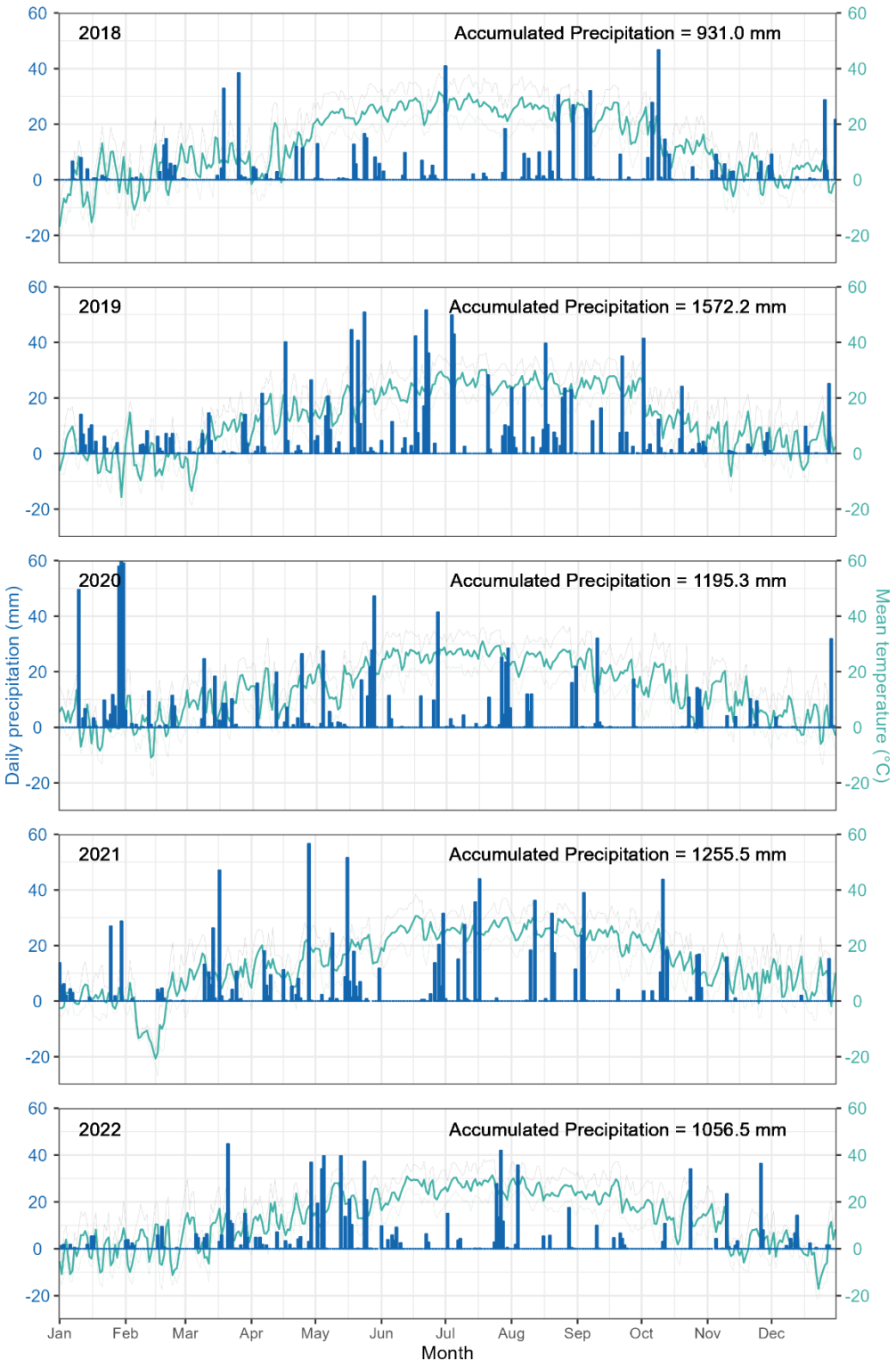


Figure S2. 1. Daily rainfall, cumulative rainfall, and maximum, average, and minimum temperatures recorded during the study years at Bucyrus, KS.

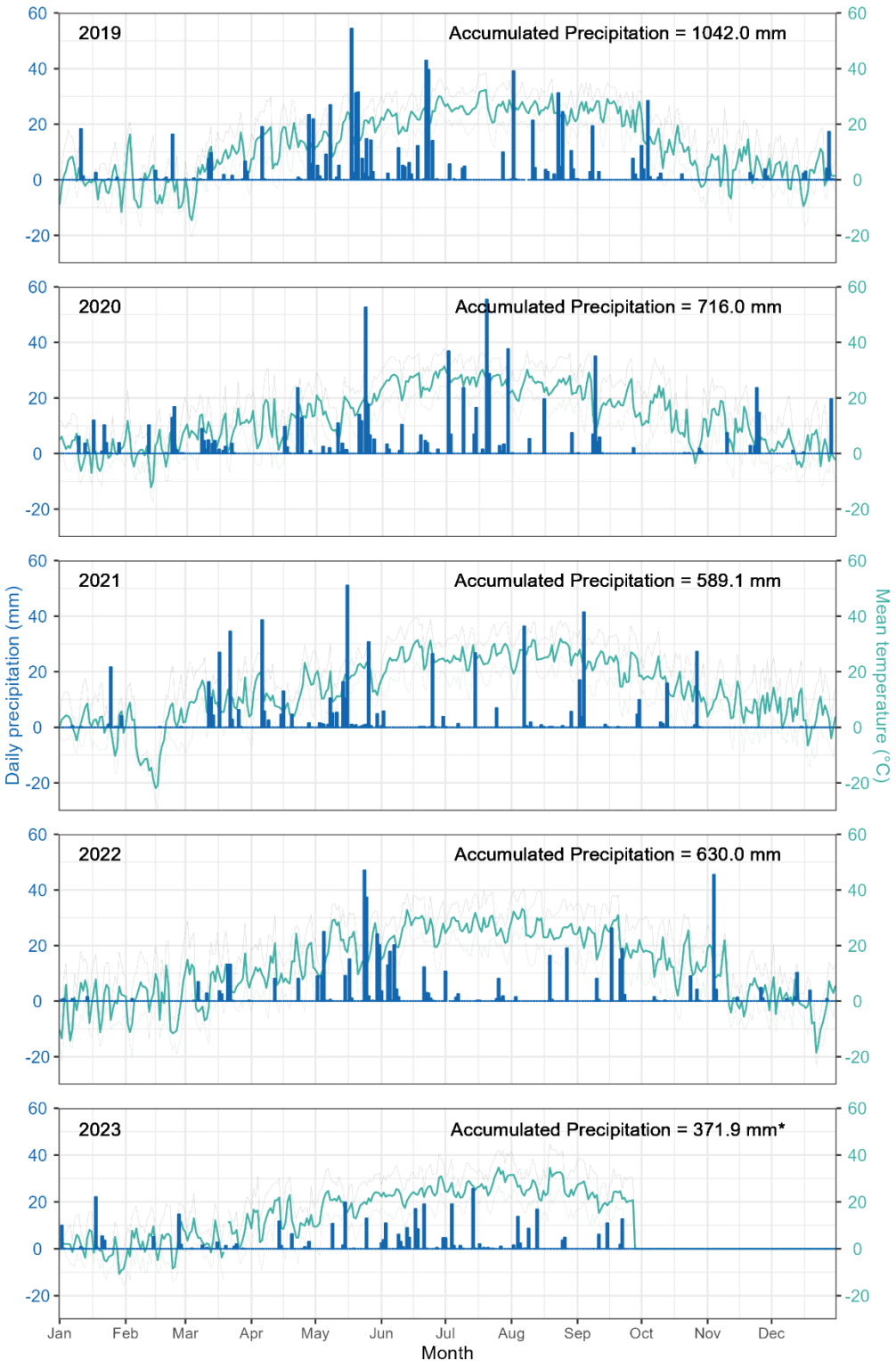


Figure S2. 2. Daily rainfall, cumulative rainfall, and maximum, average, and minimum temperatures recorded during the study years at Solomon, KS.

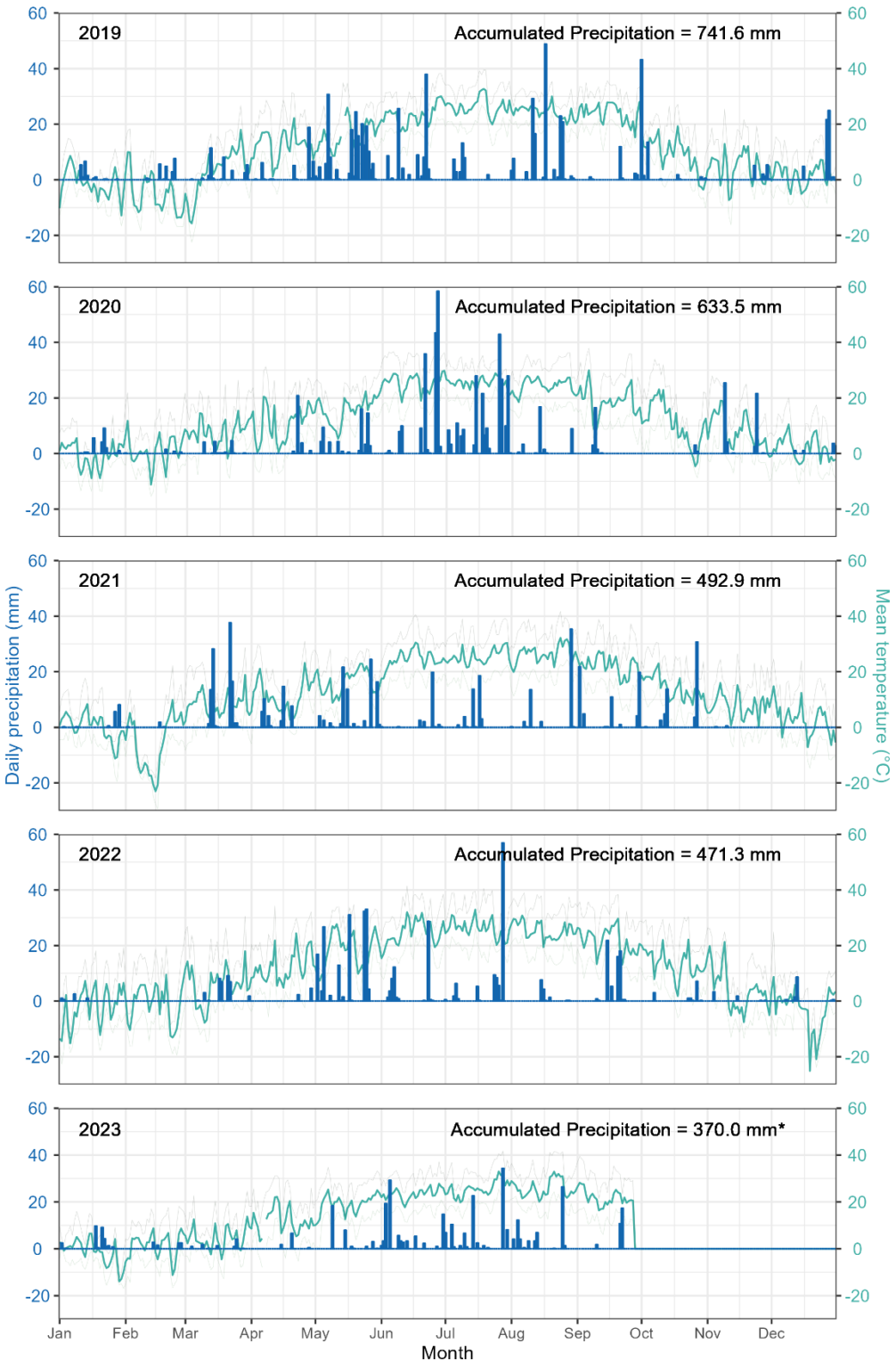


Figure S2. 3. Daily rainfall, cumulative rainfall, and maximum, average, and minimum temperatures recorded during the study years at Beloit, KS.

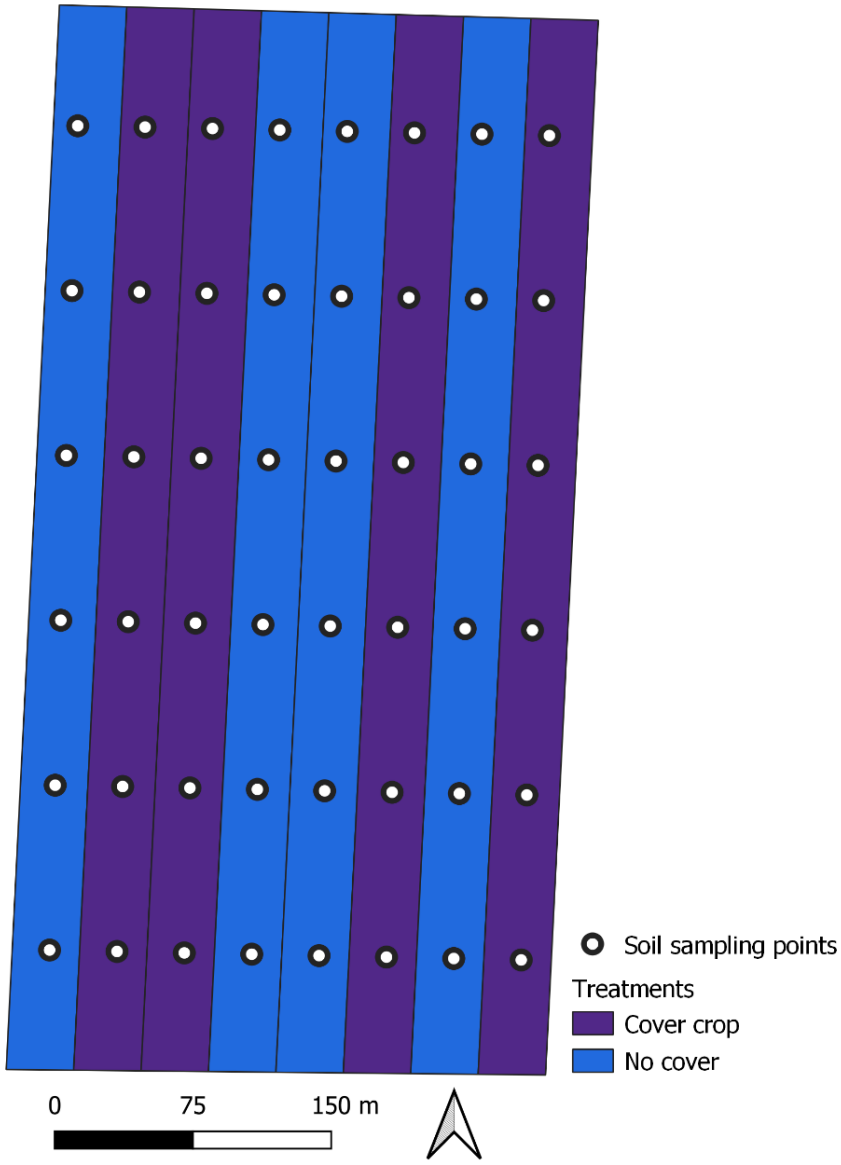


Figure S2. 4. Study experimental design. Bucyrus, Kansas.

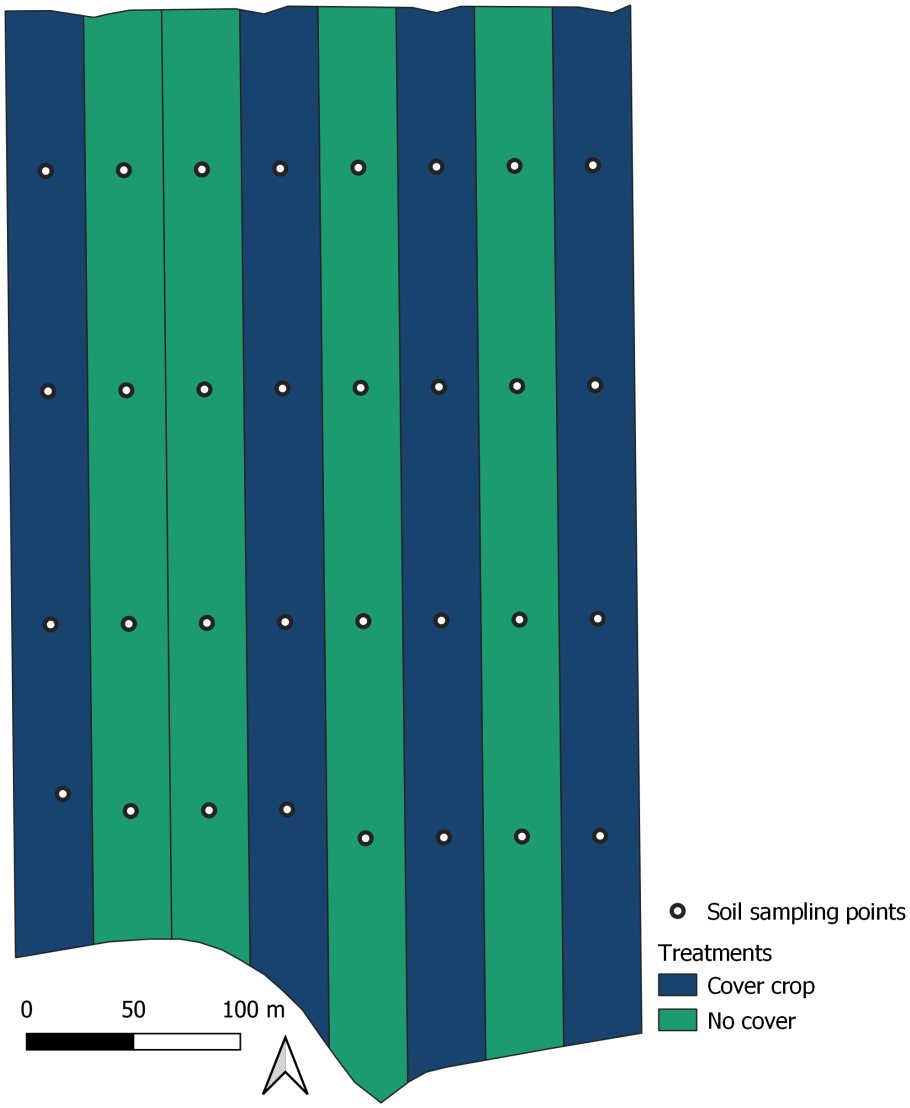


Figure S2. 5. Study experimental design. Solomon, Kansas.

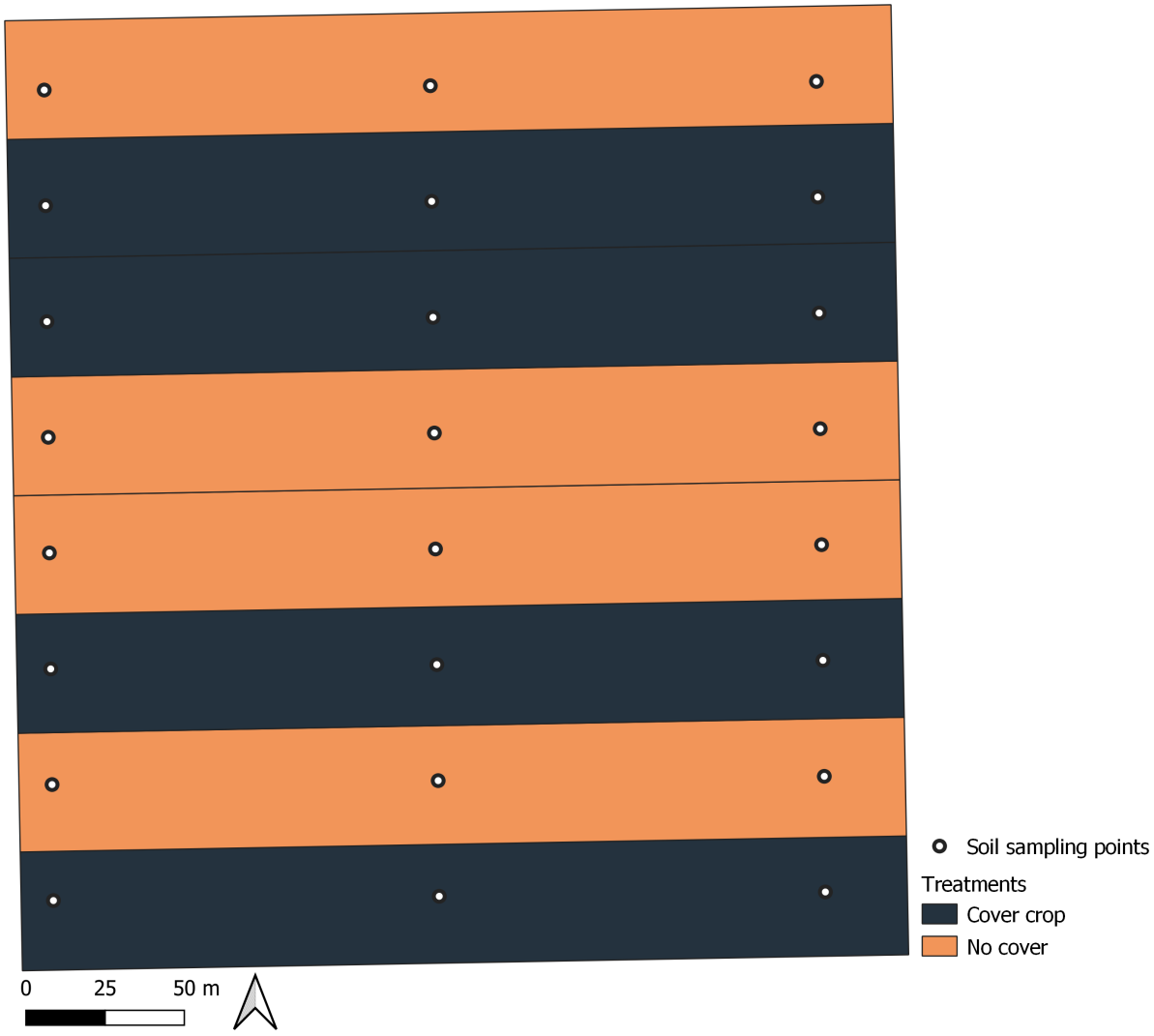


Figure S2. 6. Study experimental design. Beloit, Kansas.

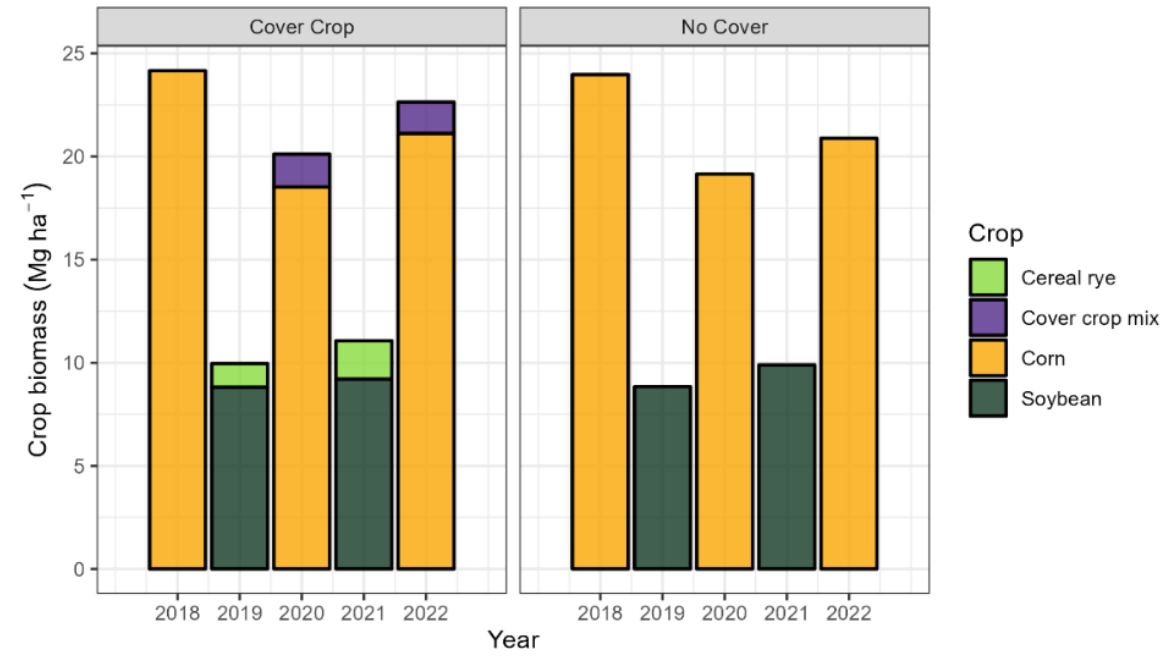


Figure S2. 7. Crop biomass by treatment and year at Bucyrus, KS.

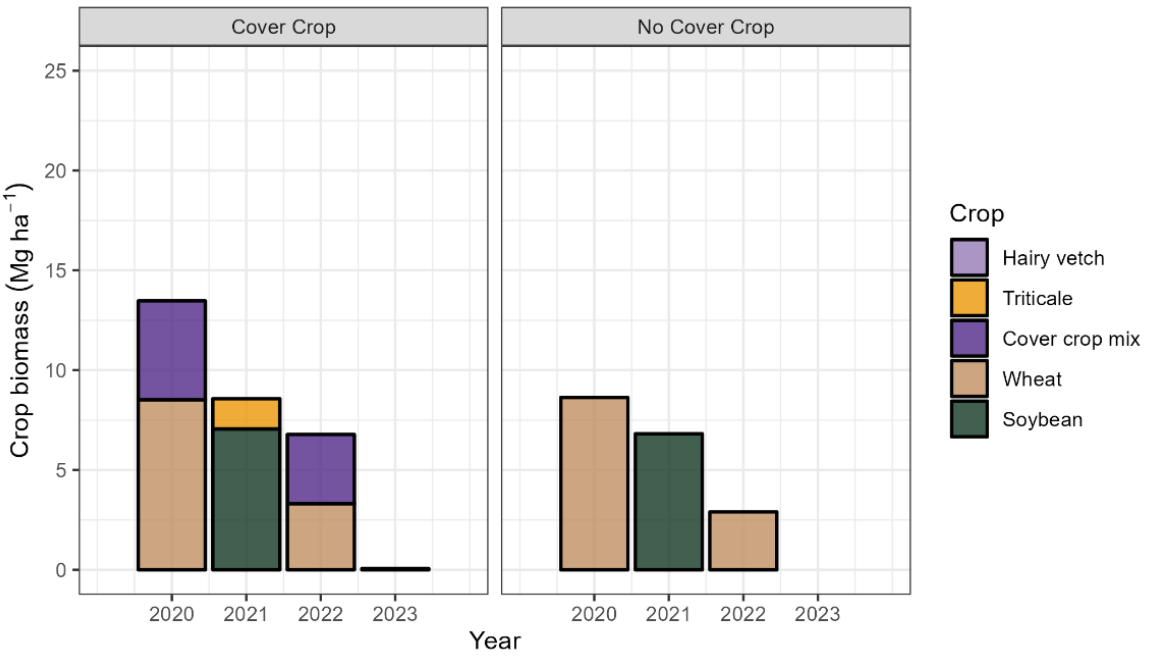


Figure S2. 8. Crop biomass by treatment and year at Solomon, KS.

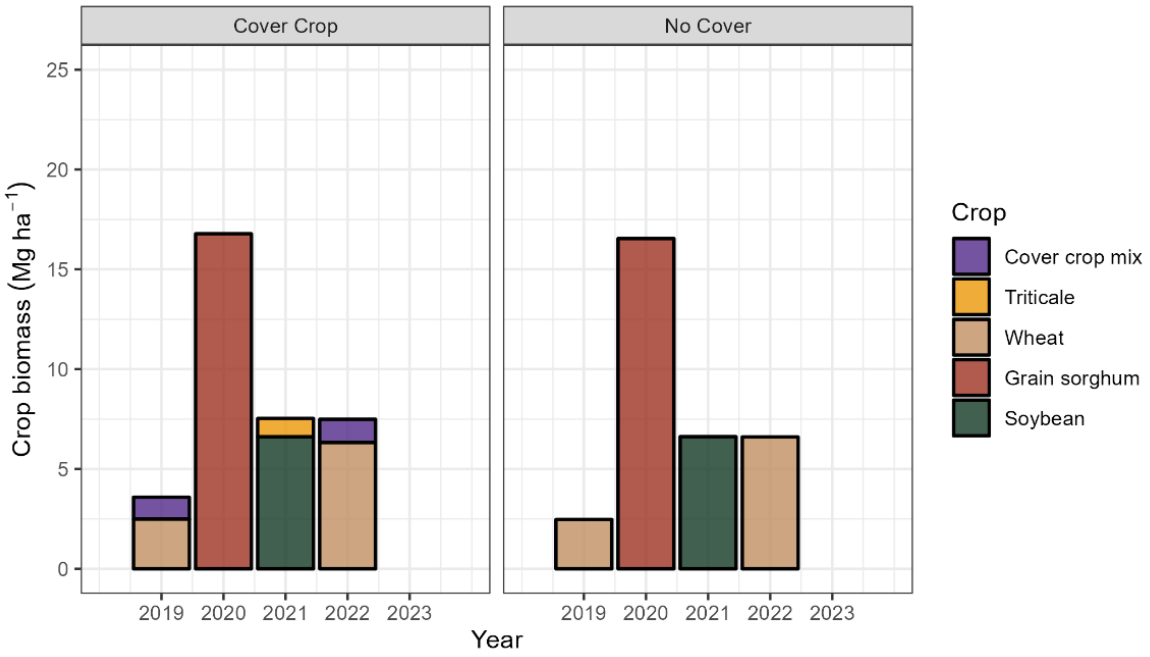


Figure S2. 9. Crop biomass by treatment and year at Beloit, KS.

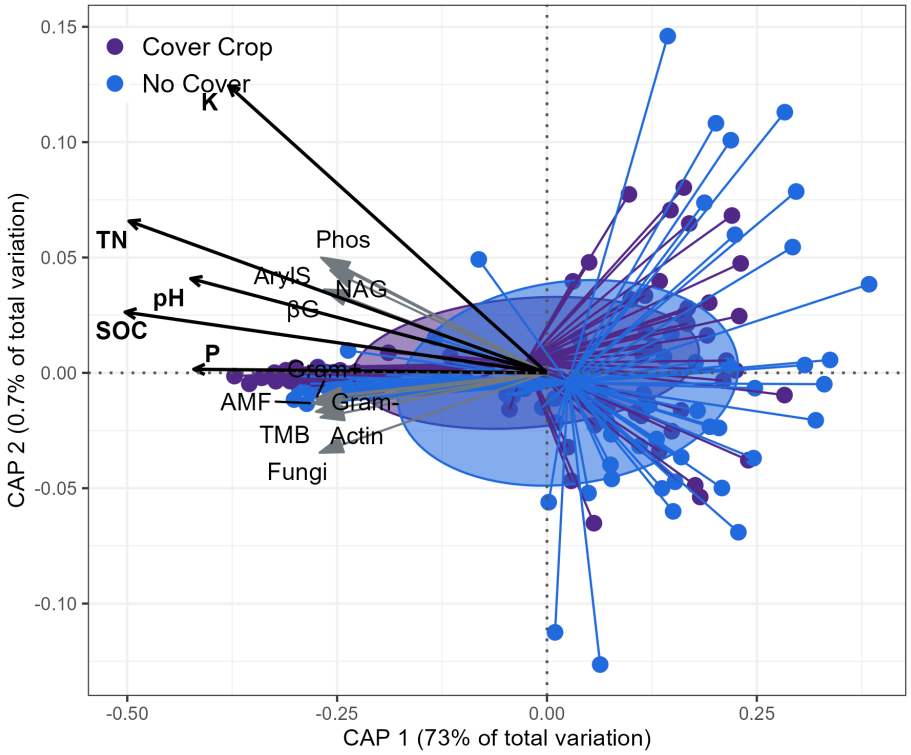


Figure S2. 10. Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activity constrained by soil chemical properties at 0-30 cm at Bucyrus. Microbial biomass (TMB), gram-positive bacteria (Gram+), gram-negative bacteria (Gram-), actinomycetes (Actin), arbuscular mycorrhizal fungi (AMF), saprophytic fungi (Fungi), β-glucosidase (βG), acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS), soil organic carbon (SOC), and total nitrogen (TN).

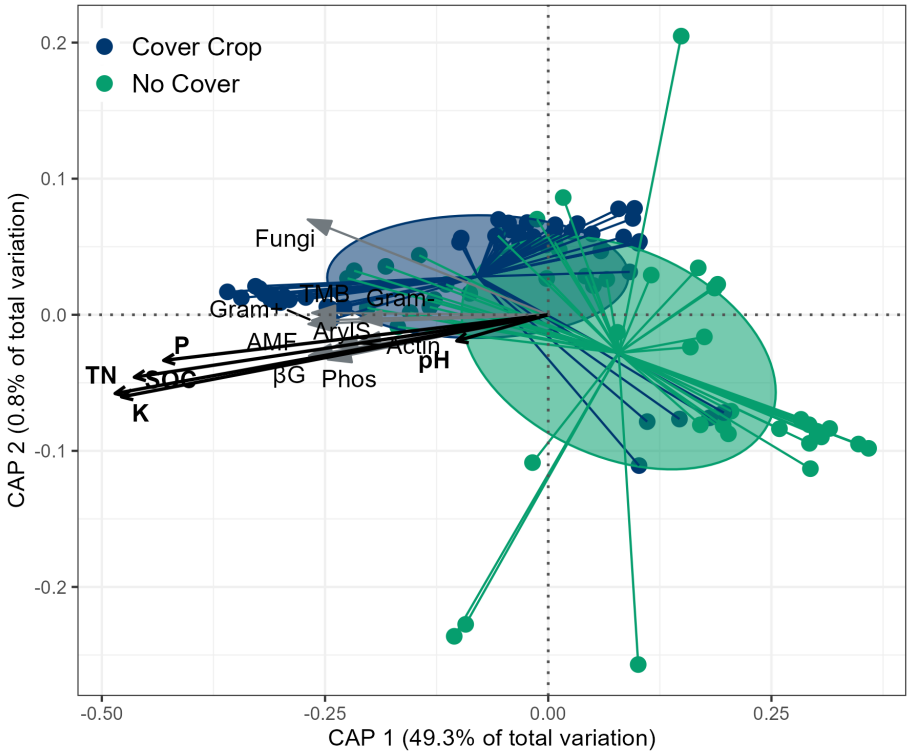


Figure S2. 11. Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activity constrained by soil chemical properties at 0-30 cm at Solomon. Microbial biomass (TMB), gram-positive bacteria (Gram+), gram-negative bacteria (Gram-), actinomycetes (Actin), arbuscular mycorrhizal fungi (AMF), saprophytic fungi (Fungi), β-glucosidase (βG), acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS), soil organic carbon (SOC), and total nitrogen (TN).

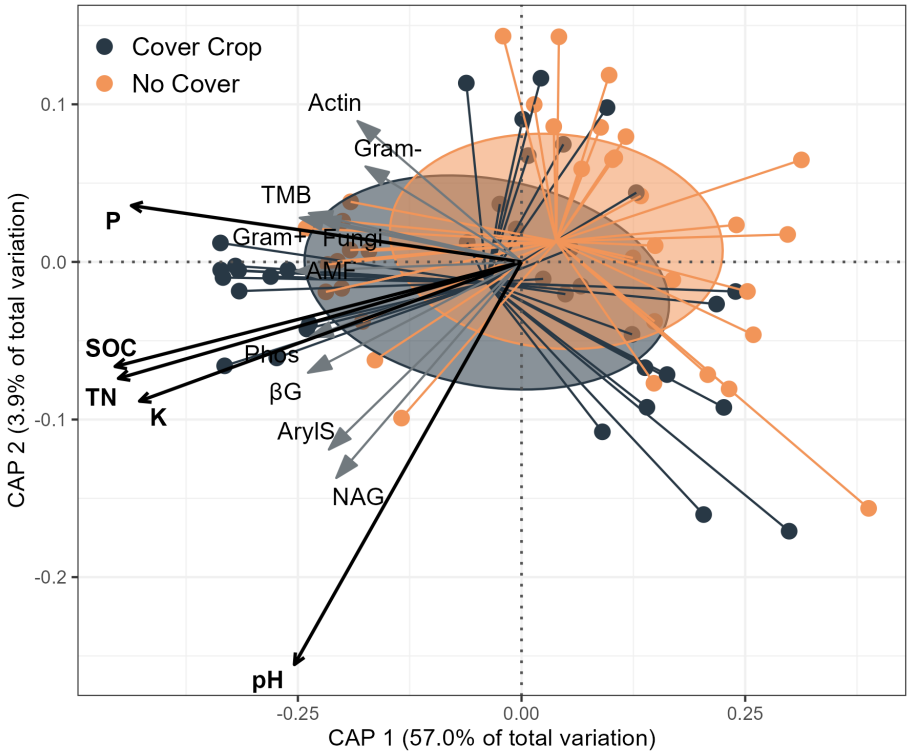


Figure S2. 12. Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activity constrained by soil chemical properties at 0-30 cm at Beloit. Microbial biomass (TMB), gram-positive bacteria (Gram+), gram-negative bacteria (Gram-), actinomycetes (Actin), arbuscular mycorrhizal fungi (AMF), saprophytic fungi (Fungi), β-glucosidase (βG), acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS), soil organic carbon (SOC), and total nitrogen (TN).

Chapter 3 - Amplifying the bond: cover crops strengthening the soil aggregates-microbial community relationship

Abstract

Agricultural practices involving continued tillage and monoculture have disrupted the soil ecosystem equilibrium, resulting in decreased soil health and a higher need for external inputs to maintain crop productivity. In response to these challenges, cover crops can significantly mitigate soil erosion, enhance nutrient cycling, promote microbial growth, and improve soil aggregation. Aggregation is a key indicator of soil structure and affects plant growth through its effect on soil moisture, oxygen diffusion, temperature, and mechanical resistance to root development. Soil microbes like fungi and bacteria are closely related to aggregation as aggregation is biota-driven. This study hypothesized that cover crops enhance the relationship between fungal and bacterial communities and macroaggregate formation. The objectives of this study were to assess (i) the effect of cover crops on soil aggregation, (ii) the effect of cover crops on fungal and bacterial biomass, and (iii) the relationship between soil microbes, cover crops, and macroaggregates. Soil aggregate stability (assessed through wet-sieving) and the biomass of fungi and bacteria (determined via PLFA) were assessed in three different on-farm studies conducted in Kansas. Cover crops consistently increased the amount of macroaggregates, fungi, and bacteria across all sites. Cover crops increased the relationship between soil microbes and aggregation in two out of three sites. Furthermore, this study revealed that the continuous presence of living roots from cover crops is critical to developing and maintaining macroaggregates as affected by increased fungi and bacterial communities.

Introduction

Concerns surrounding soil degradation and its impacts on global food security and agricultural sustainability have become increasingly relevant over the past decades (Amundson et al., 2015; Karlen & Rice, 2015). Agricultural practices involving continued tillage and monoculture have disrupted the soil ecosystem equilibrium, resulting in decreased soil health and a greater need for external inputs to maintain crop productivity (Hatfield, 2012). In response to these challenges, regenerative agricultural practices, including no-tillage and cover cropping, have gained traction (Foley et al., 2011; Pittelkow et al., 2015). Cover cropping involves growing a crop between harvest and planting of the main crop (i.e., cash crop) (Chamberlain et al., 2020), and is expected to reduce soil erosion, improve nutrient cycling, suppress weeds, increase carbon sequestration, and promote microbial growth (Blanco-Canqui, 2022; Kim et al., 2020; Pires et al., 2020).

The soil microbiome plays a crucial role in agroecosystems by influencing essential processes such as nutrient cycling, stress resilience, crop yield, and overall soil health (Gupta & Germida, 2015; Hartmann & Six, 2022). Fungi and bacteria are closely related to soil structure, especially aggregation, as structure aspect governs the flow of water, oxygen, and nutrients throughout the system (Hartmann & Six, 2022; Lehmann et al., 2017). Soil aggregation is viewed as biota-driven (Rillig & Mummey, 2006; Six et al., 2004; Wilson et al., 2009). However, most of the focus has been on management practices such as tillage and the physicochemical factors (e.g., mineralogy, soil carbon) influencing soil aggregation (Lehmann et al., 2017), with limited work on quantification of the role of cover crops on soil aggregation and its interplay with soil microbes.

Aggregation is a key indicator of soil structure (Rabot et al., 2018; Six et al., 2000) and affects plant growth through its effect on soil moisture, oxygen, temperature, and mechanical resistance to root development (Martin et al., 1955; Tonon-Debiasi et al., 2024). According to the aggregate hierarchy and pathway formation, aggregation is mediated by soil organic matter, biota, ionic bridging, clay, and carbonates (Bronick & Lal, 2005; Tisdall & Oades, 1982). Furthermore, larger aggregate units (i.e., macroaggregates) are composed of smaller aggregates (i.e., microaggregates) bonded by living bacteria and fungi, and organic molecules (Lehmann et al., 2017; Martin et al., 1955; Rillig & Mummey, 2006; Swaby, 1949; Tisdall & Oades, 1982). Moreover, roots and fungal hyphae entangle aggregates together and physically protect soil organic carbon (SOC) (Fabrizzi et al., 2009; Mikha & Rice, 2004).

In addition to providing several ecosystem services (Daryanto et al., 2018), cover crops also increase fungal and bacterial biomass through increased soil organic matter and the release of exudates, including sugar, amino acids, and organic acids (Chavarría et al., 2016; Kim et al., 2020). These processes occur mainly at the upper boundary of soil (e.g., 0-5 cm) because the surface is the interface with the atmosphere and is often considered the critical zone (Renée Brooks et al., 2010; Sprenger et al., 2017). Besides being the earliest soil layer to experience the effects of management practices, the soil surface plays a significant role in influencing water infiltration and wind and water erosion (Zhang et al., 2007). Furthermore, it holds a greater proportion of the soil nutrients (Ferreira et al., 2013; Hansel et al., 2017). Thus, we hypothesized that cover crops enhance the relationship between fungal and bacterial communities and the formation of macroaggregates at 0-5 cm soil depth. The objectives of this study were to assess (i) the effect of cover crops on soil aggregation, (ii) the effect of cover crops on fungal and bacterial biomass, and (iii) the relationship between soil microbes, cover crops, and macroaggregates.

Materials and Methods

Study site and field characteristics

On-farm research studies were conducted at three farms across Kansas, U.S., as part of the Kansas Soil Health Partnership. Research was conducted at the Bucyrus site from 2018 to 2022, whereas the Solomon and Beloit sites were between 2019 and 2023. The climate for all three sites was classified as Hot-summer humid continental (Dfa) according to (Peel et al., 2007). Soil classification (Soil Survey Staff, 2014), coordinates, elevation, average annual precipitation, and average mean annual temperature for all three sites are included in Table 3.1. For all sites, the rainfall was concentrated from March to November, with higher intensity between May and July.

Table 3. 1. Site name, location, elevation, soil classification, average annual precipitation, and average mean annual temperature.

Site	Location	Elevation	Soil taxonomy	Average annual precipitation	Average annual temperature
Bucyrus	38.7395N -94.7068W	341 m	Fine, smectitic, mesic Aquertic Argiudoll	998 mm	13°C
Solomon	38.8406N -97.3928W	381 m	Fine, mixed, superactive, mesic Pachic Argiustoll	848 mm	13°C
Beloit	39.3226N -981545W	460 m	Fine, smectitic, mesic Typic Argiustoll	700 mm	12°C

Experimental design and treatments description

The experiments were established as randomized complete block design, with two treatments [cover crop (CC) and no cover crop (NC)] and four replications (strips). Each site strip size was matched to the field and farm equipment. At Bucyrus, the field size was 21.6 ha,

with a strip size of 2.7 ha and a sampling grid of 0.45 ha. At Solomon, the field size was 13.6 ha, with a strip size of 1.7 ha and a sampling grid of 0.43 ha. At Beloit, the field had 8.8 ha, with a strip size of 1.1 ha and a sampling grid of 0.37 ha. All fields were under no-tillage management.

Throughout the five years of study, the cropping system at Bucyrus was corn (2018), cereal rye as CC (2018/2019), soybean (2019), oat, cereal rye, radish, and hairy vetch as a mix of CCs (2019/2020), corn (2020), cereal rye as CC (2020/2021), soybean (2021), cereal rye, rapeseed, winter pea, and hairy vetch as a mix of CCs (2021/2022), corn (2022), cereal rye as CC (2022/2023). Additionally, poultry litter was applied across the entire field at a rate of 600 kg ha-1. In Solomon, the cropping system was winter wheat (2019/2020), pearl millet, sudangrass, sunflower, radish, turnip, and flax as a mix of CCs (2020), triticale as CC (2020/2021), soybean (2021), winter wheat (2021/2022), pearl millet, sudangrass, sunflower, radish, turnip, and flax as a mix of CCs (2022) hairy vetch (2022/2023), and grain sorghum (2023). In Beloit, the cropping system was winter wheat (2028/2019), winter pea, oat, barley, and radish as CC (2019), grain sorghum (2020), triticale as CC (2020/2021), soybean (2021), winter wheat (2021/2022), winter pea, oat, barley, and radish as CC (2022), and grain sorghum (2023). The distinguishing factor among all the sites' treatments was the inclusion or absence of CCs. All implemented crops can be defined as Corn (*Zea mays*), cereal rye (*Secale cereale*), soybean (*Glycine max*), oat (*Avena sativa*), radish (*Raphanus sativus*), hairy vetch (*Vicia sativa*), rapeseed (*Brassica napus*), winter pea (*Pisum sativum*), pearl millet (*Pennisetum glaucum*), sudangrass (*Sorghum bicolor x S. sudanense*), sunflower (*Helianthus annus*), turnip *(Brassica rapa*), flex (Linum usitatissimum), barley (*Hordeum vulgare*), grain sorghum *(Sorghum bicolor*), and winter wheat (*Triticum aestivum*).

Soil sampling

Soil samples were taken at 0-5 cm soil depth in a GPS coordinate sampling grid for the first, third, and fifth year of the study. First year sampling was considered as the baseline. Soil samples for microbial properties were kept in a cooler (4°C) and frozen (-20°C) within 2 hrs after sampling and stored until analysis. Undisturbed samples for aggregate stability analysis were collected from a 30 x 30 x 30 cm soil pit using a shovel. Samples were then partially air-dried to avoid crushing, cleaned of coarse roots, passed through an 8-mm sieve by breaking large aggregates along natural breakpoints, and air-dried for at least 48 hrs.

Sand-free water stable aggregates analysis

To determine sand-free water stable aggregates (WSA), 100 g of soil were weighed and placed into a Yoder wet-sieving machine (Yoder, 1936) for the recovery of all particle fractions as described by Mikha & Rice (2004). Each soil sample was separated into four aggregate size classes (8-2 mm, 2-0.250 mm, 0.250-0.053 mm, and 0.053-0.020 mm diameter). The air-dried soil was placed on top of the 2-mm sieve, stacked atop the 0.250-mm sieve within an oscillation container. Subsequently, 1 liter of distilled water was added to completely immerse the soil for 10 minutes. The oscillation time was 10 minutes, stroke length was 4 cm, and frequency 30 cycles min^{-1}. Following wet-sieving, the soil from the sieves was poured into tins. The remaining water from the oscillation container was poured through 0.053-mm and 0.020-mm sieves before being collected in tins. Floating organic matter was removed in the 2-mm fraction sieve. The individual particle fractions were dried at 50°C until the water completely evaporated. Each dried fraction was then weighed to determine the percentage aggregate size fraction. Aggregates from each treatment were fractionated into macroaggregates (>2 and 0.250–2 mm) and

microaggregate (0.053–0.250 and 0.020–0.053 mm) size classes. Mean weight diameter (MWD) was calculated by the sum of the aggregate mass retained on each sieve multiplied by the mean aperture of adjacent sieves, expressed in mm. The equation below represents the MWD based on the four soil fractions used:

$$\text{MWD (mm)} = \frac{[(8 - 2\ mm\ WSA * 5) + (2 - 0.25\ mm\ WSA * 1.125) + (0.25 - 0.053\ mm\ WSA * 0.1515) + (0.053 - 0.02\ WSA * 0.0365)]}{100}$$

The percentage of sand was measured using a subsample of each aggregate size fraction. Samples were dispersed with 5 g L-1 sodium hexametaphosphate solution using an orbital shaker at 325 RPM for 5 hours. The dispersed organic matter and sand were collected on a 0.053-mm sieve, washed with distilled water, and dried at 105°C for 72 hours. The weight of the collected sand was recorded for sand correction.

Phospholipid fatty acids analysis (PLFA)

Phospholipid fatty acid (PLFA) analysis was performed with modifications to the original procedure (White & Ringelberg, 1998) to assess the soil microbial community composition. Total lipids were extracted with 10 mL of methanol, 5 mL of chloroform and 4 mL of phosphate buffer (pH 7.4) on 5 g freeze-dried soil. Water and chloroform were added after 3 hours to separate the mixture into polar and non-polar fractions. Total lipids remained in the non-polar phase. Phospholipids were isolated from neutral lipids and glycolipids by using silicic acid chromatography columns (Disposable BAKERBOND® SPE Columns, J.T. Baker®, Avantor, Radnor, Pennsylvania, USA) and eluted with methanol. The phospholipids were then saponified with KOH, methylated to fatty acid methyl esters (FAME) and analyzed by Thermo Scientific

Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Helium was used as carrier gas. FAME peaks were recognized by different retention times in comparison with the bacterial acid methyl esters mix (BAME; Matreya 1,114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). Internal standard 19:0 FAME was used to determine concentrations. A total of 30 biomarkers were identified for all samples. Microbial groups were assigned based on characteristics of the biomarkers: iso and ante-iso branches often belong to Gram-positive bacteria (Gram+); monosaturated and cyclopropyl lipids often belong to Gram-negative bacteria (Gram-); actinobacteria (Actino) have more methyl-branched fatty acids; and methyl linoleate typically belong to fungi, as describe by Pires et al. (2020). Phospholipid fatty acid abundance was reported as nmol per gram of dry soil (nmol PLFA g−1 soil). Total bacteria (TB) were the sum of Gram-positive bacteria, Gram-negative bacteria and actinomycetes. Total fungi (TF) were the sum of arbuscular mycorrhizal fungi (AMF) and saprophytic fungi.

Statistical analysis

The R Programming software (R Core Team, 2023) was used for all the analyses and figure production. Prior to statistical analysis, the dataset was spatially transformed to a sf object using x (longitude) and y (latitude) coordinates from the GPS sampling points (Pebesma & Bivand, 2023). The effect of treatment (CCs) and year, and their respective interactions (fixed effects) on the response variables was analyzed by ANOVA after fitting a linear mixed effects model using the nlme R package (Pinheiro et al., 2017) for each depth. Blocks were set as random effects. The normality of the residuals was tested using the Shapiro–Wilk test, and square-root transformation was applied when necessary. Models were tested with exponential, spherical, gaussian, and rational spatial correlation structures without nugget effect using a

maximized log-likelihood method (Pinheiro et al., 2017). Model selection for correlation structure was performed following the Akaike information criterion (AIC). Model means were extracted using the emmeans R package (Lenth, 2023) and a pairwise comparison was used to determine significant differences among treatments for all the fixed effects presenting a significance equal to or lower than 0.05 using Fisher's LSD (multcomp R package) (Hothorn et al., 2008).

A bivariate regression analysis was employed to assess the influence of the fungal and bacterial community on soil macroaggregates as affected by treatment and year. This analysis was conducted to quantitatively examine the relationship between soil microorganism levels and soil aggregation, considering the presence and impact of CCs.

Results

Aggregates size distribution

At Bucyrus, there was a two-way interaction between treatment and year for WSA >2 mm, a year effect for WSA 2-0.250 mm, and a separate effect of both treatment and year for WSA 0.250-0.053 mm and 0.053-0.020 mm (Table S3.1). While there was no increase in WSA >2 mm for NC, CC increased >2 mm aggregates by 145% from 2018 to 2022 (Figure 3.1). The 2-0.250 mm fraction increased by 24% (Figure 3.1). No cover crop also had a lower amount of 0.250-0.053- and 0.053-020 mm aggregates than CC (Figure 3.1). The increase in the first two aggregate size classes is expected to lead to a decrease in smaller classes, driven by the need for aggregate size balance.

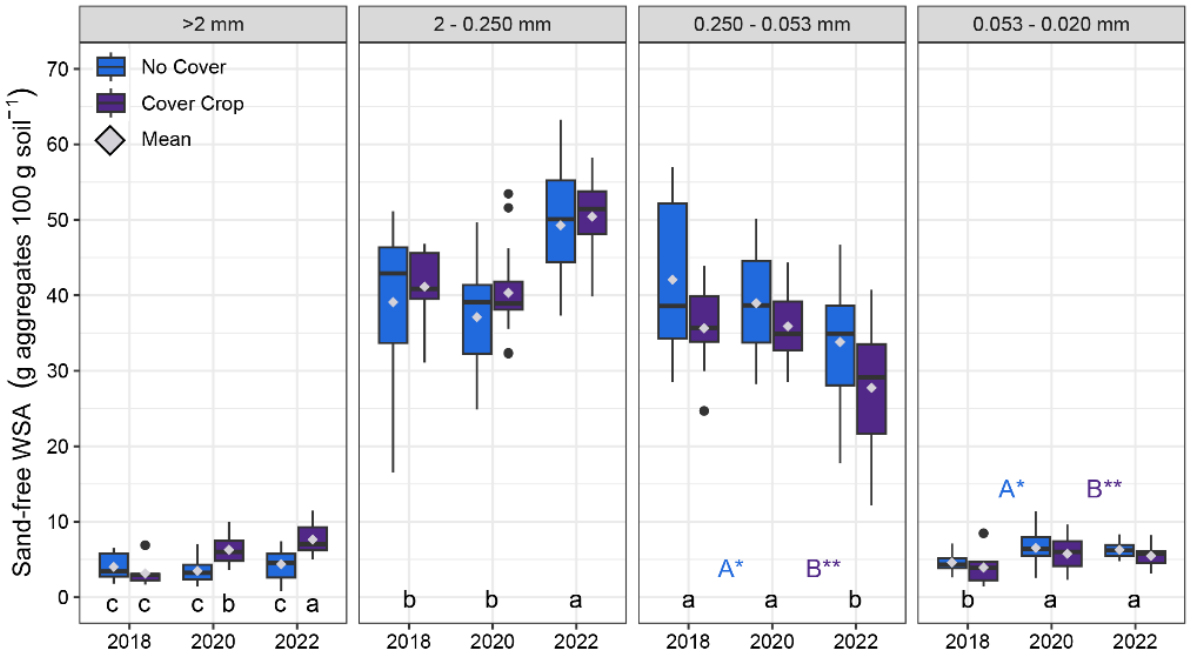


Figure 3. 1. Sand-free water stable aggregate (WSA) distribution by treatment and year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level. Lowercase letters compare the interaction or year effect, while capitalized letters compare the treatment effect: *No cover, **Cover crop.

At Solomon, there was a two-way interaction between treatment and year for WSA >2 mm, 2-0.250 mm, and 0.250-0.053 mm, and a year effect only for 0.053-0.020 mm (Table S3.2). The proportion of >2 mm and 2-0.250 mm aggregates were higher for CC than NC and increased by 47% and 25% over time, respectively (Figure 3.2). As the WSA 2-0.250 mm class increased, the WSA 0.250-0.053 mm decreased for CC. There was no significant difference in the smallest WSA class comparing 2019 and 2023 for the NC and CC treatments (Figure 3.2).

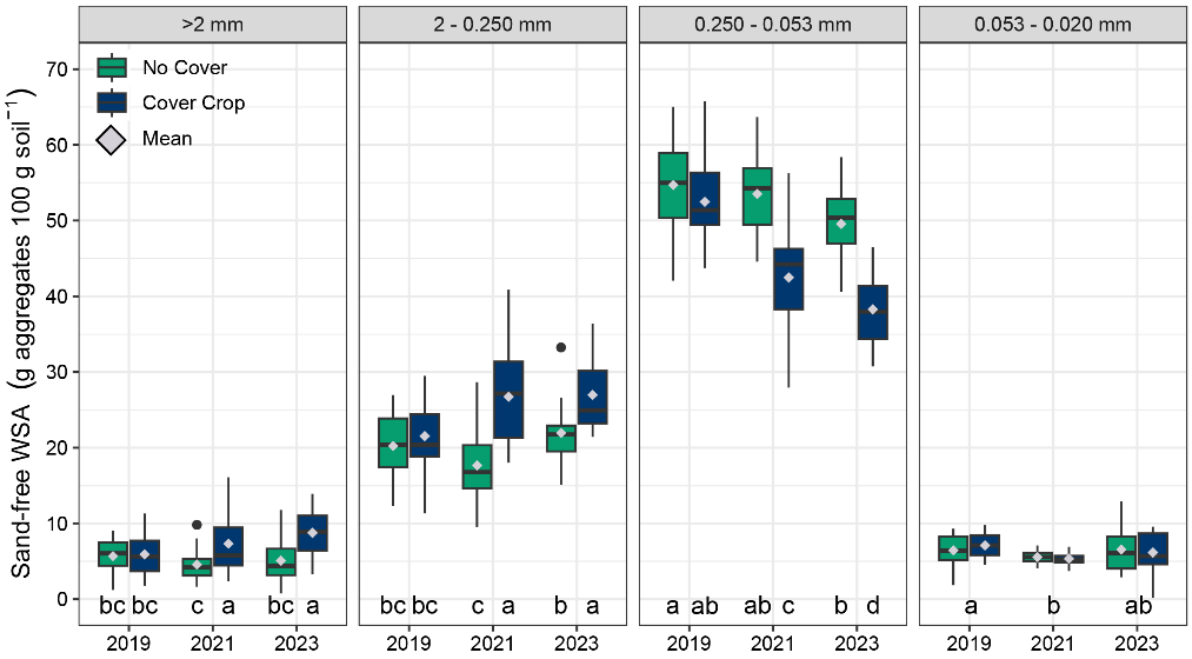


Figure 3. 2. Sand-free water stable aggregate (WSA) distribution by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Cover crops also significantly increased the amount of WSA >2 mm (385%) and 2-0.250 mm (29%) over time at Beloit (Table S3.3; Figure 3.3). Like Solomon, there was no significant difference in the smallest WSA class comparing 2019 and 2023 for both NC and CC treatments (Figure 3.3).

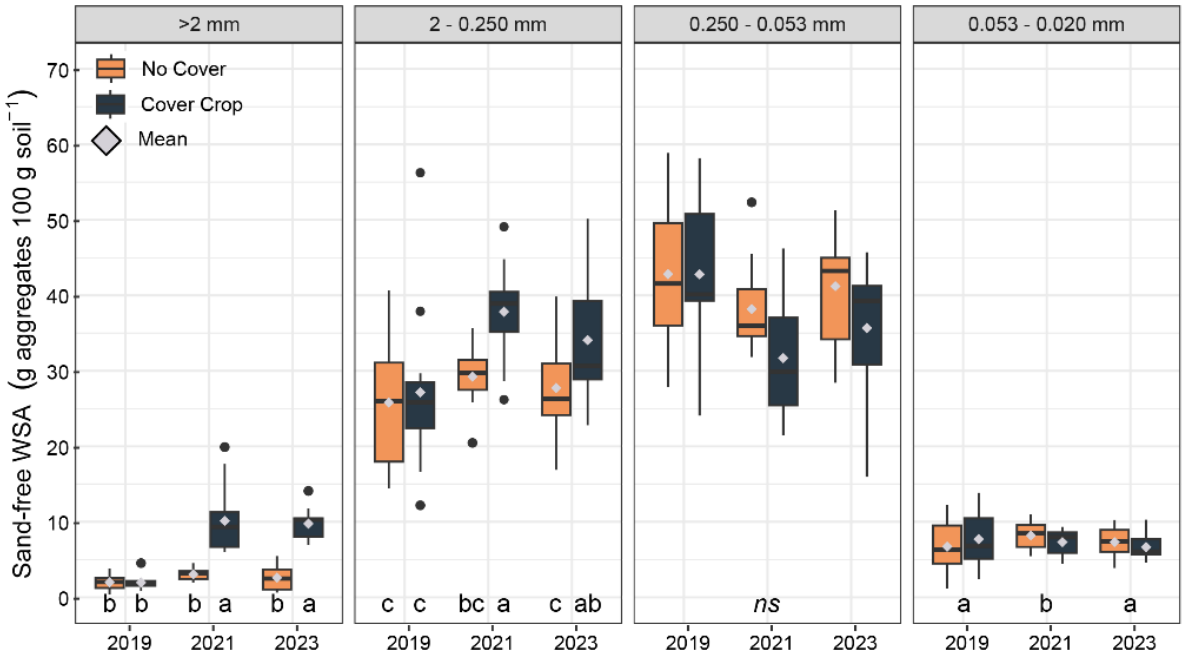


Figure 3. 3. Sand-free water stable aggregate (WSA) distribution by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Soil macroaggregates represent the sum of the WSA >2 mm and 2-0.250 mm classes. At Bucyrus, macroaggregates were significantly increased by both CC (8%) and year (28%) (Table S3.1; Figure 3.4A). For both Solomon and Beloit, a significant two-way interaction between treatment and year was observed (Table S3.1). This interaction resulted in an increase in macroaggregates with CC from 2019 to 2021, with no further increase from 2021 to 2023 (Figure 3.4B; C).

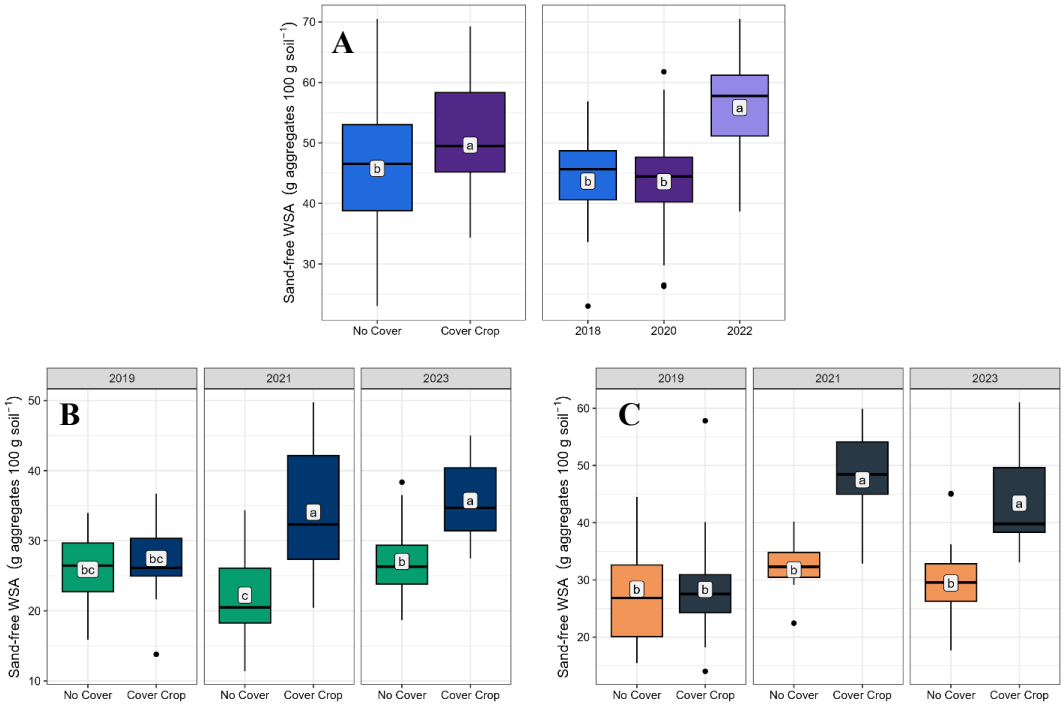


Figure 3. 4. Macroaggregates by treatment and year at 0-5 cm depth at Bucyrus (A), Solomon (B), and Beloit (C). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Soil microbial community

Similarly to macroaggregates, there was a significant increase in total fungi with CCs over time (Table S3.1) for all sites. At Bucyrus, there was a yearly increase in total fungi for NC of 2.6x, whereas for CC, there was an increase of 4.2x (Figure 3.5A). Furthermore, in 2022, total fungi were 41% greater with CC than NC (Figure 3.5A). Likewise, CCs significantly increased total fungi over time by 63% in Solomon and 55% in Beloit (Figure 3.5B; C). Total bacteria also significantly changed over time with CC for all sites (Table S3.1). At Bucyrus, total bacteria increased by 4.2x with NC and 5.3x with CC. Yet, in 2022, CC had a 24% higher total bacteria (Figure 3.6). For Solomon and Beloit, CCs increased total bacteria by 101% and 69%, respectively.

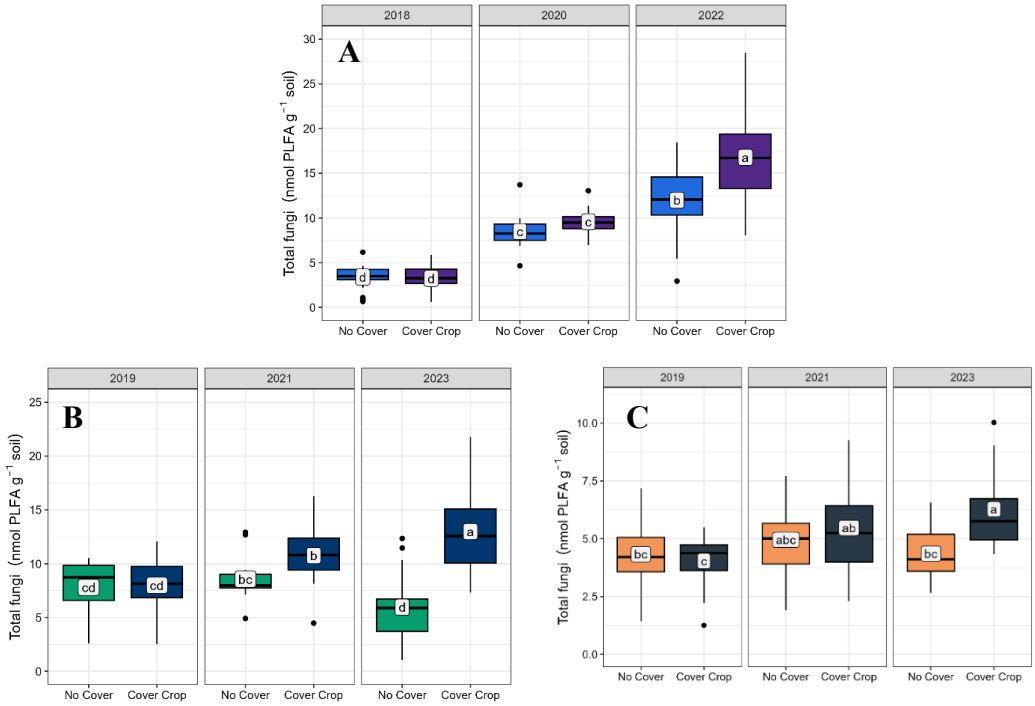


Figure 3. 5. Total fungi by treatment and year at 0-5 cm depth at Bucyrus (A), Solomon (B), and Beloit (C). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

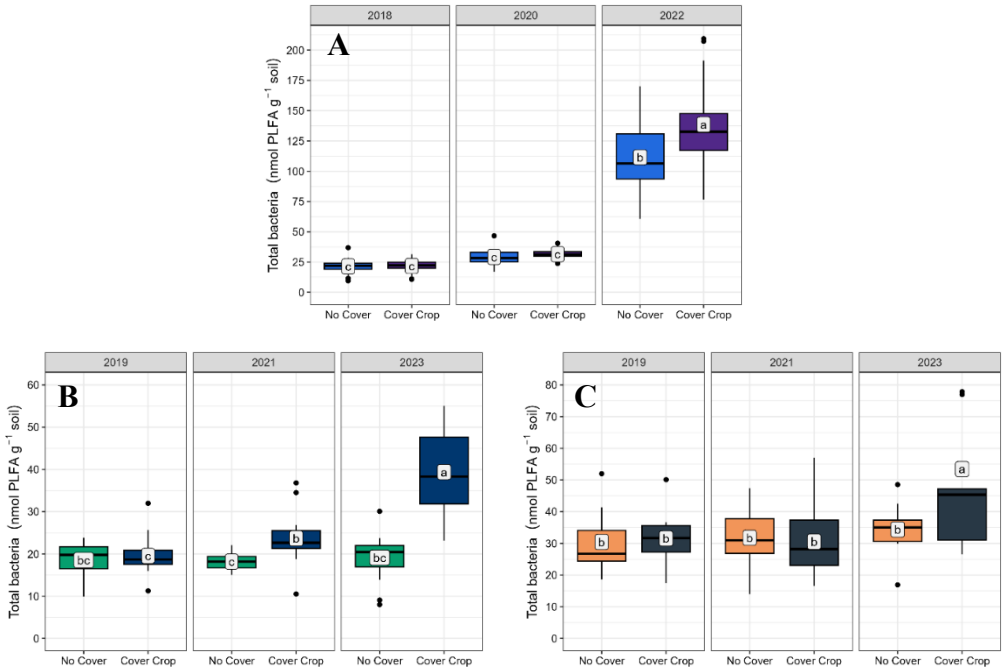


Figure 3. 6. Total bacteria by treatment and year at 0-5 cm depth at Bucyrus (A), Solomon (B), and Beloit (C). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Soil microbial community and macroaggregates relationship

Cover crops significantly strengthened the relationship between total fungi and macroaggregates at Bucyrus ($p = <0.001$; $R^2 = 0.47$, RMSE = 9.7) and Solomon ($p = 0.026$; $R^2 = 0.10$; RMSE = 7.4) (Figure 3.7A; B; Table S3.3). The linear model was not significant for Beloit (Figure 3.7C; Table S3.3). At Bucyrus, NC also increased the relationship between total fungi and macroaggregates ($p = 0.009$; $R^2 = 0.12$; RMSE = 6.0) (Figure 3.7A), whereas NC did not significantly affect this relationship in Solomon and Bucyrus (Figure 3.7B; C; Table S3.3).

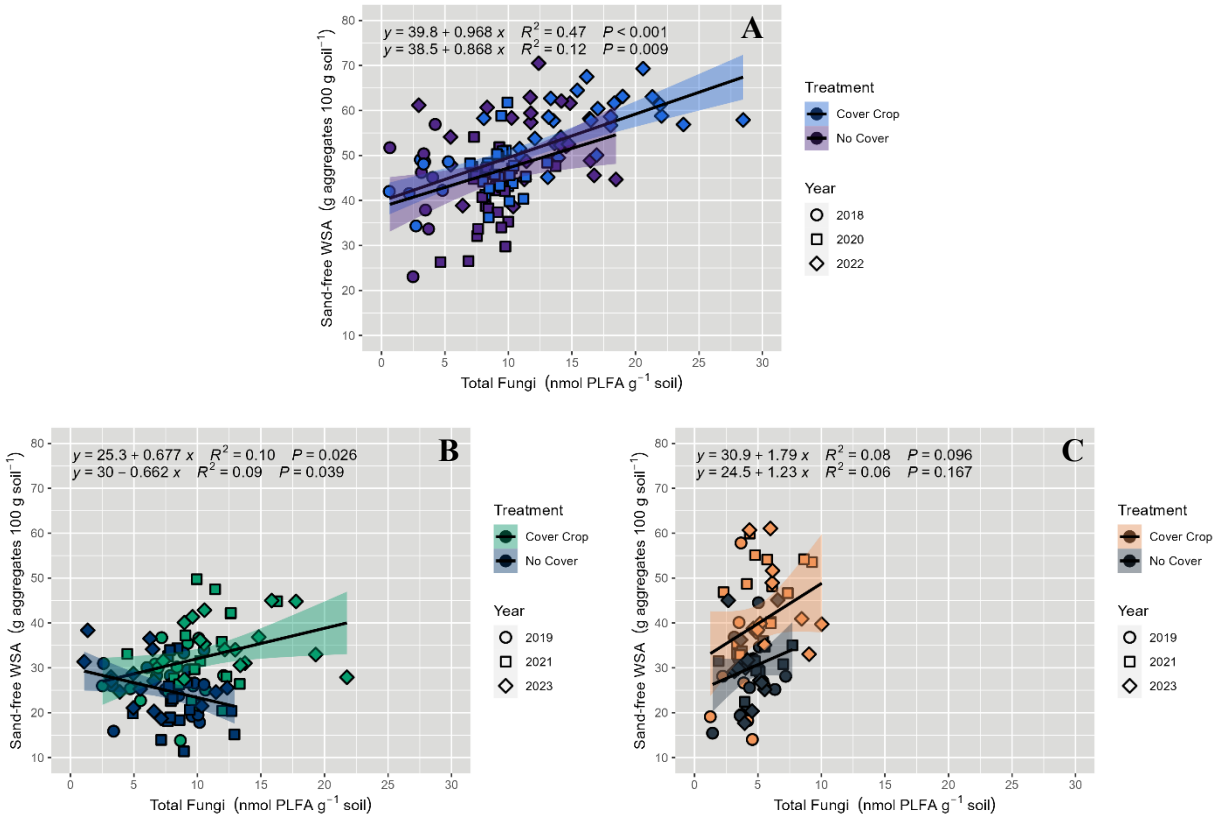


Figure 3. 7. Relationship between total fungi and macroaggregates as affected by treatment and year at 0-5 cm depth at Bucyrus (A), Solomon (B), and Beloit (C).

Similarly, CCs also improved the relationship between total bacteria and macroaggregates at Bucyrus ($p = <0.001$; $R^2 = 0.51$; RMSE = 5.8) (Figure 3.8A; Table S3.4) and Solomon ($p = 0.012$; $R^2 = 0.13$; RMSE = 7.3) (Figure 3.8B; Table S3.4). At Bucyrus, NC also significantly affected the relationship between total bacteria and macroaggregates, albeit with a lower R^2 ($p = <0.001$; $R^2 = 0.32$; RMSE = 8.5) (Figure 3.8A). No significant interaction between total bacteria, macroaggregates, and NC was found at Solomon and Beloit (Figure 3.8B; C; Table S3.4).

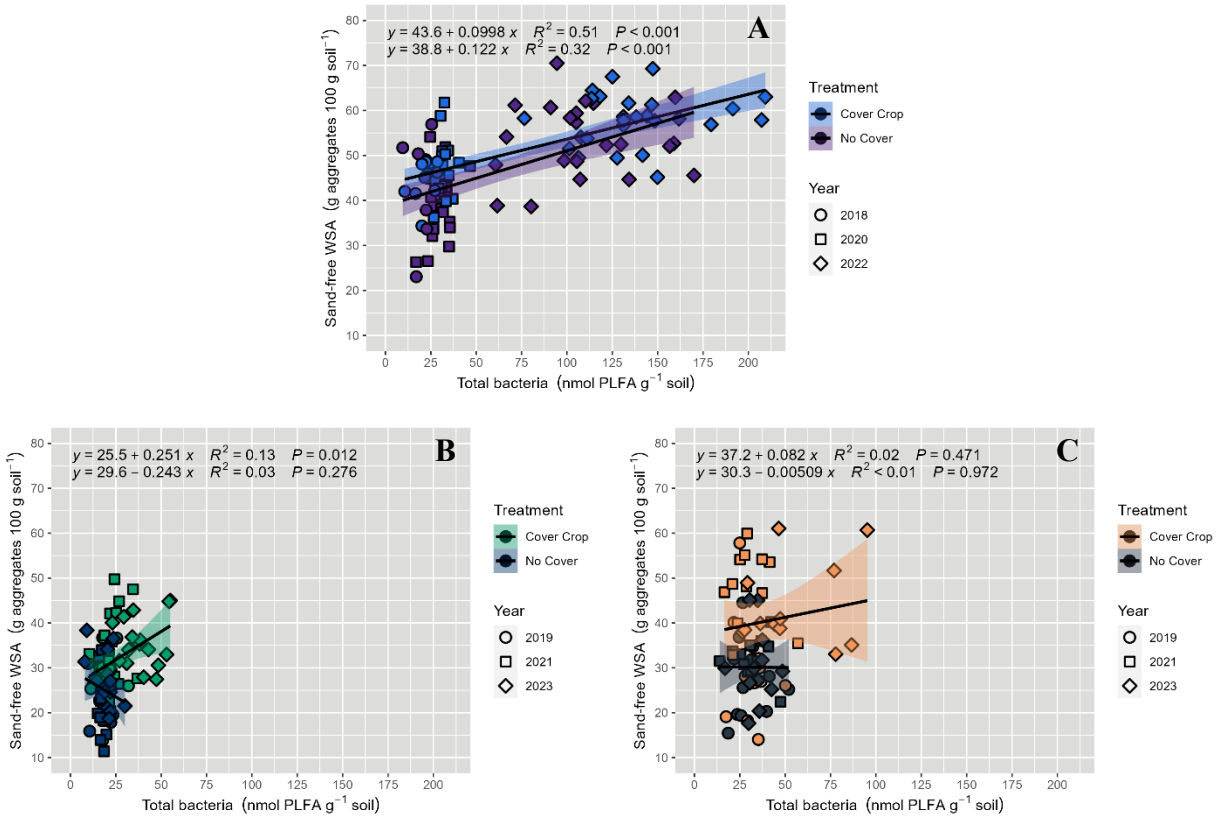


Figure 3. 8. Relationship between total bacteria and macroaggregates as affected by treatment and year at 0-5 cm depth in Bucyrus (A), Solomon (B), and Beloit (C).

All paired correlations between total fungi, total bacteria, SOC, macroaggregates, and MWD were significant ($p<0.001$) (Figure 3.9). As expected due to autocorrelation, MWD had a high correlation (0.760) with macroaggregates. Likewise, macroaggregates were positively correlated with SOC (0.620), total fungi (0.433), and total bacteria (0.646).

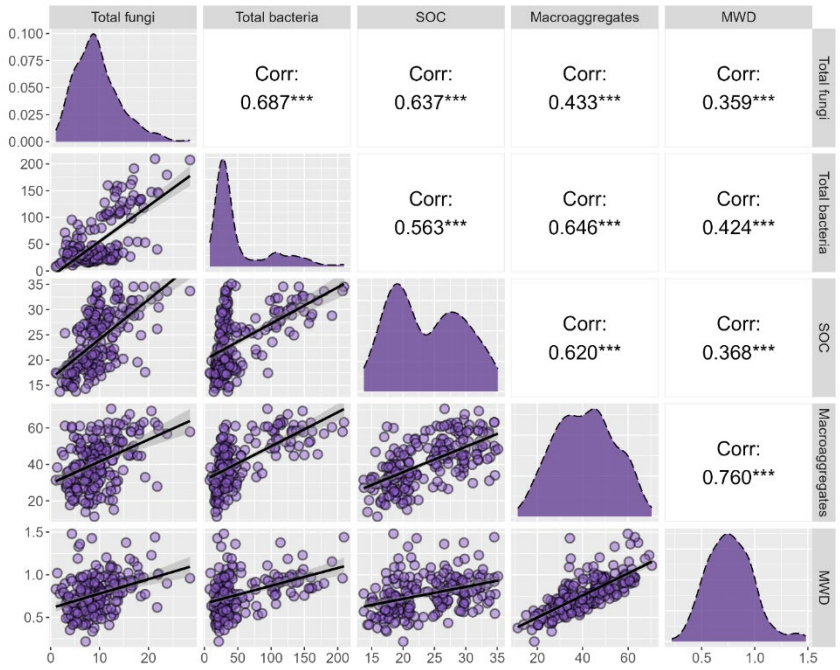


Figure 3. 9. Pearson correlation matrix between total fungi, total bacteria, soil organic carbon (SOC), macroaggregates, and mean weight diameter (MWD).

Discussion

Despite the differences in precipitation and plant biomass production among sites (Chapter 3), CC consistently increased the amount of WSA >2 mm and 2- 0.250 mm relative to NC after 5 yrs. Similar results have been reported in the literature by several authors (Blanco-Canqui & Jasa, 2019; Blanco-Canqui & Ruis, 2020; Liu et al., 2005; Nascente et al., 2015; Wood & Bowman, 2021). In a study conducted by Liu et al. (2005), CCs such as cereal rye and annual ryegrass increased the amount of WSA. Likewise, Dapaah & Vyn (1998) found that soil aggregate stability was greater following CC than under NC. In a review of CC impacts on soil physical properties, Blanco-Canqui & Ruis (2020) reported that CCs increased the percentage of macroaggregates up to 22% and MWD up to 95%. In most cases, the increase in macroaggregation was reported to be directly linked to increased SOC (e.g., humus) and root

biomass. Our study also found a positive correlation between macroaggregates and SOC and between MWD and SOC (Figure 3.9).

Cover crop species also impact soil aggregation due to different root properties, exudates, and functions (Bronick & Lal, 2005). The effect of CCs on macroaggregates and the lack of effect on microaggregates in our study may be explained by the concept of aggregate hierarchy, where roots play a bigger role in entangling microaggregates that will then form macroaggregates (Bronick & Lal, 2005; Tisdall & Oades, 1982). These results suggest that grass CCs are likely to produce more aboveground and belowground biomass than legume CCs and NC, which can be supported by the findings presented in Chapter 3. Grasses are expected to have a greater positive effect than legumes and brassicas due to their extensive and dense fibrous root system (Blanco-Canqui & Jasa, 2019; Chan & Heenan, 1999; Haynes & Beare, 1997). For instance, Sainju et al. (2022) found more WSA 0.85 – 2 mm with cereal rye than hairy vetch and crimson clover. Similar results were found by Blanco-Canqui & Jasa (2019) in a 12-year CC study in Nebraska, U.S., under a silty clay loam Mollisol. The authors reported more macroaggregates and higher MWD with grass CC than legume CC and NC.

Although most soil physical properties have slow change rates in response to soil management, wet aggregate stability often changes rapidly near the soil surface (Blanco-Canqui & Ruis, 2020). This management susceptibility may explain the increase in soil macroaggregates after two years of CCs at Solomon and Beloit and four at Bucyrus. Nonetheless, due to the poultry litter application, Bucyrus had higher macroaggregate formation. As observed by Krupek et al. (2022), Mikha & Rice (2004), and Wortmann & Shapiro (2008), manure or other sources of C-rich materials increase aggregate stability.

Soil microorganisms are pivotal in agroecosystems and are a critical component of soil health (Fierer et al., 2021). Overall, the introduction of CCs increased both total fungi and total bacteria. However, the magnitude of this change depended upon site-specific factors such as SOC and cropping system intensity and diversity (Chapter 3). The continuous presence of living roots from CC throughout most of the intercropping period provides exudates and organic compounds such as polysaccharides and proteins (Kamilova et al., 2006) that significantly contribute to increased microbial growth and biomass (Chavarría et al., 2016; Loveland & Webb, 2003). Beyond the presence of living roots, crop residue is a crucial element in fostering microbial growth by regulating soil temperature and moisture and creating a more favorable habitat for microbes (Pires et al., 2020; Wang et al., 2011). At Bucyrus, the significant increase in total fungi and total bacteria can also be attributed to the poultry litter application. In a similar study, Acosta-Martínez & Harmel (2006) also found an increased microbial population after applying poultry litter. Yet, the increase in total bacteria was higher than in total fungi. This can be explained by the better adaptability of bacteria to the metabolism of easily decomposed organic matter (e.g., poultry litter). In contrast, fungi have an advantage in soils with high contents of recalcitrant organic matter (e.g., cellulose and lignin) (X. Liu et al., 2023).

The organic compounds supplied to soil from the roots of CCs combined with the root senescence may have resulted in the production of large amounts of active glycoproteins and polysaccharide binding agents in the soil (Rillig & Mummey, 2006; Wright & Upadhyaya, 1996). Greater SOC and potentially direct plant root C inputs (Lange et al., 2015; Lu et al., 2002) supplied by CCs also promoted greater fungal and bacterial biomass, which may produce extracellular mucilaginous polysaccharide compounds that bind and stabilize soil aggregates (A. Liu et al., 2005; Roberson et al., 1991). Equally important, fungal hyphae improve aggregation

stability by reorienting clay particles, binding particles with extracellular polysaccharides, and trapping particles (Bronick & Lal, 2005). In addition to hyphae, AMF is one of the most important factors associated with greater aggregation due to the release of glomalin (Rillig & Mummey, 2006; Wilson et al., 2009). In a tallgrass prairie, Wilson et al. (2009) found a linear relationship ($p = <0.0001$; $R2 = 0.82$) between AMF and macroaggregates, and the loss of AMF resulted in a loss in soil aggregation. Arbuscular mycorrhizal fungi can form symbiotic relationships with a wide range of plant species (Bender et al., 2014; Sanders, 2002). However, some crops such as canola and forage radishes, are non-mycorrhizal (Murrell et al., 2020). The cropping system implemented in Bucyrus and Solomon had AMF-friendly crops such as sorghum, millet, cereal rye, oats, and sunflowers (Hontoria et al., 2019; Moura et al., 2022; Murrell et al., 2020; Tosi et al., 2023) and may have positively impacted AMF development and soil aggregation. The cropping system at Beloit had radish as an essential component in the rotation as part of the cover crop mix. The presence of radish may have negatively impacted AMF and, consequently, the relationship between total fungi and macroaggregates. Thus, the combination of positive factors may explain the significant correlation between both total fungi and bacteria, macroaggregates, and CCs at Bucyrus and Solomon. Moreover, more SOC and higher aggregate stability results in higher root growth, creating a win-win scenario for microbes, CCs, and aggregates (Haruna et al., 2020).

Keeping living roots in the system is crucial for maintaining and developing macroaggregates. Several authors have reported that the positive effect of CCs on WSA can be short-lived following CC termination. For instance, García-González et al. (2016) found more macroaggregates under CCs after 54 days but not after 69 days of CC termination. Blanco-Canqui et al. (2013) also found WSA to have a higher geometric mean diameter under CCs after

2 months but not after 9 months of CC termination. On the one hand, SOC, microbial products, and soil minerals have a stronger effect on microaggregates (< 0.250 mm). On the other hand, besides SOC and microbial products, macroaggregates are greatly dependent on roots and fungal hyphae. Thus, due to the temporary and transient nature of these products, macroaggregate stability is very sensitive to agricultural management practices (Chan & Heenan, 1999). In our study, the continuous presence of living roots in the soil emerged as the key factor explaining why cover crops increase soil aggregates-microbial community relationship.

Conclusion

This study has provided valuable insights into the impact of CC on soil aggregation and microbial communities, highlighting the relationship between these factors. Cover crops consistently increased the amount of macroaggregates, fungi, and bacteria across all sites. Furthermore, this study revealed that the continuous presence of living roots from CCs significantly impacted the development and maintenance of macroaggregates as affected by increased fungi and bacterial communities. Finally, our research findings can guide farmers and land managers in optimizing their CC strategies to support more resilient agricultural systems.

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Supplementary material

Table S3. 1. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on water stable aggregates (WSA) >2 mm, 2 – 0.250 mm, 0.250 – 0.053 mm, 0.053 – 0.02 mm, macroaggregates, mean weight diameter (MWD), total fungi, and total bacteria at 0-5 cm soil depth in Bucyrus.

Type III Test of Fixed Effects					
WSA >2 mm			WSA 2 – 0.250 mm		
Sf	χ^2	p-value	Sf	χ^2	p-value
Cover crop (CC)	50.1	0.0000	Cover crop (CC)	3.19	0.0737
Year (Y)	23.7	0.0000	Year (Y)	77.90	0.0000
CC*Y	16.5	0.0003	CC*Y	0.62	0.7331
WSA 0.250 – 0.053 mm			WSA 0.053 – 0.02 mm		
Cover crop (CC)	10.90	0.0009	Cover crop (CC)	5.34	0.0207
Year (Y)	28.20	0.0000	Year (Y)	14.20	0.0008
CC*Y	1.38	0.5011	CC*Y	0.02	0.9885
Macroaggregates			MWD		
Cover crop (CC)	12.60	0.0004	Cover crop (CC)	31.60	0.0000
Year (Y)	86.00	0.0000	Year (Y)	63.20	0.0000
CC*Y	1.48	0.4749	CC*Y	9.00	0.0106
Total fungi			Total bacteria		
Cover crop (CC)	10.00	0.0015	Cover crop (CC)	3.06	0.080
Year (Y)	422.00	0.0000	Year (Y)	1039.00	0.000
CC*Y	22.80	0.0000	CC*Y	17.50	0.000

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S3. 2. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on water stable aggregates (WSA) >2 mm, 2 – 0.250 mm, 0.250 – 0.053 mm, 0.053 – 0.02 mm, macroaggregates, mean weight diameter (MWD), total fungi, and total bacteria at 0-5 cm soil depth in Solomon.

Type III Test of Fixed Effects					
WSA >2 mm			WSA 2 – 0.250 mm		
Sf	χ^2	p-value	Sf	χ^2	p-value
Cover crop (CC)	9.54	0.0020	Cover crop (CC)	26.70	0.0000
Year (Y)	2.96	0.2275	Year (Y)	9	0.0122
CC*Y	8.62	0.0134	CC*Y	10.16	0.0062
WSA 0.250 – 0.053 mm			WSA 0.053 – 0.02 mm		
Cover crop (CC)	44.80	0.0000	Cover crop (CC)	0.02	0.8829
Year (Y)	46	0.0000	Year (Y)	11	0.0035
CC*Y	12.90	0.0016	CC*Y	2.84	0.2416
Macroaggregates			MWD		
Cover crop (CC)	30.30	0.0000	Cover crop (CC)	2.99	0.0836
Year (Y)	9	0.0100	Year (Y)	5.22	0.0733
CC*Y	11.00	0.0040	CC*Y	5.10	0.0778
Total fungi			Total bacteria		
Cover crop (CC)	14.40	0.0001	Cover crop (CC)	40.80	0.0000
Year (Y)	8.35	0.0154	Year (Y)	59.50	0.0000
CC*Y	27.40	0.0000	CC*Y	51.10	0.0000

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S3. 3. Analysis of variance (ANOVA) of the effect of cover crop and year and their respective interactions on water stable aggregates (WSA) >2 mm, 2 – 0.250 mm, 0.250 – 0.053 mm, 0.053 – 0.02 mm, macroaggregates, mean weight diameter (MWD), total fungi, and total bacteria at 0-5 cm soil depth in Beloit.

Type III Test of Fixed Effects					
WSA >2 mm			WSA 2 – 0.250 mm		
Sf	χ^2	p-value	Sf	χ^2	p-value
Cover crop (CC)	87.40	0.0000	Cover crop (CC)	5.47	0.0193
Year (Y)	68	0.0000	Year (Y)	13	0.0012
CC*Y	43.90	0.0000	CC*Y	7.13	0.0283
WSA 0.250 – 0.053 mm			WSA 0.053 – 0.02 mm		
Cover crop (CC)	3.52	0.0603	Cover crop (CC)	0.09	0.7609
Year (Y)	18	0.0001	Year (Y)	1.22	0.5430
CC*Y	2.90	0.2337	CC*Y	2.07	0.3537
Macroaggregates			MWD		
Cover crop (CC)	17.30	0.0000	Cover crop (CC)	4.21	0.0400
Year (Y)	31	0.0000	Year (Y)	51.80	0.0000
CC*Y	17.80	0.0001	CC*Y	5.91	0.0498
Total fungi			Total bacteria		
Cover crop (CC)	3.85	0.0496	Cover crop (CC)	4.60	0.0319
Year (Y)	7.83	0.0199	Year (Y)	16.90	0.0002
CC*Y	6.30	0.0427	CC*Y	9.40	0.0087

Source of variation (Sf); Chi-square (χ^2); p-value (p).

Table S3. 4. Model parameters of the bivariate regression between macroaggregates and total fungi.

Macroaggregates ~ total fungi						
Bucyrus						
Source of variation	p-value	R^2	Adj-R^2	AIC	BIC	RMSE
Cover crop	<0.001	0.47	0.46	417.2	423.3	9.7
No cover	0.009	0.12	0.10	364.7	370.7	6.0
Solomon						
Cover crop	0.026	0.10	0.08	332.9	338.5	7.4
No cover	0.039	0.09	0.09	313.0	318.6	6.0
Beloit						
Cover crop	0.096	0.08	0.05	286.3	291.1	12.2
No cover	0.167	0.06	0.02	249.2	253.9	7.2

Akaike information criterion (AIC); Bayesian information criterion (BIC); Root mean squared error (RMSE)

Table S3. 5. Model parameters of the bivariate regression between macroaggregates and total bacteria.

Macroaggregates ~ total bacteria						
Bucyrus						
Source of variation	p-value	R^2	Adj-R^2	AIC	BIC	RMSE
Cover crop	<0.001	0.51	0.49	360.7	366.8	5.8
No cover	<0.001	0.32	0.30	403.0	409.1	8.5
Solomon						
Cover crop	0.012	0.13	0.11	331.4	337.0	7.3
No cover	0.276	0.03	<0.01	316.3	321.9	6.2
Beloit						
Cover crop	0.471	0.02	0.01	288.7	293.5	12.6
No cover	0.972	<0.01	<0.01	251.2	256.0	7.5

Akaike information criterion (AIC); Bayesian information criterion (BIC); Root mean squared error (RMSE)

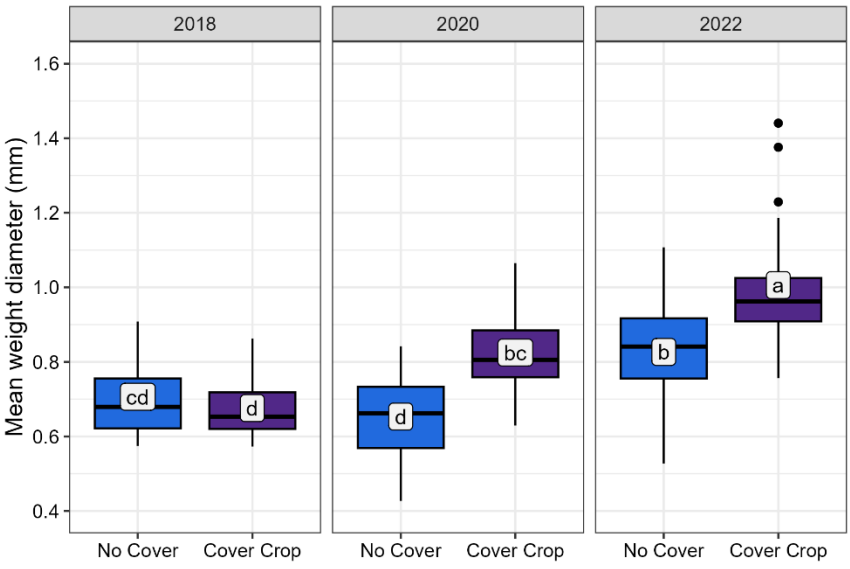


Figure S3. 1. Mean weight diameter (mm) by treatment and year at 0-5 cm depth in Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

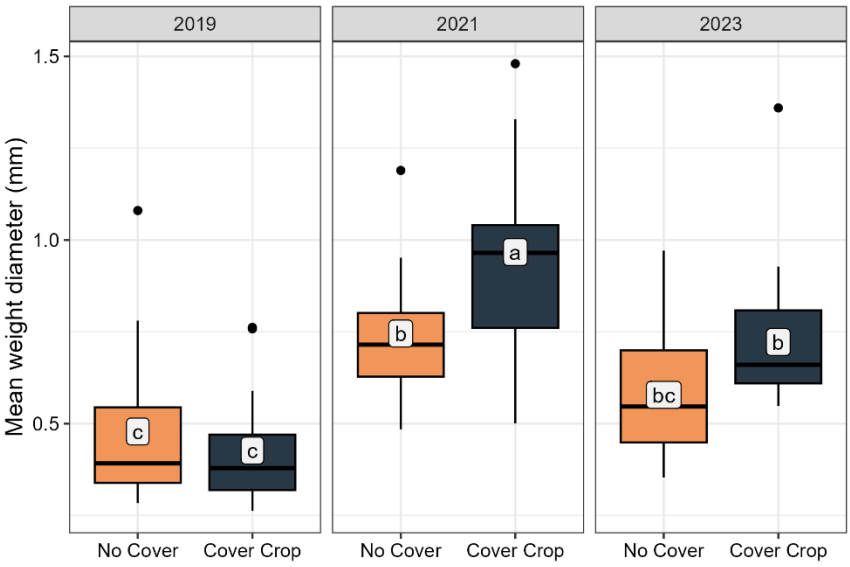


Figure S3. 2. Mean weight diameter (mm) by treatment and year at 0-5 cm depth in Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Chapter 4 - Short-term impact of nitrogen rates on potentially mineralizable nitrogen, soil organic matter, and crop yield after 25 years of on-farm regenerative agriculture

Abstract

Pioneering farmers have been using regenerative agriculture (RA) practices such as no-tillage, cover crops, and crop rotation to increase agricultural resilience and make efficient use of external inputs. Among several long-term RA benefits is the ability to efficiently manage soil nutrients. This capability raises the following questions: (i) what is the short-term impact of more conservative nitrogen (N) rates on crop yield in long-term RA? and (ii) would decreasing N rates jeopardize the long-term increase in soil organic matter (SOM)? In this study, the objectives were to (i) evaluate the effect of N rates on crop yield, and (ii) evaluate the short-term effect of N rates on soil organic carbon (SOC), total nitrogen (TN), and potentially mineralizable N (PMN). We hypothesized that implementing long-term on-farm RA practices could enable farmers to decrease N rates without compromising crop yield, as well as the short-term accumulation of SOC, TN, and PMN. Soil organic carbon (dry combustion), TN (dry combustion), PMN (laboratory incubation), and corn and winter wheat yield response were assessed in an on-farm study with a history of over 25 years of no-tillage, crop rotation, and cover crops. Treatments included four nitrogen managements with four different N rates applied in the form of urea (46-0-0): low (52 kg N ha^{-1} corn/ 0 kg N ha^{-1} wheat), conservative (104 kg N ha^{-1} corn/ 33 kg N ha^{-1} wheat), moderate (157 kg N ha^{-1} corn/ 82 kg N ha^{-1} wheat), and high (209 kg N ha^{-1} corn/ 132 kg N ha^{-1} wheat). In the short term, reducing N rates did not decrease SOC, TN, and PMN.

Moreover, in the field conditions of the study, decreasing the N rate by 48% for winter wheat production was feasible without compromising yields and SOM levels in the short term. However, reducing the N rate for corn production may lead to economic losses. Considering the growing global concern for soil health, our study highlights the need for a different approach for recommending N fertilizer for fields managed under RA.

Introduction

Adaptation to climate change and the focus on building soil organic matter (SOM) has required more dynamic and smart farming systems (Lipper et al., 2014; Okolie et al., 2022). Pioneering farmers have been using regenerative agriculture (RA) practices such as no-tillage (NT), cover crops, and crop rotation (Giller et al., 2021; Newton et al., 2020; Pittelkow et al., 2015) to increase agricultural resilience and make efficient use of external inputs (Cassman & Grassini, 2020; Tilman et al., 2002). One argument for RA is that it could be a "win-win" solution to provide climate resilience (Schreefel et al., 2020) and sustainability (Newton et al., 2020). The maintenance and improvement of SOM is an essential strategy to ensure RA implementation (Mahal et al., 2018). Among several benefits of RA is the opportunity to manage soil nutrients efficiently. This capability raises the following questions: (i) what is the short-term impact of more conservative N rates on crop yield in long-term RA? and (ii) would decreasing N rates jeopardize the SOM?

The main N sources used by crops are from (i) mineralization of soil organic matter nitrogen (SOM-N); (ii) decomposition of plant residue or organic amendments such as manure and compost; (iii) addition of N as inorganic fertilizers; and (iv) legume dinitrogen (N2) fixation (Mikha et al., 2006; Robertson et al., 2013). The second largest requirement after water in crop

production (Govindasamy et al., 2023; Robertson et al., 2013) is nitrogen (N). Nitrogen mineralization from soils with high organic matter (SOM) content has been a significant source of N for crop uptake and has been frequently associated with crop yield (Gebremedhin et al., 2020; Mahal et al., 2018). Therefore, understanding the fraction of SOM susceptible to mineralization could optimize N fertilizer management. The mineralizable fraction, commonly known as potentially mineralizable N (PMN), is defined as soil organic matter N (SOM-N) that is converted to plant-available inorganic forms (i.e., NH_4^+ and NO_3^-) under laboratory incubation with temperature, moisture, time, and aeration control (Clark et al., 2020; Mikha et al., 2006; Stanford & Smith, 1972). The accessibility of these SOM fractions to mineralization varies, ranging from labile microbial biomass and organic residues to chemically recalcitrant pools such as mineral-associated organic matter (Drinkwater et al., 1997; Villarino et al., 2023).

Since PMN measures the release of plant-available N, it has been used as a soil health indicator. However, predicting the contribution of N mineralization to crop yield has been one of the greatest challenges for improving N management in agriculture. As Clark et al. (2020) and Robertson et al. (2013) stated, N mineralization may contribute 20 to 100% of the total plant N needs depending upon soil, previous crop, weather, and N management.

While several studies have examined the impact of cover crops, crop rotation, and tillage systems on PMN (Drinkwater et al., 1997; Ghimire et al., 2019; Kuo et al., 1996; Mahal et al., 2018; Peregrina et al., 2012), there is still a lack of comprehensive investigation on the PMN response to N rates and its connection to crop yield on farm fields. Moreover, the effects of N fertilizer application on PMN and SOM are currently subjects of active debate (Poffenbarger et al., 2017). According to Mahal et al. (2018), fertilizer application influences the quantity and quality of crop residue production, which is primarily decomposed into the soil and consequently

affects PMN. Nitrogen fertilization typically increases the amount and quality of crop residue (Brown et al., 2014), leading to increased SOM and, subsequently, increased PMN. Still, N fertilizer may also lessen microbial N limitation, stimulating SOM mineralization and potentially reducing SOM, which can decrease PMN (Pikul et al., 2001; Russell et al., 2009).

Historically, high N prices have increased farmers' interest in more economically sustainable N recommendations (Watkins et al., 2010). Creating more accurate N recommendations for individual fields has been challenging because of the complexity of N gains and losses during the growing season. Adaptative management arises in this scenario of uncertainties to improve nutrient management (Morris et al., 2018). According to Morris et al. (2018), adaptative management is defined as developing on-farm management practices for efficient production and resource conservation using participatory learning. Adaptative management was designed to address the uncertainty of N required by crops and to fine-tune N recommendations. The potential of the adaptive management process in agriculture to enhance farmer practices is enormous due to farmers' direct land management and the ease of data collection (Morris et al., 2018).

The rationale for N recommendation for crops has been based only on yield goal (Stanford & Smith, 1972), with no devoted attention to the interaction between N recommendation and RA practices. Considering the history of RA practices, it is possible to conclude that previous N recommendations have been based on degraded soils primarily managed under conventional tillage with no cover crops, where crops mostly rely on external inputs. On the other hand, some institutions are developing new N recommendation systems that consider aspects such as NT, cover crops, and organic matter mineralization potential (Franzen, 2018; Morris et al., 2018; White & Spargo, 2023). In this study, the objectives were to (i)

evaluate the effect of N rates on crop yield, and (ii) evaluate the short-term effect of N rates on soil organic carbon (SOC), total nitrogen (TN), and PMN. We hypothesized that implementing long-term on-farm RA practices could enable farmers to decrease N rates without compromising crop yield, as well as the short-term accumulation of SOC, TN, and PMN.

Materials and Methods

Study site and field characteristics

On-farm field research was conducted near Glen Elder, Mitchell County, KS, U.S. (39.3552N, -98.3543W, elevation: 493m, slope: 2%) from 2019 to 2022. The soil was classified as a well-drained silt loam (Table 4.1), Harney series, fine, smectic, mesic, Typic Argiustoll (Soil Survey Staff, 2014). Soil chemical characterization is presented in Table 4.2. The climate was classified as Hot-summer humid continental (Dfa) according to Peel et al. (2007), with an average annual precipitation (1991-2020) of 700.5 mm and an average annual mean temperature of 12°C. The rainfall was concentrated from March to November, with higher intensity between May and July (Figure 4.1).

Table 4. 1. Soil texture by nitrogen management and depth (n = 4).

Nitrogen management	Clay	Silt	Sand
	-------- g kg^{-1} --------		
Low	267 ± 14	570 ± 8	162 ± 11
Conservative	272 ±11	590 ± 16	137 ± 10
Moderate	310 ± 15	562 ± 12	127 ± 12
High	312 ± 5.3	567 ± 9	120 ± 8

Means for soil texture with the standard error of the mean.

Table 4. 2. Soil chemical properties by nitrogen management and depth (n = 4).

Nitrogen management	K^+	P	Ca^{2+}	Mg^{2+}	Na^+	pH
	---------- mg kg^{-1} ----------					
			0-5 cm			
Low	514 ± 23.1	93.4 ± 6.5	1886 ± 122	293 ± 26.1	12.0 ± 1.6	4.9 ± 0.1
Conservative	518 ± 19.4	100.0 ± 6.2	1953 ± 77	341 ± 18.2	11.9 ± 1.4	4.8 ± 0.0
Moderate	544 ± 22.3	99.1 ± 6	1884 ± 126	314 ± 27.8	11.5 ± 1.5	4.8 ± 0.1
High	477 ± 19.6	107.4 ± 5.9	2002 ± 108	353 ± 23.0	11.1 ± 0.8	4.7 ± 0.0
			5-15 cm			
Low	335 ± 16.4	28.5 ± 5.1	2062 ± 128	357 ± 30.3	13.7 ± 1.3	5.1 ± 0.0
Conservative	358 ± 15.1	46.3 ± 6.3	2159 ± 90	406 ± 20.6	15.6 ± 2.0	4.9 ± 0.0
Moderate	400 ± 17.2	50.4 ± 7.1	2030 ± 141	362 ± 32.4	12 ± 1.0	4.9 ± 0.1
High	324 ± 14.9	27.7 ± 3.8	2491 ± 143	453 ± 29.6	15 ± 1.0	5.3 ± 0.1
			15-30 cm			
Low	283 ± 13.2	10.3 ± 2.6	2493 ± 144	442 ± 33.1	17.4 ± 2.2	5.7 ± 0.1
Conservative	344 ± 12.4	9.8 ± 1.4	2904 ± 91	557 ± 20.9	23.1 ± 2.3	5.7 ± 0.1
Moderate	334 ± 10.1	12.9 ± 2.2	2600 ± 135	480 ± 32.2	15.7 ± 1.6	5.5 ± 0.1
High	318 ± 12.4	13.2 ± 2.8	2967 ± 180	528 ± 34.8	20.7 ± 2.4	5.9 ± 0.2

Means for physicochemical properties with the standard error of the mean.

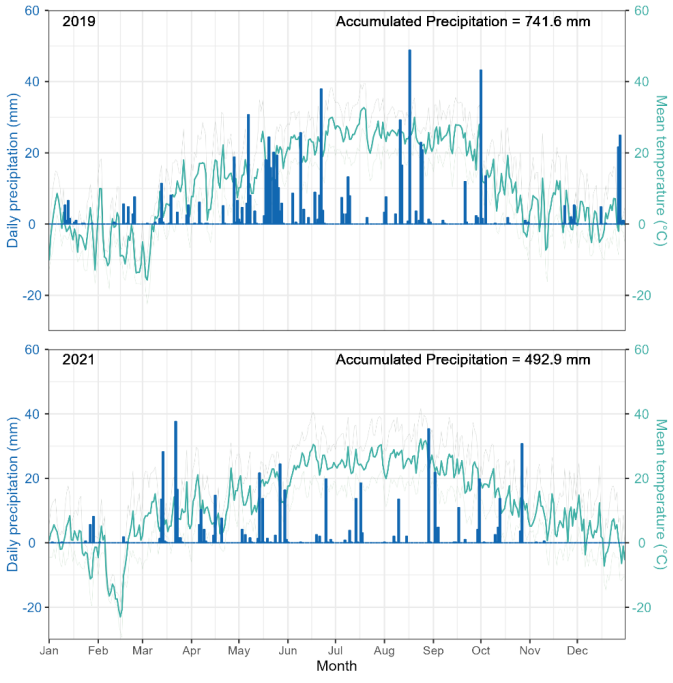


Figure 4. 1. Daily rainfall, cumulative rainfall, and maximum, average, and minimum temperatures for 2019 (corn) and 2021 (wheat).

Experimental design and treatment descriptions

The experimental site was managed with RA practices for more than 25 years. Regenerative agriculture practices implemented by the farmer included NT, cover crops, and intensified and diversified crop rotation. Cover crops included triticale *(×Triticosecale Wittmack*), oats (*Avena sativa*), sorghum sudan (*Sorghum × drummondii*), crimson clover (*Trifolium incarnatum*), radish (*Raphanus sativus*), and field peas (*Pisum sativum*). Throughout the five years of study, the crop rotation was corn (*Zea mays)* (2019), triticale as cover crops (2019/2020), soybean (*Glycine max*) (2020), winter wheat *(Triticum aestivum)* (2020/2021), double-crop grain sorghum *(Sorghum bicolor)* (2021), and soybean (2022). The 2019 corn and 2021 winter wheat were managed with variable rate (VR) nitrogen, as presented in Figure 4.2. The nitrogen management consisted of four different rates applied as N in the form of urea (46-0-0): low (52 kg N ha^{-1} corn/ 0 kg N ha^{-1} wheat), conservative (104 kg N ha^{-1} corn/ 33 kg N ha^{-1} wheat), moderate (157 kg N ha^{-1} corn/ 82 kg N ha^{-1} wheat), and high (209 kg N ha^{-1} corn/ 132 kg N ha^{-1} wheat). The VR urea was applied using a John Deere® GreenStar™ Rate Controller Dry. The moderate rate was calculated with a yield goal of 10.3 Mg ha^{-1} for corn and 4 Mg ha^{-1} for winter wheat, and was considered the recommended rate (Leikam et al., 2003). The SOM used for calculations was 5% and no previous crop adjustment was needed. The low, conservative, and high N rates were applied as a percentage of the moderate rate in a way that would make financial and technical sense for the farmer cooperator. For corn, the low rate represented 33%, the conservative rate represented 66%, and the high rate represented 133% of the moderate rate. For wheat, the low rate represented 0%, the conservative rate represented 40%, and the high rate represented 160% of the moderate rate. The experiment was established as a randomized complete block design, with four treatments (nitrogen management) and four replications (Figure

4.2). Each plot measured 1.42 ha, and the total area of the study was 22.7 ha. The plot size was equated to match all farm machinery.

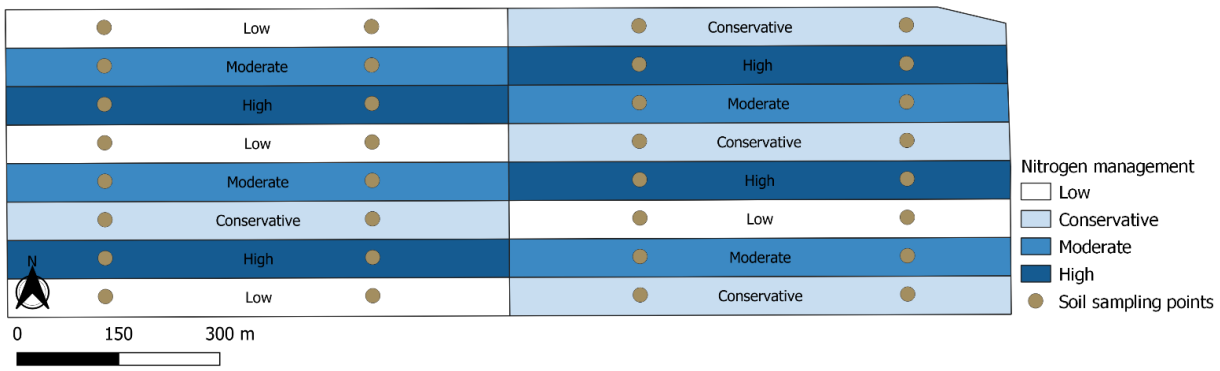


Figure 4. 2. Study experimental design. Glen Elder, Kansas, U.S.

Corn (FBN F2F1C-128A) was planted on May 25, 2019, and harvested on October 20, 2019. Corn row spacing was 76 cm and it was planted using a 16-row John Deere® 1770NT. Winter wheat (LCS Chrome) was seeded on October 12, 2020, and harvested on June 24, 2021. Wheat row spacing was 25.4 cm and was seeded using a John Deere® 1890NT air drill. Pre-emergent herbicide was applied as needed according to the farm weed control program.

Soil and yield sampling

Soil samples were taken at 0-5, 5-15, and 15-30 cm soil depth in a 0.7 ha GPS coordinate sampling grid (Figure 4.2) for SOC and TN. Soil samples for PMN were taken at 0-5 cm only. Potentially mineralizable nitrogen samples were stored in a cooler (4°C) until analysis. Samples were cleaned of roots and plant material, air-dried, ground, and passed through a 2-mm (SOC and TN) and 6-mm sieve (PMN). Corn and wheat yield was harvested with a John Deere® S680

equipped with a yield monitor and a 608C corn header for corn harvest, and a 32 Shelbourne Reynolds® stripper wheat header for wheat.

Soil measurements

Subsamples were ground again, passed through a 0.250-mm sieve, and analyzed for SOC and TN by dry combustion using a LECO® CN 828 analyzer (Leco Corp; USA). Pre-treatment for inorganic carbon removal was not required for SOC. Soil chemical properties were analyzed using the standard Mehlich-3 method (NCR221, 2015) for extractable P and ammonium acetate method for extractable K^+, Ca^{2+}, Mg^{2+}, and Na^+ (NCR221, 2015). Soil pH was determined with a 1:10 soil: water slurry method.

Potentially mineralizable nitrogen was determined by laboratory incubation. The procedure was based on the leaching method proposed by Cabrera and Kissel (1988) and modified by Garcia (1992) and Mikha et al. (2006). Soil (106 g oven dry weight) from each field replicate was packed (at field moisture) into 196 cm3 (5 cm diameter and 10 cm high) PVC cores at a bulk density of 1.05 g cm3. A 149-mm polyethylene filter (Fisher Scientific, Pittsburgh, PA) was glued to the bottom of the PVC cores to keep the soil intact. The cores were stored at 4°C until the beginning of incubation. For the leaching procedure, the cores were placed on Buchner funnels (7 cm diameter) that were attached to a side-arm 500 mL Erlenmeyer flask connected to a vacuum pump. A 10 mm nylon filter (Magna, Nylon, Osmonics Inc.) with a bubble-point pressure of 0.0685 MPa was glued to the bottom of the funnels. The bubble-point pressure of the filter allowed for soil equilibration to a water potential of -0.033 MPa. A 3 to 4 mm thick layer of solid glass spheres, 29 mm mean particle size (Potters Industries Inc., Brownwood, TX), was added to the top of the filter before leaching to ensure contact between the filter and the soil.

Each core was leached with 500 mL of 0.01 M CaCl2. Nitrate (NO_3^-) concentration was determined by cadmium reduction and colorimetric reaction (ALPKEM Corporation, 1986a; RFA Methodology No. A303-S170). Ammonium (NH_4^+) concentration was determined by an indophenol colorimetric reaction (ALPKEM Corporation, 1986b; RFA Methodology No. A303-S021). An N-free nutrient solution (50 mL) was prepared with KH_2PO_4, K_2SO_4, $MgSO_4$, and $CaSO_4$ (Cabrera & Kissel, 1988). The solution contained 0.5 mg L^{-1} of P, 4 mg L^{-1} of K^+, 113 mg L^{-1} of S, 24 mg L^{-1} of Mg^{2+}, and 100 mg L^{-1} of Ca^{2+} mg L^{-1} and was added to each core, and a vacuum of 20.033 MPa was applied for 6 h to adjust to a constant water content after leaching (Cabrera & Kissel, 1988). Subsequent leaching was performed after 7, 14, 21, 28, 42, 56, 70, 91, and 112 days. Between leaching events, soil cores were placed in 950 mL Mason jars and incubated at 35°C.

Data analysis

The R Programming software (R Core Team, 2023) was used for all the analyses and figures. Prior to statistical analysis, the dataset was spatially transformed to a sf object using x (longitude) and y (latitude) coordinates from the GPS sampling points (Pebesma & Bivand, 2023). The effect of N management, year, depth, and their respective interactions (fixed effects) on the response variables was analyzed by ANOVA after fitting a linear mixed effects model using the nlme R package (Pinheiro et al., 2017). Blocks were set as random effects. The normality of the residuals was tested using the Shapiro–Wilk test, and square-root transformation was applied when necessary. Models were tested with exponential, spherical, gaussian, and rational spatial correlation structures without nugget effect using a maximized log-likelihood method (Pinheiro et al., 2017). Model selection for correlation structure was performed

following the Akaike information criterion (AIC). Model means were extracted using the emmeans R package (Lenth, 2023) and a pairwise comparison was used to determine significant differences among treatments for all the fixed effects presenting a significance equal to or lower than 0.05 using Fisher's LSD (multcomp R pachage) (Hothorn et al., 2008).

The AONR and EONR were estimated by fitting a linear-plateau (LP) model, where "a" is the intercept, "b" is the slope during the linear phase, and "c" is the value of "x" at which the linear model reaches a plateau:

$$y = a + b * x \; if \; x \leq c$$

The AONR is defined as the lowest N rate at which no yield increase is expected if the N rate is increased (Miguez & Poffenbarger, 2022). The EONR is defined as the rate at which crop yield increase is not sufficient to cover additional N fertilizer costs (Miguez & Poffenbarger, 2022). The EONR is dependent upon crop and fertilizer prices and tends to decrease when the cost of N increases, while it increases when the price of grains rises. However, the EONR might remain constant as long as the ratio between N cost and grain price remains unchanged (Camberato & Nielsen, 2019; Zhang et al., 2023). For simplicity, in the rest of this manuscript we only consider the AONR.

Results

A LP model was fitted to estimate the AONR for corn and resulted in a $AdjR^2$ of 0.80 (Figure 4.3). The estimated AONR was 140 kg N ha^{-1} at a yield of 7.2 Mg ha^{-1} (Figure 4.3). The lower and upper AONR estimate was 112 and 174 kg N ha^{-1}, respectively (Figure 4.3). A LP model was also fitted for winter wheat ($AdjR^2$ = 0.66) and estimated an

AONR of 43 kg ha^{-1} at a yield of 4.7 Mg ha^{-1} (Figure 4.4). The lower and upper AONR estimates for winter wheat were 35 and 70 kg N ha^{-1}, respectively. The model for both corn and winter wheat demonstrated a relatively high uncertainty. The corn yield at AONR was 1.1 Mg ha^{-1} lower than the average of the county which was 8.3 Mg ha^{-1} (USDA-NASS, 2017). The winter-wheat yield at AONR was 0.9 Mg ha^{-1} higher than the average of the county which was 3.8 Mg ha^{-1} (USDA-NASS, 2017). Moreover, at AONR, corn yield was 3.1 Mg ha^{-1} lower and winter wheat 0.3 Mg ha^{-1} higher than the established yield goal.

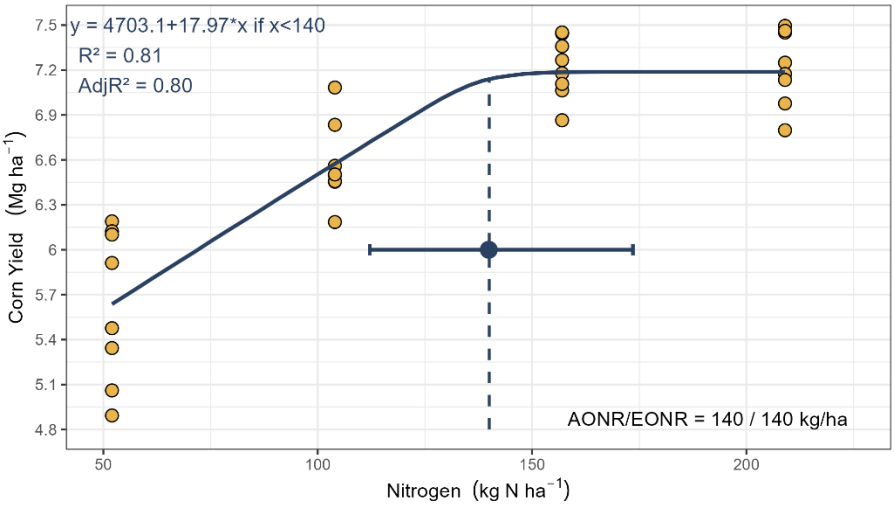


Figure 4. 3. Corn grain yield, agronomic optimum nitrogen rate (AONR), and economic optimum nitrogen rate (EONR) response to nitrogen management. Yellow dots indicate grain yield at each N rate. The AONR is represented by horizontal lines and EONR by vertical dashed lines.

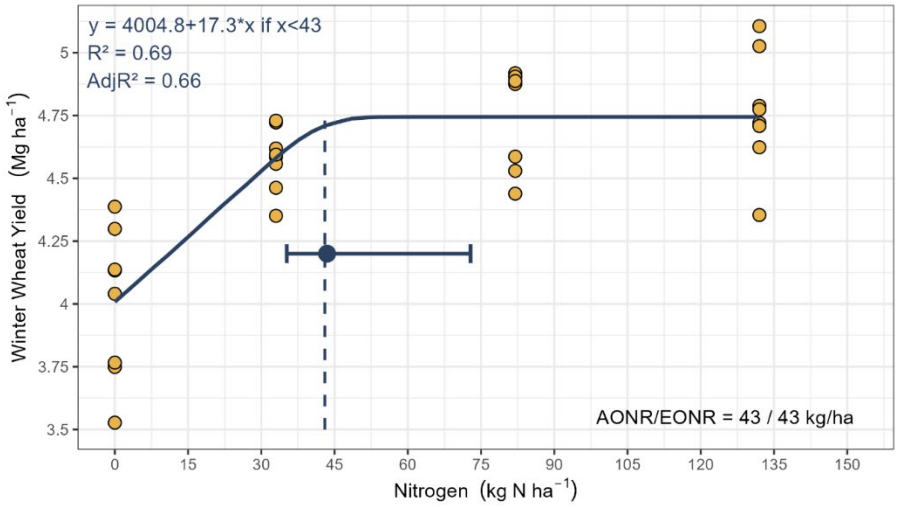


Figure 4. 4. Winter wheat yield, agronomic optimum nitrogen rate (AONR), and economic optimum nitrogen rate (EONR) response to nitrogen management. Yellow dots indicate grain yield at each N rate. The AONR is represented by horizontal lines and EONR by vertical dashed lines.

The cumulative PMN for soil samples incubated in 2019 and 2022 is presented in Figure 4.5. Nitrogen management (p = 0.22), year (p = 0.77), and the interaction (p = 0.62) had no significant effect on PMN (Table 4.3). The average accumulated PMN for 2019 samples varied among nitrogen management levels: 82 kg N ha^{-1} for low, 92 kg N ha^{-1} for conservative, 79 kg N ha^{-1} for moderate, and 76 kg N ha^{-1} for high N management. For 2022, the average accumulated PMN was 77 kg N ha^{-1} for low, 87 kg N ha^{-1} for conservative, 79 kg N ha^{-1} for moderate, and 82 kg N ha^{-1} for high N management.

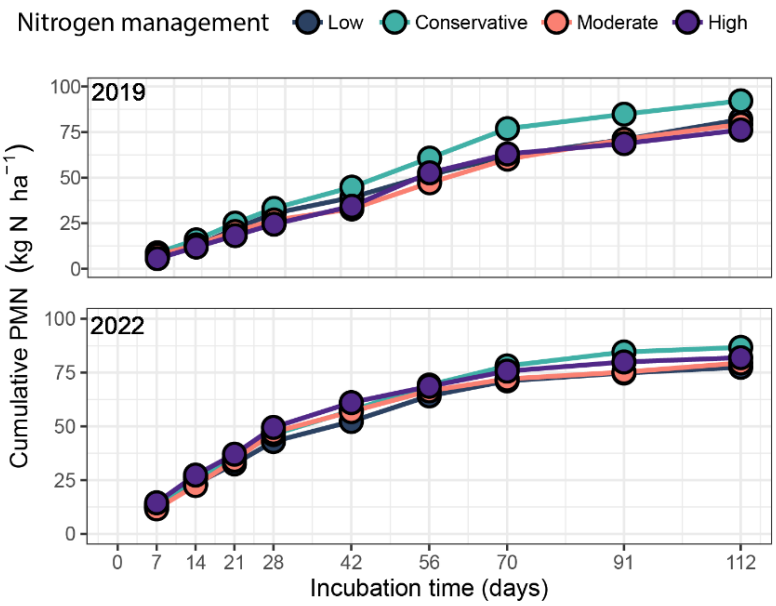


Figure 4. 5. Cumulative potentially mineralizable nitrogen (PMN) for soil samples taken in 2019 and 2022.

Table 4. 3. Analysis of variance (ANOVA) of the effect of nitrogen management, depth, year, and their respective interactions on soil organic carbon and total nitrogen. The depth effect was not tested for potentially mineralizable nitrogen.

Type III Tests of Fixed Effects					
Soil organic carbon			Total nitrogen		
Source of Variation	χ^2	p-value	Source of Variation	χ^2	p-value
Nitrogen management (NM)	2.66	0.45	Nitrogen management (NM)	1.78	0.62
Year (Y)	8.19	0.00	Year (Y)	55.95	<0.001
Depth (D)	822.78	<0.001	Depth (D)	634.67	<0.001
NM*Y	2.18	0.54	NM*Y	0.54	0.91
NM*D	0.81	0.99	NM*D	2.29	0.89
Y*D	14.66	<0.001	Y*D	2.40	0.30
NM*Y*D	1.05	0.98	NM*Y*D	1.22	0.98
Potentially mineralizable nitrogen					
Nitrogen management (NM)	4.39	0.22			
Year (Y)	0.08	0.77			
NM*Y	1.80	0.62			

χ^2 Chi-square.

A significant two-way interaction (p <0.001) between year and depth was found for SOC (Table 4.3). Soil organic carbon significantly increased by 3.5 g kg^{-1} (13%) from 2019 to 2022 (Table 4.3; Figure 4.6). Soil organic carbon was higher at 0-5 cm than 5-15 and 15-30 cm independently of year. No significant differences were found between years for 5-15 and 15-30 cm soil depth. When comparing each year vertically, significant differences were found for each depth in 2022. For 2019, 0-5 cm SOC was higher than 5-15 cm, which was not significantly different from 15-30 cm. No nitrogen management effect was found for SOC (p = 0.45).

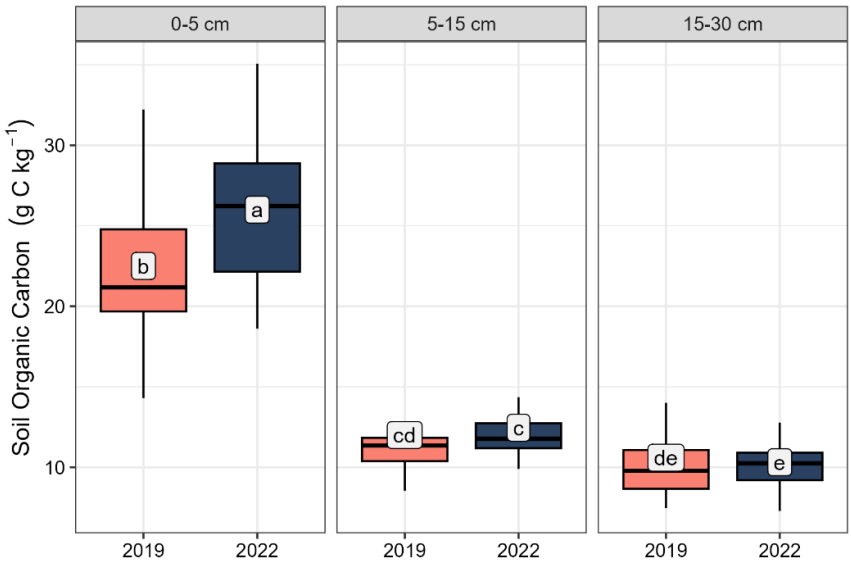


Figure 4. 6. Soil organic carbon (SOC) by year and depth. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

A significant one-way effect of year (p <0.001) and depth (p <0.001) was found for TN (Table 4.3). No nitrogen management effect was detected (p = 0.62). Following the same trend as SOC, 2022 had 0.3 kg ha^{-1} (18%) higher TN than 2019 (Figure 4.7). Regarding the depth effect, significant differences in TN were found between 0-5, 5-15, and 15-30 cm.

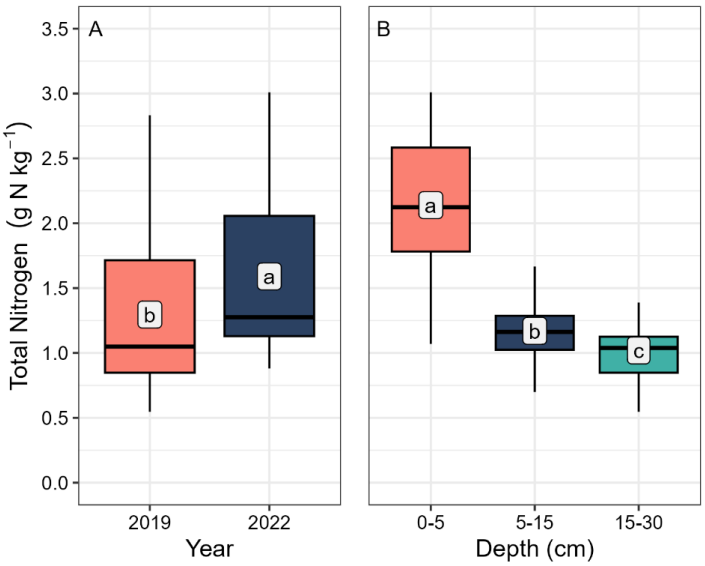


Figure 4. 7. Total nitrogen (TN) by year (A) and depth (B). Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Discussion

For 2019 corn, the difference between the recommended N rate and the AONR estimated by the LP model was 17 kg N ha^{-1}, which falls within the model uncertainty. This difference demonstrates that the moderate rate was accurate for 2019 weather conditions and yield expectations. The differences for winter wheat N recommendation were greater. While the recommended N rate was 82 kg N ha^{-1}, the estimated AONR by the LP model was 43 kg N ha^{-1}, representing a difference of 39 kg ha^{-1}. One possible reason for the difference is the amount of N mineralized from the SOM (Figure 4.5). Potentially mineralizable nitrogen was positively associated with SOM because covalent bonds among organic C and N inseparably link C and N mineralization (Drinkwater et al., 1997; Mahal et al., 2018). Although N fertilizer may reduce PMN during the growing season (Clark et al., 2019), we did not detect any difference of N rates

on PMN. This outcome may support reducing the wheat N rate without compromising the agronomic and economic return at least in the short term.

Regenerative agriculture practices such as NT and cover crops have allowed farmers to increase PMN over time, enhancing plant-available N (Mikha et al., 2006; Vyn et al., 2000; White et al., 2022). Franzen (2018) recommended reducing the N rate for spring wheat and durum by 56 kg ha^{-1} if the field had been in NT for five or more continuous years. However, if the field has been managed under NT for less than five years, the recommendation was to add 22 kg ha^{-1} of N to the application rate (Franzen, 2018). The same was true for Kansas State University, with a recommendation of adding 22 kg ha^{-1} of N (Leikam et al., 2003). Franzen (2018) also recommended reducing another 56 kg ha^{-1} for each full percent of organic matter greater than 5%.

Although there are several articles supporting that N fertilizer may increase SOM due to plant biomass production, substrate quality, and application rate (Brown et al., 2014; Mahal et al., 2018; Poffenbarger et al., 2017), there is a debate whether N fertilizer increases SOM mineralization and consequently, reduces SOM (Mulvaney et al., 2009; Osterholz et al., 2019; Russell et al., 2009). Our study revealed that in the short term, N application had no effect on SOC and TN. Although not significant for the tested N rates, SOC and TN increased over time, consistent with Mahal et al. (2019). In long-term and intense cropped soils, SOM levels tended to be more stable because N resupplies SOM-N from new crop residues as they decompose to SOM. The RA practices implemented may explain these findings due to biomass production (Blanco-Canqui, 2022; Blanco-Canqui & Lal, 2009). The cropping system of the study contained 6 crops in four years (corn-triticale-soybean-wheat-sorghum-soybean), which is considered an intensive and diverse cropping system in the Southern Great Plains (Bowles et al., 2020;

Patrignani et al., 2019; Simão et al., 2023). Highly diverse and intense cropping systems under NT generally increase SOC and TN (Nicoloso et al., 2018; Pires et al., 2020; Simão et al., 2023).

The ongoing debate and uncertainties regarding the impact of N fertilizer on SOM mineralization occur because N fertilizer can positively or negatively affect SOM mineralization through various direct and indirect pathways. One immediate positive effect is its ability to modify microbial activity and biomass. According to the stoichiometric decomposition theory, introducing inorganic nitrogen inputs can increase microbial biomass and activity when nitrogen is a limiting resource, consequently enhancing SOM mineralization (Chen et al., 2014). Additionally, N fertilizer causes an indirect positive effect on SOM mineralization by increasing net primary productivity (NPP) (Poffenbarger et al., 2017). This impact becomes especially significant in corn-based cropping systems, where inorganic N inputs have the potential to increase NPP by over 200% and covert more solar energy into chemical energy through photosynthesis (Osterholz et al., 2019; Poffenbarger et al., 2017). When there is an increase in NPP, it can also lead to an increase in SOM mineralization through a positive priming effect. This occurs because the higher NPP results in increased soil microbial biomass and enzyme activity. (Chen et al., 2014). Conversely, N fertilizer can reduce microbial activity when N is a limiting resource. This reduction occurs because inorganic N inputs decrease the decomposition of low-quality (high C: N ratio) SOM substrates that are only mineralized to access N-containing compounds (Moorhead & Sinsabaugh, 2006).

Potentially, dependent upon environmental factors such as temperature, moisture, pH, oxygen availability, and substrate quality, the accumulated PMN over 112 days of incubation from both 2019 and 2022 could satisfy 100% of the estimated winter wheat AONR. Besides substrate quality and biomass production, NT plays an important role in N mineralization and

supply. No-tillage changes the environmental conditions by regulating soil temperature and moisture as well as by increasing aeration due to improved soil structure (Fan et al., 2018; Feng et al., 2003; Lin et al., 2023; Ruan & Robertson, 2013). Furthermore, these environmental changes tend to increase N mineralization during the growing season, coinciding with the period when crops have the greatest demand and uptake of N (Pecci Canisares et al., 2021). In a study in Illinois, (Needelman et al., 1999) found 15% more SOC, 33% more particulate organic matter, and 54% more PMN in NT compared to conventional tillage systems at 0-5 cm depth. Pecci Canisares et al. (2021), in a long-term study (48 years) in Kentucky, found that the NT system had 1000 kg N ha^{-1} greater total soil N stocks in the top 20 cm, mineralized 65 kg ha^{-1} more N during corn growth, and resulted in 22-71 kg N ha^{-1} and 780-1800 kg ha^{-1} greater N uptake and grain yield across N rates, respectively, than the plowed system. Similar results were reported by Doran (1987) and Acir et al. (2022).

In Kansas, N uptake and growth of winter wheat managed under NT is synchronized with increased microbial activity, precipitation, temperature, and soil moisture. Based on our study, this synchronization could account for the 48% reduction in the AON. In contrast, when there is high N demand by crops, soils managed under conventional tillage and low residue systems tend to be drier and deficient in mineralized N (Pecci Canisares et al., 2021). However, a well-managed 10-year NT system with N fertilizer application overestimated SOM-N contribution by about 27% in Kansas (Mikha et al., 2006). On the other hand, an NT system with no N fertilizer application overestimated SOM-N by more than 100% (Cabrera & Kissel, 1988; Mikha et al., 2006). Most of these variations were attributed to differences in soil temperature. Nevertheless, Campbell et al. (1988) modeling estimated lower N mineralization compared with in-situ N mineralization. The reason behind the additional N mineralization, not accounted for by their

model, was attributed to the alternating wet and dry conditions experienced in the field. These fluctuations in moisture levels led to increased N mineralization. When assessing PMN, an additional factor that might lead to overestimation is soil disturbance during laboratory incubation preparation. Long-term incubation could also contribute to the breakdown of soil aggregates and the subsequent release of protected SOM for microbial decomposition. These processes could inadvertently inflate the measured PMN levels beyond their actual values (Mikha, 2003).

Conclusion

This research significantly contributes to the existing knowledge on N management for corn and winter wheat under RA practices, offering valuable insights to enhance agricultural sustainability. In the short term, reducing N rates did not decrease SOC, TN, and PMN. Moreover, in field conditions, decreasing the N rate by 48% for winter wheat production was feasible without compromising yields and SOM levels in the short term. However, reducing the N rate would not be advisable for corn production as it may lead to economic losses. Considering the growing global concern for soil health, our study highlights the need for a different approach in recommending N fertilizer for fields managed under RA. This approach would empower farmers to take advantage of the SOC and TN (e.g., SOM) built over time and use external inputs more efficiently. Although our study provides crucial outcomes, it also has limitations. To address these, future recommendations would include delineating field N mineralization zones instead of employing a randomized complete block design for assigning different N rates. Such an approach would mitigate uncertainty and variation due to SOM variability in the field. Implementing N mineralization zones and adopting precision agriculture technologies, such as

variable rate fertilizer application, could greatly benefit farmers. By identifying specific areas with varying N mineralization capabilities, farmers can tailor their N fertilizer application according to the unique needs of each zone. This targeted approach could optimize N use efficiency and enhance overall crop productivity while promoting sustainable agricultural practices. Moreover, long-term on-farm studies are needed to comprehensively understand the impact of N rates on PMN and SOM's temporal variability. It is essential to acknowledge that the short duration of this study represents a significant limitation, as several years of reduced N rates might lead to a decline in SOM. Engaging in long-term on-farm research would significantly contribute to the continued progress of RA.

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Chapter 5 - Perspective of U.S. farmers on collaborative on-farm agronomic research

This chapter is a manuscript that has been submitted for publication in Agronomy Journal. The co-authors on this manuscript include Fernanda S. Krupek, Gabriela I. Carmona, Osler A. Ortez, Laura Thompson, Daniel J. Quinn, Andre F.B. Reis, Rodrigo Werle, Péter Kovács, Maninder P. Singh, J.M. Shawn Hutchinson, Dorivar Ruiz Diaz, Charles W. Rice, and Ignacio A. Ciampitti.

Abstract

On-farm research has emerged in recent years as a unique approach to involve farmers and other agricultural stakeholders as active participants in knowledge development and as an effective method of technology and innovation transfer across farms. This study assessed the perspective and knowledge of U.S. farmers regarding on-farm research via the implementation of a 24 question-survey distributed across most of the U.S. Midwest and South-Central regions. We found that farmers are generally willing to engage with universities to conduct on-farm research and were 40% more likely to adopt practices supported by on-farm research findings than research not conducted on-farm. Notably, a shift towards conservation practices was made, with cover crops and no-till at the forefront. Insights of this nature have implications for fostering collaborations, addressing constraints, and maximizing the impact of on-farm research, offering guidance for sustainable agriculture progress in the U.S. and beyond. Results from this research survey could be used to initiate much-needed policies to promote on-farm research. Further, information on the benefits and drawbacks of on-farm research could be used in the development of studies that benefit both the farmer and researchers.

Introduction

On-farm research has emerged in recent years as a unique approach to involve farmers and other agricultural stakeholders as active participants in knowledge development and as an effective method of technology and innovation transfer across farms (Wood et al., 2014). Additionally, farmers worldwide are placing growing emphasis on improving agricultural practices to enhance sustainability and build resilience in the face of various environmental and production challenges (Pittelkow et al., 2015; Jayaraman et al., 2021). Consequently, there is a need for decentralized, inclusive, diverse, and networked approaches to agricultural research, outreach, service, and extension to support farmers in their decision-making processes to achieve their goals (Ponzio et al., 2013; Wood et al., 2014; Lacoste et al., 2022; Jackson-Smith and Veisi, 2023). Sackett (2013) recommended participatory research with farmer cooperators to develop peer-to-peer networking. Their study indicated that outreach should engage technical assistance providers with locally relevant information. In this scenario, on-farm research can help reduce the risk that research leads towards outputs important to scientists rather than primary users and decision-makers such as farmers (Cook et al., 2013; Lacoste et al., 2022). The shared desire to work together toward a common goal is an important factor in supporting effective learning between farmers and researchers (Hardie Hale et al., 2022). Experimentation on farms can represent a 'multi-win' approach to ensure that researchers focus on relevant topics and that farmers benefit from the knowledge gained from the applied research (Thompson et al., 2019).

On-farm research is broadly defined, according to the Sustainable Agriculture Research and Education (SARE) program, as a study with straightforward experimental design, replications, and statistical analysis conducted as part of a farm using plots large enough to allow the use of farmer's machinery (Chaney, 2017). The fundamental concept of on-farm research is a

partnership between the researcher and the farmer(s) throughout the project. This innovative process brings agricultural stakeholders together around mutually beneficial experimentation to support farmers' management decisions (Wood et al., 2014). Chaney (2017) and Lacoste et al. (2022) acknowledged that on-farm research is generally supported by three mechanisms that build on the complex and interconnected histories of formal and farmer participatory research: (1) farm fields at meaningful scales (real world conditions/field-scale) rather than in controlled environments or small experimental plots that are designed in a small part of a field or externally (e.g., industry or university plots); (2) the interests of farmers and/or surrounding community are explicitly acknowledged to allow for building productive relationships; and (3) on-farm research is understood as a process of partnership between researchers and other stakeholders with farmers that pursue shared goals. On-farm research can be initiated through a variety of approaches.

Thompson et al. (2019) highlighted the three main approaches for on-farm research programs: (1) researcher-initiated; (2) industry-initiated; and (3) farmer-initiated. In the first approach, researchers propose a research topic and recruit farmers to participate. In the second approach, the industry may desire a third-party test of a product or practice, and in the third approach, the research question is generated by a farmer or group of farmers. In reality, most of the on-farm research may be a combination of these approaches and should clearly demonstrate a compelling engagement of farmers in the process.

Despite increasing enthusiasm around the concept of on-farm research within the agricultural community, there are still uncertainties regarding its effectiveness in helping farmers in their day-to-day decisions. Creativity and innovation are always welcome in designing an experiment that will eventually lead to improvements in management decisions and the

sustainability of production systems, even if creativity and innovation come with a reasonable level of risk (Krupek et al., 2021). Therefore, the objective of this study was to assess and understand the perspective and knowledge of U.S. farmers regarding on-farm research via the implementation of a survey instrument distributed across most of the U.S. Midwest and South-Central regions. We hypothesized that farmers are eager for partnerships and implementation of on-farm research to facilitate their learning and decision-making and that they consider this an important research and extension service to their operations.

Materials and Methods

A qualitative research questionnaire was deployed in the United States (Figure 5.1) containing 3 open- and 21 closed-ended questions (Table 5.1) to assess farmers' perception of what on-farm research entails, the importance of and their willingness to participate in on-farm research and to understand better farmers' farming infrastructure and how it can influence on-farm research capacity. In the case of open-ended questions, participants were instructed to provide written or typed responses, while for closed-ended questions, they were only asked to select from predefined options (Connor Desai and Reimers, 2019).

The questionnaire received approval from the Kansas State University Institutional Review Board to conduct the study (IRB-11084). The farmers were invited to answer the questionnaire during field days (paper or online) and through online links (e.g., social media, listservs, direct email) shared by researchers and extension agents across the U.S. from March to July 2022. The survey was deliberately shared to encompass a wide spectrum of farmers without making any distinction based on their pre-existing familiarity with on-farm research. This approach was adopted to ensure outreach to a diverse and inclusive farming community. On the

approach and invitation, farmers were briefly contextualized on the research objective, promoting entities, and the confidentiality of personal data. Upon agreement of participation, the process of answering the questions was self-instructed, with no influence or assistance from any research and extension staff. The participant could choose not to answer any of the questions. When the questionnaire was answered in paper form, we transcribed the information to the online system to secure the database. The involvement in this survey was completely optional, and participants did not receive any compensation for completing the survey. There were no expected risks or benefits associated with participating.

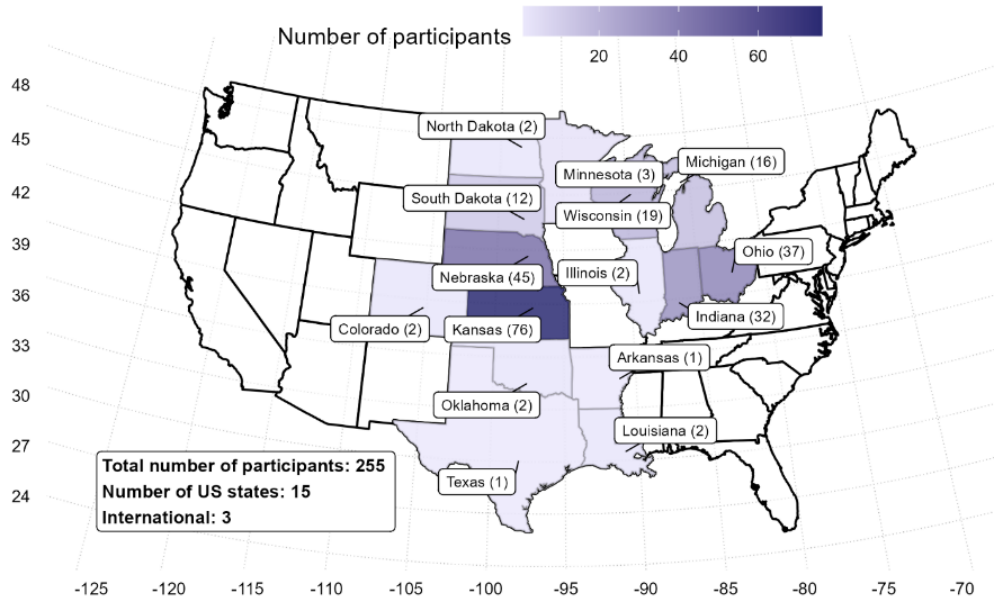


Figure 5.1. Number of participants by U.S. states that completed the on-farm research survey.

Table 5.1. Questions description, identification number, and number of responses recorded for all 24 questions.

ID	Question	Type	Number of answers
1	What is your age?	Closed	255
2	How long have you been in production?	Open	234
3	What state is your operation located?	Open	252
4	What is the average size, in acres, of cropland in your operation?	Closed	255
5	Is on-farm research a new concept for you?[a]	Closed	251
6	In your opinion, what best describes on-farm research?	Closed[b]	251
7	Have you participated in on-farm research?	Closed	251
8	How likely are you to collaborate with a university (e.g., researchers and extension educators) for conducting on-farm research?	Closed	250
9	How likely are you to adopt practices recommended based on an on-farm study?	Closed	251
10	How likely are you to adopt practices recommended based on a research study other than on-farm study?	Closed	250
11	I consider on-farm research/experimentation an important way to strengthen relationships between producers and a university.	Closed	247
12	In your opinion, what are the top 3 on-farm research limitations?	Closed[b]	246
13	In your opinion, what are the top 3 on-farm research strengths?	Closed[b]	247
14	How do you compare on-farm research to other educational opportunities (e.g., greenhouse research, small plots research) available to you?	Closed	239
15	I am satisfied with the investment Universities makes in outreach and extension.	Closed	240
16	How many field days do you attend annually?	Closed	242
17	What type of extension events do you usually attend?	Closed[b]	232
18	On-farm research can foster innovation (use of cover crops, nutrient management, precision agriculture, etc.) to my farm.	Closed	238
19	How often do you consult field information sources?	Closed	235
20	What is your primary source for field information?	Closed[b]	237
21	What has been and/or would be your primary motivator for doing on-farm research?	Closed[b]	239
22	Do you have any major changes to your farming in the last 10 years? Examples: added cover crops, added irrigation, moved to no-tillage, etc.).	Open	174
23	Do you have a yield monitor in your combine?	Closed	234
24	Do you use available precision agriculture technologies?	Closed[b]	233

The questions along with their responses were exported from Qualtrics as a comma-separated value (.csv) text file and manipulated using the R software (R Core Team, 2023). The

data was cleaned to remove invalid entries and figures were built using the ggplot2 (Wickham, 2016) and wordcloud (Fellows, 2018) packages. Absent answers were expressed as NA.

Results

Among respondents, the average age was 50 years, years in production was 25, and cropland area was 610 acres (Table 5.2). The category with the largest number of respondents was 55-64 years old (n=65). Approximately half of the responses were concentrated in the categories at or above 45 years of age, reflecting the current population of farmers managing fields within the U.S. (USDA NASS, 2019). Regarding years in production, the highest class was between 21 and 40 years (n=81), which represented about 32% of the total responses, whereas 54% of the farmers respondents had at least 21 years in production (Table 5.2). Among the surveyed population, 96 out of the 255 respondents (38% of the population) farmed more than 1,000 acres.

Table 5. 2**.** Surveyed farmer profiles related to age, years in production, and cropland area expressed in acres.

Age			Years in production			Cropland area (hectares)		
Response	N	%	Response	N	%	Response	N	%
< 18	6	2.4	< 5	17	6.7	< 20	23	9.0
18-24	37	14.5	6-10	36	14.1	20-40	18	7.1
25-34	44	17.3	11-20	43	16.9	40-202	70	27.5
35-44	47	18.4	21-40	81	31.8	202-405	48	18.8
45-54	55	21.6	> 40	57	22.4	> 405	96	37.6
55-64	65	25.5	NA	21	8.2			
>65	6	0.4						
NA	1	2.4						

For 79% of the respondents, on-farm research was not a new concept (Figure 5.2a), suggesting that there is strong familiarity among the farming community in the surveyed population. Of all respondents, 132 had participated in on-farm research and 119 had not (Figure 5.2b). When asked what best describes on-farm research, the most selected answers were *side-by-side* (n=159), *plots* (n=140), *real-scale* (n=139), and *replication* (n=139) (Figure 5.2c). *Precision agriculture* and *partnership* had the lowest scores. Among the "others" answer, farmers mentioned "*action research that is realistic for farmers to complete"*, "*experimental"*, "*livestock"*, and "*collaboration"*, representing some of the perceptions that farmers currently have about on-farm research.

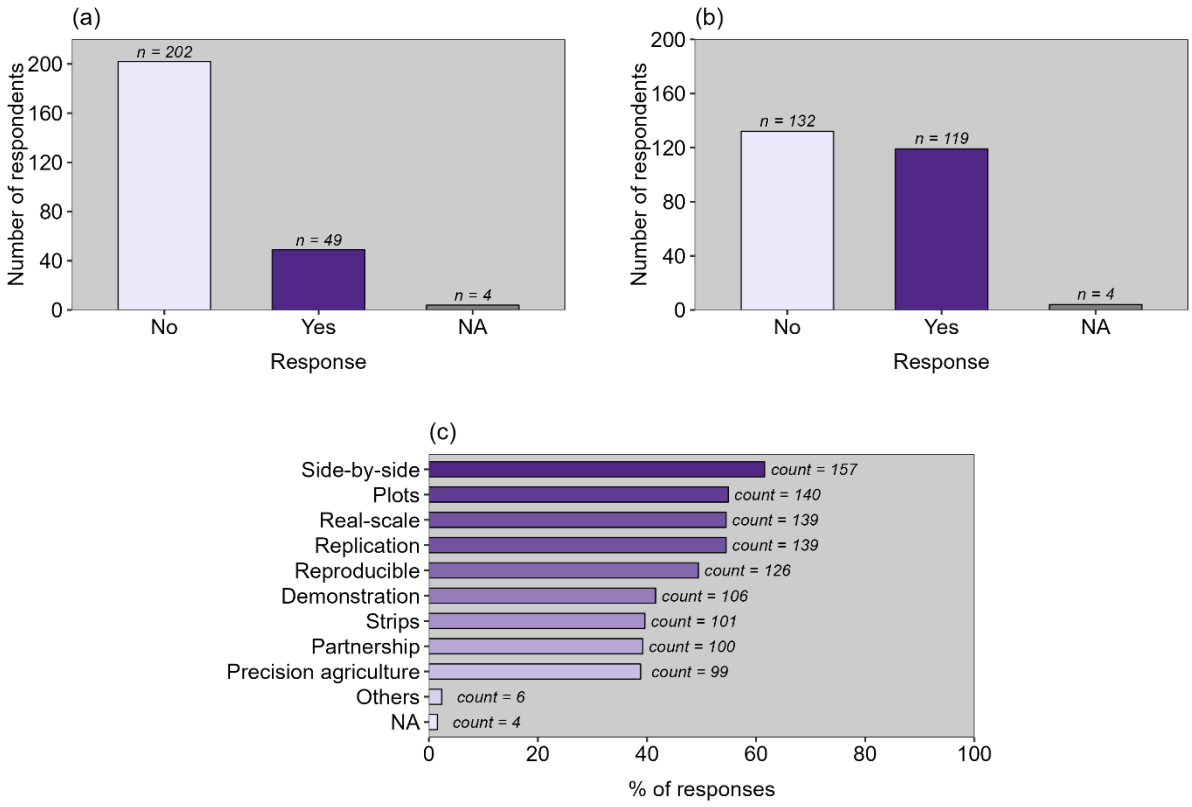


Figure 5. 2**.** Farmers response to: Is on-farm research a new concept for you? (a). Have you participated in on-farm research? (b). In your opinion, what best describes on-farm research? (c).

The participants were also asked about the likelihood of collaborating with a university to conduct on-farm research (Figure 5.3a), adopting practices based on findings from an on-farm study (Figure 5.3b), and adopting practices based on a research study other than an on-farm study. Most respondents were likely to collaborate with universities for conducting on-farm research, demonstrating farmers' readiness for collaboration. Considering the likelihood categories of 8 or higher, farmers were 40% more likely to adopt practices based on on-farm studies (66%) compared to other than on-farm studies (26%; Figure 5.3c). Additionally, farmers were asked about on-farm research as an important way to strengthen relationships between producers and a university. The results showed that 88% of the respondents (n=224) agreed or strongly agreed that on-farm research was important to strengthen relationships between producers and universities (Figure 5.3d).

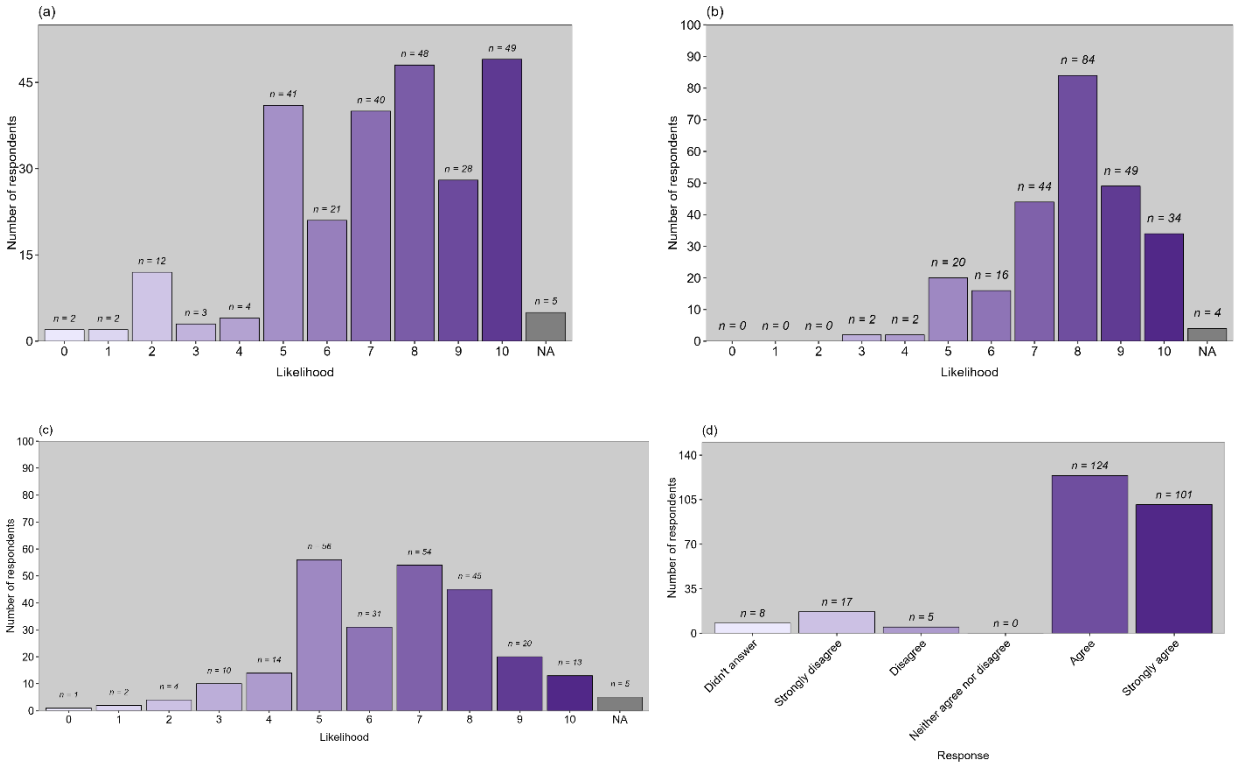


Figure 5. 3**.** Farmers response to: How likely are you to collaborate with a university (e.g., researchers and extension educators) for conducting on-farm research? (a). How likely are you to adopt practices recommended based on an on-farm study? (b). How likely are you to adopt practices recommended based on a research study other than on-farm study? (c). I consider on-farm research/experimentation an important way to strengthen relationships between producers and universities (d).

When questioned about their opinion on the limitations (Figure 5.4a) and strengths (Figure 5.4b) of on-farm research, time management emerged as the main limitation (n=195). Another significant limitation was the plot setup. Other limitations mentioned by the respondents were "*farmer transferring agronomic data"*, "*year-to-year variability"*, "*data collection"*, "*cost of input for research", "conflict in farmer and university schedule"*, "*funding"*, "*realistic plots that are not in the way of the rest of the operation"*, "*cleaning out drill or planter for each variable"*, "*monetary compensation for time"*, and "*distance to university"*. Knowledge gained was the main strength (n=207; Figure 5.4b) along with access to unbiased research information, and applied research. Other strengths of on-farm research mentioned by the respondents were "*more localized environments"* and "*first-hand knowledge of person to contact"*.

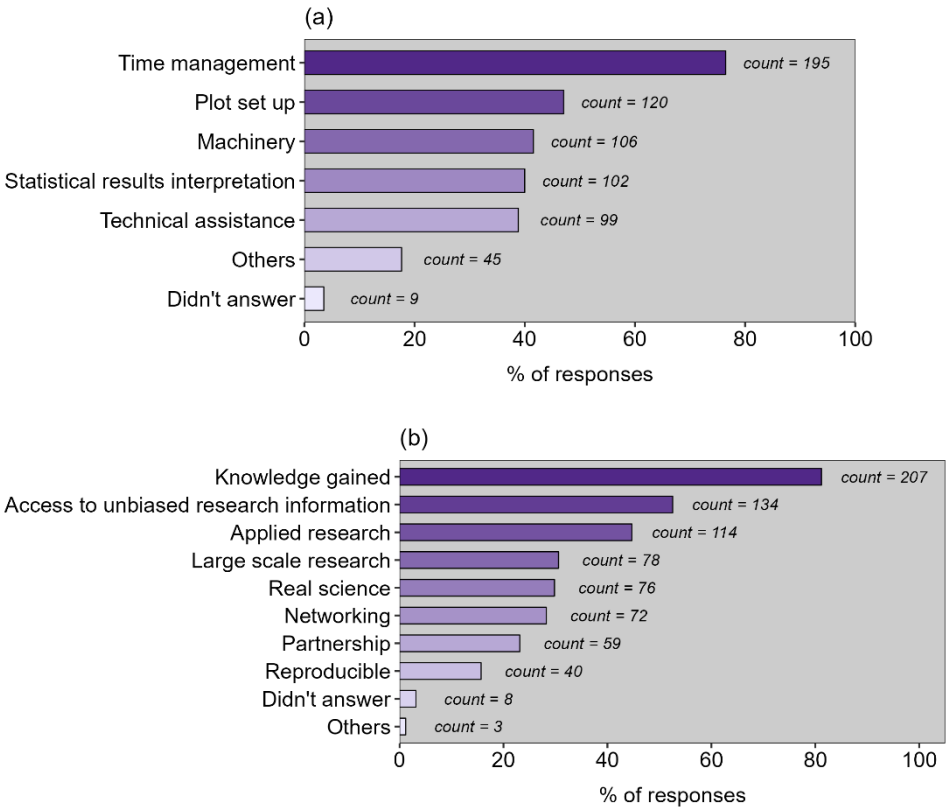


Figure 5. 4**.** Farmers response to: In your opinion, what are the top 3 on-farm research limitations? (a). In your opinion, what are the top 3 on-farm research strengths? (b).

Furthermore, 89.8% of the respondents considered on-farm research above average (n=128) or one of the best (n=101) education opportunities when compared to greenhouse or small plots studies (Table 5.3). Additionally, 85.9% of the respondents agree (n=127) or strongly agree (n=92) that on-farm research can foster innovation within their farms; only 6.7% disagree (n=1) or strongly disagree (n=16; Table 5.3).

Table 5. 3. Farmers response to: How do you compare on-farm research to other education opportunities (e.g., greenhouse research, small plots research) available to you? On-farm research can foster innovation (cover crops, nutrient management, precision agriculture, etc.) to my farm.

How do you compare on-farm research to other education opportunities available to you?			On-farm research can foster innovation to my farm.		
--- Response ---	--- N ---	--- % ---	--- Response ---	--- N ---	--- % ---
Above average	128	50.2	Strongly agree	92	36.1
One of the best	101	39.6	Agree	127	49.8
Below average	10	3.9	Neither agree nor disagree	2	0.8
One of the worst	0	0.0	Disagree	1	0.4
NA	16	6.3	Strongly disagree	16	6.3
			NA	17	6.7

Upon being asked about their primary motivators for doing on-farm research, farmer responses were productivity and profitability (72.9%, n=186), get some questions answered (51.4%, n=131), environmental stewardship (36.9%, n=94), receive recommendations (33.3%, n=85), and understand crop functioning (31.4%, n=80; Table 5.4). Enabling digital agriculture (7.5%, n=19) and food security (9%, n=23) were not primary motivators. Among the other options, farmers mentioned "*to advance soil health research"*, "*help university with research"*, "*seeing new things being tried without taking financial risks"*, "*reducing need of inputs", "to improve soil health and water quality"*, "*manage climate change"*, "*maintain extension relations"*, and "*support to learn and understand"*.

Table 5. 4**.** Farmers response to: What has been and/or would be your primary motivator for doing on-farm research?

What has been and/or would be your primary motivator for doing on-farm research?		
--- Response ---	--- N ---	-- %[a] --
Productivity and profitability	186	72.9
Enabling digital agriculture	19	7.5
Food security	23	9.0
Networking and sharing	74	29.0
Environmental stewardship	94	36.9
Get some questions answered	131	51.4
Receive recommendations	85	33.3
Understand crop functioning	80	31.4
Others	15	5.9
NA	16	6.3

[a] Percentage of multiple-choice questions is related to each possible answer.

Once queried about their satisfaction with university's investment in outreach and extension, 13.3% responded that they strongly agree that they are satisfied, 42% agree, 9.4% neither agree nor disagree, 5.9% disagree, and 23.5% strongly disagree (Table 5.5). Undoubtedly, the most extension events attended by the respondents were field days (65.5%, n=167), followed by meetings (45.1%, n=115), conferences (40.4%, n=103), and workshops (43.5%, n=111; Table 5.5). The majority of the respondents (59.2%) attend 1 (n=68) or 2 (n=83) field days per year, and 12.5% do not attend any field day (Table 5.5). Other types of events referenced by the respondents were "*ranch tours"*, "*webinars"*, "*reading online"*, and "*Zoom calls".*

Table 5. 5. Farmers response to: I am satisfied with the investment universities make in outreach and extension. What type of extension events do you usually attend? How many field days to you attend annually.

I am satisfied with the investment universities make in outreach and extension.			What type of extension events do you usually attend?			How many field days do you attend annually?		
Response	N	%	Response	N	%*	Response	N	%
Strongly agree	34	13.3	Field days	167	65.5	None	32	12.5
Agree	107	42.0	Workshops	111	43.5	One	68	26.7
Neither agree nor disagree	24	9.4	Seminars	90	35.3	Two	83	32.5
Disagree	15	5.9	Meetings	115	45.1	Three	19	7.5
Strongly disagree	60	23.5	Summits	19	7.5	> 4	40	15.7
NA	15	5.9	Conferences	103	40.4	NA	13	5.1
			Others	23	9.0			
			NA	23	9.0			

*Percentage of multiple-choice questions is related to each possible answer.

According to farmers responses, their main source of field information were universities (n=164) and private industry (n=145), with public institutions like the USDA ranking third (n=81) in the list (Figure 5.5a). Other sources of information given by the participants were "*other producers"*, "*seed companies"*, "*growers associations"*, "*crop consultants"*, "*magazines"*, "*private groups"*, "*newsletters", "industry trade groups"*, and "*books"*. Most of the respondents consult information sources less than once a month (n=66), once a month (n=71), and once a week (n=63; Figure 5b).

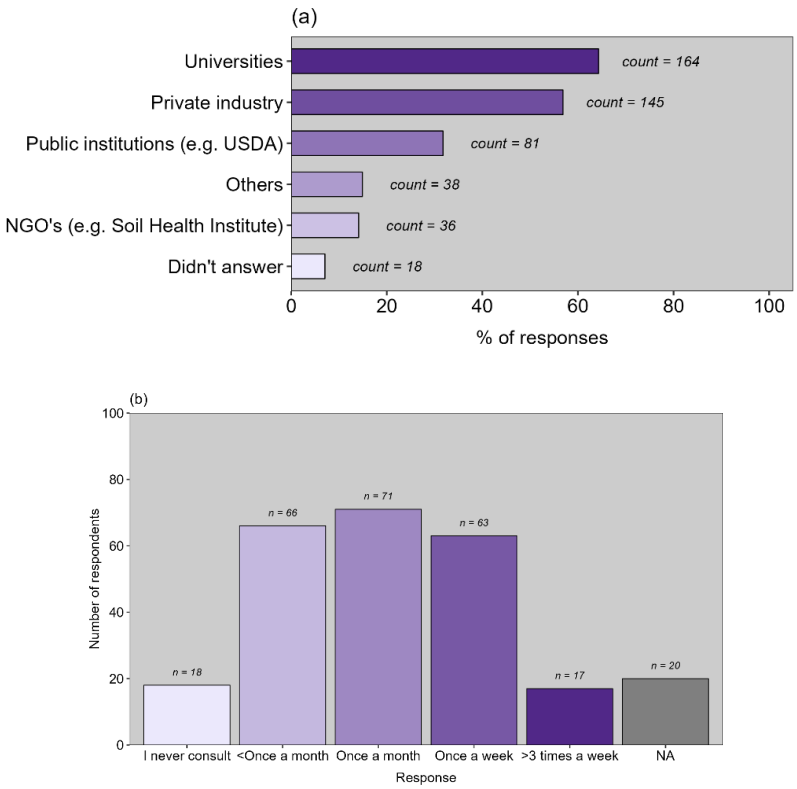


Figure 5. 5**.** Farmers response to: What is your primary source for field information? (a). How often do you consult field information sources? (b).

To understand what is new regarding management practices, the farmers were questioned about the major changes in their farming in the last 10 years (Figure 5.6). Out of the 255 farmers, 41.6% (n=106) of them cited cover crops and 17.6% (n=45) no-till as major changes to their farming, followed by irrigation (5.5%, n=14), precision agriculture (5.5%, n=14), diverse crop rotation (4.3%, n=11), and reduced tillage (3.9%, n=10). Ninety farmers reported no changes (35.2%). Understanding that precision agriculture is one of the main new approaches to agriculture, farmers were asked whether they use available precision agriculture technologies (Figure S5.1); 54 responded that they do not use precision agriculture technologies; 131 use grid soil sampling; 124 variable rate fertilizer, and 94 variable rate seeding. Since combine-mounted

yield monitors are one of the proctors for implementing on-farm research, the farmers were asked whether they have it available; 171 farmers have yield monitors available (Figure S5.2).

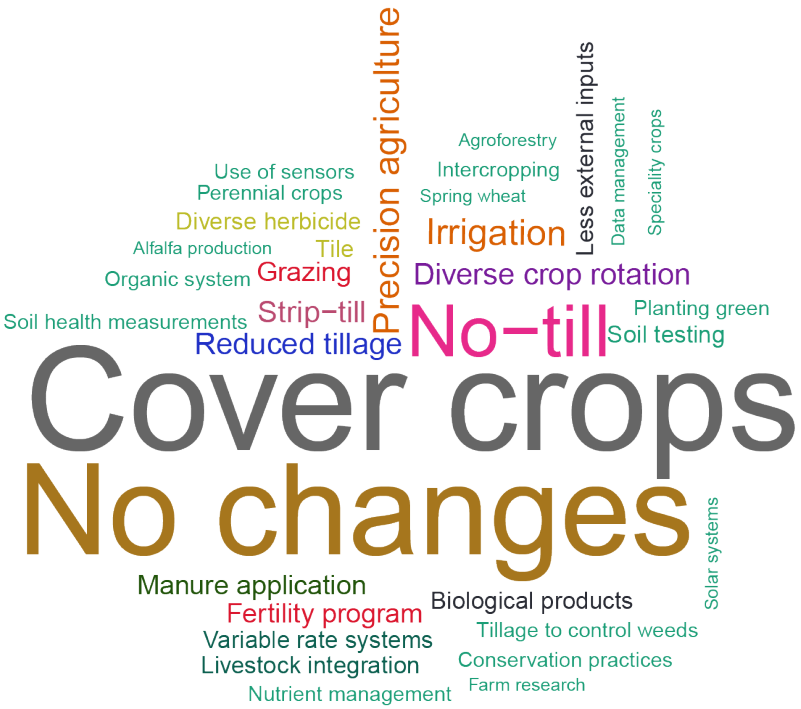


Figure 5. 6. Farmers response to: Did you have any major changes to your farming in the last 10 years? Minimum and maximum frequency: 1, 106.

Discussion

This study provides unique evidence on the overall perspective of U.S. farmers regarding on-farm research. Although our sample size was relatively small compared to the total number of farmers in the U.S., the survey effectively covered a large geographical area. It revealed that on-farm research is a relevant topic for many U.S. farmers. The average age of the survey respondents was 50, which falls close to the average age of farm producers (57) reported in the 2017 Census of Agriculture (USDA NASS, 2019). Approximately 34% of the respondents were less than 35 years old (n=87), representing a younger generation of farmers. Young farmers may act as bridges linking distinct groups of farmers and help disseminate critical information, such

as adopting new practices or adapting to new challenges (Parks, 2022). The average cropland area from the survey (610 acres) approximates the average U.S. farm size reported by the USDA (446 acres) (USDA NASS, 2023). The data presented in Figure 5.5a suggests that on-farm research is not a new or unfamiliar concept for most of the surveyed population. This finding aligns with Kyveryga (2019), who stated that on-farm research rapidly expanded during the last two decades, especially in developed countries like the U.S. For instance, the Nebraska on-farm research network completed 101 on-farm studies from 1990 to 2019. One of the main reasons for this increase was the use of precision agriculture tools such as global navigation systems, variable rate devices, and yield monitoring (Bullock et al., 2019; Kyveryga, 2019). Precision agriculture was considered by 99 farmers as one of the best describers for on-farm research. Although most of the surveyed farmers were familiar with on-farm research, they had a split level of involvement. One of the explanations for the varying degree of engagement was that participatory research applies more to resourceful farmers than to marginal ones (Witcombe, 1999). On-farm research is often, but not only, adopted by farmers that use modern technology and can overcome labor and time management challenges with geographic information system (GIS) and precision agriculture systems. These farmers may use global navigation satellite systems-driven equipment for plot establishment, planting, and harvesting (Bullock et al., 2019). Similarly, variable rate systems and sprayers with section control may be used and help to overcome time management, the main on-farm research limitation highlighted in this study. It is also important to consider that although modern technology can certainly help the on-farm research process regarding time and labor management, farmers deciding whether to engage in on-farm research may go beyond the availability of precision agriculture systems and farm

machinery. Also, systems-thinkers and risk-taking farmers might be more willing to conduct on-farm research and adopt new practices (Church et al., 2020).

The terms that best described on-farm research included side-by-side, plots, real-scale, and replication, indicating that the respondents associate on-farm research with practices that involve comparing different treatments or interventions under actual farming conditions and observing their effects in a replicated manner. This finding underscores the importance of conducting research on actual farms to achieve scalable results and perhaps the adoption of certain practices (Lacoste et al., 2022). The concept of partnership, which signifies a connection between farmers and research institutions such as universities, received one of the lowest scores among the respondents. This suggests that the current perceived level of collaboration between farmers and research institutions might be relatively low, or farmers do not believe they need partnerships for conducting on-farm research. Despite this result, the respondents also valued on-farm research as a way to build connections with researchers and extensionists. It would be imperative for researchers trying to establish on-farm research to articulate and develop the partnership concept better. Thompson et al. (2019) underlined that positive experiences in the Nebraska on-farm research program were primarily credited to interactions between the university and farmers. Even though the data suggests that the current level of collaboration is relatively low, the survey data indicate that most respondents expressed a positive inclination towards collaboration with universities for on-farm research. This suggests that although some farmers may be skeptical, most tend to be open to partnering with universities to conduct research activities on their farms. This willingness for collaboration highlights the potential for productive interactions between farmers and researchers (Adamsone-Fiskovica and Grivins, 2022; Toffolini and Jeuffroy, 2022). Further exploration of this aspect could provide value for

improvement in fostering more robust partnerships between farmers and research institutions in the U.S., while serving as a model example for other regions across the globe.

Our data revealed that a substantial percentage of respondents indicated a high likelihood of adopting practices recommended based on on-farm studies. This shows a high level of trust and confidence among farmers in the findings and recommendations derived from on-farm research. In Nebraska, 75% of farmers interviewed by Thompson et al. (2019) had put their research results into practice in their farm operations, either by making a change based on on-farm research results or by not making a change as the research confirmed better results using their current practices. It is important to note that farmers recognize the relevance and applicability of on-farm studies and their willingness to implement the practices suggested by such research to improve their farming operations. In the study by Thompson et al. (2019), a farmer who was interviewed mentioned having intentions of making a change. However, the research outcomes ultimately provided the necessary confidence to carry out the proposed practice. Our own findings, consistent with other research, suggest that researchers should deepen their understanding, educate students, and actively work towards expanding networks for on-farm research. By doing so, the adoption of new practices can be accelerated and enhanced. The notably higher likelihood of adoption observed through participation in on-farm research is a robust indicator of its potential impact. This, in turn, should play a crucial role in shaping decisions made by both university administrations and government policymakers. Additionally, it may assist in promoting more targeted outreach and extension efforts.

Furthermore, the adoption rate of practices not tested with on-farm research (e.g., small plots and greenhouse) is significantly lower, and only 25% of the recommended practices from non-on-farm studies are likely to be adopted by farmers. This disparity highlights the perceived

value and credibility that farmers place on on-farm research compared to other types of research. It also underscores the importance of conducting research directly on farms to generate recommendations that farmers find more relevant and applicable to their specific contexts and environments (Koehler-Cole et al., 2023; Snapp et al., 2023). On-farm research, hence, has the potential to become a leading catalyst for helping farmers to adopt sustainable practices. Nevertheless, the importance of small plots and greenhouse studies cannot be understated in the context of agricultural development. Such efforts are crucial in refining the list of potential practices that can realistically be subjected to on-farm comparisons. Another outcome of this work is that 88% of the farmers that agreed or strongly agreed that on-farm research is important for strengthening relationships between producers and universities serve as an indication that farmers recognize the potential benefits of on-farm research beyond the specific research outcomes. Previous research has indicated that farmers value the collaborative aspect of on-farm research and acknowledge its role in fostering stronger connections and partnerships between themselves and research institutions (Snapp et al., 2023). The mutual understanding of the importance of on-farm research for relationship building further emphasizes the potential for fruitful collaborations between farmers and universities across the U.S.

When considering the limitations of on-farm research, the respondents identified time management as the main challenge. Allocation of time is a significant constraint when engaging in on-farm research activities. Another noteworthy limitation mentioned by the respondents was the plot setup, which implies that establishing and maintaining research plots on farms can pose practical challenges. On the other hand, the respondents recognized several strengths of on-farm research. The most prominent strength mentioned was the knowledge gained from conducting research on their farms. This finding highlights the value of on-farm research in generating

practical knowledge and insights directly applicable to farmers' operations. Access to unbiased research information and the opportunity to conduct applied research was also emphasized as strengths of on-farm research. In-depth interviews with farmers from the Nebraska On-Farm Research Network revealed that farmers conducted on-farm research mainly to increase profitability and to fulfill their need for unbiased results (Thompson et al., 2019). Moreover, the farmers responded that they consider on-farm research one of the best education opportunities, demonstrating its educational value and effectiveness as a learning-by-doing opportunity for farmers (Krupek et al., 2021; Snapp et al., 2023). This also indicates a positive attitude towards the impact of on-farm research in driving and supporting farm-level innovation.

On-farm research is demand-driven (Lacoste et al., 2022). Unsurprisingly, the primary motivator for doing on-farm research was productivity and profitability (n=186), which underscores the practical and economic considerations that drive farmers to conduct research on their farms. Farmers recognize that implementing research-based practices can increase productivity and profitability, making it a crucial motivation for their involvement in on-farm research activities. Another significant motivator identified by 131 farmers was the desire to get some questions answered, which most time is a shared goal between farmers and researchers. This suggests farmers engage in on-farm research to address specific questions or uncertainties about their farming practices, which can align well with entities assisting in the research process (e.g., educators, universities). Another important motivator was environmental stewardship. This motivator can be directly linked to the major changes that the respondents had on their farms. Unquestionably, the recent adoption of cover crops and no-tillage (Figure 5.6) pointed farmers toward increased agricultural sustainability, resilience, and environmental stewardship. In the "other" category, farmers also cited that "advancing soil health research", "reducing the need for

inputs", "improving soil health and water quality", and "managing climate change" are important motivators. The survey did not identify enabling digital agriculture and food security as primary motivators. While these aspects are important (Bullock et al., 2019), they may not be the primary driving forces behind farmers' engagement in on-farm research activities across our surveyed population.

Field days were the most popular events attended by farmers, followed by meetings, conferences, and workshops. This reveals that farmers actively engage in these face-to-face events to acquire knowledge and stay updated with the latest information available to manage their fields (Heiniger et al., 2002). It is worth noting that a surprising number of respondents, 32 in total, reported not attending any field day, indicating a potential gap in their access to this type of extension activity. Field days are an important opportunity to facilitate the adoption of desired farming practices and have been a staple of agricultural organizations for a long time (Singh et al., 2018). However, highlight the changes and alternatives for knowledge delivery used by a certain group of farmers, and the extension delivery approach should constantly explore alternative methods.

Both universities and private industry serve an important role as sources of field information for farmers and were cited as the most influential by the farmers, followed by public institutions like USDA. This result brings the opportunity to trusted organizations such as Land Grant Universities to foster networking opportunities between well- and less-connected farmers to guide new trusting relationships of knowledge exchange. The adoption of practices by farmers can be increased when using a knowledge network supported by programs and resources that incorporate technical, social, and experiential learning pathways (Wick et al., 2019). Respondents also mentioned other valuable sources, such as other producers, seed companies,

and grower associations. Friendly communication with neighbors or trusted partners tends to be more readily trusted than information from outsider sources (Hoffmann et al., 2007). In the United Kingdom, farmers were more willing to try a new sustainable agricultural practice when influenced by another farmer or an advisor they knew, trusted, and had a long-term relationship (Rust et al., 2021). This highlights the importance of establishing trust via on-farm research networks to involve the farming community and tackle critical concerns related to production, profitability, and the environment. Farmers are usually more open to new practices when they learn them through observation, trials, and two-way dialogue (Tarnoczi and Berkes, 2010). Importantly, our study revealed that farmers rely on diverse sources to gather information and knowledge about their farming practices and are regularly interested in accessing new knowledge and staying informed about management practices, just as suggested by Rust et al., 2022. The frequency (e.g., once a month or better) at which farmers use field information is a robust opportunity for researchers and universities to influence and support the decision-making process by farmers. Although most respondents are satisfied with university investment in research and extension, the data indicated some level of dissatisfaction among some farmers. Augmented investment could potentially become imperative to align with farmers' expectations and requirements, as well as to facilitate the nationwide utilization of on-farm research to enhance adoption rates.

Regarding major changes in their farming practices in the last 10 years, cover crops and no-till dominated the responses. This reflects a significant shift towards conservation-oriented practices to improve soil health and reduce environmental impacts (Pires et al., 2020; Bowman et al., 2022). Other changes mentioned included irrigation, precision agriculture, diverse crop rotations, and reduced tillage. Our results may be reflecting the current research agenda and

investments that conservation agriculture (e.g., cover crops, no-till) research has received in recent years. Increased funding and resources have become available in recent years for agricultural producers to improve soil health and prevent nutrient loss and erosion (Popovici et al., 2023). Notably, 35% (n=90) of the farmers reported no changes, indicating a potential resistance or slower adoption of new practices. Alternatively, it may represent that some producers have already incorporated these practices into their regular operation for more than 10 years. For late adopters, mentoring from a trusted source could be critical to facilitate the adoption of new practices (Bagnall et al., 2020). In terms of precision agriculture, our study suggests that while some farmers are adopting new agriculture technologies, a portion of the farming community still has not embraced these advanced tools. McFadden et al. (2023) surveyed with U.S. farmers to understand the adoption of precision agriculture in the digital area. The authors reported that in many cases, the adoption decisions were based on their expectation of how the tools would affect their farming operation performance. In addition, the availability of combine-mounted yield monitors throughout the survey community was high. Thus, a considerable number of farmers have the necessary tools for collecting yield data, which can contribute toward implementing on-farm research (Gauci, 2022) and address productivity and profitability concerns. These were primary motivators for farmers to engage in participatory on-farm research.

Overall, this study features farmers' high trust in on-farm research outcomes, influencing practice adoption. Limitations like time management and plot setup were identified, while strengths included practical knowledge gained and unbiased insights. Notably, a shift towards conservation practices was evident, with cover crops and no-till at the forefront. Insights of this nature have implications for fostering collaborations, addressing constraints, and maximizing the

impact of on-farm research, offering guidance for sustainable agricultural progress in the U.S. and potentially beyond.

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Supplementary material

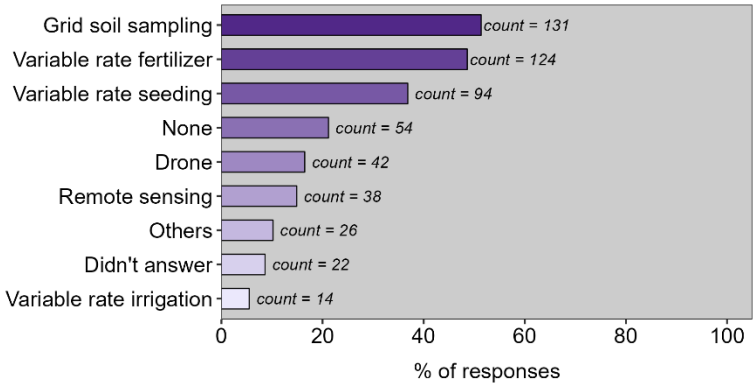


Figure S5. 1. Do you use available precision agriculture technologies?

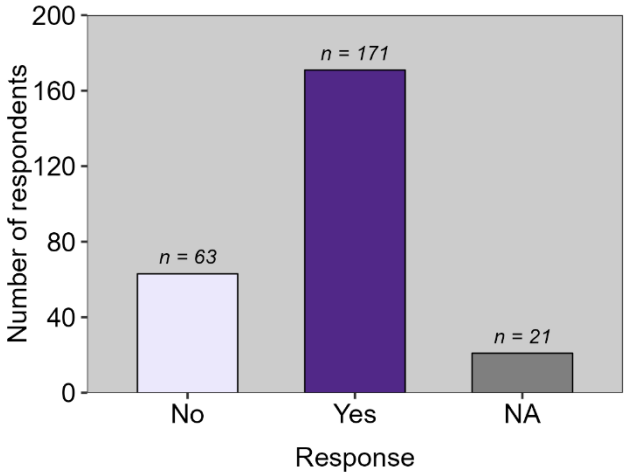


Figure S5. 2. Do you have yield monitor in your combine?

Chapter 6 - Conclusion

The overall goal of this research was to explore on-farm sustainable agricultural practices to design farming systems for sustaining soil health management in Kansas. These chapters quantified the impact of cover crops and nutrient management on biological, chemical, and physical soil health indicators at the farm level. Chapter two underscored the importance of CCs in improving soil health, as evidenced by the increase in soil microbial biomass, bacteria, fungi, SOC, βG, and many other biological soil health indicators (Figure 6.1). Overall, the extent of these changes occurred in response to the precipitation patterns across sites. Our findings support the hypothesis that increasing SOC levels and stimulating soil microbial growth and activity under water-limited environments requires a longer time. This also implies that in water-limited environments, CCs are crucial to augment aboveground plant biomass production. In contrast, in non-water-limited environments like eastern Kansas, CCs may not substantially impact total biomass production due to the already high-yielding nature of cash crops.

In chapter three valuable insights were gained on the impact of CC on soil aggregation, highlighting the relationship between CCs, aggregation, and soil microbes. This study found that the continuous presence of living roots from CCs significantly impacted the development and maintenance of macroaggregates as affected by increased fungi and bacterial communities.

The information in chapter four contributed to the existing knowledge on N management for corn and winter wheat under RA practices, offering valuable insights to enhance agricultural sustainability. For the field conditions of the study, decreasing the N rate by 48% for winter wheat production was feasible without compromising yields and SOC levels in the short term. However, reducing the N rate would not be advisable for corn production as it may lead to economic losses. This approach can empower farmers to take advantage of the mineralized N

from the SOM increased over time and use external inputs more efficiently, ultimately promoting soil health.

Farmers' high trust in on-farm research outcomes can influence the adoption of sustainable practices (Chapter 5). Limitations like time management and plot setup were identified in on-farm research, while strengths included practical knowledge gained and unbiased insights. Notably, a shift towards conservation practices has been made in the last 10 years, with cover crops and no-till at the forefront of these changes. We also found that farmers were 40% more likely to adopt practices supported by findings of on-farm research compared to off-farm ones, and farmers' primary motivators for doing on-farm research were productivity and profitability. Insights of this nature have implications for fostering collaborations, addressing constraints, and maximizing the impact of on-farm research, offering guidance for sustainable agriculture progress in the U.S. and beyond. Results from this research survey can drive further policy and initiatives promoting on-farm research.

Ultimately, the diverse array of scientific methods employed in this work, from laboratory-based analysis to on-farm research and nationwide survey, significantly contributed to advancing the scientific understanding of how agricultural practices impact soil-crop outcomes and how farmers' perspectives about on-farm research impact their decision-making. Overall, this research offered a strong foundation for practical applications that can contribute to the development of more resource-efficient, profitable, resilient, and healthy agricultural systems. This research can potentially guide farmers and land managers in optimizing their farming strategies for a more sustainable future in agriculture.

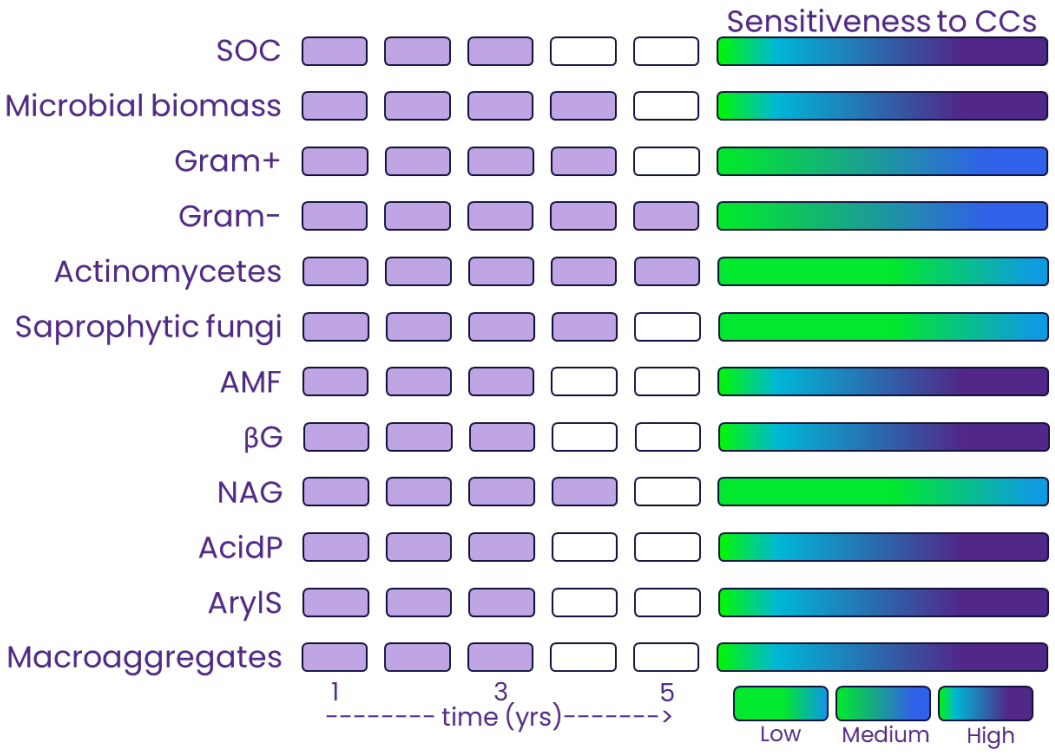


Figure 6. 1. Time scale changes and sensitiveness of biological soil health indicators to cover crops (CCs).

Future Research and Considerations

Future prospects from this research could involve further refining CC use to different agricultural environments and conditions, potentially leading to more sustainable and resilient farming systems. Future studies could explore additional strategies and crop choices that optimize soil health in regions facing water scarcity. For nutrient management, further studies could explore the long-term effects of reduced N rates on soil health and crop productivity after long-term RA. Moreover, it is necessary to include more site-years to cover a broader range of soil types and climate patterns.

This research demonstrated opportunities to integrate soil health research and outreach and extension programming. Applied research that combines extension services has increasingly concentrated on the production of high-value or commodity crops, often neglecting diversified and alternative cropping systems. Consequently, there is a growing need for more participatory on-farm research to increase knowledge of soil health. Expanding on-farm research in soil health calls for establishing farmer-to-farmer mentoring programs, facilitated by extension services, to reinforce soil health knowledge and disseminate innovative practices. Future prospects may involve developing mechanisms to facilitate and incentivize on-farm research, enabling more farmers to confidently adopt sustainable practices. In summary, for extension services to play a valuable role in soil health, they must embrace a holistic approach that extends beyond the dissemination of information. This approach entails recognizing existing knowledge, fostering partnerships, and addressing the complex socio-economic factors that shape the adoption of soil health practices. Finally, it is crucial to realize that the timelines of researchers and farmers may not always align. For the establishment and sustainability of last-longing partnerships, developing on-farm soil health programs that deliver timely information is imperative, effectively supporting decision-making processes. In essence, these programs should be designed to bridge the gap between the often-divergent timelines of research and the practical needs of farmers, ensuring that research findings are readily available when farmers require them for informed decision-making. This synchronization is fundamental to the success and longevity of collaborative efforts in soil health management.

Appendix A – Chapter 2

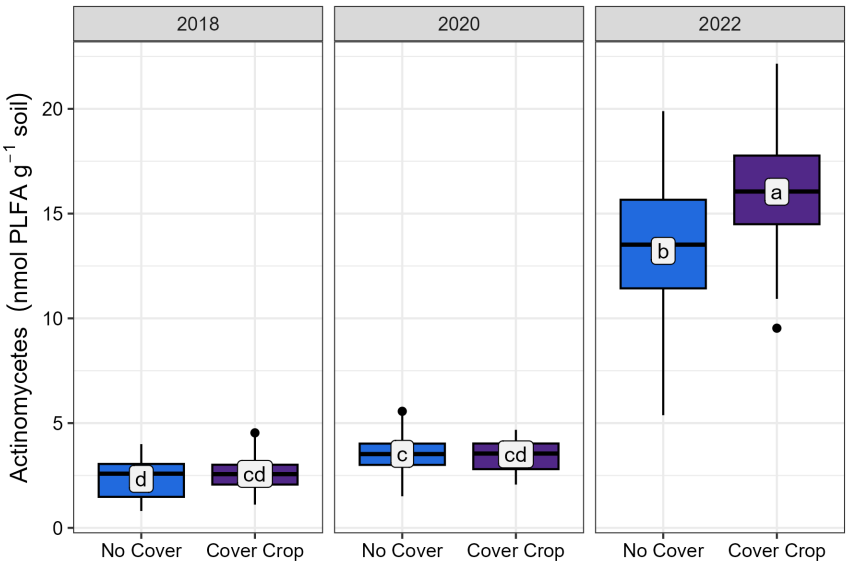


Figure A.1. Actinomycetes by treatment and year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

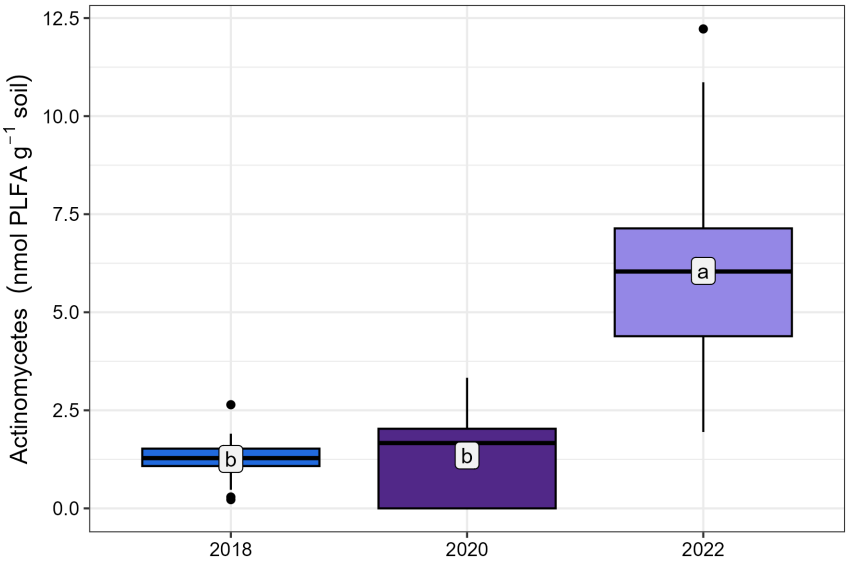


Figure A.2. Actinomycetes by treatment and year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

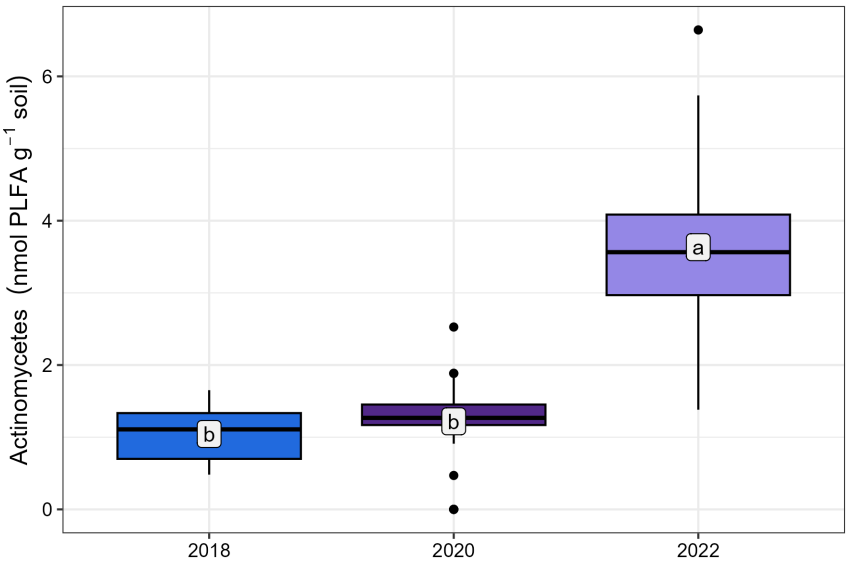


Figure A.3. Actinomycetes by treatment and year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

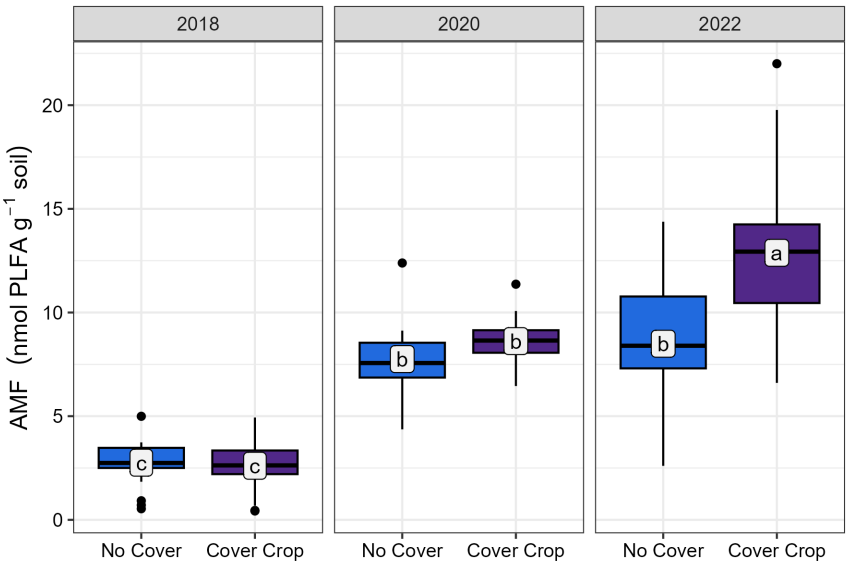


Figure A.4. Arbuscular mycorrhizal fungi (AMF) by treatment and year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

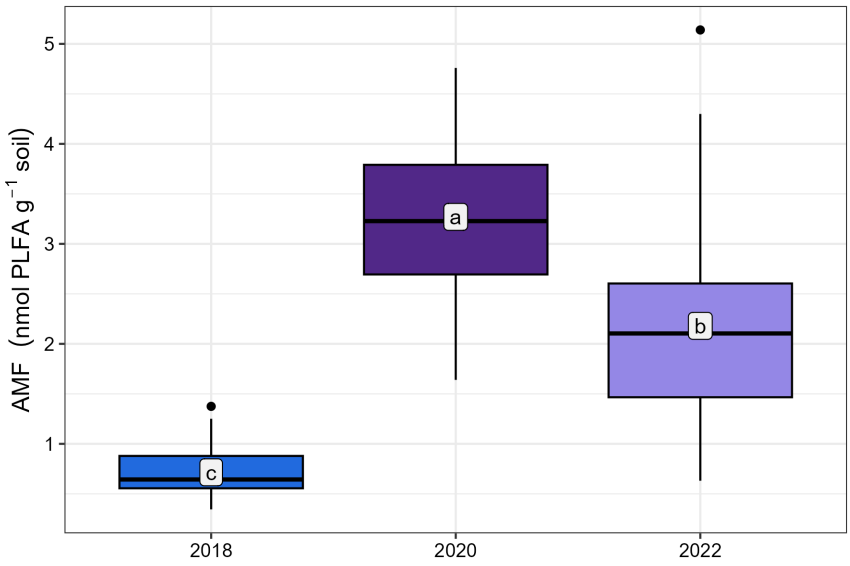


Figure A.5. Arbuscular mycorrhizal fungi (AMF) by year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

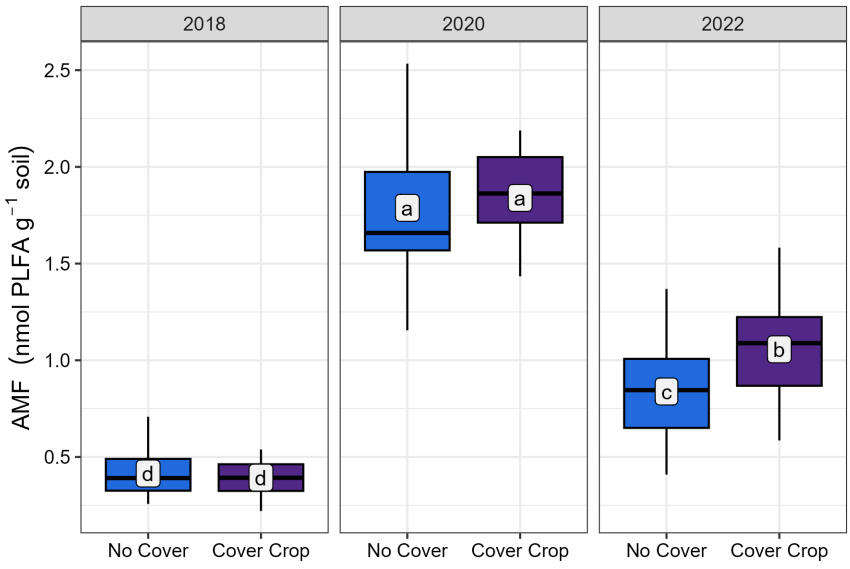


Figure A.56. Arbuscular mycorrhizal fungi (AMF) by treatment and year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

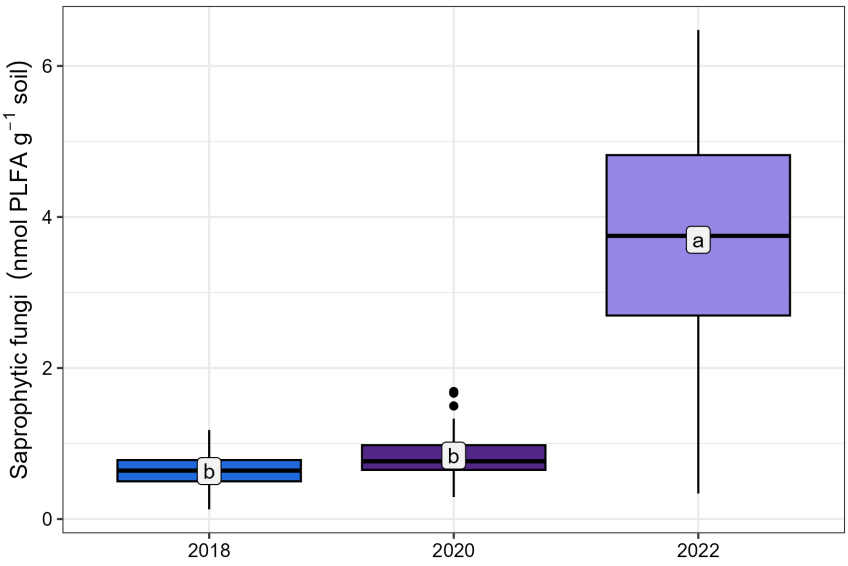


Figure A.7. Saprophytic fungi by year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

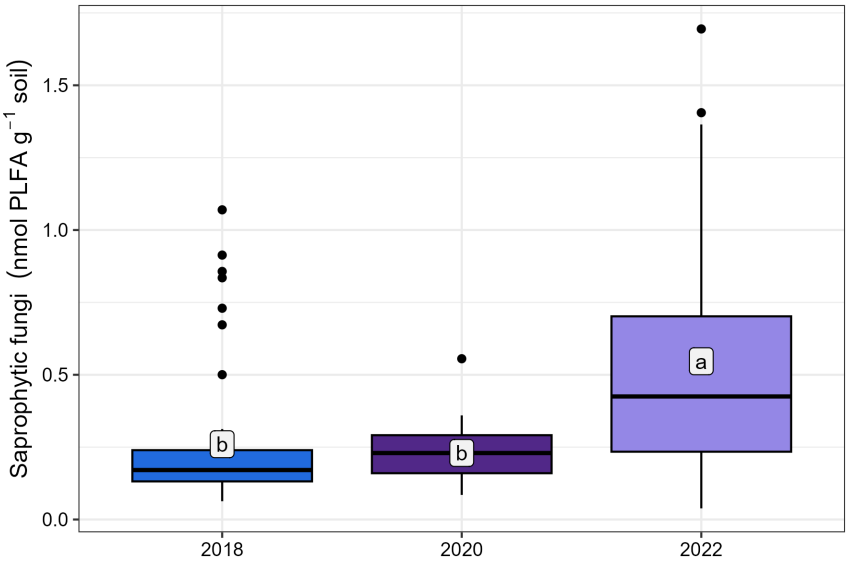


Figure A.8. Saprophytic fungi by year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

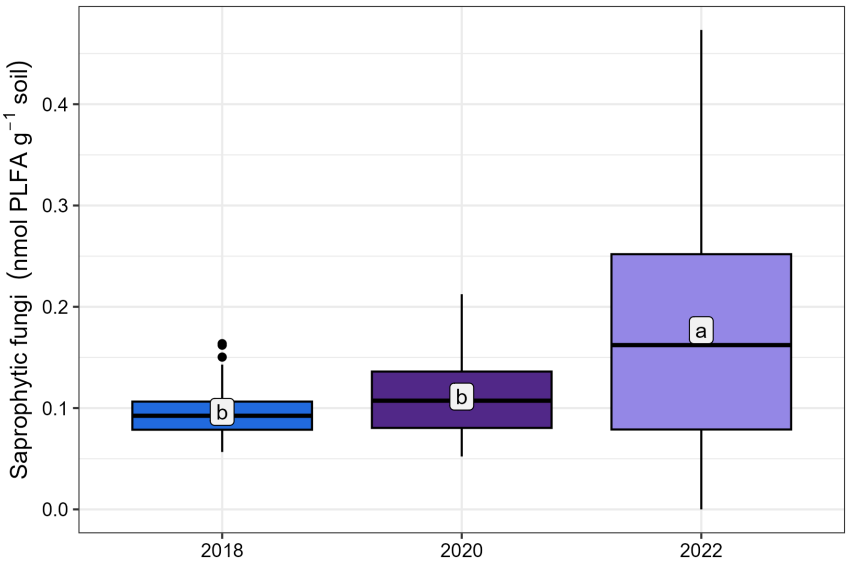


Figure A.9. Saprophytic fungi by year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

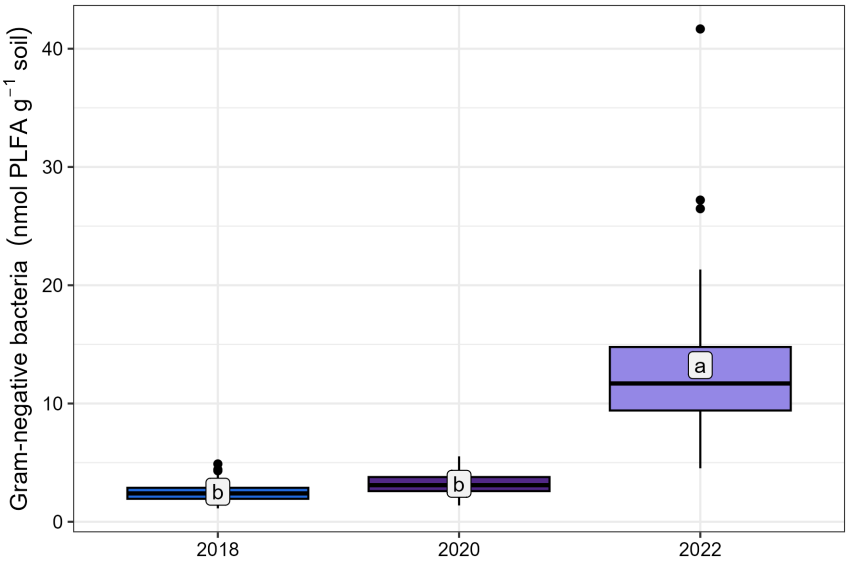


Figure A.10. Gram-negative bacteria by year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

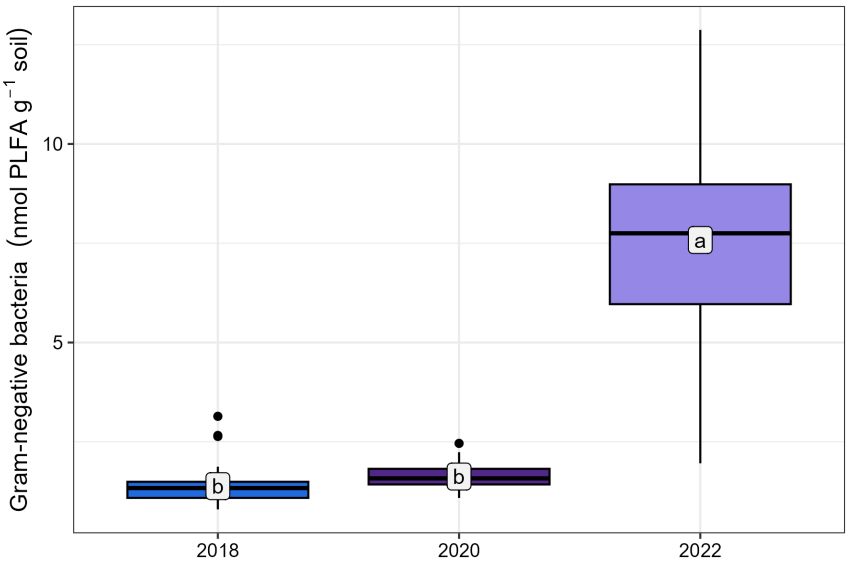


Figure A.11. Gram-negative bacteria by year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

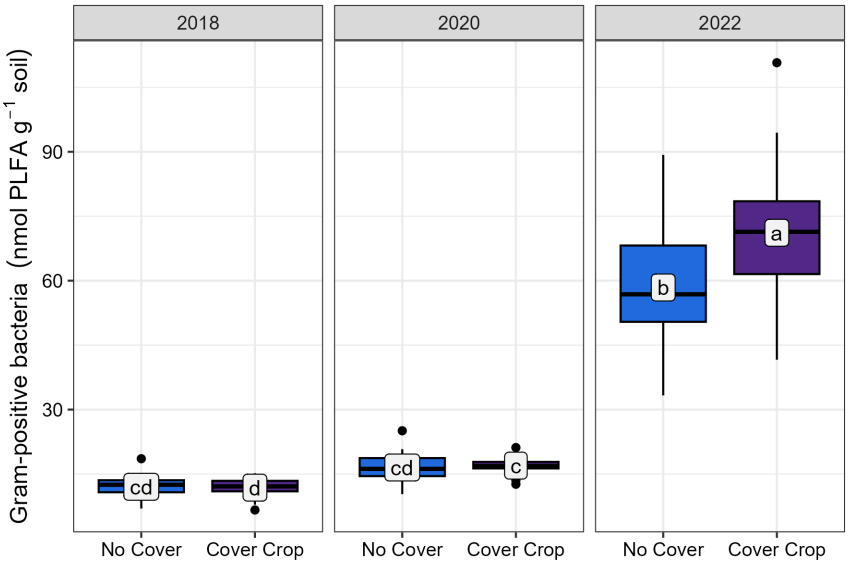


Figure A.12. Gram-positive bacteria by treatment and year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

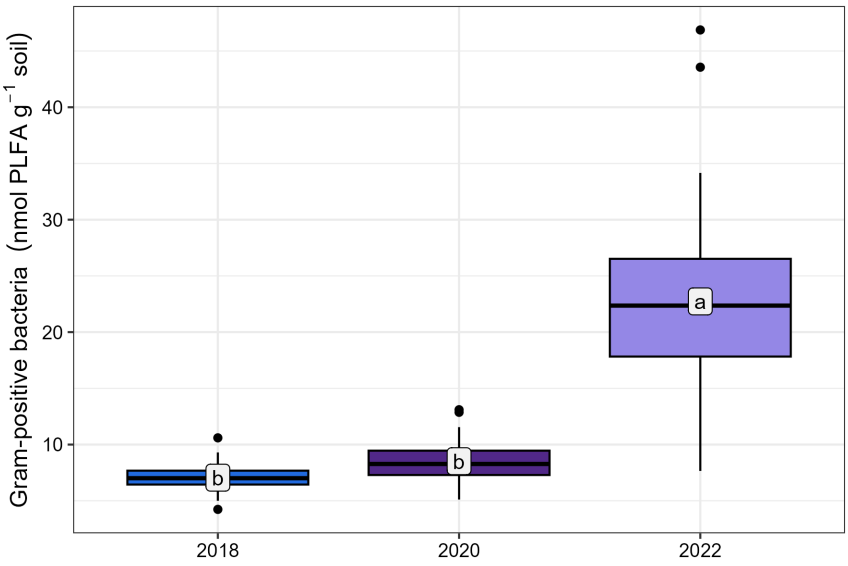


Figure A.13. Gram-positive bacteria by year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

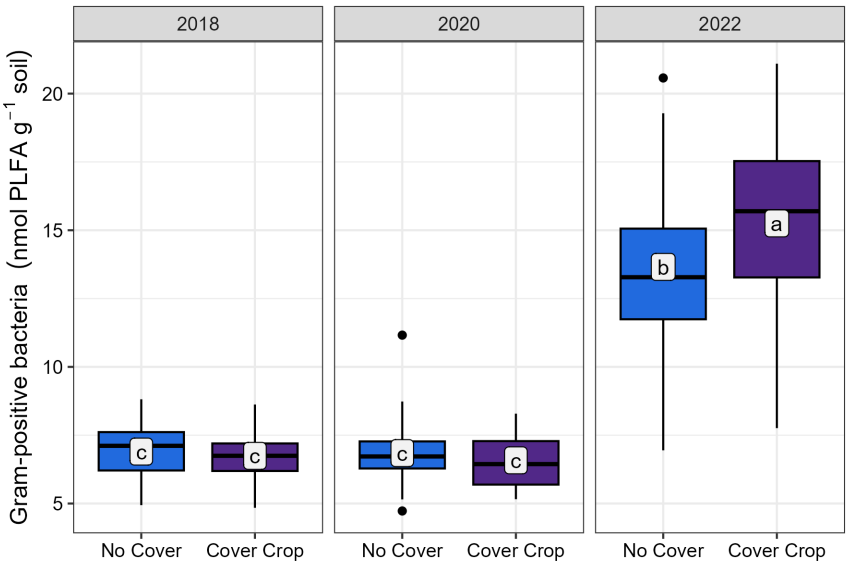


Figure A.14. Gram-positive bacteria by treatment and year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

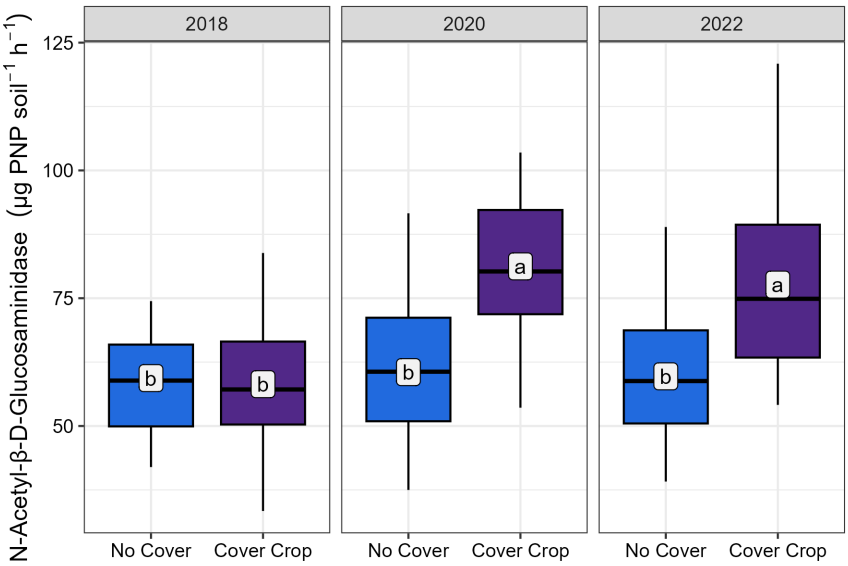


Figure A.15. N-acetyl-β-D-Glucosaminidase activity by treatment and year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

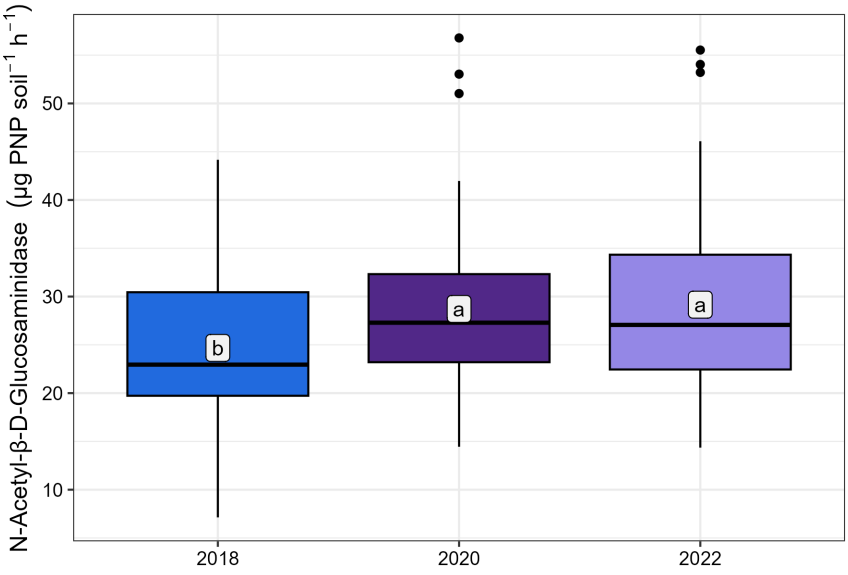


Figure A.16. N-acetyl-β-D-Glucosaminidase activity by year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

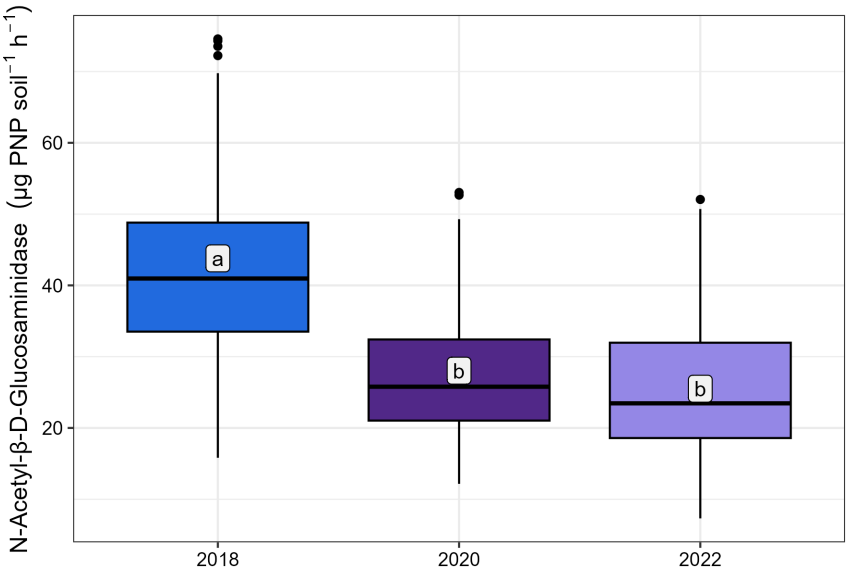


Figure A.17. N-acetyl-β-D-Glucosaminidase activity by year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

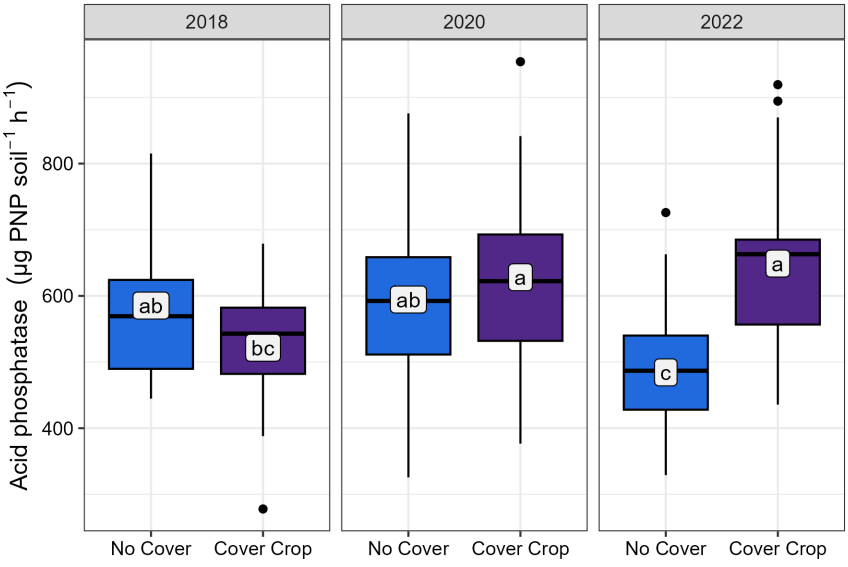


Figure A.18. Acid phosphatase activity by treatment and year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

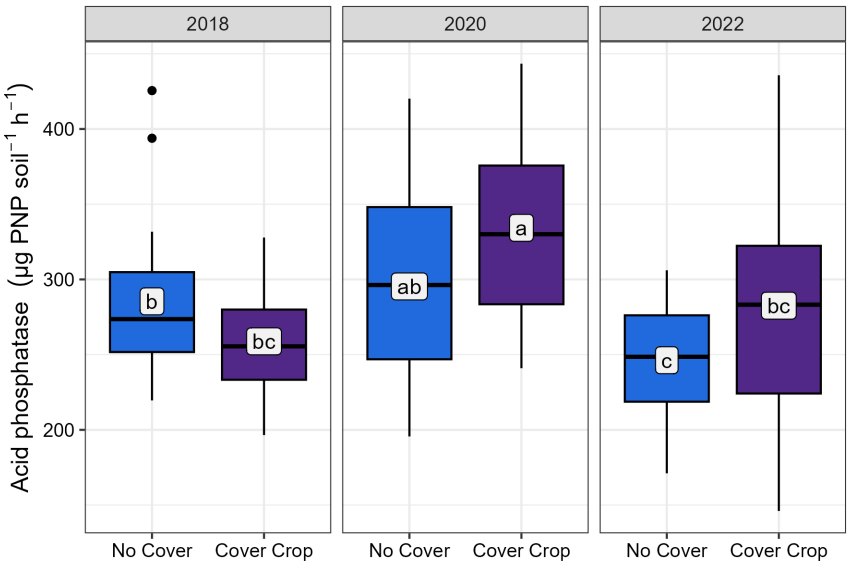


Figure A.19. Acid phosphatase activity by treatment and year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

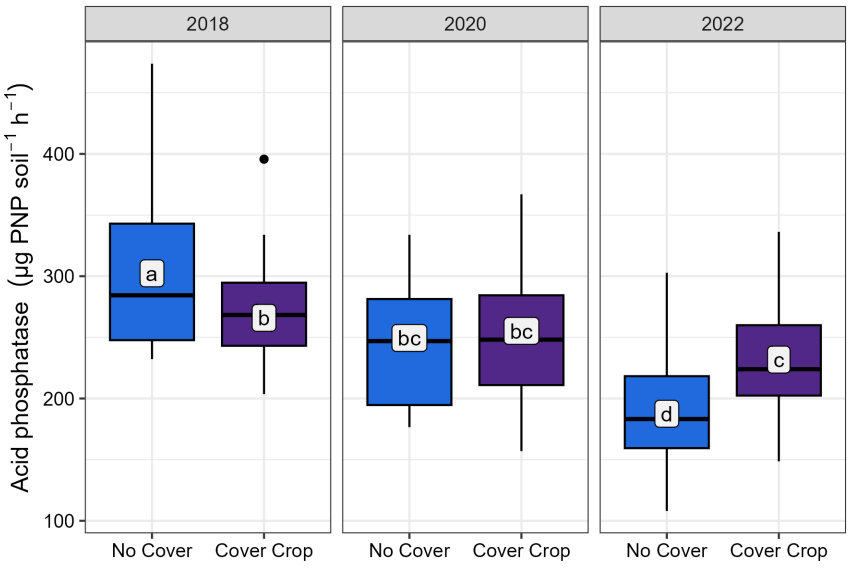


Figure A.20. Acid phosphatase activity by treatment and year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

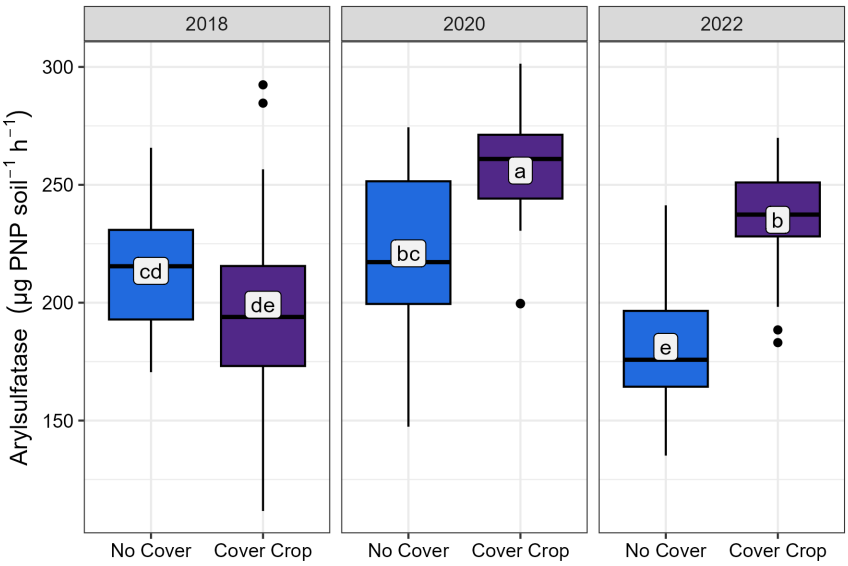


Figure A.21. Arylsulfatase activity by treatment and year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

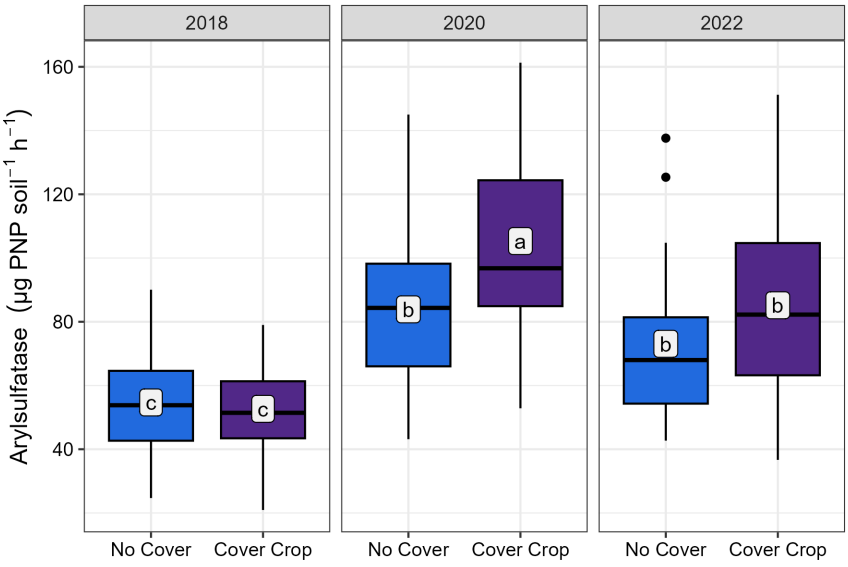


Figure A.22. Arylsulfatase activity by treatment and year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

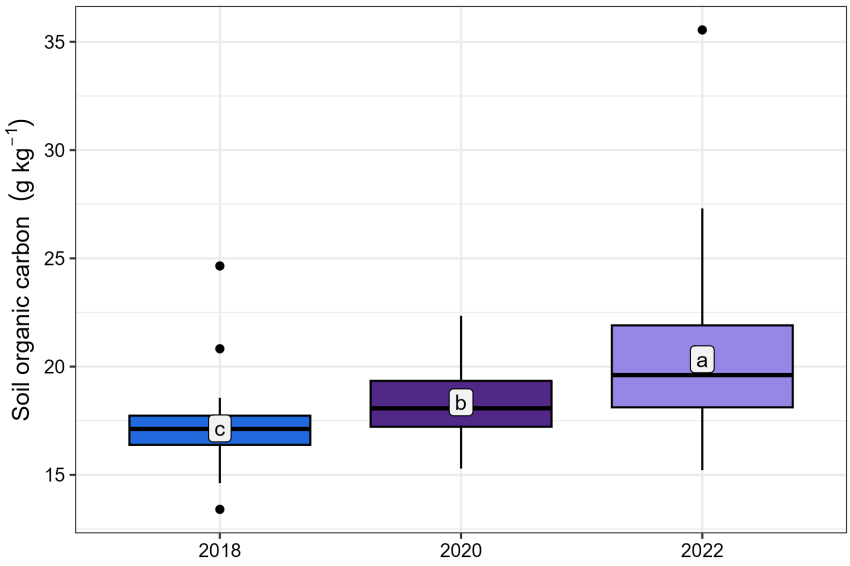


Figure A.23. Soil organic carbon by year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

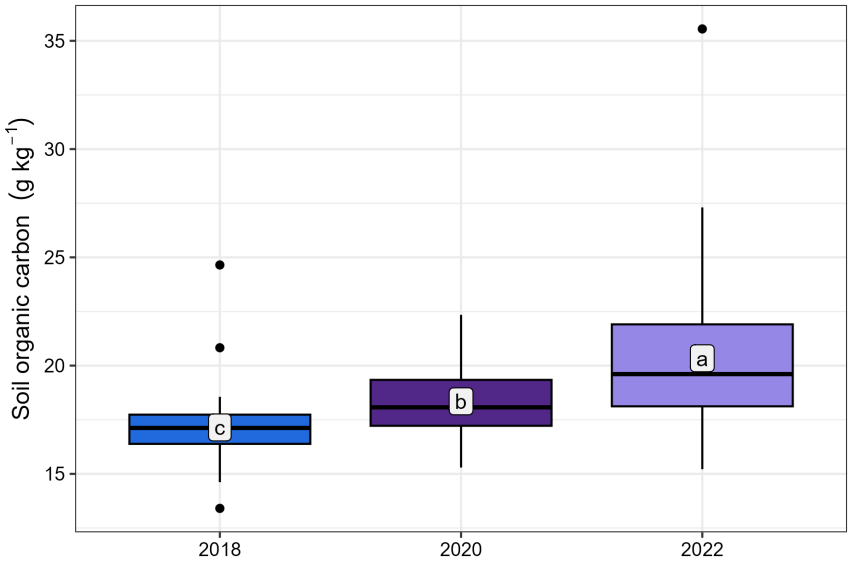


Figure A.24. Soil organic carbon by year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

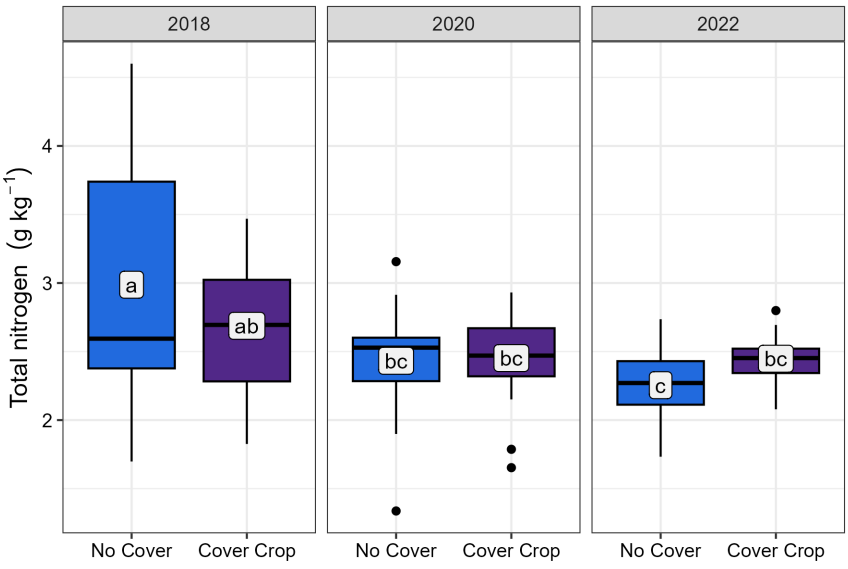


Figure A.25. Total nitrogen by treatment and year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

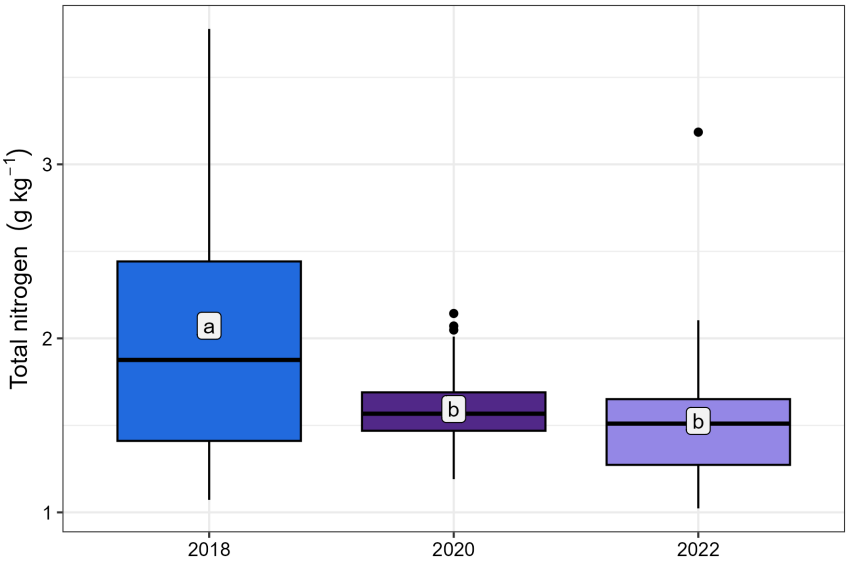


Figure A.26. Total nitrogen by year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

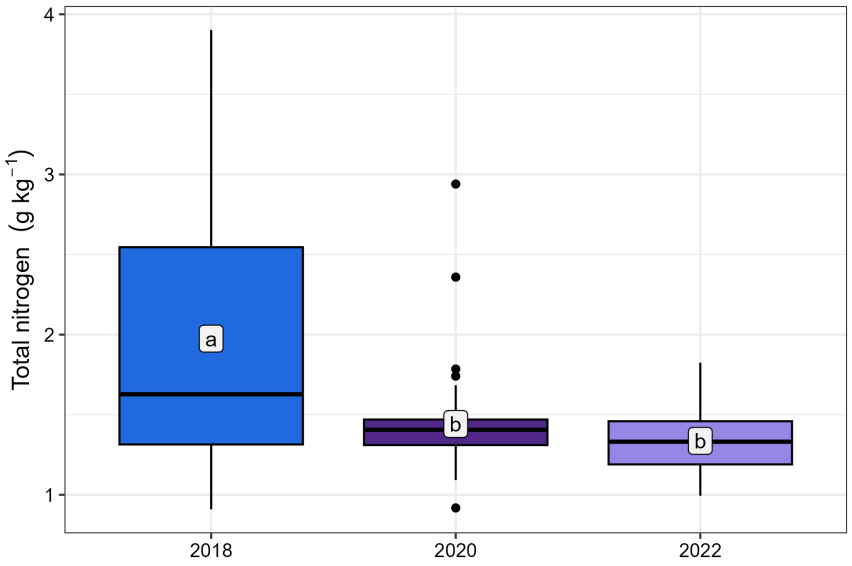


Figure A.27. Total nitrogen by year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

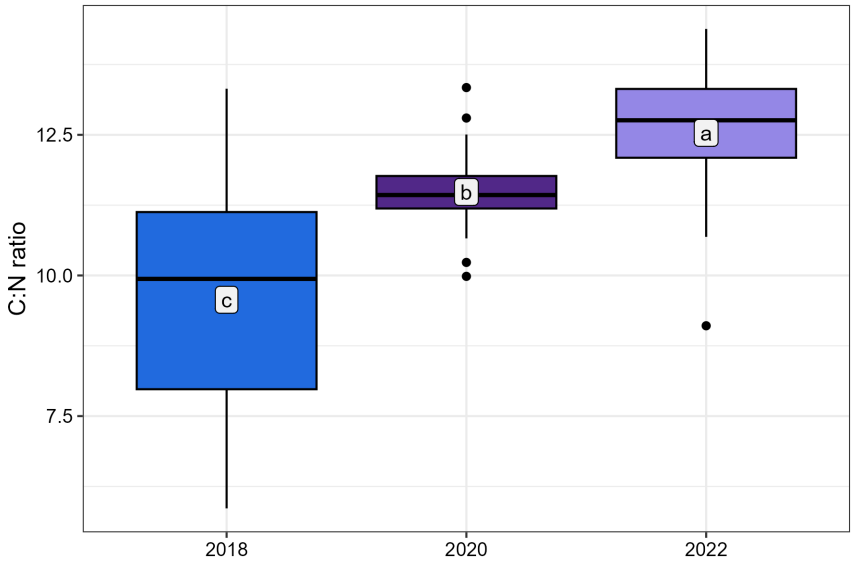


Figure A.28. C:N ratio by year at 0-5 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

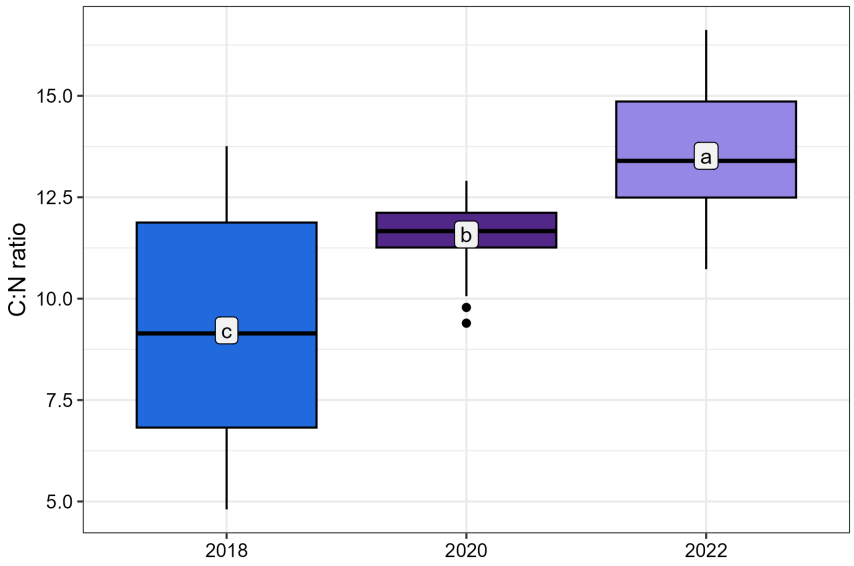


Figure A.29. Carbon: nitrogen ratio by year at 5-15 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

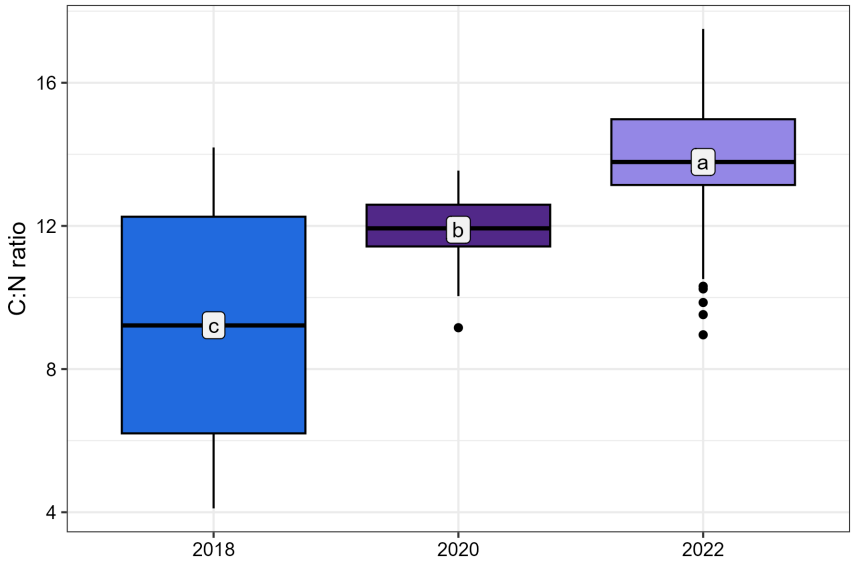


Figure A.30. Carbon: nitrogen ratio by year at 15-30 cm depth at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

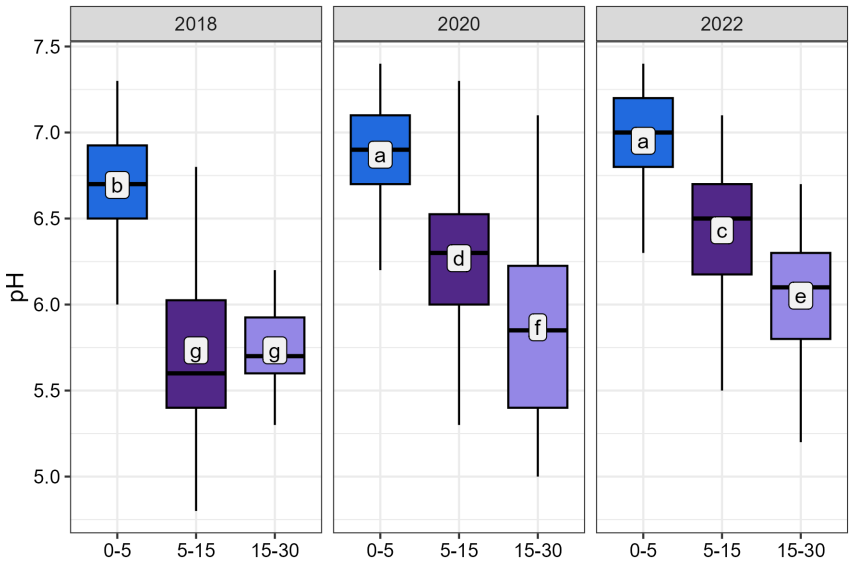


Figure A.31. Soil pH by depth and year at Bucyrus. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

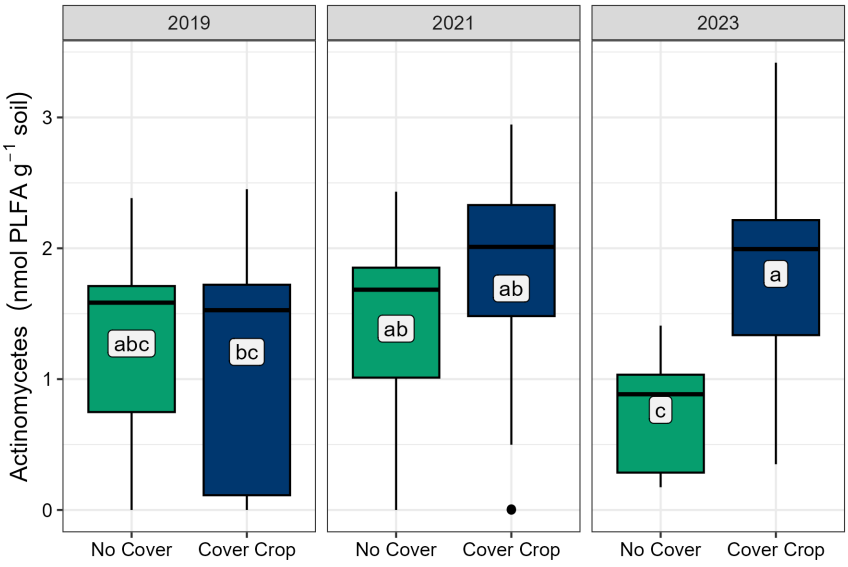


Figure A.32. Actinomycetes by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

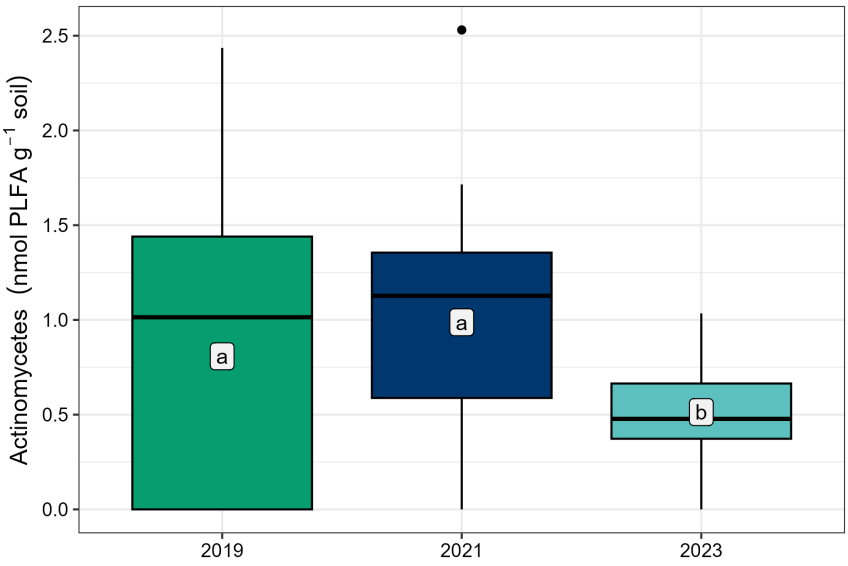


Figure A.33. Actinomycetes by year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

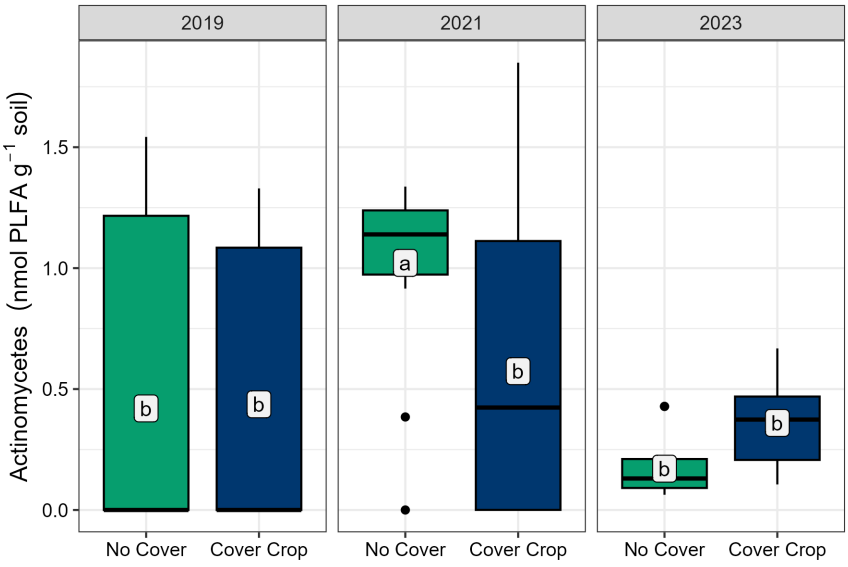


Figure A.34. Actinomycetes by treatment and year at 15-30 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

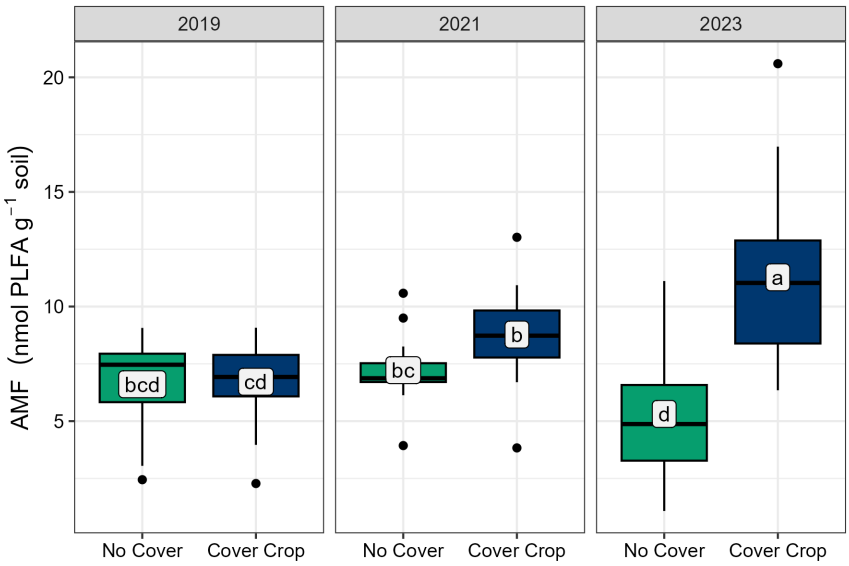


Figure A.35. Arbuscular mycorrhizal fungi (AMF) by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

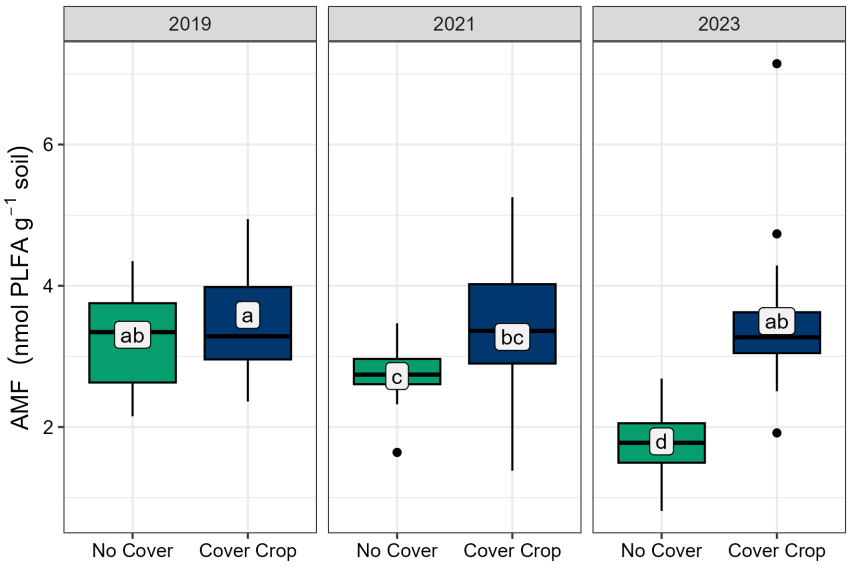


Figure A.36. Arbuscular mycorrhizal fungi (AMF) by treatment and year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

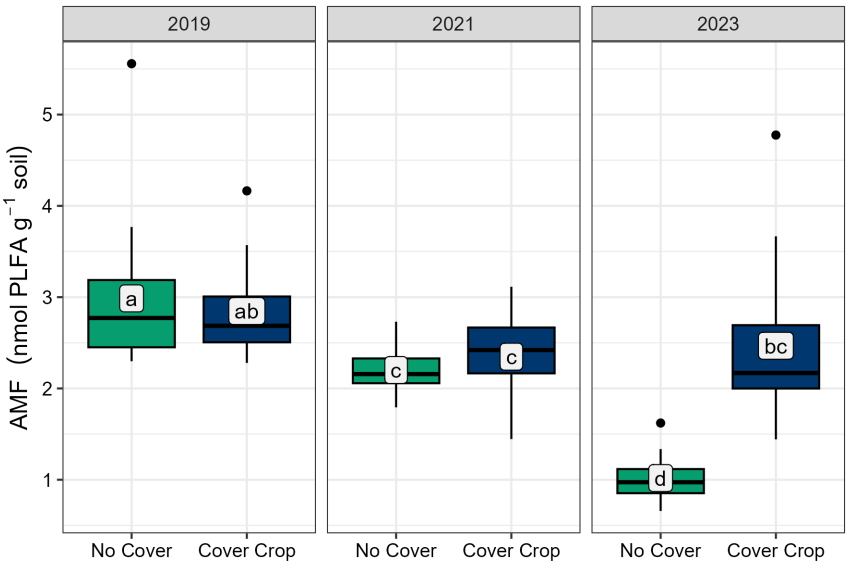


Figure A.37. Arbuscular mycorrhizal fungi (AMF) by treatment and year at 15-30 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

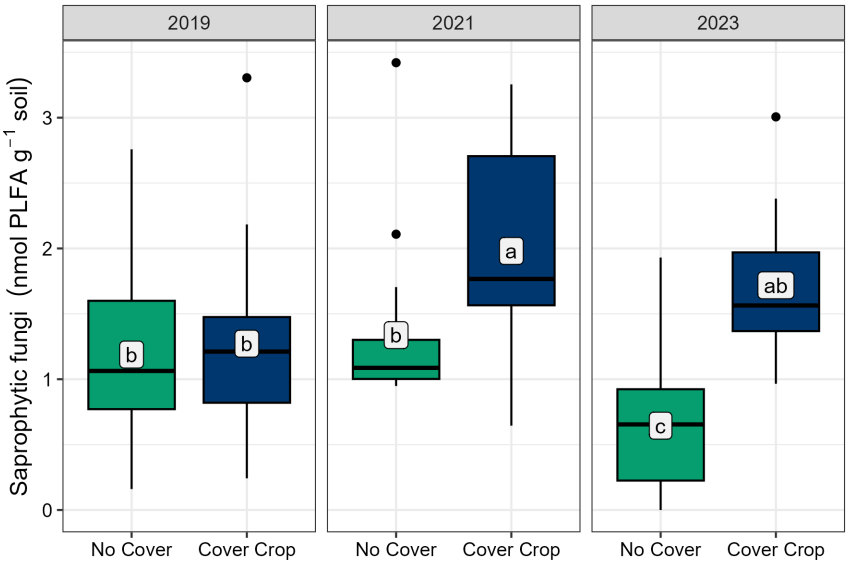


Figure A.38. Saprophytic fungi by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

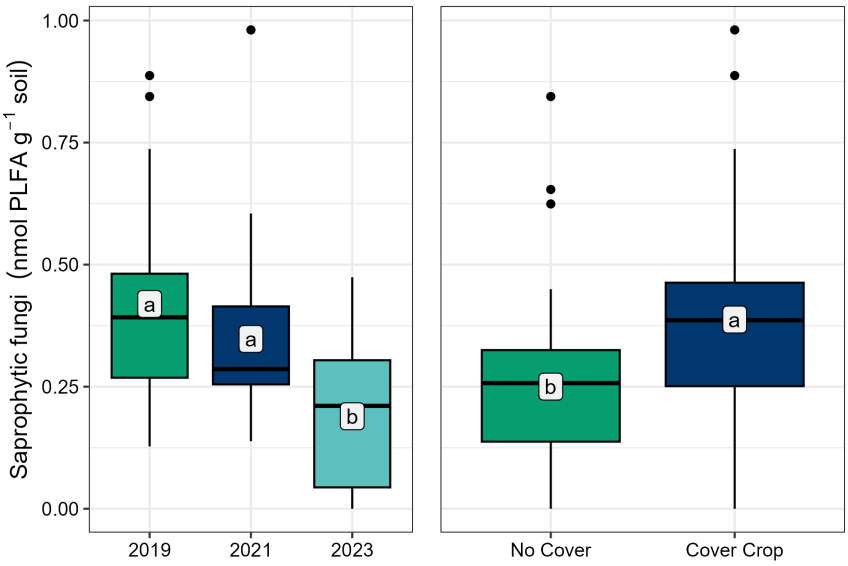


Figure A.39. Saprophytic fungi by treatment and year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

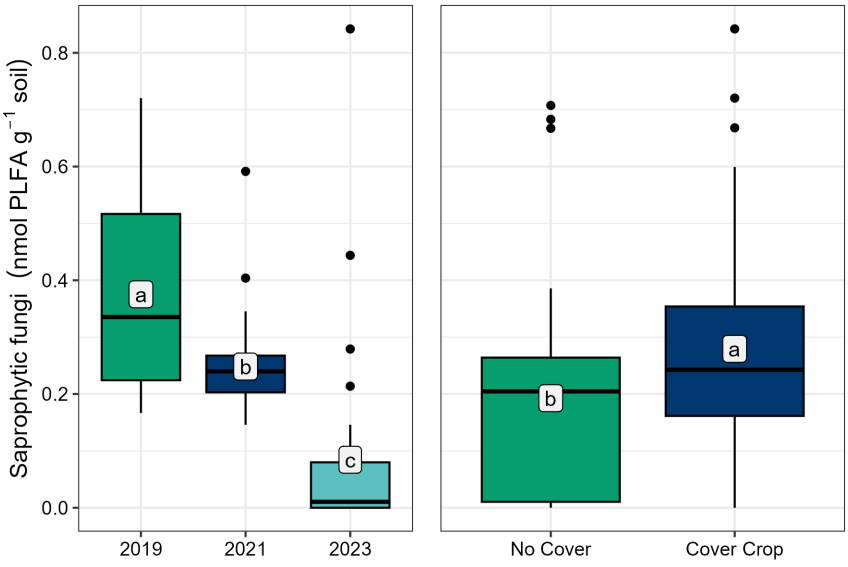


Figure A.40. Saprophytic fungi by treatment and year at 15-30 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

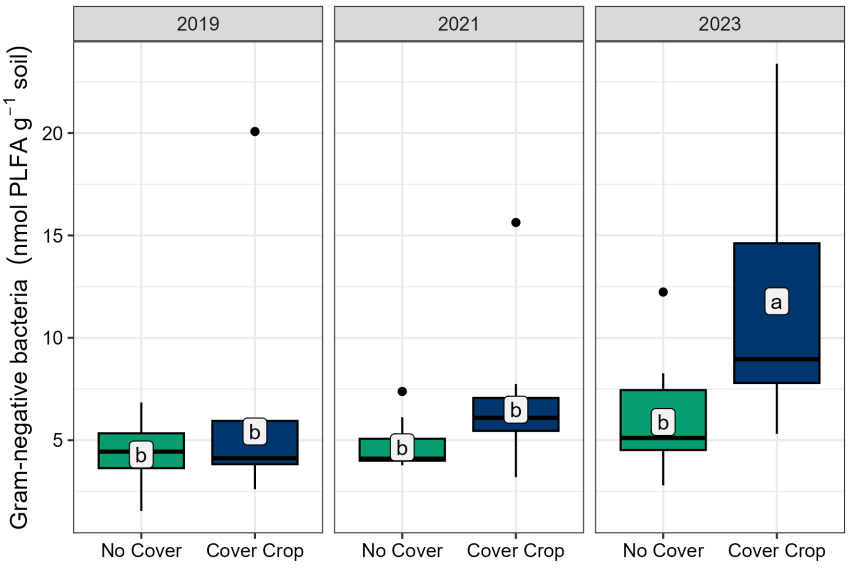


Figure A.41. Gram-negative bacteria by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

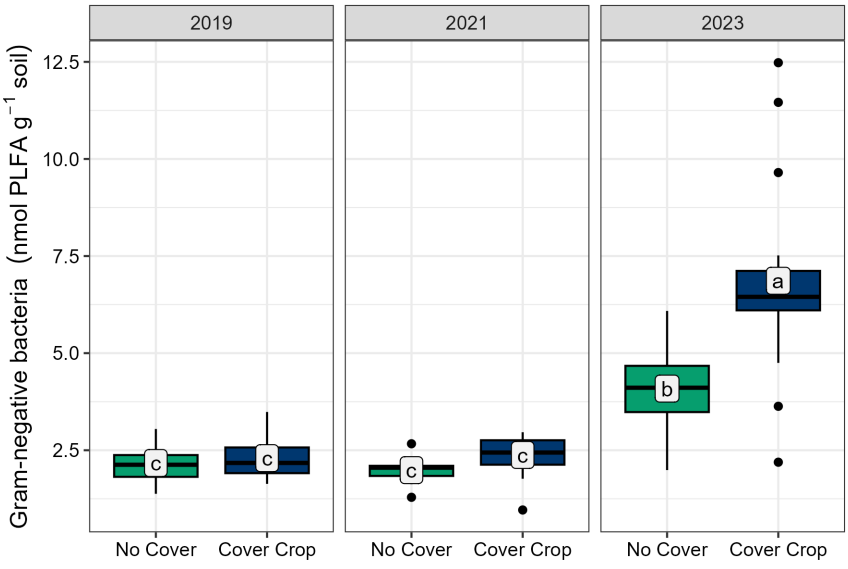


Figure A.42. Gram-negative bacteria by treatment and year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

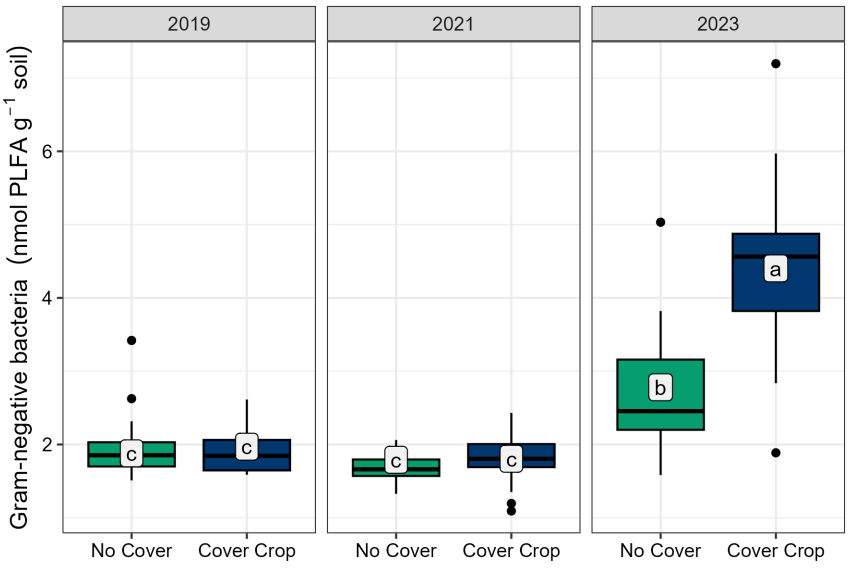


Figure A.43. Gram-negative bacteria by treatment and year at 15-30 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

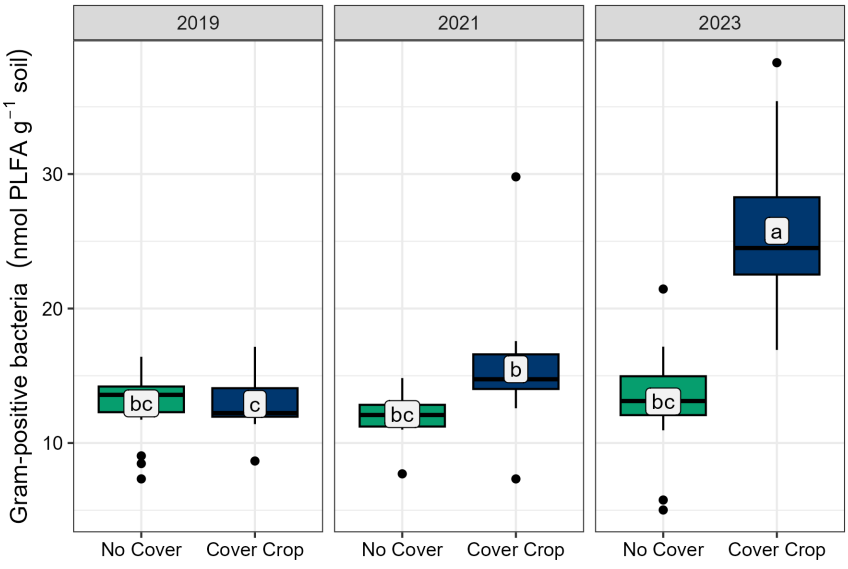


Figure A.44. Gram-positive bacteria by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

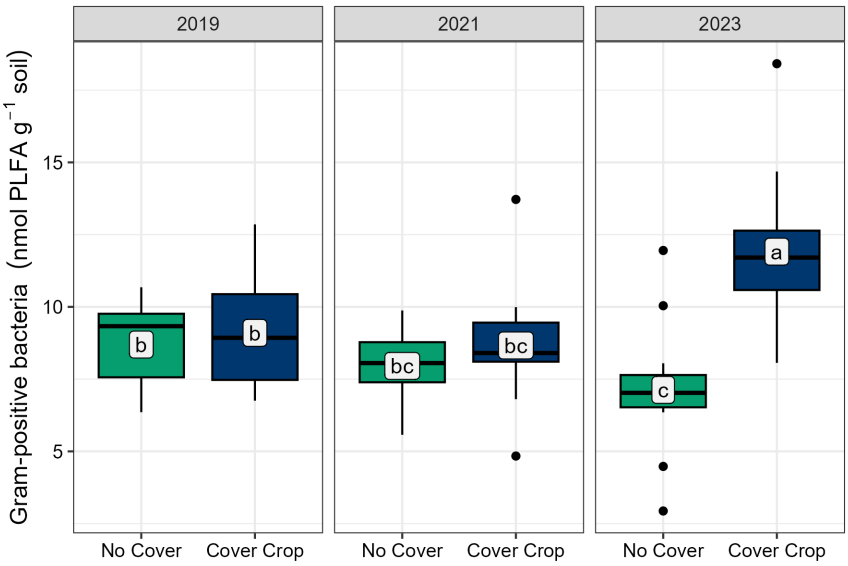


Figure A.45. Gram-positive bacteria by treatment and year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

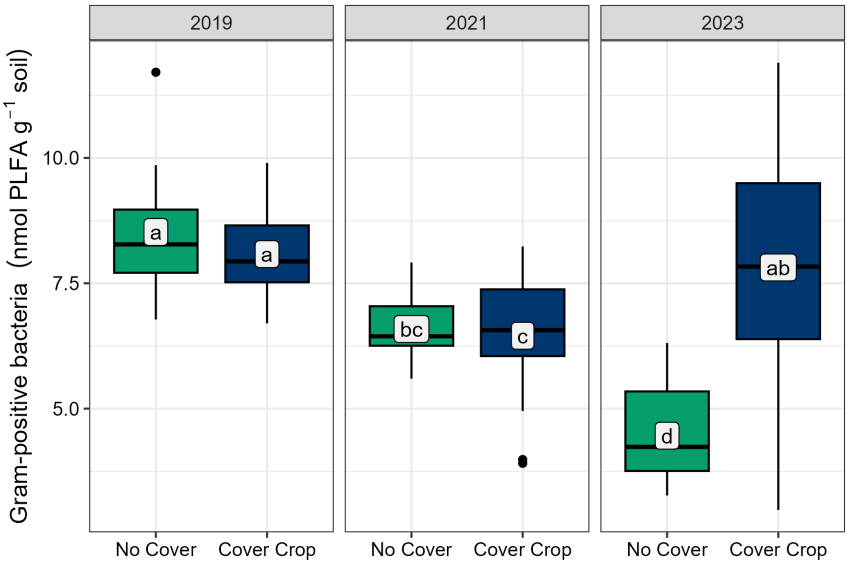


Figure A.46. Gram-positive bacteria by treatment and year at 15-30 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

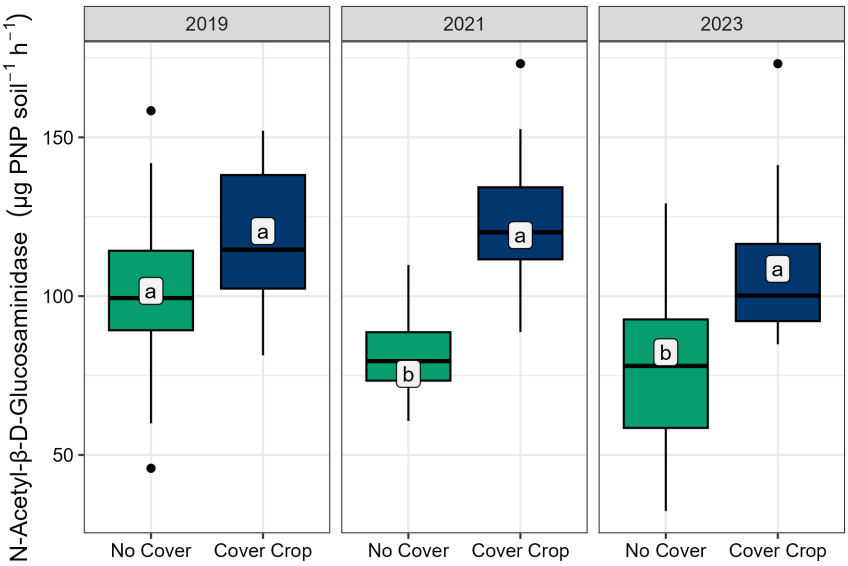


Figure A.47. N-acetyl-β-D-Glucosaminidase activity by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

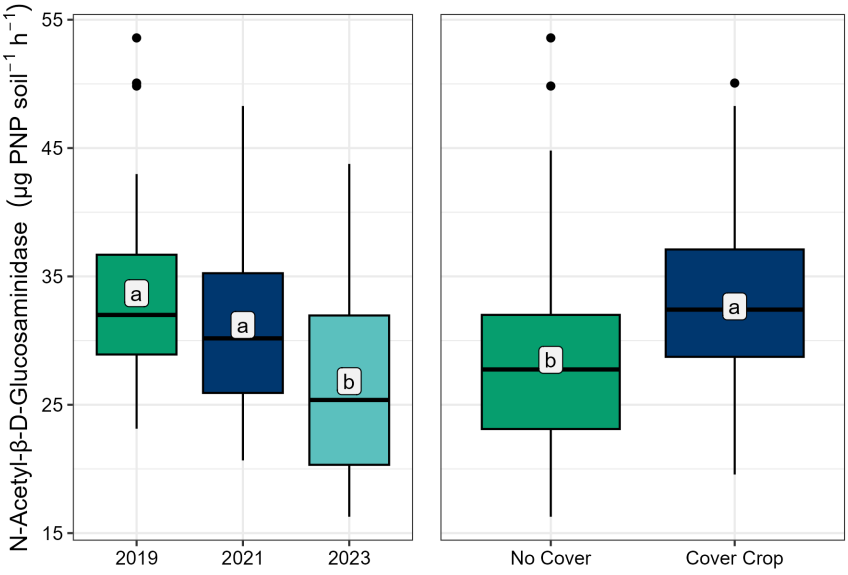


Figure A.48. N-acetyl-β-D-Glucosaminidase activity by treatment and year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

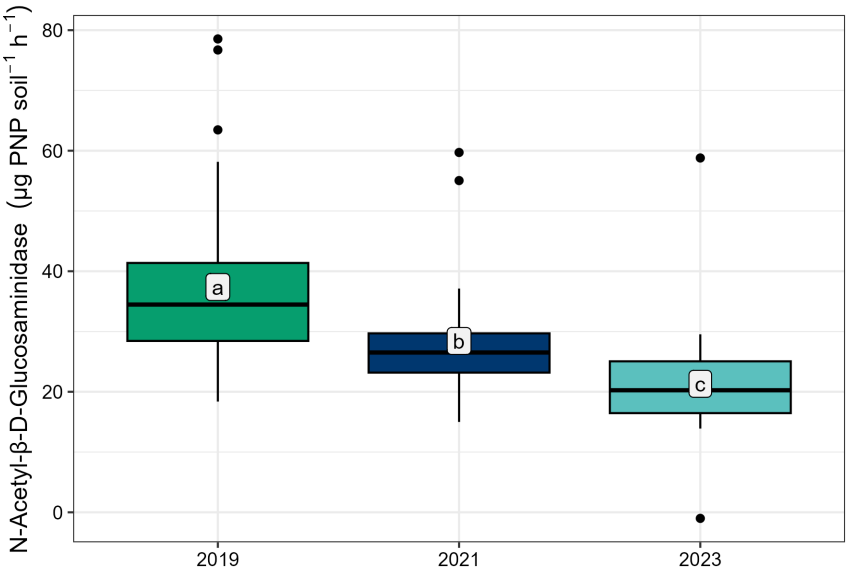


Figure A.49. N-acetyl-β-D-Glucosaminidase activity by year at 15-30 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

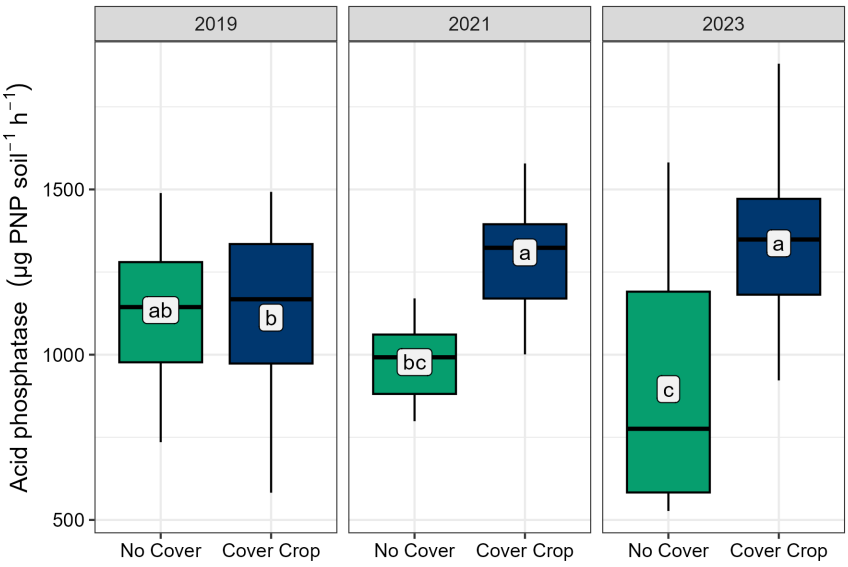


Figure A.50. Acid phosphatase activity by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

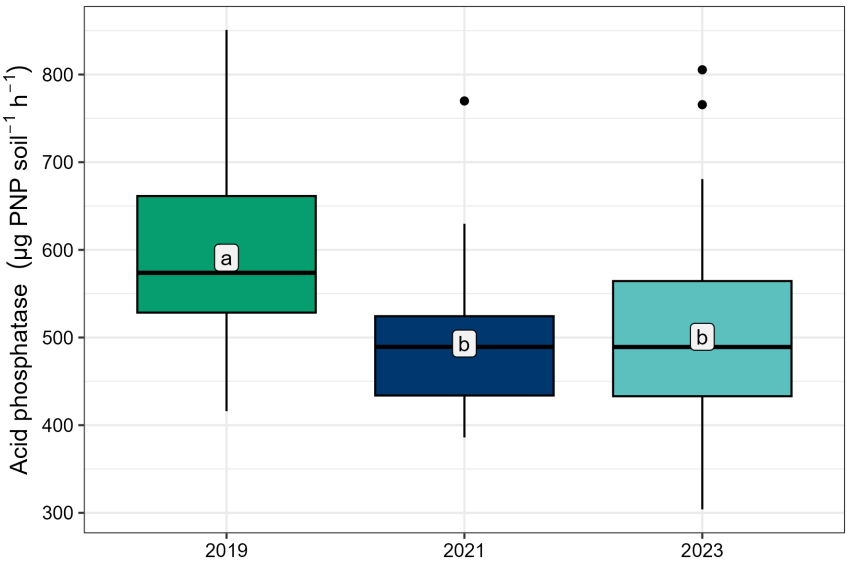


Figure A.51. Acid phosphatase activity by treatment and year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

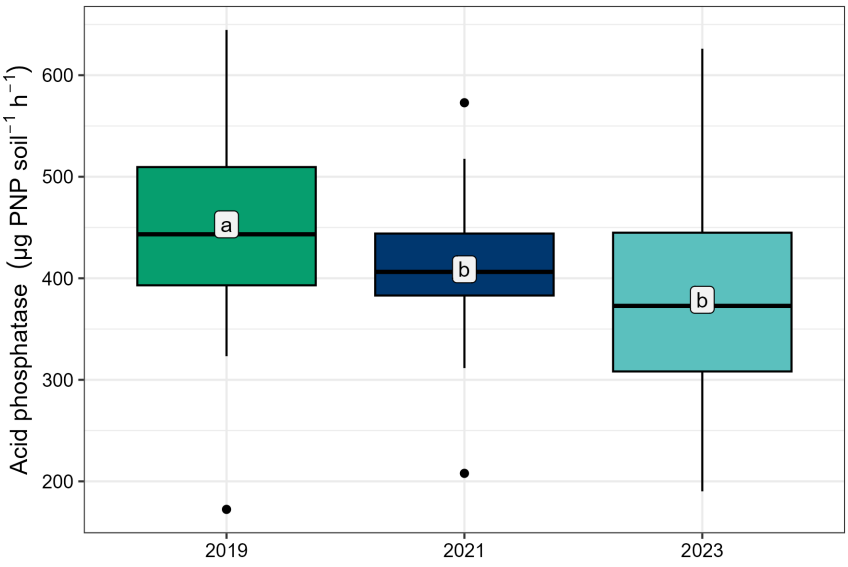


Figure A.52. Acid phosphatase activity by treatment and year at 15-30 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

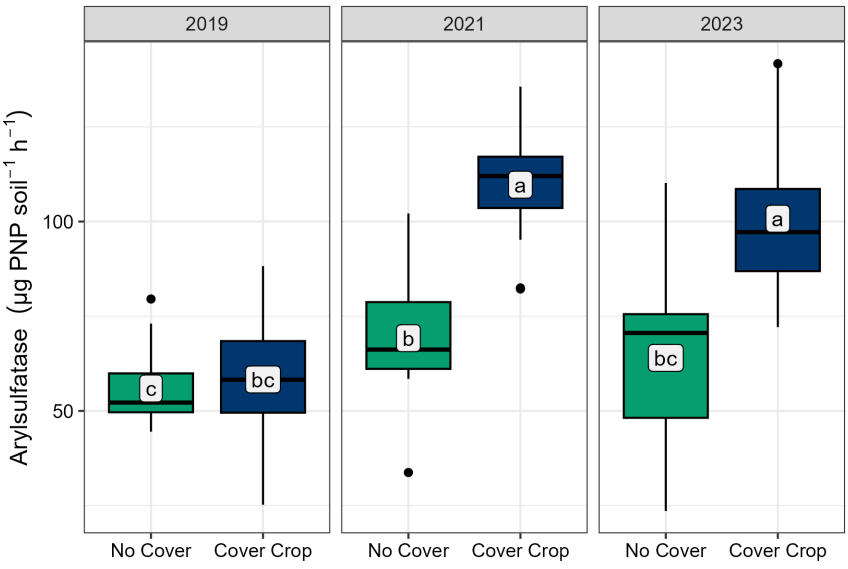


Figure A.53. Arylsulfatase activity by treatment and year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

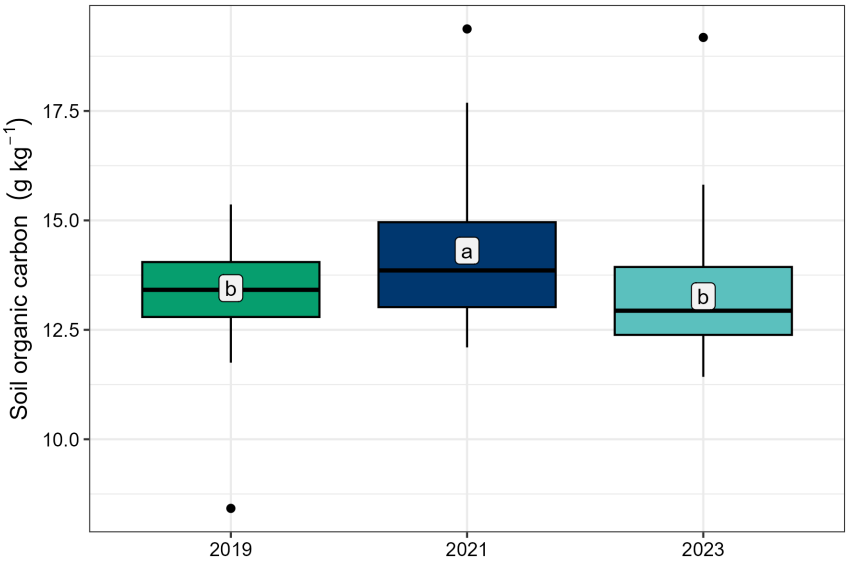


Figure A.54. Soil organic carbon by year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

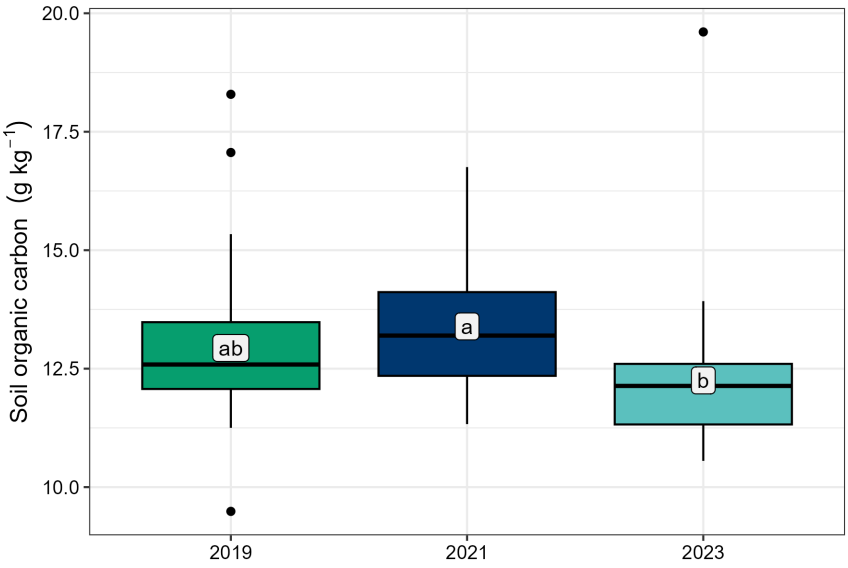


Figure A.55. Soil organic carbon by year at 15-30 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

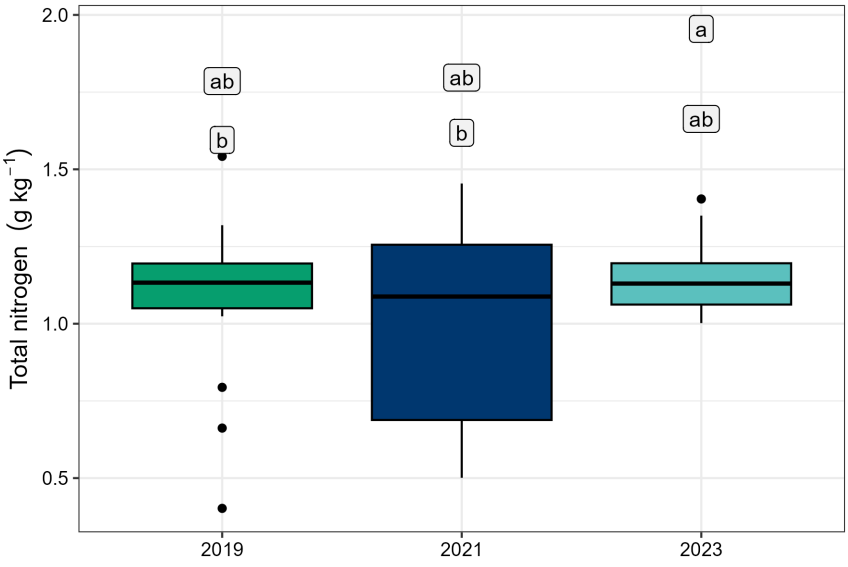


Figure A.56. Total nitrogen by year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

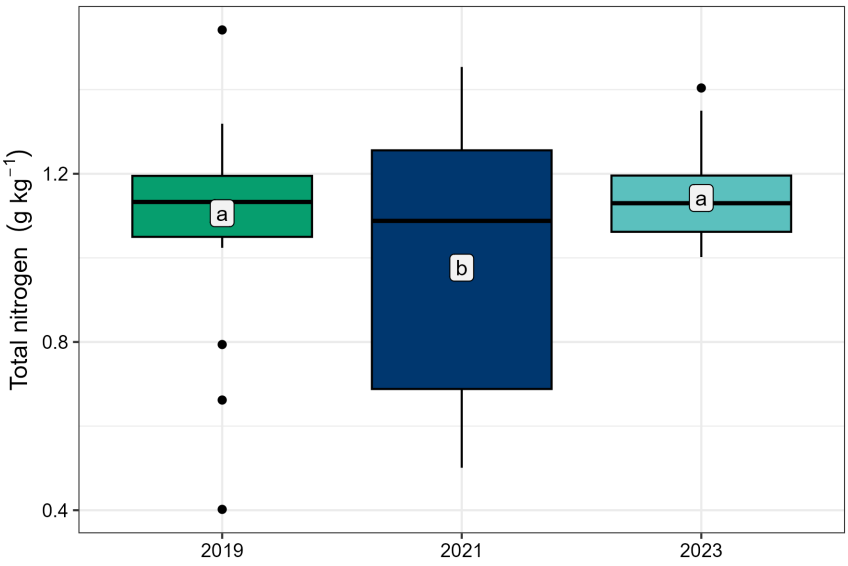


Figure A.57. Total nitrogen by year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

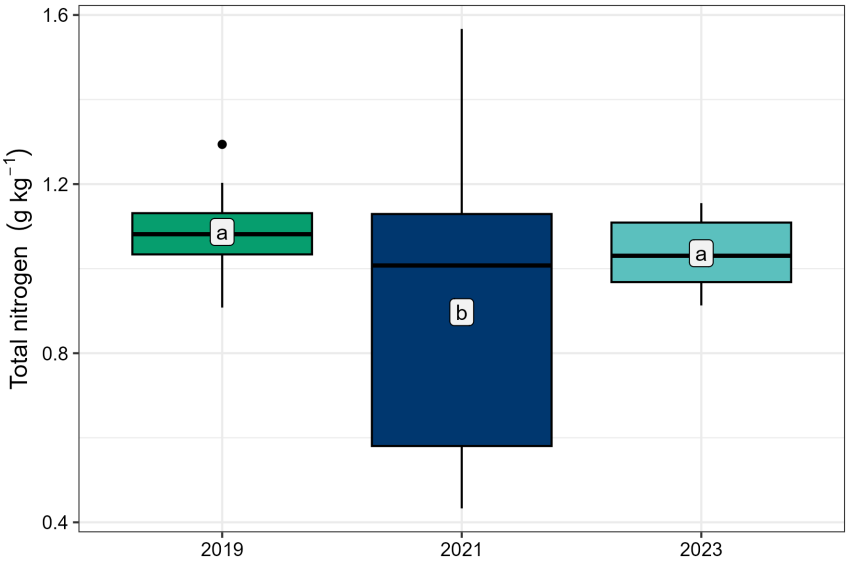


Figure A.58. Total nitrogen by year at 15-30 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

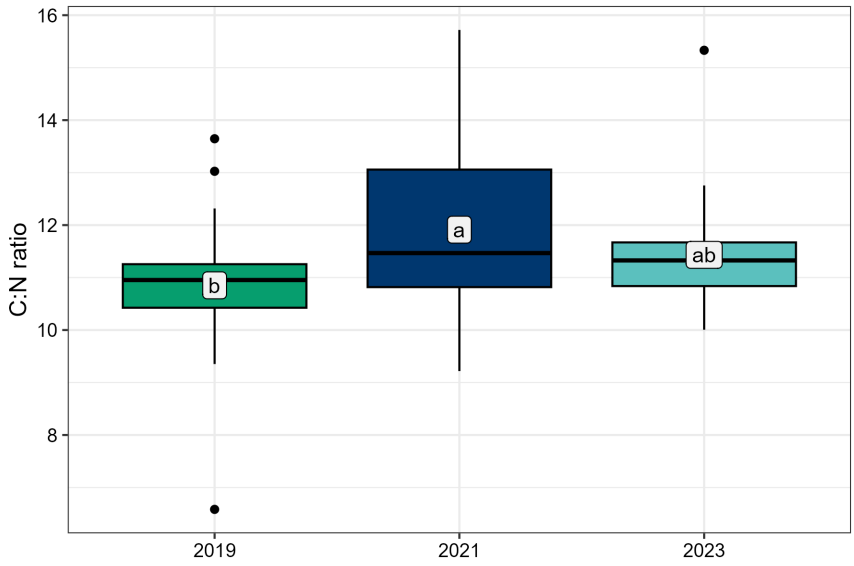


Figure A.59. Carbon: nitrogen ratio by year at 0-5 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

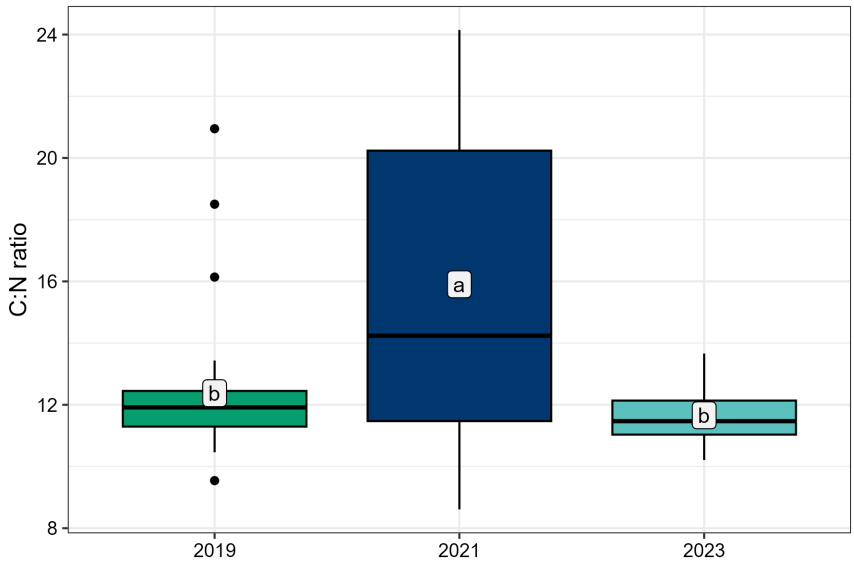


Figure A.60. Carbon: nitrogen ratio by year at 5-15 cm depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

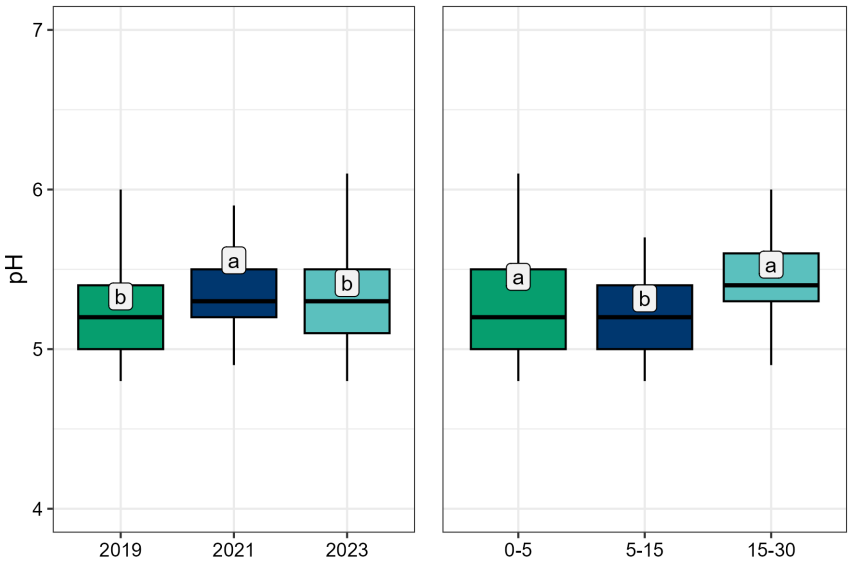


Figure A.61. Soil pH by year and depth at Solomon. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

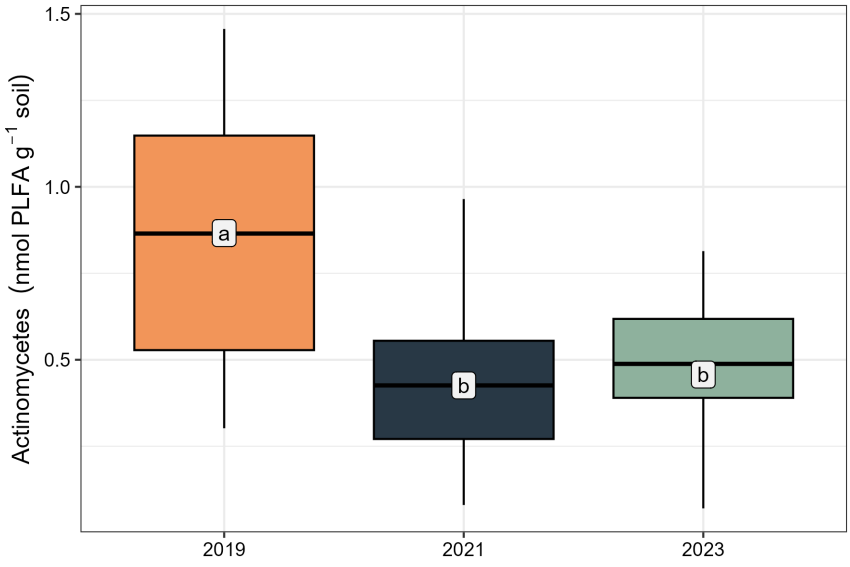


Figure A.62. Actinomycetes by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

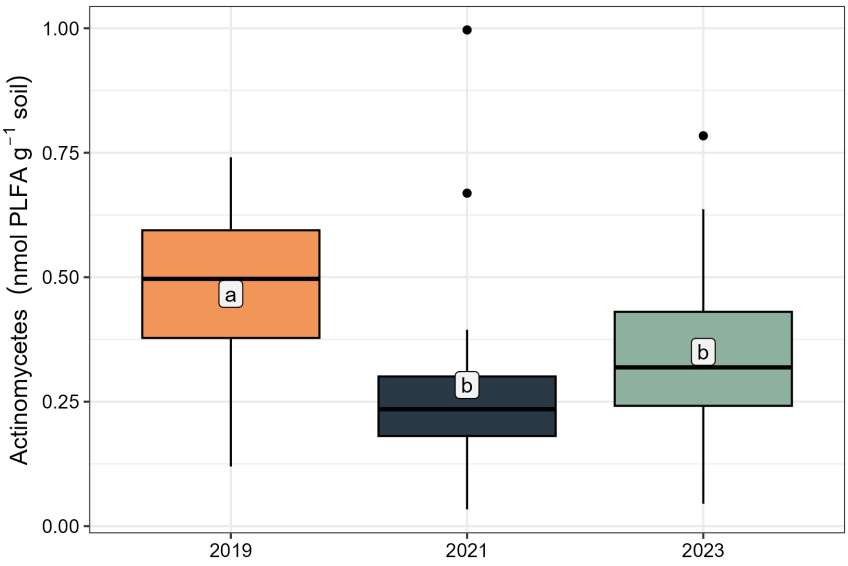


Figure A.63. Actinomycetes by treatment and year at 5-15 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

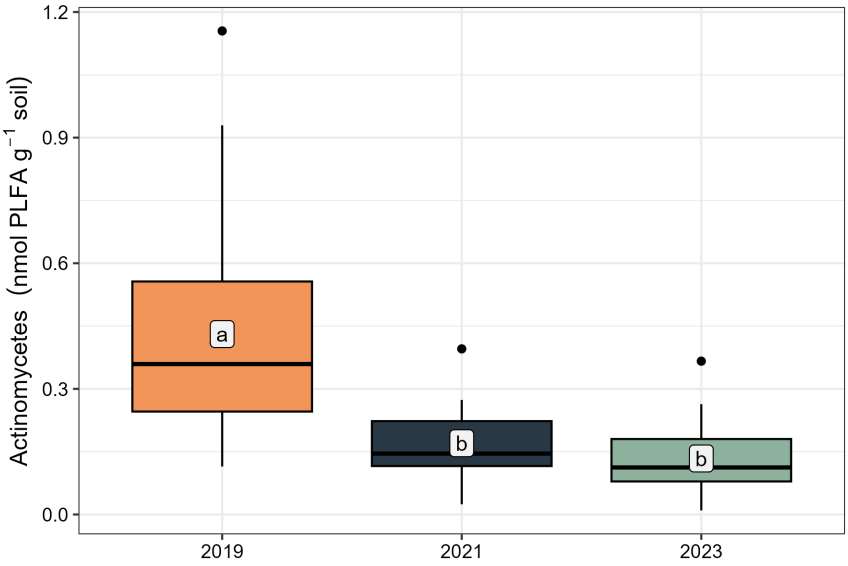


Figure A.64. Actinomycetes by treatment and year at 15-30 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

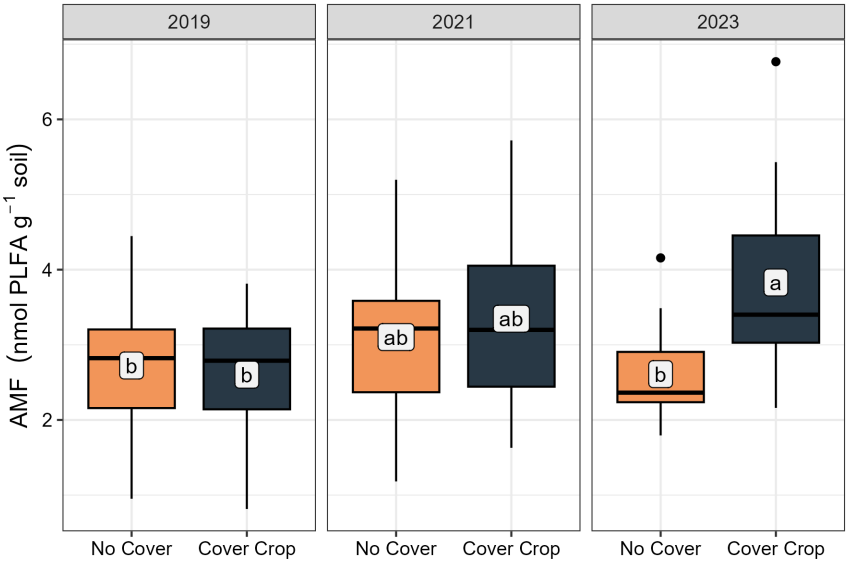


Figure A.65. Arbuscular Mycorrhizal fungi (AMF) by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

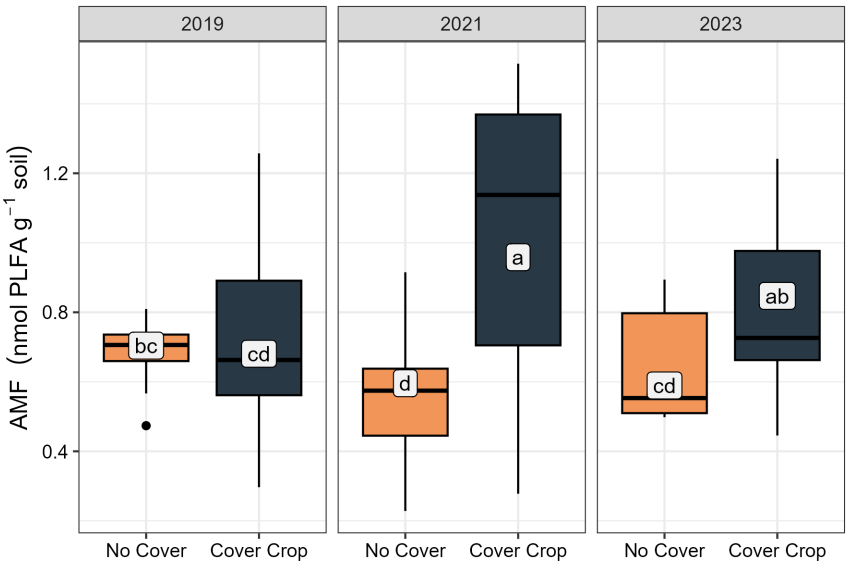


Figure A.66. Arbuscular Mycorrhizal fungi (AMF) by treatment and year at 5-15 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

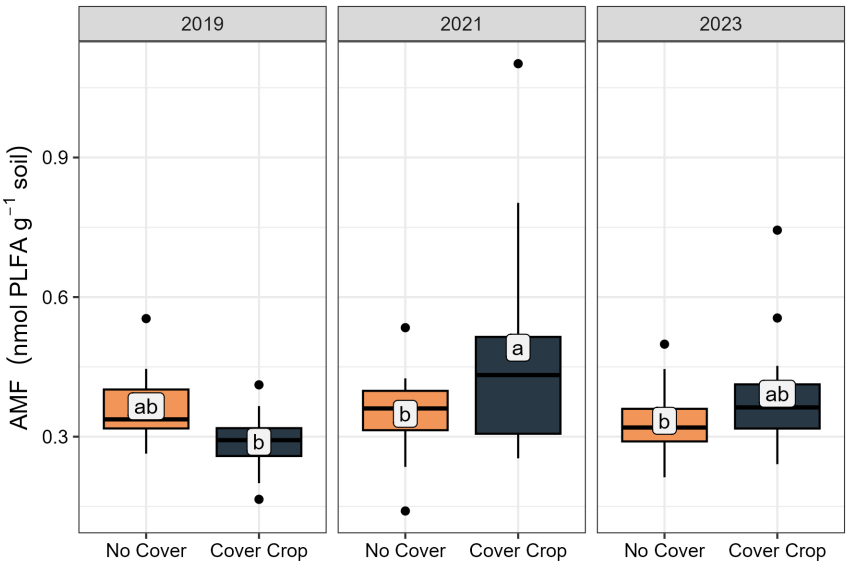


Figure A.67. Arbuscular Mycorrhizal fungi (AMF) by treatment and year at 15-30 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

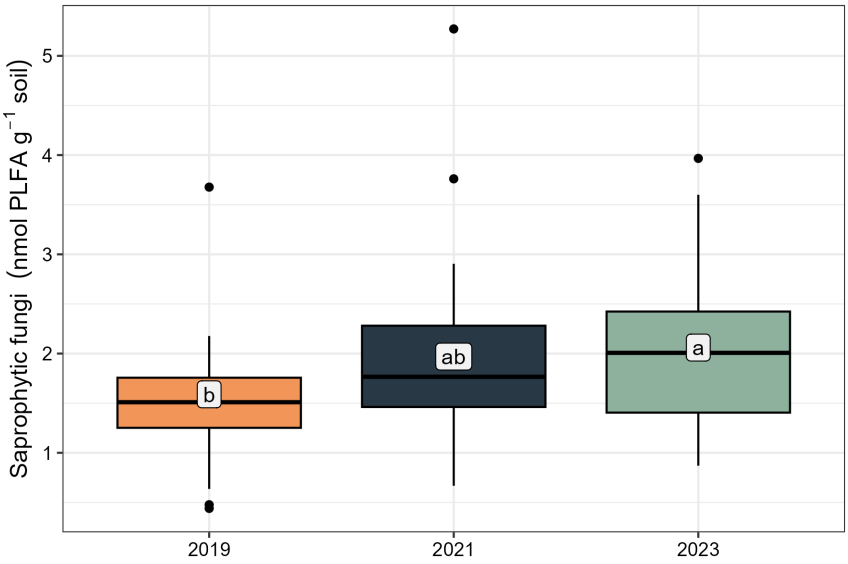


Figure A.68. Saprophytic fungi by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

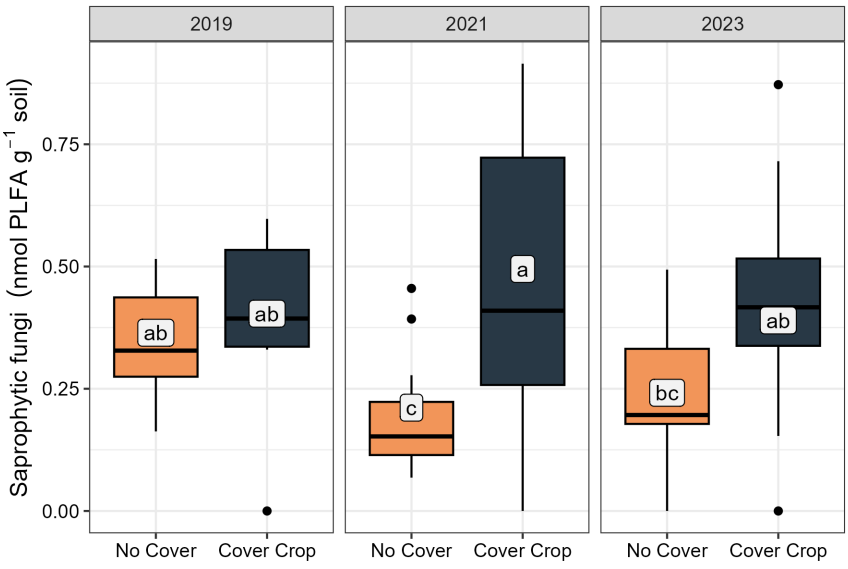


Figure A.69. Saprophytic fungi by treatment and year at 5-15 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

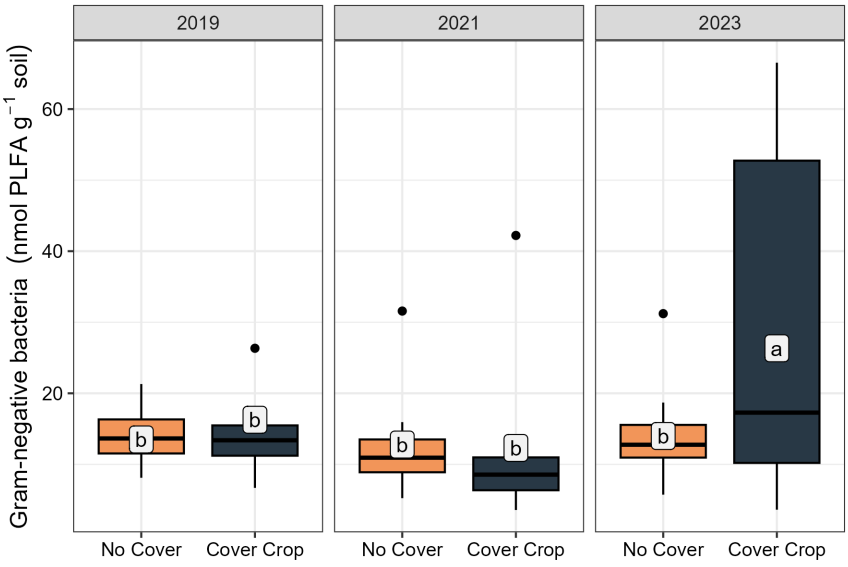


Figure A.70. Gram-negative bacteria by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

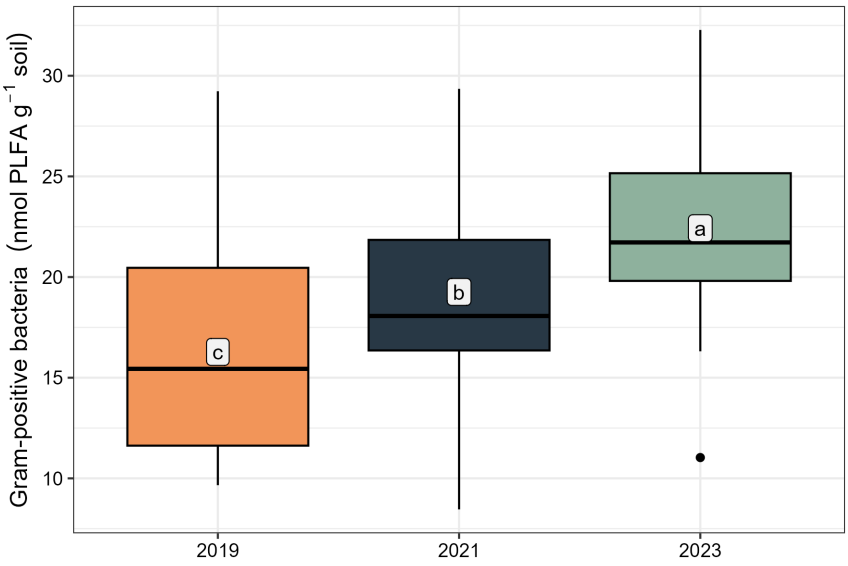


Figure A.71. Gram-positive bacteria by year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

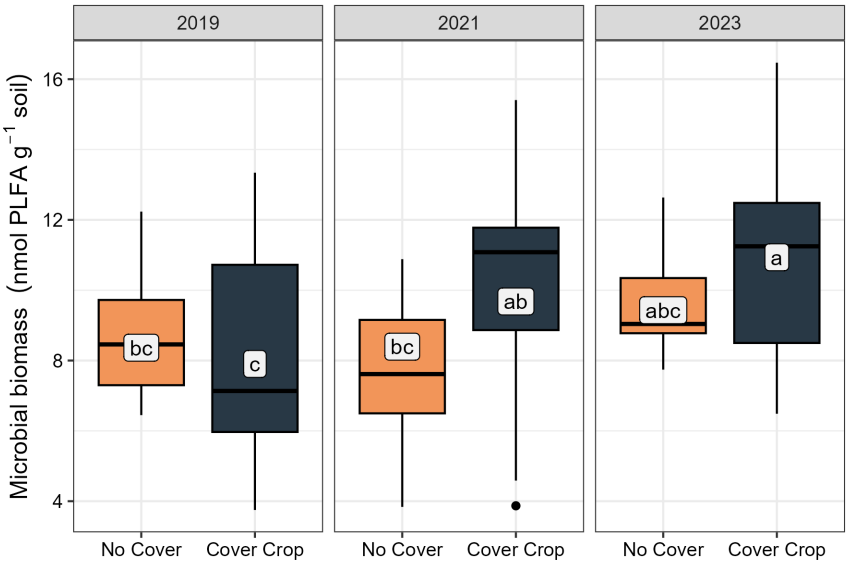


Figure A.72. Gram-positive bacteria by treatment and year at 5-15 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

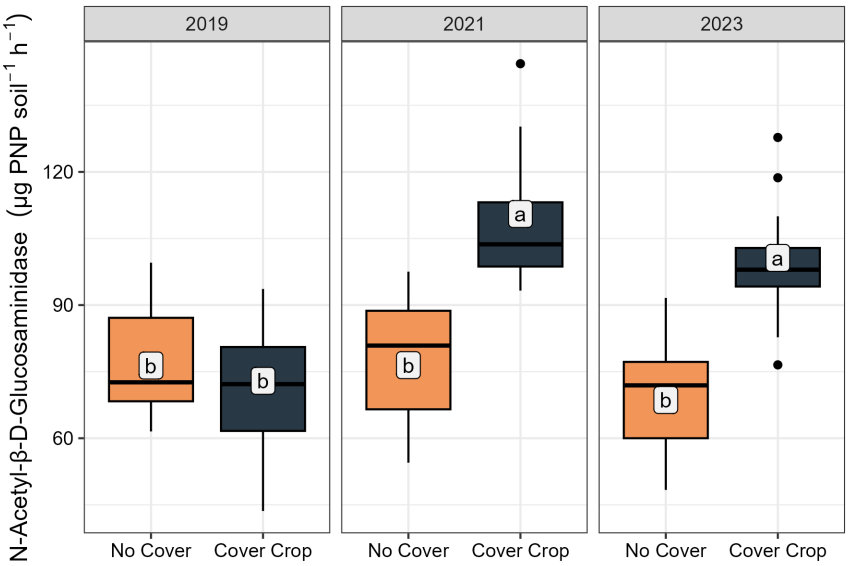


Figure A.73. N-acetyl-β-D-Glucosaminidase activity by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

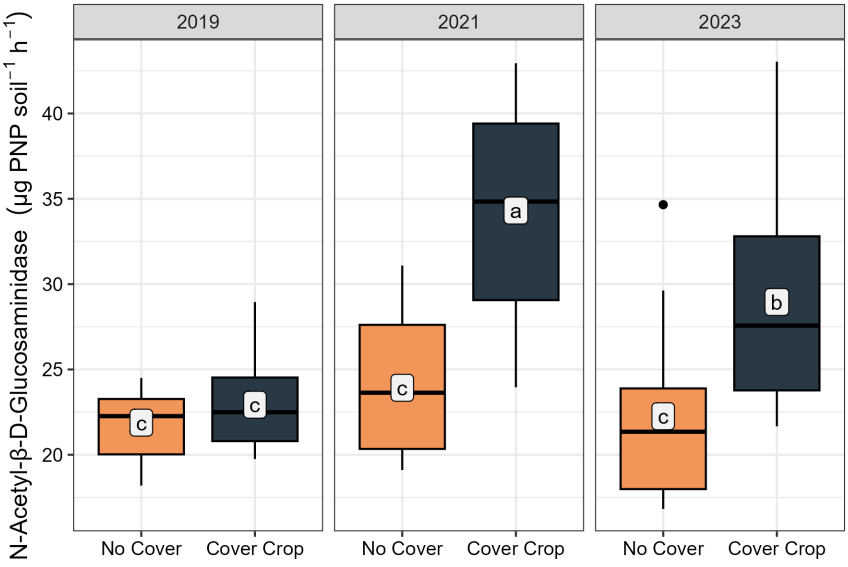


Figure A.74. N-acetyl-β-D-Glucosaminidase activity by treatment and year at 5-15 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

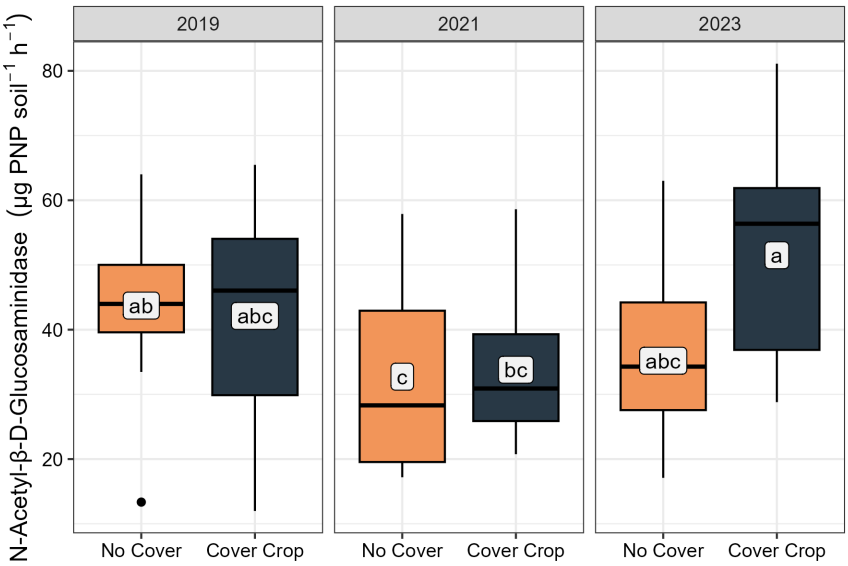


Figure A.75. N-acetyl-β-D-Glucosaminidase activity by treatment and year at 15-30 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

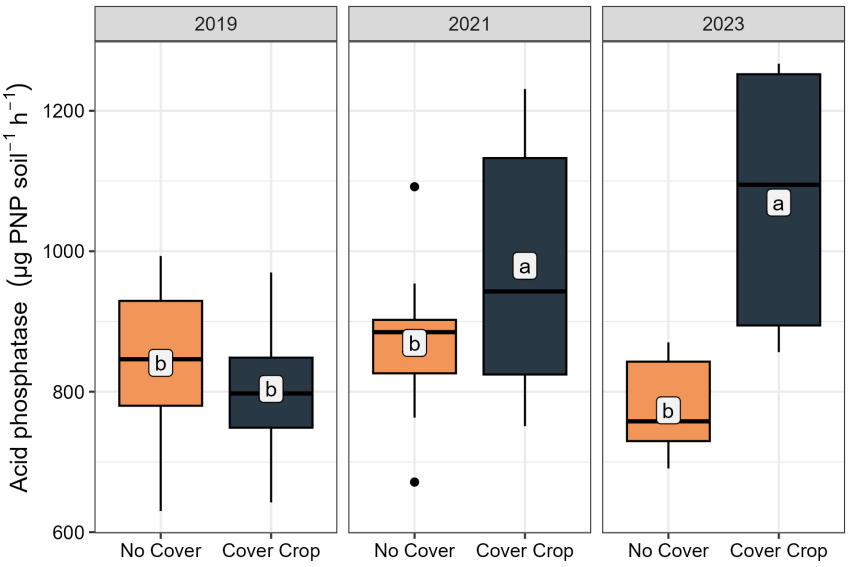


Figure A.76. Acid phosphatase activity by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

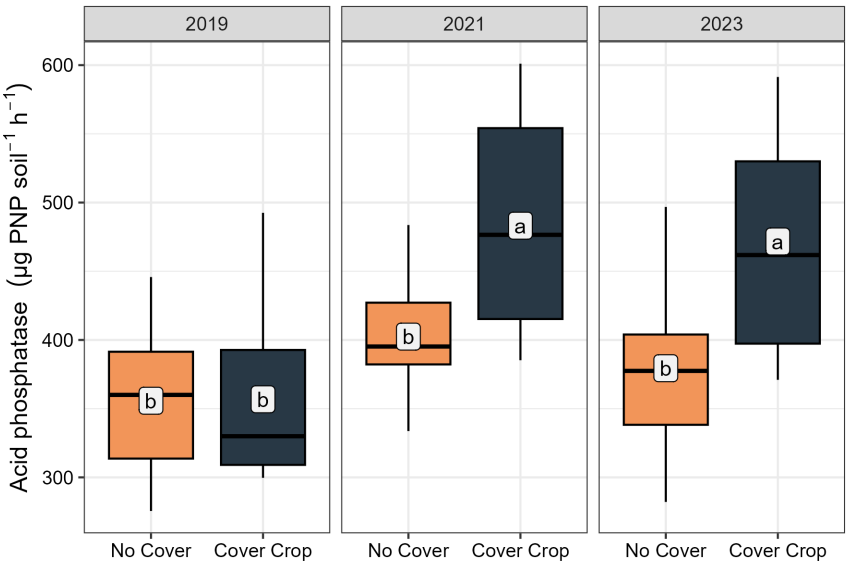


Figure A.77. Acid phosphatase activity by treatment and year at 5-15 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

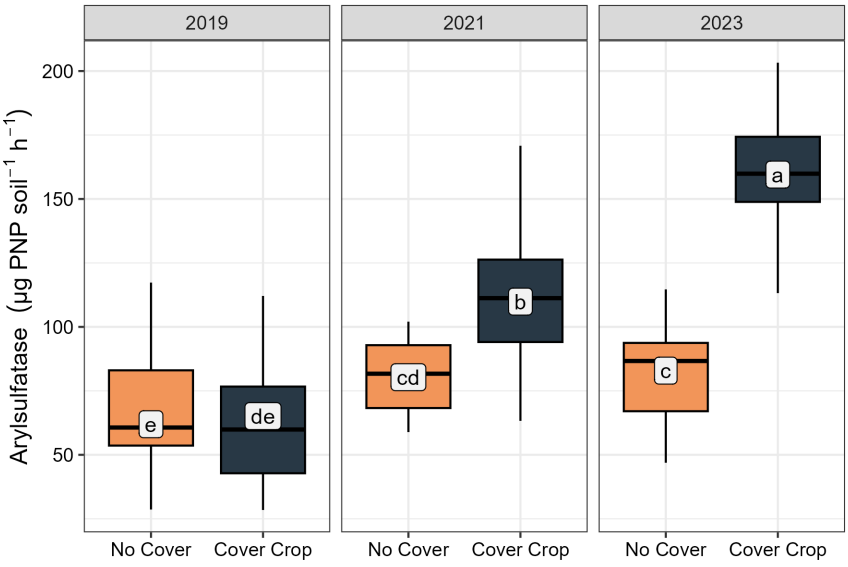


Figure A.78. Arylsulfatase activity by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

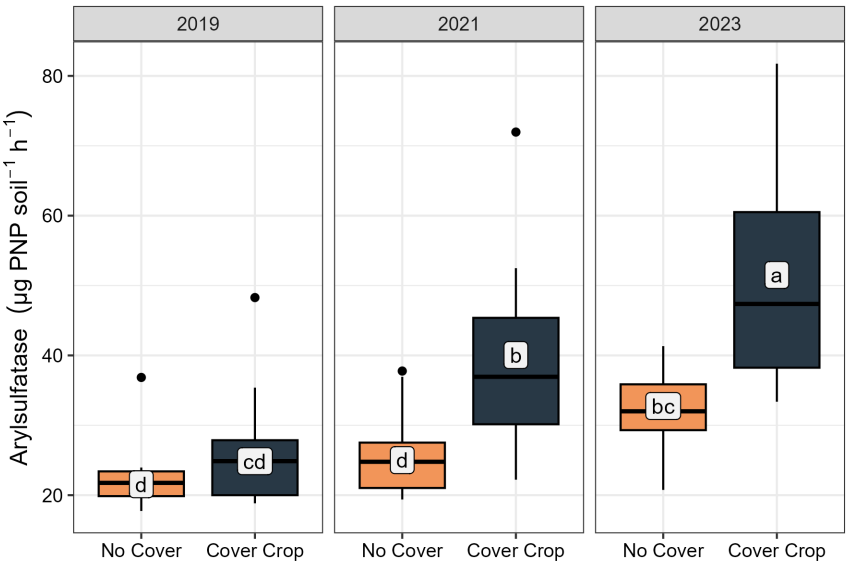


Figure A.79. Arylsulfatase activity by treatment and year at 5-15 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

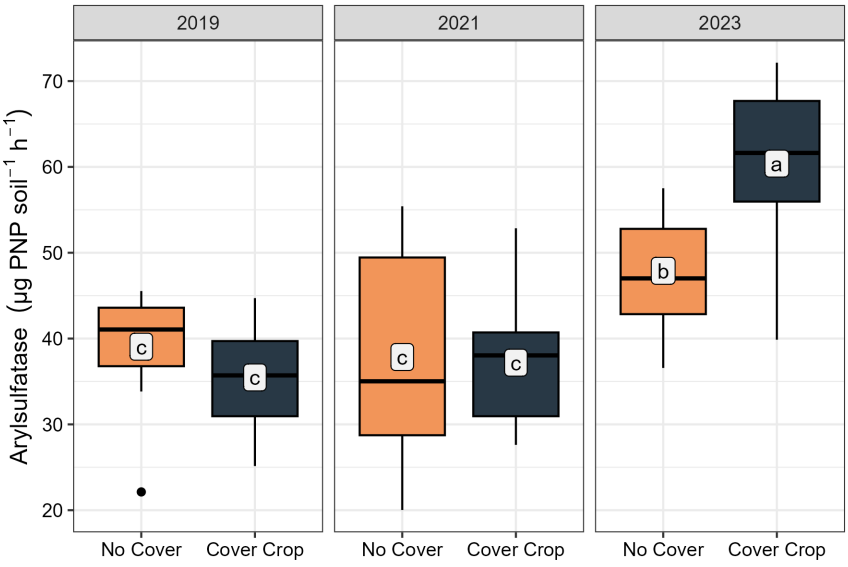


Figure A.80. Arylsulfatase activity by treatment and year at 15-30 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

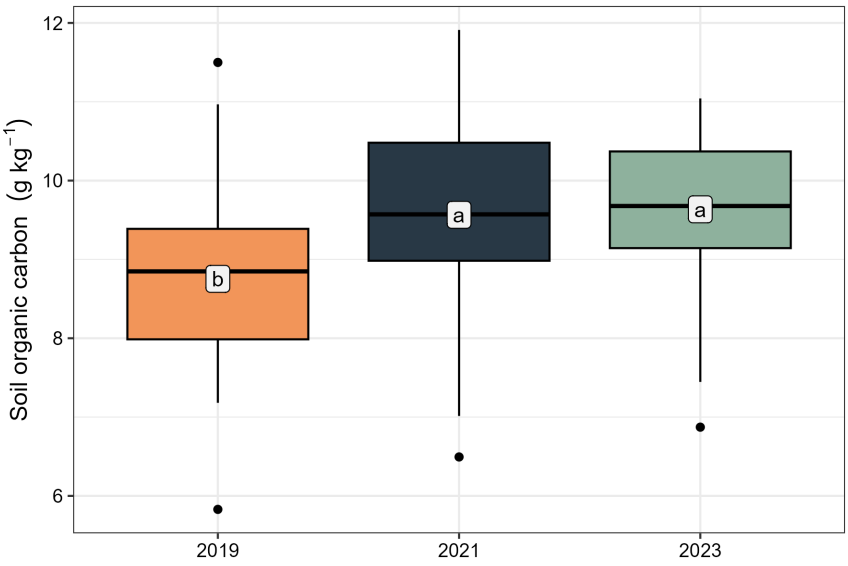


Figure A.81. Soil organic carbon by year at 15-30 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

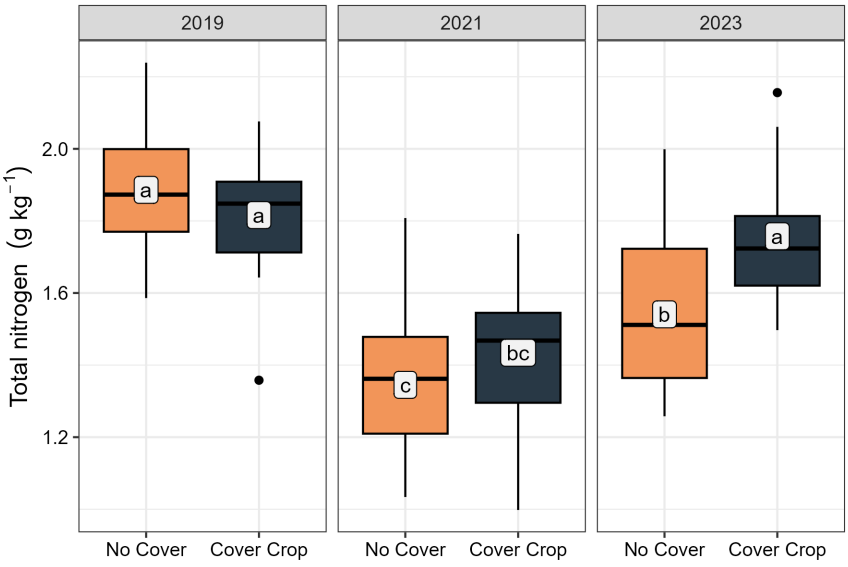


Figure A.82. Total nitrogen by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

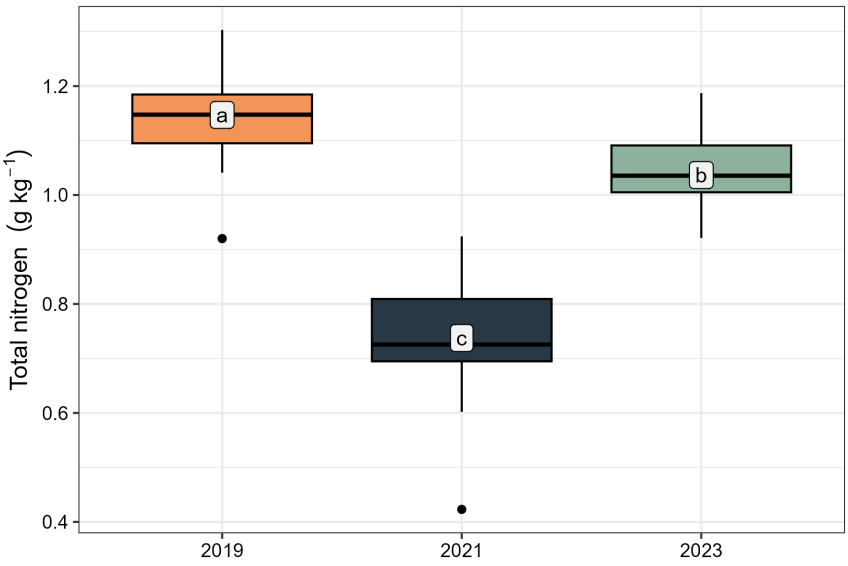


Figure A.83. Total nitrogen by treatment and year at 5-15 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

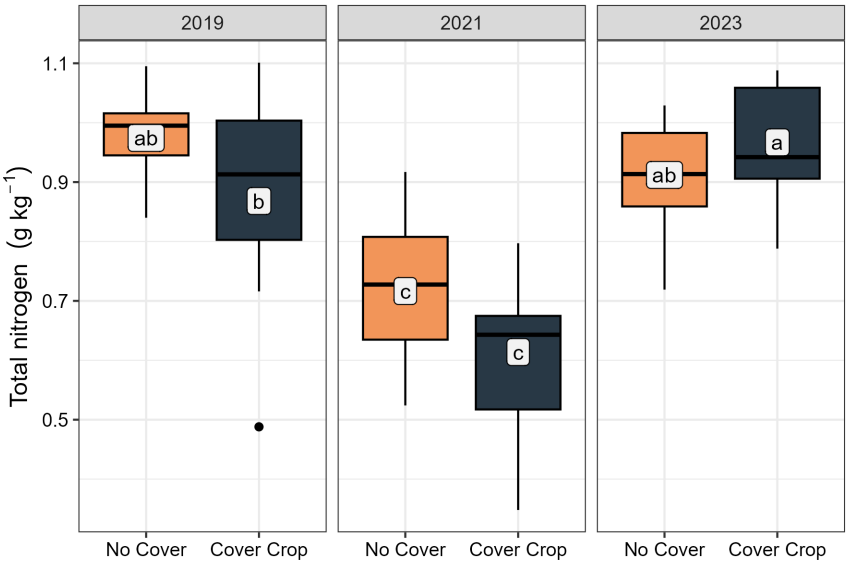


Figure A.84. Total nitrogen by treatment and year at 15-30 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

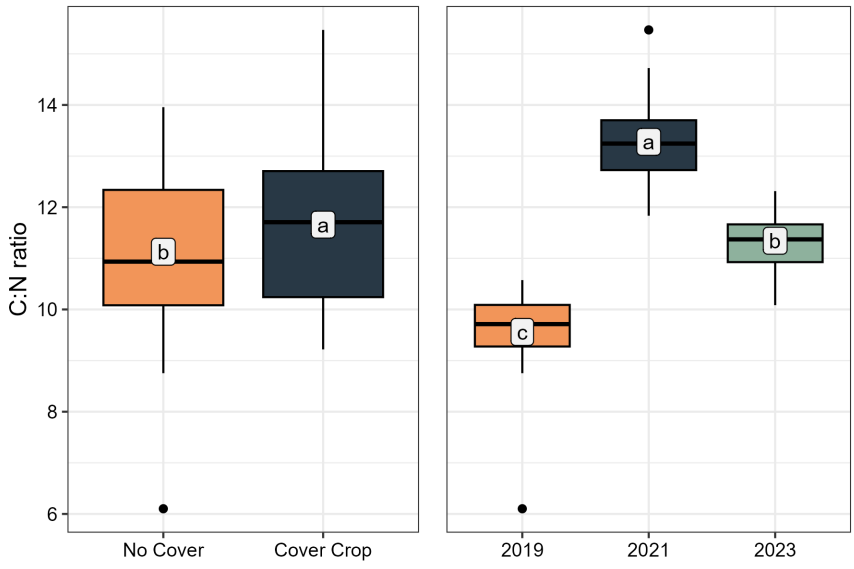


Figure A.85. Carbon: nitrogen ratio by treatment and year at 0-5 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

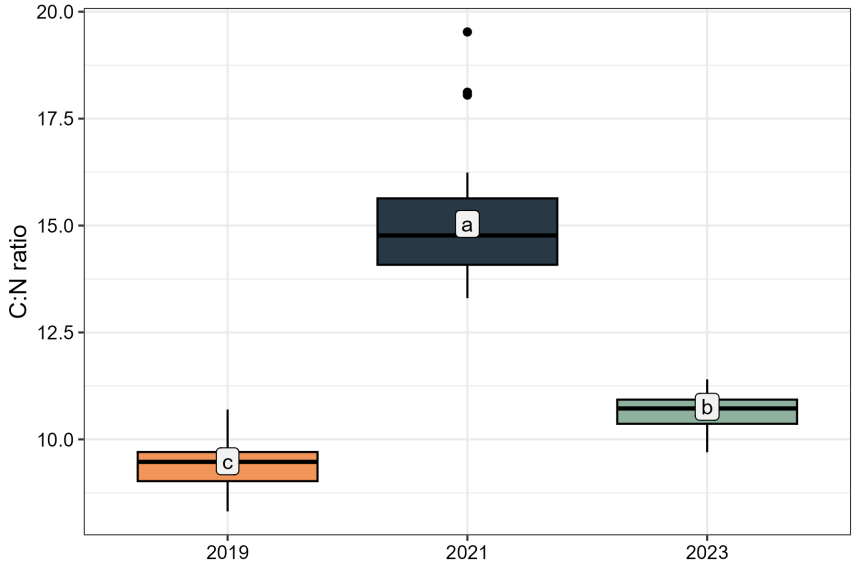


Figure A.86. Carbon: nitrogen ratio by year at 5-15 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

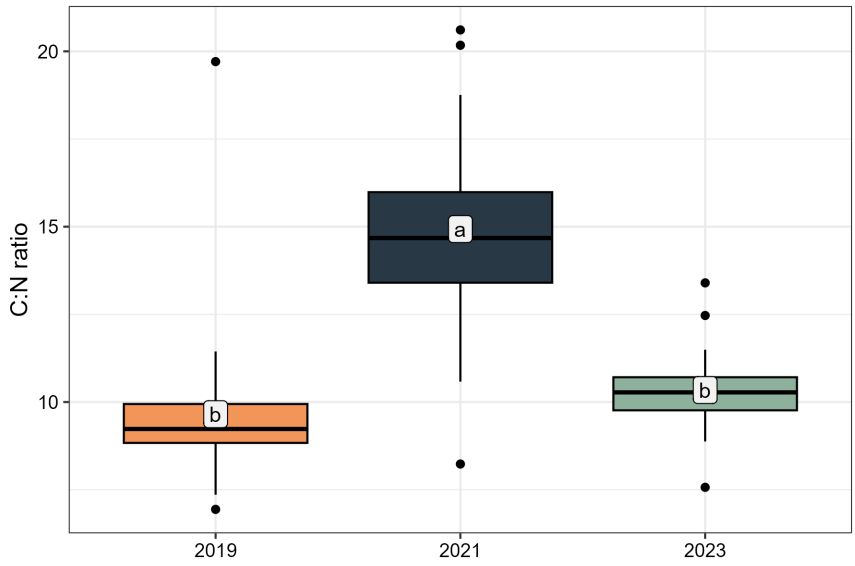


Figure A.87. Carbon: nitrogen ratio by year at 15-30 cm depth at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

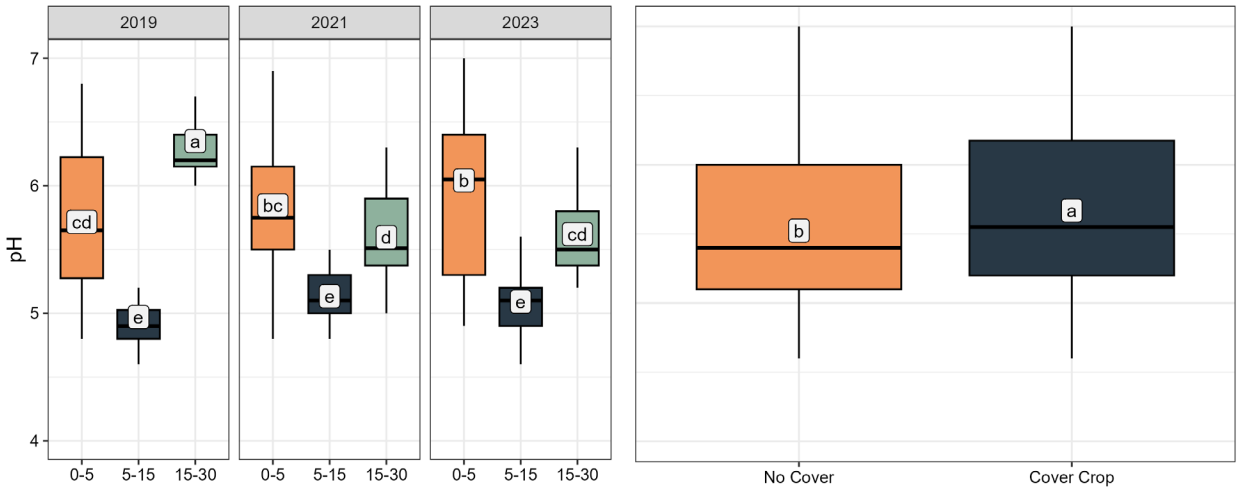


Figure A.88. Soil pH by year and depth, and by treatment at Beloit. Means followed by the same letter are not different according to Fisher's LSD test at the 5% level.

Table A1. Selected spatial correlation structure for linear mixed effects models for each response variable, location, and depth. No spatial correlation structure (1), exponential (2), spherical (3), gaussian (4), and rational (5).

Bucyrus					
Response variable	Model	Response variable	Model	Response variable	Model
------- 0-5 cm -------		------- 5-15 cm -------		------- 15-30 cm -------	
TMB	1	TMB	1	TMB	1
Gram+	1	Gram+	1	Gram+	1
Gram-	1	Gram-	4	Gram-	1
Actino	1	Actino	1	Actino	1
AMF	1	AMF	1	AMF	1
Fungi	1	Fungi	1	Fungi	1
βG	2	βG	1	βG	1
AcidP	2	AcidP	1	AcidP	2
NAG	4	NAG	1	NAG	1
ArylS	4	ArylS	1	ArylS	2
SOC	4	SOC	5	SOC	5
TN	1	TN	1	TN	1
Solomon					
TMB	1	TMB	4	TMB	1
Gram+	1	Gram+	1	Gram+	1
Gram-	1	Gram-	4	Gram-	4
Actino	1	Actino	4	Actino	1
AMF	1	AMF	4	AMF	1
Fungi	1	Fungi	1	Fungi	1
βG	4	βG	1	βG	3
AcidP	1	AcidP	2	AcidP	1
NAG	4	NAG	1	NAG	2
ArylS	1	ArylS	4	ArylS	4
SOC	4	SOC	2	SOC	2
TN	1	TN	1	TN	1
Beloit					
TMB	4	TMB	1	TMB	1
Gram+	4	Gram+	1	Gram+	1
Gram-	4	Gram-	3	Gram-	1
Actino	4	Actino	4	Actino	1
AMF	1	AMF	4	AMF	1
Fungi	3	Fungi	1	Fungi	1
βG	1	βG	1	βG	1
AcidP	1	AcidP	1	AcidP	1
NAG	1	NAG	1	NAG	1
ArylS	4	ArylS	1	ArylS	1
SOC	1	SOC	2	SOC	1
TN	4	TN	2	TN	2

Microbial biomass (TMB), gram-positive bacteria (Gram+), gram-negative bacteria (Gram+), actinomycetes (Actino), arbuscular mycorrhizal fungi (AMF), and saprophytic fungi (Fungi). β-glucosidase (βG), Acid phosphatase (AcidP), N-acetyl-β-glucosaminidase (NAG), arylsulfatase (ArylS).