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Comparative Multifunctionality of Dryland Annual and Perennial Grain Production Systems in a Mediterranean Climate

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Table of Contents

Acknowledgements iii

Abstract ix

Introduction 1

Chapter 1: Comparing perennial to annual grain crops reveals that plant productivity and no-till underpin soil carbon stabilization and microbial biomass in a Mediterranean climate

Abstract 7

1 Introduction 8

2 Materials and methods 11

2.1 Field Location and Management 11

2.2 Experimental Design and Sampling 12

2.3 Statistical Analyses 13

3 Results 16

3.1 Temperature and precipitation 16

3.2 Aboveground biomass productivity 17

3.3 Soil microbial biomass 19

3.4 Soil Carbon 21

3.5 Soil carbon mineralization versus soil carbon stabilization potential 25

4. Discussion 28

4.1 Crop type determines total aboveground biomass yield 29

4.2 Tillage and crop effects on total microbial biomass 30

4.3 Tillage, crop, and aboveground biomass productivity effects on soil carbon 31

4.4 Soil carbon stabilization versus mineralization mechanisms and the relationship to microbial biomass 32

5 Conclusion 34

6 Appendices 35

7 Acknowledgements 37

8 References 38

Chapter 2: Multifunctionality of annual and perennial grain production systems in a Mediterranean climate

Abstract 42

1 Introduction 44

2 Materials and methods 48

2.1 Experimental location and management 48

2.2. Experimental design 48

2.2.1 Grain and biomass harvest 49

2.2.2 Soil sampling and processing 50

2.3 Calculations and statistical analyses 51

3 Results 55

3.1 Temperature and precipitation 55

3.2 Grain yield stability 56

3.3 Nitrogen use efficiency (NUE) in aboveground biomass and grain 57

3.4 Soil carbon mass as affected by crop type and nitrogen fertilization 58

4 Discussion 62

4.1 Grain yield stability is greater in annual than perennial grain cropping systems 62

4.2 Aboveground nitrogen use efficiency trends higher in annual than perennial grain cropping systems 64

4.3 Cropping system and nitrogen effects on soil carbon storage 65

4.4 Multifunctionality and ecosystem services framework: tradeoffs in tilled wheat, no-till wheat and IWG systems 68

5 Conclusion 69

6 Appendices 70

7 Acknowledgements 71

8 References 72

Chapter 3: Soil carbon storage and compositional responses of soil microbial communities under perennial grain IWG vs. annual wheat

Abstract 77

1 Introduction 78

2 Materials and methods 82

2.1 Soil sampling and processing 82

2.2 DNA extraction 83

2.3 Amplicon sequencing 84

2.4 Bioinformatic analysis 85

2.5 Soil microbial biomass and carbon sampling, measurements, & analyses 85

3 Results 87

3.1 Effect of crop type, soil compartment and soil depth on soil microbial diversity and composition of soil bacterial and fungal communities 87

3.2 Soil chemical and biological properties and soil microbial community composition 91

3.3 PLFA fungi : bacteria ratio and soil carbon mass 93

3.4 Fungal taxonomy in tilled annual wheat and IWG at depth 97

4 Discussion 99

4.1 The effects of crop type on soil microbial diversity metrics at various soil depths 99

4.2 Fungal and bacterial community composition cluster differently among crop types 100

4.3 Fungal and bacterial community composition cluster differently among soil depths 102

4.4 pH, labile soil carbon and active microbial biomass relationship to soil microbial community composition 104

4.5 Differences in fungal community composition and F/B ratio in IWG vs. annual wheat does not correspond to greater soil carbon at depth in IWG 104

4.6 Annual wheat has high abundance of fungal taxa associated with total soil organic carbon and higher soil carbon at depth 106

5 Conclusion 107

6 Data Accessibility Statement 107

7 Appendices 108

8 Acknowledgements 111

9 References 112

Dissertation conclusion 118

Abstract

Agricultural stakeholders are becoming increasingly aware of the need for sustainable food systems. In a shift from an agricultural paradigm that prioritizes yield, there is now growing interest in multifunctional food systems that simultaneously promote environmental integrity while also providing adequate yield and nutrition. An investigation into how current and proposed alternatives to annual grain systems address the multifaceted objectives of food system sustainability is necessary given that annual grains currently comprise nearly 70% of earth's cultivated land and provide majority of the world's food. Furthermore, it is crucial to conduct research on these grain systems in highly productive and economically valuable agroecological regions, such as California (CA). As the largest and most diverse agricultural state in the U.S., CA provides market opportunities while also heralding what current and future food systems must overcome to maintain food supply amid projected water and weather extremes. Thus, we investigated the multifunctionality of a tilled annual wheat system and two proposed alternatives, no-till annual wheat production and novel perennial grain production, in the Mediterranean climate of California. We measured plant and soil parameters for three years in intermediate wheatgrass or IWG, no-till annual wheat, and tilled annual wheat at four nitrogen rates. IWG had significant fluctuations in aboveground biomass (AGB) and had the highest soil carbon (C) mineralization at each soil depth. No-till wheat had stable AGB and the highest soil C stabilization and microbial biomass in the topsoil, which suggests plant productivity and a lack of soil disturbance over time are key factors underpinning enhanced soil C stabilization and gains in microbial biomass in the topsoil. Yield stability, soil carbon storage and nitrogen use efficiency (NUE) were then compared among the three systems using a multifunctionality framework. IWG had large interannual fluctuations in grain yield and NUE, while annual wheat (till and no-till) had stable grain yields and higher NUE than IWG. Soil carbon gains in IWG and no-till wheat relative to the tilled wheat were contingent upon N fertilization and constrained to the topsoil, while tilled wheat stored carbon at depth and across the whole soil profile. Finally, soil microbial community composition and soil carbon were compared in tilled annual wheat and IWG during the final year of the experiment. Bacterial community composition was more sensitive to soil depth than crop type, whereas fungal community composition was influenced largely by crop type. While the fungi : bacteria ratio was higher in IWG at deep soil depths, tilled annual wheat had a higher abundance of fungal taxa known to positively correlate with soil carbon and higher soil carbon mass than IWG at the 60-90 cm soil depth. The two major takeaways from this three-year field experiment were: 1) sampling the subsoil (> 30 cm) is crucial when comparing system-level soil carbon storage potential of annual and perennial grain systems; and 2) plant productivity underpins numerous components of agricultural multifunctionality and thus plant adaption is of utmost importance when designing and implementing future food systems.

Introduction

Agricultural stakeholders worldwide are becoming increasingly aware of the need for sustainable food systems. There is growing interest in shifting from an agricultural paradigm that prioritizes yield to one that prioritizes multifunctional food systems that simultaneously promote environmental integrity while also providing adequate yield and nutrition. Current and proposed alternatives to annual grain systems should be investigated with respect to how they address agricultural multifunctionality given that annual grains currently comprise nearly 70% of earth's cultivated land and provide majority of the world's food. Furthermore, it is crucial to conduct research on these grain systems in highly productive and economically valuable agroecological regions, such as California (CA). As the largest agricultural state in the U.S., CA provides diverse market opportunities while also heralding what current and future food systems must overcome to maintain food supply amid expected water and weather extremes (Pathak et al., 2018). Three important grain production systems whose multifunctionality are of interest in the Mediterranean climate of California are tilled annual wheat and two proposed alternatives, novel perennial grain production and no-till annual wheat production.

After the 1930's Dust Bowl in the U.S., a shift towards conservation tillage practices occurred in response to the severe soil degradation witnessed across significant portions of agricultural land. No-till, a form of conservation tillage (Archer et al., 2017), has now been adopted on more than 125 million hectares worldwide (Pittelkow et al., 2015a). In comparison to conventional tillage practices, no-till agriculture performs best in dry climates under rainfed conditions (Pittelkow et al., 2015b). Cereal crop yields appear to be only moderately reduced by no-till practices, with impacts on annual wheat (*Triticum aestivum*; Pittelkow et al., 2015b) among the most minor. Another benefit of converting from tillage to no-tillage practices has been improvements in soil health indicators in the topsoil (Nunes et al., 2018; Nunes et al., 2020). Annual systems managed with reduced tillage have shown increases in soil organic matter (SOM) and improved soil chemical, biological and physical properties (Nunes et al., 2018; Nunes et al., 2020). These benefits have also been associated with perennial cropping systems (Nunes et al., 2018; Nunes et al., 2020) and there has been increasing interest in whether novel perennial grain crops can also improve SOM and other various soil properties.

The perennial grain crop that has gained most attention as a potential alternative to annual grain production is intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey; trademarked Kernza®) or IWG. IWG is a domesticated cool-season perennial wheatgrass that has been bred for grain production (DeHaan and Ismail, 2017) and as of today is being cultivated on approximately 1,400 hectares of cropland in the U.S. (The Land Institute, 2022). IWG produces a high-quality forage (Favre et al., 2019), grain yields of 300 to 1500 kg ha^{-1} (Culman et al., 2013; Jungers et al., 2017; Pugliese, 2019; Tautges et al., 2018) and biomass yields of up to 12,000 kg ha^{-1} (Jungers et al., 2017) or more. Recent studies comparing soils under perennial versus annual grains have reported higher carbon fluxes and soil carbon (C) and nitrogen (N) storage in soils after IWG production compared to annual monocultures and restored native vegetation systems (Means et al., 2022). However, if considering IWG as a sustainable alternative to annual grain production, such benefits must be considered alongside its aboveground productivity and stability relative to annual grain crops. For instance, IWG yields only 30% of the grain of annual wheat (Culman et al., 2013; Cassman et al., 2022), has a harvest index of 0.06-0.1 (Dick et al., 2018; Hunter et al., 2020; Culman et al., 2013) and may experience yield instability due to interannual variation in nutrient and water supply, leading to large fluctuations in IWG production (Loomis et al., 2022). These are all important considerations when comparing perennial and annual grain crop production.

Some of the reported belowground benefits of IWG production, such as increased values of labile carbon and soil carbon concentration relative to annual wheat, are promising (Culman et al., 2013; Means et al., 2022; Audu et al., 2022). However, it remains unclear whether these benefits stem from aboveground productivity, perennial root presence or, more indirectly, from the lack of tillage associated with perennial production since tillage and aboveground biomass yield have not been controlled for in previous studies. In addition, comparisons of annual wheat and IWG have primarily been conducted in a relatively narrow set of U.S. climates and ecosystems (i.e., Midwest and Northeast USA). Finally, there remains considerable discussion whether soil health and soil carbon storage are higher under no-tillage systems (annual or perennial) versus conventionally tilled systems across the entire soil profile (Luo et al., 2010; Bai et al., 2019; Page et al., 2020). For instance, the significance and magnitude of soil health responses in tilled versus no-till systems in California's San Joaquin Valley has shown to vary by soil depth (Mitchell et al., 2017).

Over the course of three years, the study reported in this dissertation investigated aboveground productivity, soil carbon, and soil microbial communities in tilled annual wheat, no-till annual wheat, and IWG in the Mediterranean climate of California through a series of agronomic measurements, soil assessments, and genomic analyses.

Chapter 1 compares aboveground biomass productivity in these three cropping systems while controlling for the effect of tillage and determines the relative importance of perennial root presence and soil disturbance on active soil microbial biomass (PLFA), mineralizable soil carbon (MinC), and permanganate oxidizable carbon (POXC) at different soil depths. It also investigates whether aboveground biomass productivity influences MinC and POXC and determines how these three cropping systems differ in their potential for soil carbon mineralization and soil carbon stabilization. By tracking aboveground biomass yields and measuring soil carbon (POXC and MinC) and microbial biomass to a depth of 60 cm in these three cropping systems, we were able to identify factors that underpin aboveground productivity and soil carbon mineralization and stabilization in the Mediterranean climate of California.

Chapter 2 compares the ability of IWG, no-till annual wheat, and tilled annual wheat to address consumer-oriented provisioning services, regulating services and disservices in a Mediterranean climate based on a multifunctionality and ecosystem service framework proposed by Huang et al. (2015). The three measurements we took over the course of three years to investigate these three services were grain yield stability, soil carbon storage to a 90 cm soil depth, and NUE. Yield stability was assessed by measuring grain yield every year in each cropping system, while soil carbon mass was monitored from 2017-2020 to a depth of 90 cm throughout the entire experiment and analyzed using the equivalent soil mass (ESM) method. Nitrogen use efficiency was assessed through measurements of nitrogen fertilizer recovery efficiency (NUE) in grain and aboveground biomass. By tracking these measurements and subsequently analyzing them through this integrated framework, we were able to identify tradeoffs in consumer-oriented provisioning services, regulating services, and disservices among tilled wheat, no-till wheat, and IWG in a Mediterranean climate.

Chapter 3 measures if and how perennial and annual grains (IWG and tilled annual wheat) affect soil microbial diversity and whether they select for distinct microbial (fungal and bacterial) community compositions within the rhizosphere and bulk soil. It also determines which soil physiochemical properties influence soil microbial community composition and compares the fungi: bacteria (F/B) ratio and soil carbon storage in IWG and tilled annual wheat. Through a combination of genomic analyses (16S and ITS), active microbial biomass measurements (PLFA), and soil carbon mass assessments using the equivalent soil mass method down to a depth of 90 cm, we identified trends in microbial diversity and composition, microbial biomass, and soil carbon storage under tilled annual wheat and IWG in the Mediterranean climate of California.

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Chapter 1: Comparing perennial to annual grain crops reveals that plant productivity and no-till underpin soil carbon stabilization and microbial biomass in a Mediterranean climate

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Abstract

As interest in the production of perennial grain crops increases, it is critical to quantify differences in aboveground biomass (AGB) productivity and soil carbon stabilization potential between annual and perennial grain crops, and to understand the factors determining these relationships. This study measured the effects of crop type, tillage, and nitrogen (N) fertilization on AGB, soil microbial biomass, and soil carbon in annual and perennial grain crops in a Mediterranean climate. AGB and associated soil samples from tilled wheat *(Triticum aestivum*), no-till wheat, and intermediate wheatgrass [Kernza ® (*Thinopyrum intermedium*)] (IWG) were collected annually across three years under various N fertilization rates. We quantified AGB yields, soil microbial biomass (via phospholipid fatty acid analysis), permanganate oxidizable carbon (POXC) and mineralizable soil carbon (MinC). Across N rates and years, IWG yielded 28% less AGB than annual wheat ($p < .001$). A lack of soil disturbance (no-tillage vs. tillage) contributed foremost to greater microbial biomass abundance in the topsoil by year three of the experiment ($p < 0.01$), while crop type (annual vs. perennial) had the greatest influence on

whether microbial communities tended toward stabilizing versus mineralizing soil carbon ($p<.001$). Higher stabilization processes were primarily driven by the increased plant productivity of annual wheat compared to IWG, while lack of tillage contributed secondarily. Overall, the no-till annual wheat treatment achieved an average of 14% greater microbial biomass and higher soil carbon stabilization potential ($p \leq 0.04$) in the top 30 cm of soil than the IWG and tilled annual wheat systems by matching tilled annual wheat in plant vigor and IWG in lack of soil disturbance. Our results suggest that no-till annual wheat is better suited than tilled wheat or IWG in providing gains in soil microbial biomass and soil carbon stabilization, while still maintaining stable aboveground biomass yields overtime.

1. Introduction

Intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey; trademarked Kernza®) or IWG is a domesticated cool-season perennial wheatgrass that has been bred for grain production (DeHaan and Ismail, 2017). As of today, IWG is being cultivated on approximately 1,400 hectares of cropland in the United States (The Land Institute, 2022) and the palatable IWG grain is used in beer, bread, and cereal production (DeHaan & Ismail, 2017; Broom, 2019). Recent studies comparing soils under perennial versus annual grains have reported higher carbon fluxes and soil carbon (C) and nitrogen (N) storage in soils after IWG production than in annual monocultures and restored native vegetation systems (Means et al., 2022). IWG also produces a high-quality forage (Favre et al., 2019), grain yields of 300 to 1500 kg ha^{-1} (Culman et al., 2013; Jungers et al., 2017; Tautges et al., 2018; Pugliese, 2019) and biomass yields of up to 12,000 kg ha^{-1} (Jungers et al., 2017) or more. However, biomass yield potential and the associated ecosystem services of IWG production must be considered alongside its grain productivity and yield stability relative to annual grain crops if considering it a sustainable alternative to annual grain production. For instance, IWG yields approximately 30% of the grain of annual wheat, (Culman et al., 2013; Cassman et al., 2022), has a harvest index of 0.06-0.1 (Culman et al., 2013; Dick et al., 2018; Hunter et al., 2020a), and may experience yield instability due to interannual variation in nutrient and water supply, leading to large fluctuations in IWG production (Loomis et al., 2022). These are all important considerations when comparing perennial and annual grain crop production.

In addition to productivity, tillage is an important consideration when comparing relative benefits of perennial versus annual grain cultivation. In annual grain cropping systems and more broadly, there has been a transition from intensive tillage to conservation tillage practices in recent decades (USDA NASS, 2017). Prompted by the 1930's Dust Bowl in the U.S., shifts towards reduced tillage practices occurred because of extreme degradation across significant portions of agricultural land. No-till, a form of conservation tillage, is now widely implemented on a global scale with more than 125 million hectares in no-till production worldwide (Pittelkow et al., 2015a). In comparison to conventional tillage practices, no-till agriculture has been shown to perform best in dry climates under rainfed conditions (Pittelkow et al., 2015b). Yields for cereal crop production have recently shown to be moderately impacted by no-till practices, with the negative impacts associated with no-till being smallest for annual wheat (*Triticum aestivum*; Pittelkow et al., 2015b). In addition to only modest yield reductions with no-till agriculture, converting from moldboard plow to no-tillage has been shown to significantly increase soil health indicators in the topsoil, including increased soil organic matter (SOM) and improved soil chemical, biological and physical properties in both annual and perennial crop types (Nunes et al., 2018; Nunes et al., 2020).

Given the inherent differences in photosynthate allocation and soil disturbance frequency between annual versus perennial grain systems, tradeoffs in aboveground productivity and ecosystem services, particularly with respect to soil carbon are likely. For instance, relative to annual grains (i.e., wheat, maize, rye), IWG colonizes a significantly greater percentage of soil area with root biomass at soil depths greater than 60 cm (Duchene et al., 2020), has greater coarse and fine root biomass (Sprunger et al., 2019) and can decrease nitrate leaching by 86-99% (Culman et al., 2013; Jungers et al., 2019). This increased root colonization of the soil profile could be one explanation for why IWG has been found to have a significantly greater amount of active fungal microbial biomass (determined via fungal phospholipid fatty acids or PLFA) in the topsoil as compared to annual wheat. Furthermore, studies have shown that IWG production significantly increases mineralizable soil carbon (MinC), a labile soil carbon pool measurement that reflects soil carbon mineralization and soil nutrient availability (Hurisso et al., 2016; Wade et. al., 2019), in the top 10 cm of soil relative to tilled annual wheat after two years (Culman et al., 2013). Conversely, permanganate oxidizable carbon (POXC) is a more processed pool of soil carbon that is an indicator of soil organic matter stabilization or accumulation (Hurisso et al., 2016) and has mixed outcomes when measured in IWG and annual wheat systems (Culman et al., 2013 ; Sprunger et al., 2019). Additionally, in another recent study, IWG and annual wheat had similar amounts of labile and stable soil carbon when measured as large and medium sized fractions of particulate organic matter-carbon, respectively (Sprunger et al., 2018a). As a result, it remains unclear if IWG is more likely than annual wheat to promote soil carbon mineralization or soil carbon stabilization across soil depths. In addition, while recent studies comparing IWG and annual wheat have provided valuable insight into how annual and perennial grain systems differ above and belowground, the effect of tillage and aboveground biomass (AGB) has not been controlled for in published studies. Moreover, comparisons of annual wheat and IWG production in previous studies have largely been made in a relatively narrow set of U.S. climates and ecosystems (i.e., Midwest and Northeast USA).

To better understand potential tradeoffs between above and belowground productivity among IWG and annual wheat, we measured plant and soil outcomes over the course of three years in a Mediterranean climate from three cropping systems: IWG, no-till annual wheat, and tilled annual wheat. Our objectives were to: 1) compare AGB in these three cropping systems while controlling for the effect of tillage; 2) determine the relative importance of perennial root presence and soil disturbance on active microbial biomass, MinC, and POXC at different soil depths; 3) determine whether AGB productivity influences soil carbon (POXC, MinC); and 4) determine whether these cropping systems differ in their potential for soil carbon stabilization. We hypothesized that: 1) AGB productivity between annual wheat and IWG would be comparable; 2) perennial root presence would more strongly influence microbial biomass, MinC and POXC than lack of soil disturbance, particularly at depths exceeding 30 cm; 3) AGB productivity would influence MinC and POXC; and 4) no-tillage systems (IWG and no-till wheat) would contribute more to soil carbon stabilization while tilled systems would contribute more to soil carbon mineralization.

2. Materials and methods

2.1. Field Location and Management

The experiment was conducted at the UC Davis Russell Ranch Sustainable Agriculture Facility in Yolo County (38°32’27.30" N, 121°52’7.39” W). At an elevation of 16m, this experimental site is in the northern Central Valley of California with wet winters and hot, dry summers - typical of semiarid and Mediterranean climates. The soil type is a Yolo silt loam (Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). Prior to the establishment of the experiment, the area was an unmanaged grassland for 10 years. Prior to planting in 2017, a starter NPK fertilizer (11-52-0) was applied at 135 kg ha^{-1}. Intermediate wheatgrass (IWG) was initially planted in December 2017, but then replanted in January 2018 due to failed crop establishment. Annual wheat was planted in December 2017, December 2018, and November 2019. Nitrogen fertilization for select treatments was broadcast-applied as urea annually during early vegetative growth stages for each crop type. Urea was always applied ahead of a rainfall event sufficient to ensure soil incorporation (≥ 13mm). To control weed pressure, select IWG plots were hand-weeded annually each spring and Glyphosate was applied during the winter of

2019 at labeled rates to all IWG plots when IWG was dormant and thus not actively growing. Osprey was applied to all non-actively growing main plots in winter 2019.

2.2. Experimental Design and Sampling

The experimental design was a split plot design with four replications. The main plots were 12m x 6m and planted in 15 cm rows to one of three cropping system types; no-till annual wheat (Hard Red Spring wheat, var. WB 9229), tilled annual wheat (Hard Red Spring wheat, var. WB 9229), or IWG (*Thinopyrum intermedium*; 5th selection cycle, The Land Institute, Kansas, USA). Annual wheat in tilled and no-till plots was seeded at a rate of 135 kg ha^{-1} (3.5 million seeds ha^{-1}) and IWG was seeded at a rate of 11.2 kg ha^{-1} (1.9 million seeds ha^{-1}). All plots were disked to a depth of approximately 20-30 cm when establishing the experiment. In subsequent years, only the tilled annual wheat plots were disked and rototilled each fall to a depth of 15-25 cm prior to soil sampling and planting operations. Standing biomass that remained after harvest was mowed to a height of approximately 6 cm and the biomass was removed. In this way, belowground carbon outcomes in each system were independent of confounding effects of large amounts of aboveground biomass incorporation. Four nitrogen rates were then randomly assigned to 3m x 6m sub-plots within each plot. N rates were 0, 56, 112, or 168 kg ha^{-1} and were broadcast-applied as urea (46-0-0).

For biomass sampling, total biomass (grain and vegetative biomass) was hand harvested to a stubble height of approximately 6 cm from a representative $1m^2$ area in each subplot (Table S1.1). All biomass samples were weighed in the field and then dried in an oven at approximately 60 degrees Celsius until samples reached a stable weight. Total biomass yields were then reported on an oven dry basis. For soil sampling, one soil sample was taken with a Geoprobe to a depth of 1.2 meters with a 4.3 cm diameter probe from three field replicates of each cropping system type at the 0 and 112 kg ha^{-1} nitrogen rates on 19 October 2018, 15 October 2019, and 21 September 2020. Soil cores were immediately stored on ice and refrigerated until processed in the lab. Upon processing, moist soil cores were divided into 0-15, 15-30, and 30-60 cm increments with a linear correction to account for soil core compaction. Each soil depth increment was then either freeze-dried for PLFA analysis or air dried, sieved to 2mm, weighed, and stored in falcon tubes. All prepared soil samples were sent directly to Microbial I.D. for PLFA analysis (active soil microbial biomass) and to the Ohio State University Soil Fertility laboratory for MinC and POXC analyses. Mineralizable soil carbon was measured as the burst of CO^2 from dried soil over the course of 24 hours after the soil sample had been rewetted with water. POXC measurements were carried out with a weak oxidizing solution as described by Weil (2003) and Culman et al. (2012) PLFA analysis was performed following the methods described by Buyer & Sasser (2012).

2.3. Statistical Analyses

Mixed linear models were fitted using the lme function from the nlme package (version 3.1 - 152) (Pinheiro et al., 2021 in R version 4.0.3; R Core Team, 2020). Standard errors and estimated marginal means (EMMs) (Lenth, 2021), were derived from mixed linear models. Assumptions of normality and homogeneity of variance were met for biomass yield. A log transformation was necessary for MinC, POXC, and microbial biomass to meet these assumptions. The relationships between crop type (annual, perennial), tillage (no-tillage, tillage), year, nitrogen fertilization and total biomass were modeled with the equation:

Equation 1: Biomass Yield

$Y = A + A^2 + B + B^2 + C;$

Where:

Y = Biomass Yield

A = Nitrogen rate nested within year nested within crop type

B = Nitrogen rate nested within year nested within tillage

C = Random intercept of nitrogen rate nested within year nested within block

For total biomass yields, the interaction between till and no-till wheat was not significant, so the effects were combined when generating EMMs and standard errors.

The relationship between crop type, tillage, year, nitrogen fertilization, soil depth and: 1) mineralizable carbon; 2) POXC; and 3) microbial biomass (as determined by PLFA) were modeled with the equation:

Equation 2: MinC, POXC, microbial biomass

Z = A + B + C;

Where:

Z = MinC, POXC, or microbial biomass

A = Soil depth nested within nitrogen rate, nested within year, nested within crop

B = Soil depth nested within nitrogen rate, nested within year, nested within tillage

C = Random intercept of soil depth nested within nitrogen rate, nested within year, nested within block

Each soil depth increment was subset (0-15,15-30,30-60 cm) and the relationship between crop type, tillage, year, nitrogen fertilization, aboveground biomass yield and: 1) MinC; and 2) POXC were modeled with the equation:

Equation 3: MinC and POXC

Z = A + B + C;

Where:

Z = MinC, POXC

A = Biomass yield nested within nitrogen rate, nested within year, nested within crop

B = Biomass yield nested within nitrogen rate, nested within year, nested within tillage

C = Random intercept of biomass yield nested within nitrogen rate, nested within year, nested within block

The function r.squaredGLMM in the MuMIn package (version 1.43.17) (Barton, 2020) was used to calculate Pseudo R^2 for the mixed linear models. We used these values to infer the variation explained by each model. The effects of crop, tillage, year, and nitrogen on biomass yield were tested using a type three ANOVA in the car package (Fox & Weisberg, 2019). Because nitrogen fertilizer treatments did not significantly affect the response variable estimates in Equation 2, effects were averaged across nitrogen rates when creating estimates for these variables (Figures 1.3, 1.4, and 1.5).

To determine cropping system effects on soil carbon stabilization versus mineralization, we utilized the framework developed by Hurisso et al. (2016). Specifically, we extracted residuals from a linear regression model of the relationship between POXC and MinC values measured from the same soil sample. POXC observations greater than predicted by the linear model resulted in positive residuals, while MinC observations greater than predicted had negative residuals. Residuals specific to samples from each cropping system treatment and depth were derived from the equation:

Equation 4: Linear relationship between POXC and MinC (adopted from Hurisso et al., 2016)

Z = A*B + C;

Where:

Z = POXC

A = Logged mineralizable carbon

B = Soil depth nested within nitrogen rate, nested within year

C = Random effect of soil depth nested within biomass, nested within nitrogen rate, nested within year, nested within block

As a factor, there were instances where all three soil depths were included in the model, or the data was subset to 0-15 and 15-30 cm depths to determine crop type effects on only the top 30 cm of soil.

The relationship between crop, year, soil depth and residuals generated from the linear model in Equation 4 were modeled with the equation:

Equation 5: Residuals as affected by cropping system type or crop, year, and soil depth

Z = A;

Where:

Z = Residuals generated from linear model in Equation 4

A = Soil depth nested within year, nested within cropping system type (tilled wheat, no-till wheat, IWG) or crop (annual/perennial)

3. Results

3.1. Temperature and precipitation

The cumulative precipitation totals for the first (2017-2018) and third (2019-2020) experimental seasons were 55% and 51% of the 20-year average annual precipitation of 479 mm (2000-2020) for this experimental location, respectively (Fig. 1.1). Conversely, the second (2018-2019) experimental season had 65% more precipitation than the 20-year average, with cumulative totals approximately triple those observed in the first or third year of the experiment. Additionally, recorded maximum temperatures during the 2018-2019 growing season were 40%, 15%, and 14% lower in February, March, and May, respectively, than maximum temperatures during the same periods for the first and third year of the experiment.

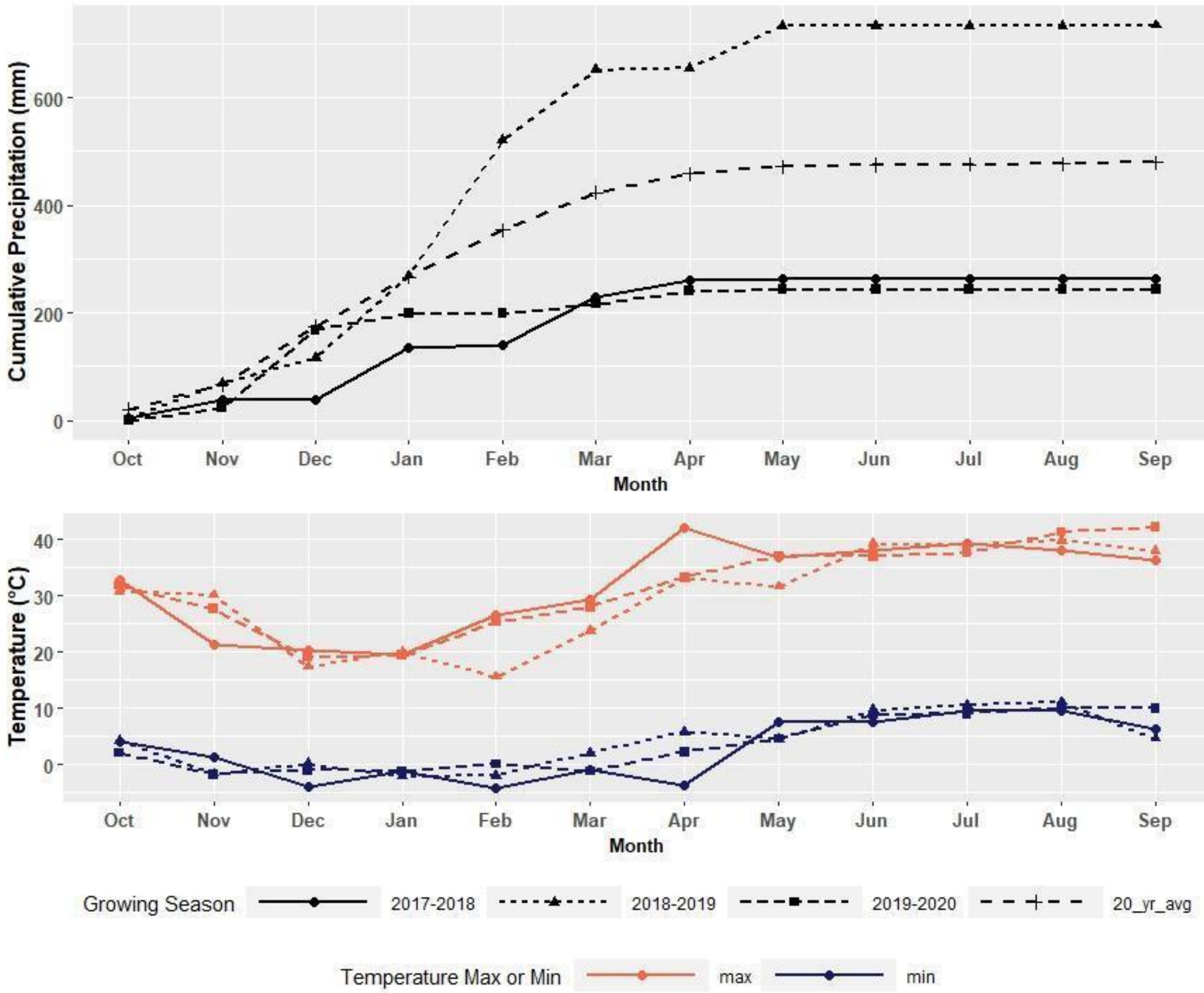

Figure 1.1 Cumulative precipitation (mm), minimum temperature (°C), and maximum temperature (°C) for the three experimental growing seasons between 2017- 2020 and cumulative precipitation (mm) average for 2000-2020 in Yolo County.

3.2. Aboveground biomass productivity

Approximately 79% of variation in total aboveground biomass yield (ABG) was explained by the combined effects of crop, tillage, year, and nitrogen rate (Eq. 1). Over the course of three seasons, nitrogen fertilization significantly affected AGB ($p < 0.001$) and explained 19% of the variation in fixed effects for AGB (Fig. 1.2). Meanwhile, tillage did not significantly affect AGB. Therefore, AGB estimates for annual wheat were based on the combined effects of no-till and tilled wheat.

Across N rates and years, the total AGB yield for IWG was 28% less than annual wheat ($p<0.001$). More specifically, annual wheat yielded 8,309 kg ha^{-1} on average (Fig. 1.2; Table S1.2) of AGB, while IWG yielded 5956 kg ha^{-1} .The relationship between annual wheat and IWG AGB yields varied across years. Annual wheat had nearly triple the AGB yield of IWG in 2018 and 2020, and its AGB yields were significantly greater at every nitrogen rate ($p<0.001$).

Conversely, in 2019, IWG had significantly higher AGB than annual wheat ($p<0.001$) at all but the nitrogen rate of 0 kg ha^{-1} (Fig. 1.2). For a given N rate (0 kg N ha^{-1} or 112 kg N ha^{-1}), AGB in annual wheat varied by approximately 15% over the course of three years, while IWG exhibited greater variation across years and N rates. For instance, in 2019, AGB yield for IWG was 265% more than 2018 and nearly 400% more than 2020, when it received 112 kg N ha^{-1}.

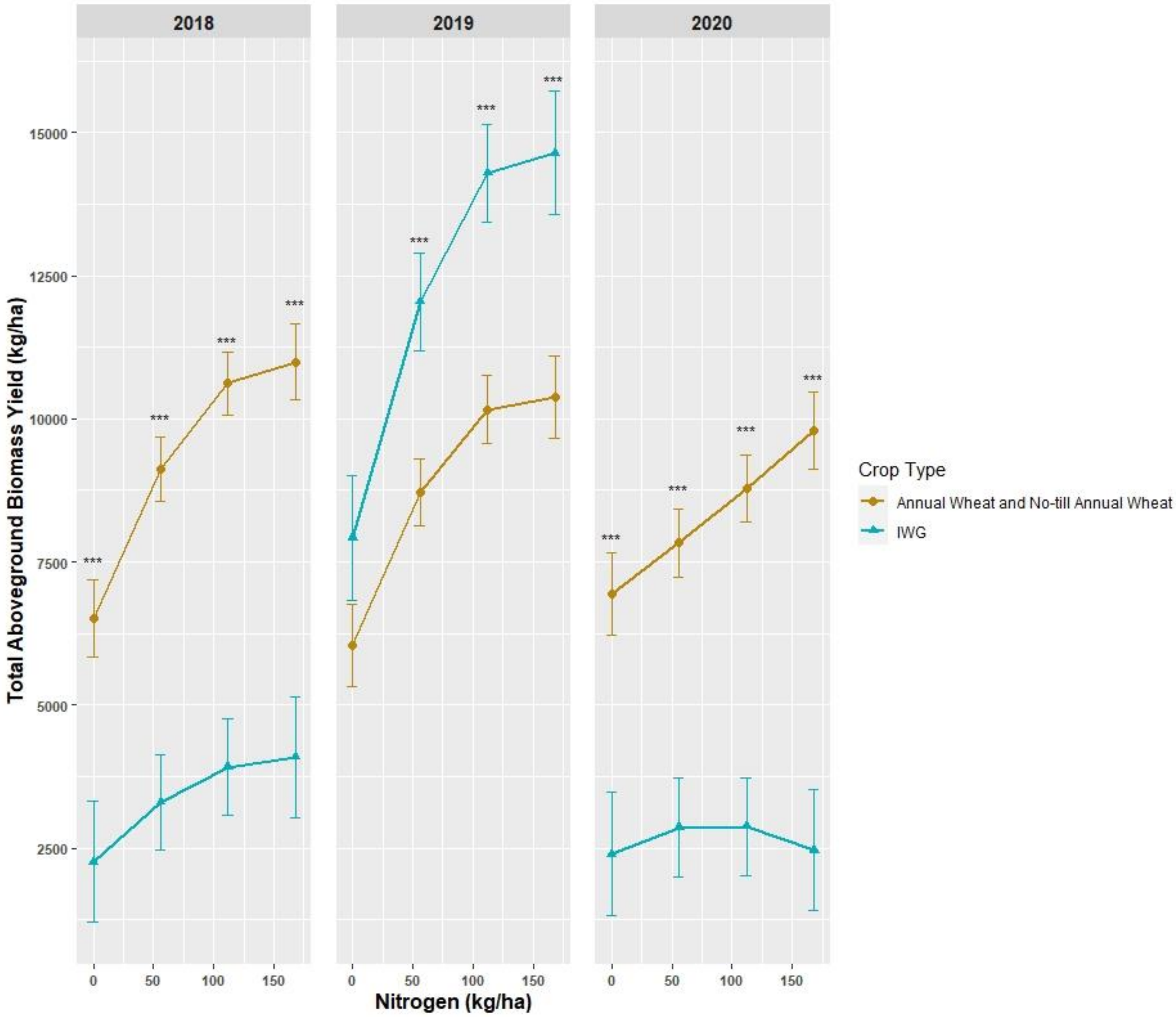

Figure 1.2 Total aboveground biomass yield (kg ha^{-1}) for IWG and annual wheat (tilled and no-till wheat) crop types across three years (2018,2019,2020) and four nitrogen rates. Error bars represent ± one SE from the mean value derived from the linear model in Equation 1. Asterisks represent significant pairwise differences between crop types within a given year and nitrogen rate, where *** $p < 0.001$.

3.3. Soil microbial biomass

The effects of crop, tillage, year, nitrogen rate, and soil depth explained 77% of variation in microbial biomass (Eq. 2). When compared across years and soil depths, IWG had equivalent microbial biomass as the annual systems ($p = 0.83$) (Fig. 1.3a). Conversely, the no-tillage systems (IWG and no-till annual wheat combined) had approximately 15% higher microbial biomass as compared to tilled annual wheat across years and soil depths ($p < 0.01$) (Fig. 1.3b).

However, the effects of crop type and tillage on microbial biomass varied by depth and time. Specifically, the effect of tillage largely occurred in the top 0-30 cm (Fig. 1.3b), where no-tillage systems resulted in higher microbial biomass by the last year of the experiment as compared to tilled annual wheat (Fig. 1.3b). In contrast, IWG had significantly more microbial biomass than annual wheat at the 30-60 cm depth, but these differences were confined to the first two years of the experiment (Fig. 1.3a). When each of the three cropping systems were compared on an individual basis (Eq. S1.1, Table S1.3) in 2020, the no-till annual wheat system had greater microbial biomass than tilled annual wheat across all depths ($p < 0.05$) as well as greater microbial biomass than IWG in the top 0-30 cm.

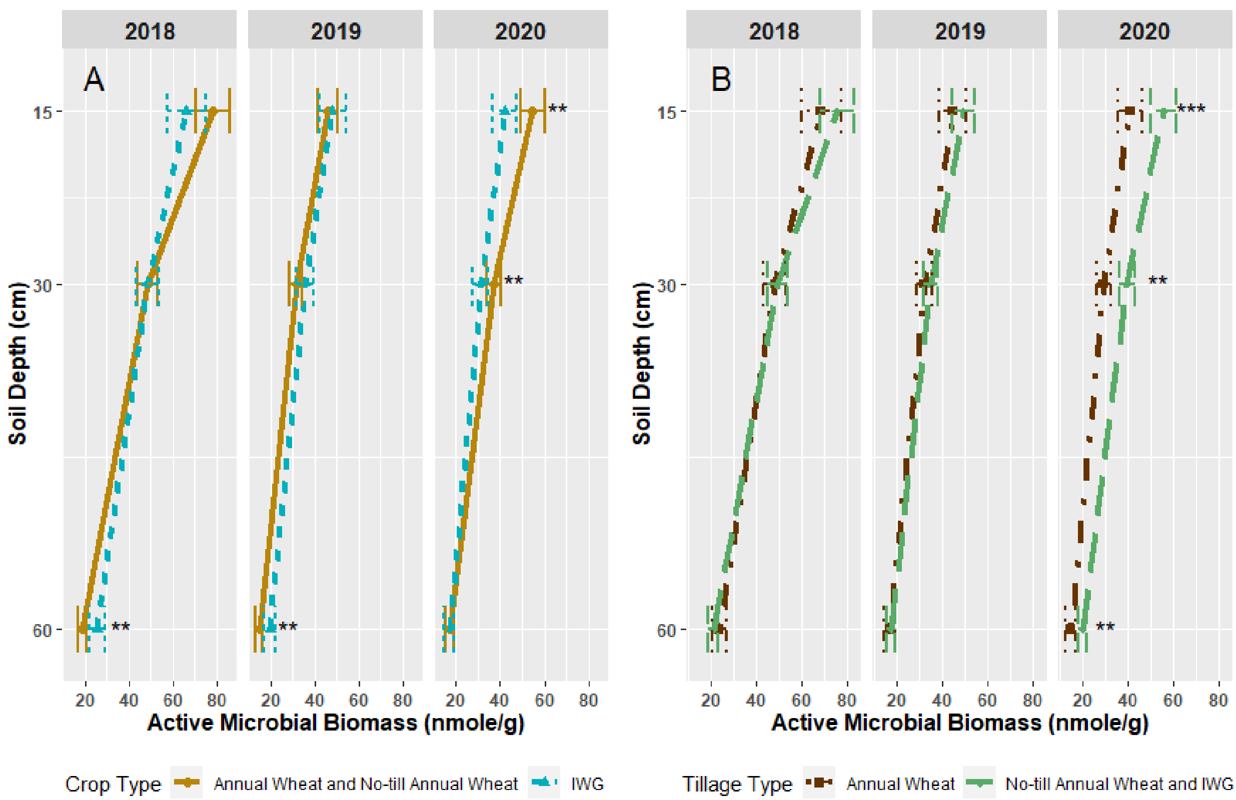

Figure 1.3 Active microbial biomass (as determined by phospholipid fatty acids or PLFA): A) the effect of crop types (intermediate wheatgrass versus tilled and no-till annual wheat) and B) tillage types (tilled annual wheat versus no-till annual wheat and intermediate wheatgrass) for three years (2018, 2019, 2020) at three depths (0-15, 15-30, 30-60 cm) across two nitrogen rates (0, 112 kg N ha^{-1}). Error bars represent ± one SE from the mean. Asterisks represent significant pairwise differences between crop types or tillage types within a given year and soil depth, where * p <0.10, ** p <0.05, and *** p <0.001.

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3.4. Soil Carbon

3.4.1. POXC

The no-till annual wheat system had higher POXC than IWG and tilled annual wheat in the top 30 cm every year (Fig.1.4, Table S1.3). To determine how tillage or ABG productivity influenced POXC outcomes at a given soil depth, we investigated the linear relationship between ABG and POXC while controlling for the effect of tillage. We found that while POXC generally had a positive linear relationship with AGB overall, this relationship was strongest at the shallowest soil depth ($p=0.14$). At this depth, ABG accounted for 20% of the variation in POXC (Eq. 3). To illustrate this effect further: without considering the effects of AGB (Eq. 2), annual wheat resulted in higher POXC than IWG at the 0-15 cm depth in the two years (2018 and 2020) when its AGB yield greatly exceeded IWG (Fig. 1.2; Fig. 1.4a). As such, once AGB was included as an explanatory variable (Eq. 3), POXC differences between the crop types were no longer significant within individual years or across the experiment.

Similarly, the effect of tillage on POXC at the shallowest soil depth (Fig. 1.4b) differed depending on whether AGB was included as an explanatory factor in the statistical model. Without AGB considered, the untilled treatments (no-till annual wheat and IWG) resulted in over 50 mg kg^{-1} higher POXC in the last two years of the experiment compared to tilled annual wheat ($p < 0.10$). However, if the effect of AGB was included as an explanatory factor, the effect of tillage no longer explained differences in POXC. Therefore, the differences in POXC values among the crop types at the 0-15 and 15-30 cm depths (Fig. 1.4a) appear to be due in part to the differences in biomass productivity between IWG and annual wheat.

Since estimates of the effect of tillage (Fig. 1.4b) include both crop types in the no-till group (and are therefore partly confounded by the effect of AGB), comparing the no-till and tilled annual wheat systems in isolation is necessary to understand the effect of tillage on POXC independent of AGB effects. When these systems were compared, no-till wheat resulted in 52 mg kg^{-1} higher POXC than tilled wheat across the experiment ($p = 0.01$) despite having nearly the same AGB. Additionally, no-till wheat had significantly higher POXC than IWG across the experiment ($p=0.02$) and this difference was largest by 2020 ($p=0.01$). Taken together, these results indicate that POXC concentrations were higher as a function of both greater AGB production and a lack of soil disturbance. At the 15-30 cm depth these effects were similar but accounted for a smaller proportion of the total variance in POXC as compared to the 0-15 cm depth. Meanwhile, at the 30-60 cm depth, POXC did not differ whether AGB was included as an explanatory factor. Likewise, at this depth, IWG had higher POXC by the last year of the experiment than annual wheat. However, this was primarily due to lower POXC values in the no-till annual wheat at this depth, which had less POXC than both IWG ($p= 0.17$) and tilled annual wheat ($p=0.21$) by the final year of the experiment (Eq. S1.1, Table S1.3).

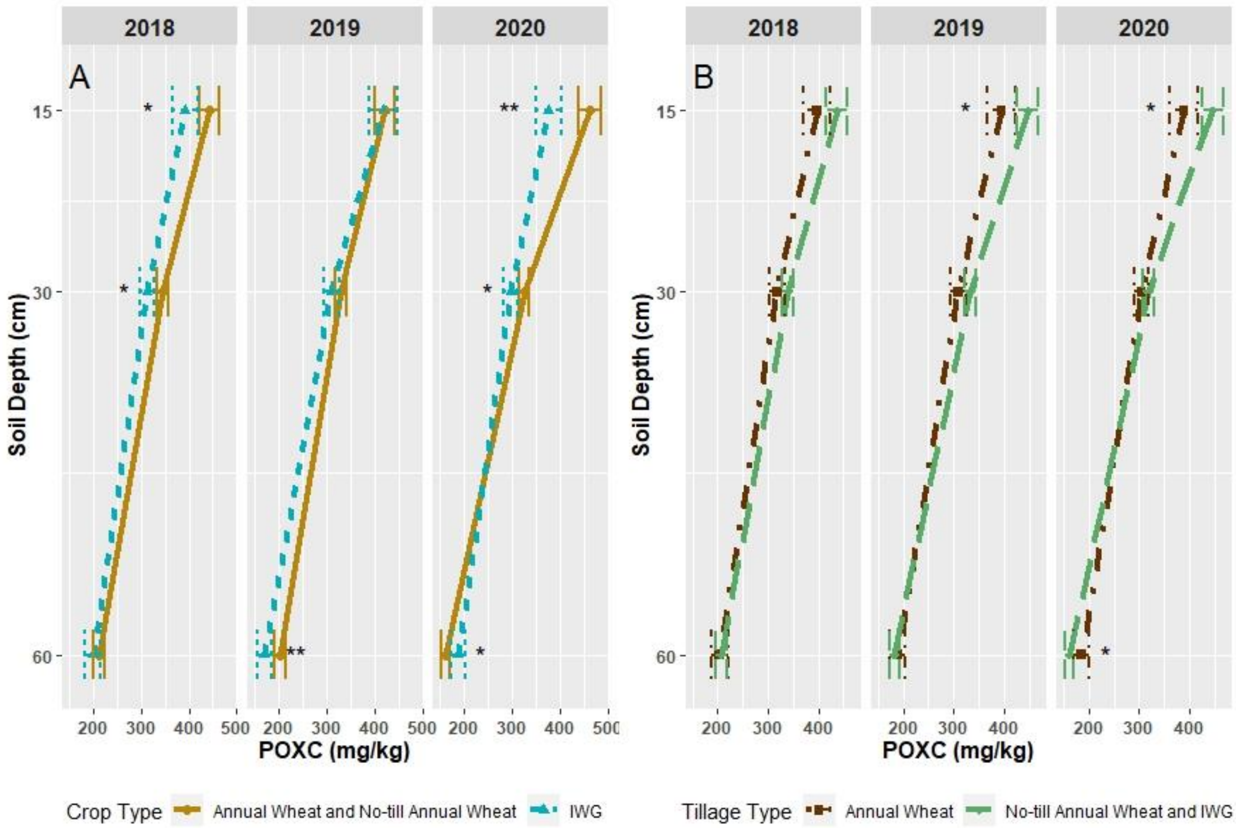

Figure 1.4 Permanganate oxidizable carbon (POXC) for A) the effect of crop types (intermediate wheatgrass versus tilled and no-till annual wheat) and B) tillage types (tilled annual wheat versus no-till annual wheat and intermediate wheatgrass) for three years (2018, 2019, 2020) at three depths (0-15, 15-30, 30-60 cm) across two nitrogen rates (0, 112 kg N ha^{-1}). Error bars represent ± one SE from the mean. Asterisks represent significant pairwise differences between crop types or tillage types within a given year and soil depth, where * p <0.10, ** p <0.05, and *** p <0.001.

3.4.2. MinC

Whereas the largest differences in POXC among cropping systems occurred in the top 0-15 cm (Fig. 1.4), the largest and most consistent system differences in MinC occurred below this depth (Fig. 1.5). IWG had significantly higher MinC than annual wheat at the 30-60 cm depth and these effects were consistent across time (Fig. 1.5a). Likewise, IWG had higher MinC than annual wheat at the 15-30 cm depth in the first two seasons, but this difference was no longer significant by the third season (Fig. 1.5a). Unlike POXC, AGB had a negative relationship with MinC across years and soil depths. AGB accounted for 39% of the variation in MinC at the 30-60 cm depth and explained more of the variation in MinC at this depth than any other.

Similarly, AGB explained 25% of the variation in MinC at the 15-30 cm depth. In addition, the direction of the relationship between AGB and MinC varied across seasons in a manner that tracked the AGB productivity of IWG (Fig. 1.2). Specifically, in 2018 and 2020, there was a negative relationship between AGB and MinC ($p < 0.05$); whereas in 2019 there was a positive relationship ($p < 0.10$). As such, once AGB was included as an explanatory variable (Eq. 3), MinC differences between the crop types were no longer significant within individual years or across the experiment.

In contrast, the effect of tillage on MinC (Fig. 1.5b) did not differ whether AGB was included as an explanatory factor. When considering the effect of tillage, including both IWG and no-till annual wheat (Fig. 1.5b), the MinC in the untilled treatments trended higher in the 0-15 and 15-30 cm soil depth, but these differences were only statistically significant at the 15-30 cm depth in the final season ($p = 0.09$). Likewise, when MinC from the no-till and tilled annual wheat systems were compared without IWG considered (Eq. S1.1,Table S1.3), the no-till annual wheat generally had higher MinC, and this difference was primarily a function of higher MinC at the 0-30 depth. Therefore, the lack of soil disturbance appeared to influence MinC, but this effect was secondary to the effects of AGB productivity of the cropping system.

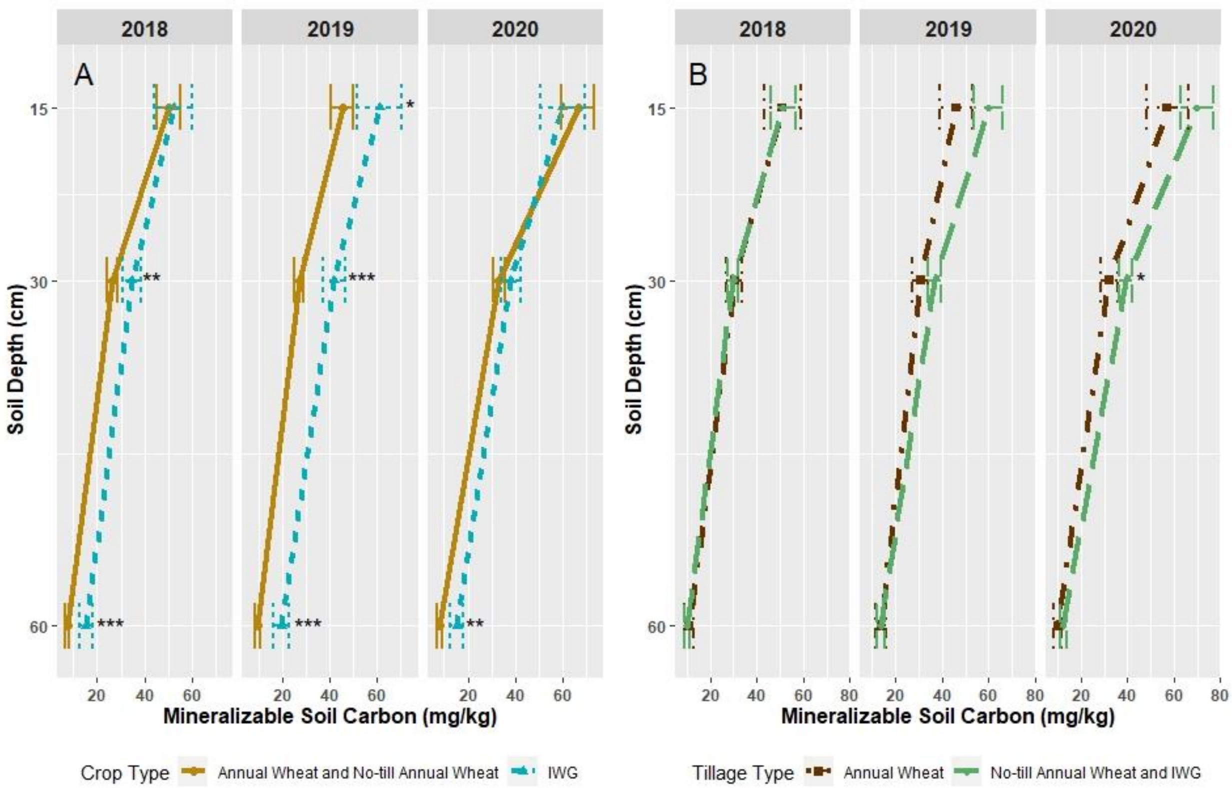

Figure 1.5 Mineralizable soil carbon (MinC) for: A) the effect of crop types (intermediate wheatgrass versus tilled and no-till annual wheat) and B) tillage types (tilled annual wheat versus no-till annual wheat and intermediate wheatgrass) for three years (2018, 2019, 2020) at three depths (0-15, 15-30, 30-60 cm) across two nitrogen rates (0, 112 kg N ha^{-1}). Error bars represent ± one SE from the mean. Asterisks represent significant pairwise differences between crop types or tillage types within a given year and soil depth, where * p <0.10, ** p <0.05, and *** p <0.001.

3.5. Soil carbon mineralization versus soil carbon stabilization potential

The relative relationship between POXC and MinC measurements recorded within the same soil sample can be used as a comparative indicator of soil carbon stabilization versus mineralization across cropping system treatments (Hurisso, et al., 2016). In this framework, POXC values that exceed the general linear relationship (positive residuals, Eqns. 4,5) indicate greater soil carbon stabilization potential, while MinC values that fall below the general linear relationship (negative residuals, Eqns. 4,5) indicate greater soil carbon mineralization potential. IWG had significant soil carbon mineralization potential (negative residuals, Eqns. 4,5) at every soil depth across all years (Fig. 1.6). Among IWG observations, those with the highest soil carbon mineralization potential were treatments where no N fertilizer had been applied and for measurements recorded during the final year of the experiment (data not shown). Across all years of the experiment, tilled annual wheat had significant soil carbon mineralization potential in the top 0-15 cm, while at the 30-60 cm depth, tilled annual wheat had greater soil carbon stabilization potential (positive residuals, Eq. 4 & 5, Fig.1.6, Table 1.1). Meanwhile, no-till annual wheat had significant soil carbon stabilization potential in the top 0-30 cm across all years (Fig. 1.6, $p < 0.01$) with the exception of the 30-60 cm depth in year three (Table 1.1, $p = 0.34$). At this given time point and depth, tilled annual wheat was the only system with significant soil carbon stabilization potential (Fig.1.6, Table 1.1, $p = 0.06$). Across time, the no-till annual wheat system had significantly higher soil carbon stabilization potential ($p \leq 0.04$) in the top 30 cm of soil than did IWG and tilled annual wheat systems (Eq. 4,5).

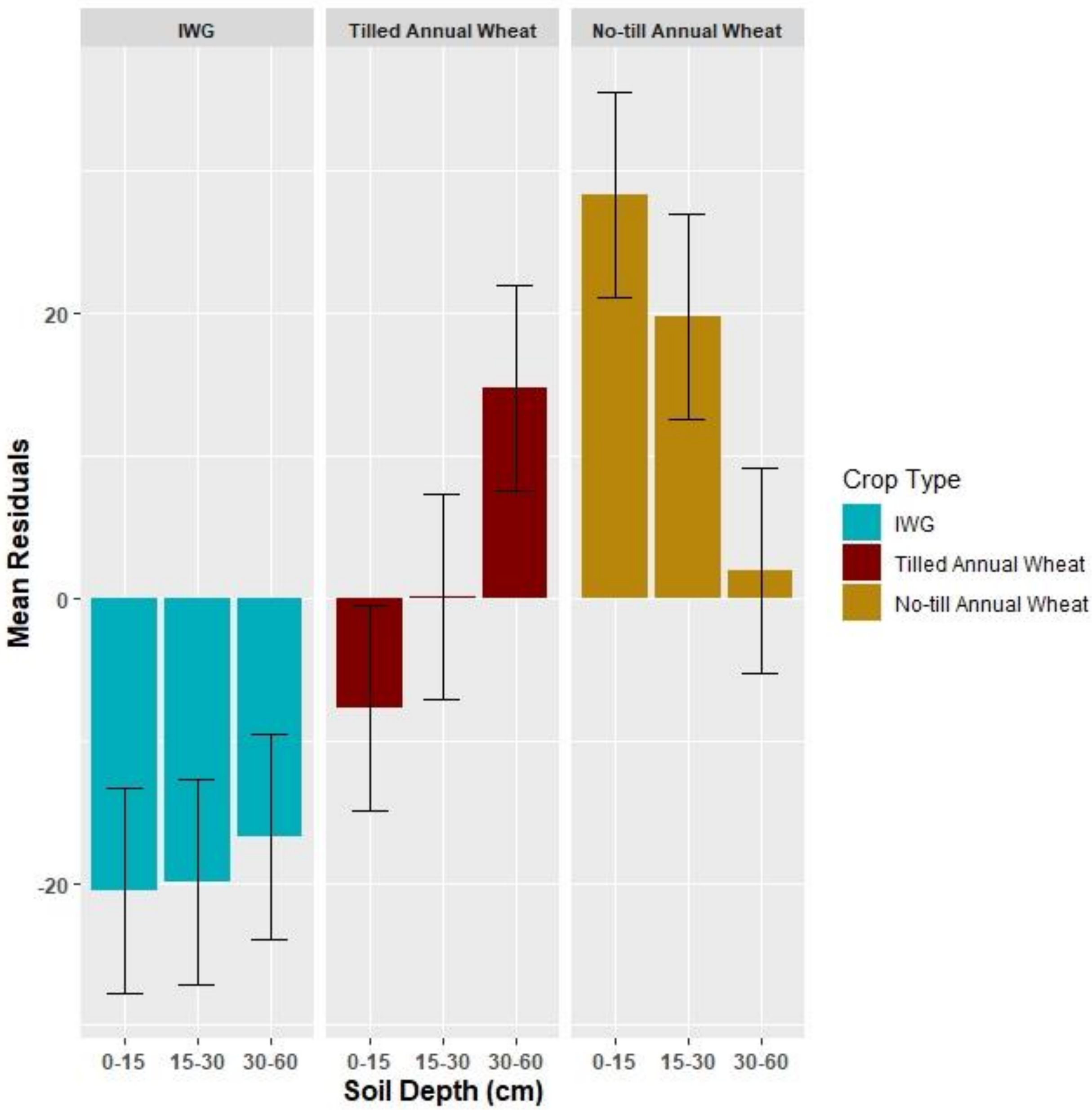

Figure 1.6 Mean residuals from linear regression model (Eqns. 4,5) to assess cropping system (IWG, no-till annual wheat, tilled annual wheat) effects on permanganate oxidizable carbon (POXC) versus mineralizable carbon (MinC) at each soil depth (0-15, 15-30, 30-60 cm) across years (2018,2019,2020). Error bars represent ± one SE from the mean.

Crop Type	Soil Depth (cm)	Experimental Year	Avg. Residuals	p-value
IWG	0-15			
		2018	-7.57	0.546
		2019	-3.35	0.789
		2020	-50.74	<0.001
	15-30			
		2018	-35.93	0.005
		2019	-11.56	0.357
		2020	-12.40	0.323
	30-60			
		2018	-13.68	0.276
		2019	-23.00	0.054
		2020	-12.21	0.330
No-till Annual Wheat	0-15			
		2018	28.19	0.026
		2019	20.08	0.110
		2020	36.60	0.004
	15-30			
		2018	26.26	0.038
		2019	15.99	0.203
		2020	17.25	0.170
	30-60			
		2018	9.51	0.448
		2019	8.48	0.499
		2020	-12.02	0.338
Tilled Annual Wheat	0-15			
		2018	-20.62	0.101
		2019	-16.74	0.183
		2020	14.14	0.260
	15-30			
		2018	9.67	0.440
		2019	-4.43	0.723
		2020	-4.86	0.698
	30-60			
		2018	4.17	0.739
		2019	15.87	0.206
		2020	24.23	0.055

Table 1.1 Mean residuals and SE (± 12.5) from linear regression model (Eqns. 4,5) to assess cropping system (IWG, tilled annual wheat, no-till annual wheat) effects on soil carbon stabilization or mineralization at each soil depth (0-15, 15-30, 30-60 cm) and year (2018,2019,2020). P-values indicate whether residuals for the specific crop type, year, and soil depth treatment combination are significantly different from the population average in positive (stabilizing) or negative (mineralizing) directions.

4. Discussion

Our results indicate that differences in soil microbial biomass and soil carbon observed between annual wheat and IWG are partly a function of differences in AGB productivity between the two crop types alongside inherent differences in soil disturbance in tilled annual crops versus perennial crop production. This has important implications for tradeoff accounting between crop productivity and potential ecosystem benefits for annual and perennial crops and more broadly.

4.1. Crop type determines total aboveground biomass yield

Although we hypothesized that the two crop types would produce similar AGB, across the experiment IWG produced 28% less AGB than annual wheat, with large interannual fluctuations (Fig. 1.2). In contrast, annual wheat produced stable amounts of AGB across the three seasons. These differences in AGB yield stability may reflect differences in adaptation to a semi-arid Mediterranean climate between the two crop types. In this regard, the annual wheat variety grown in this experiment has been specifically selected for the experimental environment, whereas IWG is native to Eurasia and the variety used in this experiment was selected in the Midwest U.S.A. Further, unlike annual wheat, IWG exhibited green cover much later into the season, which may have resulted in differences in soil water supply (Loomis, 2022) that accumulated across the three seasons of the experiment. Given the significant fluctuations in annual precipitation and extended dry periods observed during the experiment, which are typical for the climatic region, these differences in climatic adaptation, growth habit and soil water use could underpin the lower ABG productivity in IWG reported here.

Despite the climatic differences between this experiment and those where the majority of IWG productivity data has been collected (i.e., the Midwest U.S.), IWG AGB in year 2 of 3 (2019) was comparable to previously reported yields (Culman et al., 2013; Jungers et al., 2017; Sprunger et al., 2018b; Tautges et al., 2018; Pugleise et al., 2019 ; Fernandez et al., 2020; Hunter et al., 2020b; Zimbric et al., 2020). In 2018 and 2020, AGB yields of IWG were lower than these referenced studies, but similar to IWG forage biomass yields recently reported in Denmark (Clément et al., 2022). The interannual variation in IWG AGB suggests it depended to a large extent on total annual precipitation (Fig. 1.1, Fig. 1.2). The lowest productivity occurred in 2018 and 2020, when precipitation was approximately 50% less than the 20-year average

(Fig. 1.1). Conversely, productivity was highest in 2019 when annual precipitation was 65% higher than the 20-year average and maximum temperatures during critical IWG growth periods were lower than the first and last year of the experiment. While previous work has suggested that IWG yield decline over time could be attributed to nitrogen limitations (Tautges et al., 2018), IWG becoming sod bound (Culman et al., 2013), or an increase in plant density (Jungers et al., 2017), these causes did not appear to influence the reduction in productivity between years two and three in this experiment.

4.2. Tillage and crop effects on total microbial biomass

As hypothesized, IWG generally increased microbial biomass at depth relative to annual wheat (Fig. 1.3a). However, a lack of tillage more strongly influenced the amount of microbial biomass both overall and by the last year of the experiment at every soil depth (Fig. 1.3b). The observed increase in microbial biomass in the no-till annual wheat system versus the tilled annual wheat system aligns with findings from other studies (van Groenigen et al., 2010; Helgason et al., 2010; Mathew et al., 2012; Li et al., 2020; Sun et al., 2020), but our study reports observations that extend deeper into the soil profile than in previous work. Specifically, these studies observed relative increases in microbial biomass in no-till versus tilled systems at depths of 0-10 cm, while we found that this effect extended to a soil depth of 60 cm. Total microbial biomass has recently been shown to relate positively to large soil macropores (Sun et al., 2020). Thus, the increase in microbial biomass may be partly explained by the development of large soil macropores and increased microhabitats for microbial populations (Li et al., 2020 ; Sun et al., 2020) as a result of reduced soil disturbance. Furthermore, the mulched surface of no-till systems prevents crusting and promotes water infiltration, oxygen diffusion, and dissolved organic carbon movement through the soil profile via improved pore connectivity in the top 10 cm of soil (Pires et al., 2017). These proposed mechanisms are not inherently related to crop rooting patterns or belowground biomass allocation, which may explain why, overall, microbial biomass was more strongly influenced by tillage than crop type in the topsoil by the final year of our study.

4.3. Tillage, crop, and aboveground biomass productivity effects on soil carbon

The soil carbon measurements utilized in this study are widely used indicators of a processed and stabilized labile soil carbon pool (Culman et al., 2012) and a soil carbon pool highly accessible to microbial activity (Haney et al. 2008) in the case of POXC and MinC, respectively. While both are thought to reflect labile forms of soil carbon, POXC is chemically defined and has shown to indicate soil carbon stabilization while MinC is a biological indicator more strongly associated with soil carbon mineralization and nutrient availability (Hurisso et al, 2016).

In our study, both plant productivity and tillage affected POXC concentrations. These effects were greatest and most consistently observed at the 0-15 cm soil depth, but they were also measurable at 15-30 cm (Fig. 1.4). Interestingly, at the 30-60 cm soil depth, there were inconsistent results in POXC such that the effect of crop type varied from 2019 to 2020 and the tilled annual wheat system had the highest POXC values by the end of the experiment (Fig. 1.4b). Regarding tillage, POXC has been found to respond positively to reduced tillage in other regions (De Moraes Sá et al., 2014; Pheap et al., 2019; Liptzin et al., 2022). In addition, a correlation between higher crop productivity and increased POXC has been shown previously in a no-till wheat system to a soil depth of 20 cm (De Moraes Sá et al., 2014). When interpreting the influence of crop productivity on POXC, it is important to note that AGB was a covariate of nitrogen fertilization (Fig. 1.2) in this experiment. Thus, while we did not find evidence that N rates influenced the POXC outcomes in this study per se ($p = 0.8$), the differences in crop vigor resulting from added N availability does appear to have resulted in higher POXC.

Whereas POXC was influenced both by crop type and tillage effects, and system-level differences were primarily observed in shallow soil depths, for MinC crop type had the largest effect and this effect was the most consistently observed at the 30-60 cm soil depth (Fig. 1.5a). IWG had significantly more MinC than annual wheat at the 30-60 cm soil depth, and IWG MinC values tracked its seasonal AGB trends across all depths. Given this, MinC differences might have been partly related to differences in IWG root exudation and rooting depth. While root biomass was not measured in this study, other recent studies have confirmed that as much as 30% of the IWG root system may be found in deeper (100-160 cm) soil layers (versus 16% for annual grains; Duchene et al., 2020). Additionally, IWG has been found to produce more coarse and fine roots than annual wheat (Sprunger et al., 2019), which could result in increased soil carbon inputs (Kumar et al., 2006). More broadly, cultivation of perennial plants has resulted in higher MinC values compared to annual systems in a variety of other agroecosystems (Culman et al., 2013; Diederich et al., 2019; Audu et al., 2022). Meanwhile, at shallower soil depths, lack of soil disturbance resulted in marginally higher MinC values by the final season of the experiment (Fig. 1.5b). Though these differences were smaller and less consistent than those related to crop type, they agree with a study by Kainiemi et al. (2015), which measured lower short-term soil respiration in moldboard plow tillage treatments compared to no or shallow tillage treatments and attributed this effect to differences in soil temperature, soil moisture and depth distribution of crop residues.

4.4. Soil carbon stabilization versus mineralization and the relationship to microbial biomass

When a cropping system trends towards greater MinC values versus POXC values (normalized to other experimental factors, Eqns. 4, 5), the interpretation is that, in aggregate, soil carbon mineralization processes exceed soil carbon stabilization processes for a given soil sample. In contrast, the reverse is true when POXC values exceed MinC values according to the Hurisso et al. (2016) framework. While the promotion of soil carbon mineralization versus carbon stabilization processes under IWG cultivation across years and soil depths (Fig.1.6,

Table 1.1) is contrary to our hypothesis, another recent study found split results for various perennial grass monocultures. More specifically, switchgrass was more closely associated with soil carbon mineralization processes while miscanthus was more closely associated with soil carbon stabilization processes (Sprunger et al., 2020). In the same study, a restored prairie system with substantial fine root biomass production also promoted soil carbon mineralization, which the investigators propose may have been driven by a lack of fertilization, hindering C stabilization. This explanation aligns with our observation that IWG receiving no N fertilizer had greater soil carbon mineralization potential than the IWG treatment receiving 112 kg N ha^{-1}. Taken together, perhaps a lack of residual N in the soil solution (Culman et al., 2013; Jungers et al., 2019), increased rooting depth (Duchene et al., 2020) or decreased overall AGB (Fig. 1.2) relative to the annual wheat system led to increased soil carbon mineralization within the IWG system.

Observed carbon stabilization trends in the top 30 cm of soil in our study could be explained by the no-till system having similar carbon inputs as the tilled system yet without mineralization of soil organic carbon via tillage activities. Our results corroborate recent studies that have found systems managed with conservation tillage practices result in soil carbon stabilization in surface soils, while conventionally tilled systems generally result in soil carbon mineralization (Pheap et al., 2019; Thoumazeau et al., 2020). Our results also strongly agree with a study conducted in a similar agroecosystem (Hurisso et. al., 2016), where two systems with similar amounts of cover crop-derived biomass inputs had different soil carbon stabilization potentials when the effect of tillage was controlled for. However, these studies measured POXC and MinC only in the top 30 cm of soil whereas our observations also include measurements at 30-60 cm deep. At this depth, the relationship between tilled and no-till annual wheat systems is reversed, which could be a result of organic matter burial (i.e., residual straw and root crown) via mechanical disturbance of the topsoil and residual straw in the tilled wheat system overtime (Button et al., 2022). Carbon from buried organic matter may have later leached deeper into the soil profile (>30 cm) as dissolved organic carbon, particularly after the above-average precipitation in 2019.

Based on the Hurisso et al. (2016) framework describing the relationship between POXC and MinC within a given cropping system, we can indirectly infer the activity (i.e., stabilizing or mineralizing soil carbon) of the active microbial communities we measured in our systems to draw two overarching conclusions. First, a lack of soil disturbance contributed foremost to greater microbial abundance in the topsoil by the final season of the experiment (Fig. 1.3). Next, crop type had the greatest influence on whether microbial communities trended toward stabilizing versus mineralizing soil organic matter. This is evidenced by the fact that higher stabilization processes were primarily driven by the increased plant productivity of annual wheat compared to IWG (Fig. 1.6, Table 1.1). To a lesser extent, tillage also affected soil carbon stabilization potential as evidenced by the contrast between the two annual wheat treatments (which had equivalent crop productivity). In this case, lack of soil disturbance increased soil carbon stabilization in the top 0-30 cm of soil, while soil disturbance increased soil carbon stabilization at the depth of 30-60cm. Thus, soil carbon stabilization potential appears to be governed by primary productivity more so than conservation processes (i.e., no-tillage), although conservation also appears to have played a significant role. The integration of these effects can be observed in the outcomes measured in the no-till annual wheat treatment, which combined greater overall microbial biomass with higher SOM stabilization potential as compared to IWG and the tilled annual wheat systems by matching tilled annual wheat in AGB productivity and IWG in lack of soil disturbance.

5. Conclusion

In our three-year study conducted in a Mediterranean climate, IWG exhibited significant interannual variation in aboveground biomass productivity (28% less than annual wheat), increased soil microbial biomass at depth within the first two years, and increased soil carbon mineralization processes across time and soil depths. Conversely, no-till annual wheat had stable aboveground biomass yields comparable to those of tilled annual wheat and had the highest soil microbial biomass, POXC, and MinC in the top 0-30 cm of soil by the final year of the experiment. We found that plant vigor (i.e., aboveground biomass productivity) and a lack of soil disturbance were likely underpinning soil carbon stabilization in the no-till annual wheat system. Our results suggest that no-till annual wheat is better suited than tilled wheat or IWG in providing gains in soil microbial biomass and soil carbon stabilization, while still maintaining stable aboveground biomass yields overtime.

6. Appendices

Crop	Planting date	Nitrogen fertilization date	Harvest date
2018			
Annual Wheat	11 Dec. 2017	28 Feb. 2018	July 2018
IWG	17 Jan. 2018	28 Feb. 2018	October 2018
2019			
Annual Wheat	13 Dec. 2018	22 Feb. 2019	July 2019
IWG	-	31 Jan. 2019	August 2019
2020			
Annual Wheat	25 Nov. 2019	6 March 2020	May (biomass) June (grain) 2020
IWG	-	6 March 2020	July 2020

Supplementary Table 1.1. Planting, nitrogen fertilization, and harvest dates for annual wheat (tilled and no-till) and intermediate wheatgrass (IWG) during the three experimental years.

Year	Total Biomass Yield	
	IWG	Annual Wheat
	kg ha^{-1}	
2018		
0 kg N ha^{-1}	2261 ± 1056	6520 ± 673
112 kg N ha^{-1}	3919 ± 835	10615 ± 560
2019		
0 kg N ha^{-1}	7915 ± 1084	6035 ± 716
112 kg N ha^{-1}	14289 ± 856	10163 ± 592
2020		
0 kg N ha^{-1}	2401 ± 1083	6945 ± 715
112 kg N ha^{-1}	2882 ± 855	8782 ± 589

Supplementary Table 1.2. Mean +/- one SE reported for total biomass yields (kg ha^{-1}) for each crop (annual wheat or intermediate wheatgrass), year (2018, 2019, 2020), and nitrogen rate (0, 112 kg N ha^{-1}) combination.

Supplementary Equation 1.1: MinC, POXC, microbial biomass

Z = A + B

Where:

Z = MinC, POXC, or microbial biomass

A = Soil depth nested within nitrogen rate, nested within year, nested within cropping system (IWG, tilled wheat, no-till wheat)

B = Random intercept of soil depth nested within nitrogen rate, nested within year, nested within block

For certain instances, data was subset such that only 0-15 and 15-30 cm depth increments were analyzed to form conclusions regarding how crop type affected the top 30 cm of soil.

Soil Measurement	2018			2019			2020		
	IWG	No-Till Wheat	Tilled Wheat	IWG	No-Till Wheat	Tilled Wheat	IWG	No-Till Wheat	Tilled Wheat
Microbial Biomass									
0-15	69.52 ± 8.0	81.82 ± 9.4	74.2 ± 8.5	50.47 ± 5.8	48.33 ± 5.6	43.73 ± 5.0	49.13 ± 5.7	63.67 ± 7.3	46.66 ± 5.4
15-30	48.83 ± 4.9	49.35 ± 24.9	48.17 ± 4.8	37.26 ± 3.7	33.07 ± 3.3	30.38 ± 3.0	36.22 ± 3.6	43.43 ± 4.3	31.89 ± 3.2
30-60	24.09 ± 3.0	17.95 ± 2.2	20.30 ± 2.5	20.30 ± 2.5	15.48 ± 1.5	9.57 ± 1.4	16.88 ± 2.5	9.03 ± 1.4	7.5 ± 1.1
POXC									
0-15	410.70 ± 24.6	463.43 ± 27.8	421.77 ± 25.3	446.30 ± 26.8	448.37 ± 26.9	395.93 ± 23.7	402.01 ± 24.1	494.75 ± 29.7	431.70 ± 25.9
15-30	323.33 ± 13.7	357.37 ± 15.2	334.30 ± 14.2	321.93 ± 13.7	342.37 ± 14.5	318.72 ± 13.5	304.17 ± 12.9	332.47 ± 14.1	317.98 ± 13.5
30-60	200.40 ± 13.7	212.51 ± 14.5	210.02 ± 14.4	167.49 ± 11.5	199.63 ± 13.7	206.54 ± 14.1	174.13 ± 11.9	150.14 ± 10.3	172.52 ± 11.8
MinC									
0-15	52.45 ± 6.9	50.38 ± 6.6	50.00 ± 6.6	69.75 ± 9.2	51.62 ± 6.8	39.60 ± 5.2	66.35 ± 8.7	73.56 ± 9.7	60.17 ± 7.9
15-30	34.26 ± 3.2	26.12 ± 2.5	26.69 ± 2.5	45.79 ± 4.3	29.60 ± 2.8	24.67 ± 2.3	42.04 ± 4.0	36.56 ± 3.5	29.98 ± 2.8
30-60	14.62 ± 2.2	7.02 ± 1.1	7.61 ± 1.1	19.73 ± 3.0	9.73 ± 1.5	9.57 ± 1.4	16.88 ± 2.5	9.03 ± 1.4	7.5 ± 1.1

Supplementary Table 1.3 Mean +/- one SE reported for microbial biomass (nmole/g), POXC (mg kg^{-1}), and MinC (mg kg^{-1}) for each cropping system type (IWG, no-till wheat, tilled wheat), year (2018, 2019, 2020), and soil depth averaged across nitrogen rates. Mean values based on Supplementary Equation 1.

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Chapter 2: Multifunctionality of annual and perennial grain production systems in a Mediterranean climate

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Abstract

Over the last 60 years, adoption of conservation agricultural practices has greatly increased and been shown to improve the multifunctionality and ecosystem services of grain production. Two conservation agriculture alternatives to tilled annual wheat production are no-till and adoption of novel perennial grain production. We implemented a multifunctionality and ecosystem service framework to compare the ability of IWG, no-till annual wheat, and tilled annual wheat at different levels of nitrogen (N) fertilization to address consumer-oriented services, regulating services and disservices in a Mediterranean climate. We measured grain yield, soil carbon storage to a 90 cm soil depth, and fertilizer-N recovery efficiency (an indicator of nitrogen use efficiency) over three growing seasons. While consumer-oriented provisioning services such as grain yield and disservices such as nitrogen use efficiency (NUE) have been compared among tilled and no-till wheat systems, there has yet to be a direct comparison of these services among tilled wheat, no-till wheat and the novel perennial grain known as intermediate wheatgrass

(IWG; trademarked Kernza ®) in a Mediterranean climate. Furthermore, it remains unclear how nitrogen fertilization affects regulating services, such as soil carbon (C) storage, and whether perennial grains can increase carbon storage throughout the soil profile (i.e., > 30 cm) relative to annual systems when the effect of tillage is controlled for. IWG grain yield varied substantially and was 35% of that of annual wheat systems in the single year it produced grain. Conversely, tilled and no-till annual wheat exhibited the highest and most stable grain yields, aboveground NUE and grain NUE overtime. In the top 30 cm of soil and only when fertilized, the two conservation systems (IWG and no-till annual wheat) had an average of 17% greater soil carbon mass than tilled wheat by the third year of the experiment. At this same depth and when fertilized, the two systems had an average of 5.5 Mg ha^{-1} more in soil carbon gains (Δ soil carbon mass) within 4 years relative to tilled wheat. However, below 60 cm and across N rates, tilled annual wheat contained 40-48% more soil carbon mass than the conservation systems. Furthermore, it was the only system that significantly gained soil carbon mass across N rates and the whole soil profile within four years ($p = 0.02$). Our results highlight that the two conservation systems, no-till wheat and IWG, only partially addressed regulating services, as soil carbon gains in these two systems relative to the tilled system were contingent upon nitrogen fertilization and constrained to the top of 30 cm of soil. In contrast, we found the tilled annual wheat system provided greater climate regulation services by storing carbon in the subsoil and across the whole profile regardless of N fertilization. Therefore, plant vigor, likely as a function of plant adaptation in this study, was critical for increasing provisioning services and regulating services and decreasing disservices, as measured by increased grain yield stability, soil carbon storage, and NUE. Our results underscore the importance of measuring subsoil carbon and conducting long-term studies in a variety of climates before novel cropping systems are adopted in new region.

1. Introduction

Integrated frameworks that consider yield in tandem with other ecosystem services should inform the design and implementation of sustainable cropping systems. A conceptual and integrated framework of multifunctional agriculture and ecosystem services proposed by Huang et al. (2015) distinguishes three groups of services from agriculture: consumer-oriented services, regulating services, and disservices (Fig. S2.1). Consumer-oriented services focus on the provisioning of food and fiber, while regulating services include climate mitigation and soil retention. Disservices include nutrient runoff that may negatively affect surrounding bodies of water. Ideally, a given cropping system would maintain or increase consumer-oriented provisioning services and regulating services, all the while decreasing disservices. Three measurements that directly address these three groups of services are yield stability, soil carbon storage, and nitrogen use efficiency (NUE), respectively.

Stability, not just the magnitude, of a crop's yield is an important agroecosystem provisioning service that ensures consistency and resilience to environmental variation (i.e., a decreased genotype-by-environment interaction; Desclaux et al., 2008). Temporal yield stability can be directly quantified (Tilman et al., 2006) or discussed more broadly as changes in grain yield over time. Stabilization of crop yields can be improved via breeding strategies, specific management practices, and cultivar choice (Costanzo & Bàrberi, 2014). Soil carbon storage, mediated by soil microbial communities (Six et al., 2006, Liang et al., 2017), is a critical regulating ecosystem service that addresses soil retention and climate regulation, and is strongly associated with water regulation, water retention, habitat provisioning and nutrient cycling (Adhikari & Hartemink, 2016). With respect to NUE, nitrogen (N) losses to the environment are a disservice from agriculture and pose a major challenge to sustainability (Zhang et al., 2015). NUE can measured through indicators such as fertilizer-N recovery efficiency (Congreves et al., 2021) and can be improved by increasing nitrogen uptake efficiency via total plant N and nitrogen utilization efficiency via grain yield N (Barraclough et al.

2010). Collectively, yield stability, soil carbon storage, and NUE are valuable measurements that can help elucidate the multifunctionality of important food crops such as grains.

Grain crops occupy approximately 70% of the world's agricultural land and provide 80% of the world's food (Pimentel et al., 2012), and thus have significant impact on global food markets and the environment. Annual grains currently dominate global grain production (Pimentel et al., 2012) and grain crop production can oxidize soil organic carbon in the tillage layer and increase wind erosion via tillage, resulting in losses of up to 5 gigatons of soil yr^{-1} (Montanarella et al., 2015). Degradation of soil, in turn, decreases microbial biomass (Dupont et al., 2010), depletes soil nutrients and organic matter, and destroys soil structure (den Biggelaar et al., 2001). With respect to disservices, annual grain crops (i.e., rice, wheat, maize) have been shown to recover an estimated 46-65% of applied fertilizer N (Ladha et al., 2005). Remaining N in these systems may become immobilized in soil organic N pools or be subject to potential losses via denitrification and volatilization or contaminate waterways and groundwater through leaching (Cassman et al., 2002). Over the last 60 years, production of annual grains has increased, as have conservation agricultural practices that aim to satisfy humans need for food while maintaining quality of the environment (Soto-Gomez & Perez-Rodríguez, 2022). Two agricultural practices that address most conservation agriculture principles (i.e., minimal soil disturbance and maintain permanent soil cover) and show promise in improving the multifunctionality and ecosystem services for wheat or wheat-like crops are no-till and adoption of novel perennial grain crops.

Conservation tillage emerged during the 1930's U.S. Dust Bowl (Baveye et al., 2011) during which vast expanses of agricultural land were degraded. No-till, a form of conservation tillage (Archer et al., 2017) , has now been adopted on more than 125 million hectares worldwide (Pittelkow et al., 2015a). No-till practices appear to be most beneficial in dry climates under rainfed conditions (Pittelkow et al., 2015b) and negative impacts of no-till on provisioning services (i.e., yield) appear to be less in annual wheat (*Triticum aestivum*; Pittelkow et al., 2015b) than other crops. Converting from moldboard plow to no-tillage may enhance regulating services by significantly improving soil chemical, biological, and physical properties in both annual and perennial crops (Nunes et al., 2018; Nunes et al., 2020). However, whether soil C storage is higher under no-till versus conventional tilled systems across the entire soil profile remains a point of considerable discussion with variability between regions and grain systems (Luo et al., 2010; Bai et al., 2019; Page et al., 2020). With respect to disservices, no-till wheat systems have shown to have comparable (Dalal et al., 2011) or higher (Ernst et al., 2020) NUE than tilled annual wheat systems in certain instances.

While only recently released to the market, perennial grain production is a conservation practice that involves minimal soil disturbance and permanent soil cover and thus may improve multifunctionality within grain production systems. The novel perennial grain intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey; trademarked Kernza®) or IWG, is a domesticated cool-season perennial wheatgrass bred for grain production (DeHaan and Ismail, 2017) and, as of today, cultivated on 1,400 hectares of U.S. cropland. Emerging literature has compared the provisioning services of annual wheat and IWG and found that while IWG can produce comparable or higher biomass yields than annual wheat (Culman et al., 2013, Sprunger et al., 2018b), it yields approximately 30% of the grain of annual wheat (Culman et al., 2013; Cassman et al., 2022). Furthermore, IWG has shown greater instability in aboveground biomass yield than annual wheat in a Mediterranean climate (Fig. 1.2) and some growers have concern about yield loss overtime (Wayman et al., 2019). With respect to regulating services, it has been postulated that IWG has an extensive root system can deposit carbon deep in soil where decomposition rates are lower and exudates can stabilize via binding to available mineral surfaces (van der Pol et al., 2022); however, results testing these ideas are mixed. IWG has been reported to be associated with increased particulate organic matter carbon or POM relative to annual wheat (Audu et al., 2022; van der Pol et al., 2022), while other studies have found no difference between the two systems (Sprunger et al., 2018a). Finally, studies in the Midwest U.S. that have compared disservices associated with annual wheat and IWG have reported that relative to annual wheat, IWG has a higher whole-crop (roots + aboveground biomass) NUE (Sprunger et al., 2018b) and can decrease nitrate leaching by 86-99% (Culman et al., 2013; Jungers et al., 2019). While these recent studies provide valuable insight into how IWG and annual wheat compare with respect to provisioning and regulating services and disservices, these observations have largely been made in a relatively narrow set of U.S. climates and ecosystems (i.e., Midwest and Northeast USA). Furthermore, there has been no control for the effect of tillage when comparing soil carbon storage at depth in annual wheat and IWG.

To better understand how IWG and annual wheat impact consumer-oriented provisioning services, regulating services and disservices while controlling for the effect of tillage, we compared IWG, no-till annual wheat, and tilled annual wheat at two different nitrogen rates (0 and 112 kg N ha^{-1}) in a Mediterranean climate based on a multifunctionality and ecosystem service framework. We measured three components to compare these services including grain yield stability, soil carbon mass to a 90 cm soil depth, and nitrogen use efficiency (NUE) as measured by fertilizer-N recovery efficiency. We hypothesized that: 1) annual wheat systems will have greater grain yields and grain yield stability than IWG at both N rates; 2) N fertilization will increase soil carbon storage; 3) the highest soil carbon will be in the topsoil (0-30 cm) of the two conservation systems (no-till wheat and IWG), but only IWG will increase soil carbon across depths (0-90 cm); 4) both annual wheat systems will have greater grain NUE, but similar aboveground biomass NUE, compared to IWG.

2. Materials and methods

2.1. Experimental location and management

The experiment was conducted at the UC Davis Russell Ranch Sustainable Agriculture Facility in Yolo County (38°32’27.30" N, 121°52’7.39” W). At an elevation of 16m, this experimental site is in the northern Central Valley of California with wet winters and hot, dry summers - typical of semiarid and Mediterranean climates. The soil type is a Yolo silt loam (Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). Prior to the establishment of the experiment, the area was an unmanaged grassland for 10 years. Prior to planting in 2017, a starter NPK fertilizer (11-52-0) was applied at 135 kg ha^{-1}. Intermediate wheatgrass (IWG) was initially planted in December 2017, but then replanted in January 2018 due to failed crop establishment. Annual wheat was planted in December 2017, December 2018, and November 2019. Nitrogen fertilization for select treatments was broadcast-applied as urea annually during early vegetative growth stages for each crop type. Urea was always applied ahead of a rainfall event sufficient to ensure soil incorporation (≥ 13mm). To control weed pressure, select IWG plots were hand-weeded annually each spring and Glyphosate was applied during the winter of 2019 at labeled rates to all IWG plots when IWG was dormant and thus not actively growing. Osprey was applied to all non-actively growing main plots in winter 2019.

2.2. Experimental design

The experimental design was a split plot design with four replications. The main plots were 12m x 6m and planted in 15 cm rows to one of three cropping system types; no-till annual wheat (Hard Red Spring wheat, var. WB 9229), tilled annual wheat (Hard Red Spring wheat, var. WB 9229), or IWG (*Thinopyrum intermedium*; 5th selection cycle, The Land Institute, Kansas, USA). Annual wheat in tilled and no-till plots was seeded at a rate of 135 kg ha^{-1} and IWG was seeded at a rate of 11.2 kg ha^{-1}. All plots were disked to a depth of approximately 20-30 cm when establishing the experiment. In subsequent years, only the tilled annual wheat plots were disked and rototilled each fall to a depth of 15-25 cm prior to soil sampling and planting operations. In each main plot, standing biomass that remained after harvest was mowed to a height of approximately 6 cm and the biomass was removed. Two nitrogen rates were randomly assigned to 3m x 6m sub-plots within each plot. N rates were 0 and112 kg ha^{-1} and were broadcast-applied as urea (46-0-0).

2.2.1 Grain and biomass harvest

Grain was harvested using a Wintersteiger Classic small plot combine adjusted to minimize grain loss from an area of approximately 9 m^2 per plot. For annual wheat, approximately 8% and 15% of the plots were negatively affected by rodent damage in 2018 and 2020, respectively. For these, grain yield at the plot scale was estimated via hand harvest on an area basis. Grain was weighed in the field, subsampled for cleaning, and then corrected to 12% moisture content to determine final yields. Grain yields reported for IWG include the lemma and palea.

Total plant biomass (grain and vegetative biomass) was cut to a stubble height of approximately 6 cm in each sub plot from a representative 1m^2 area. All biomass samples were weighed in the field and then dried in an oven at approximately 60 degrees C until samples reached a stable weight. Once reaching a stable weight, samples were ground to 4 mm using a Wiley Mill Grinder (Model 4, Thomas Scientific, Philadelphia, PA, USA) for non-grain biomass and total plant biomass, while a coffee grinder was used for grain. All grain and biomass samples were further ground into a fine powder using a ball mill before being analyzed with a near-infrared spectrometer calibrated via ad hoc calibration using continuous flow continuous flow isotope ratio mass spectrometer (IRMS) (Sercon Ltd., Cheshire, UK).

2.2.2 Soil sampling and processing

Nine baseline soil samples were taken with a Geoprobe to a depth of 90 cm in 2017 prior to planting the experiment. Baseline sample locations within the experimental site were designed to capture variation in soil properties across the field. For soil sampling within experimental plots from 2018 to 2020, one soil sample was randomly taken with a Geoprobe to a depth of 2.4 meters with a 4.3 cm diameter probe from three field replicates of each cropping system type at the 0 and 112 kg ha^{-1} nitrogen rates on 19 October 2018, 15 October 2019, and 21 September 2020. Soil cores were immediately stored on ice and refrigerated until processed in the lab. Upon processing, soil cores were divided into 0-30, 30-60, 60-90 cm increments with a linear correction to account for soil core compaction. Soil samples, void of rock fragments, were divided by depth layers versus by soil horizons because the soil at this site is very young (<6,000 years) and thus horizons are still considered relatively homogenous (Tautges et al., 2019).

For soil carbon sample processing, each soil depth increment was air-dried, sieved <2mm, and archived in plastic bags at room temperature. Subsamples were then collected from each air-dried and sieved soil depth increment, all visible plant material was removed, and it was oven dried at 60°C for 72 hours and ball mill ground for a minimum of 24 hours. Total C and N were then determined for each subsample by dry combustion with a Costech ESC 4010 Elemental Analyzer (Valencia, CA, USA). Because it is negligible in our soils, we did not directly analyze inorganic carbon, thus we use the term soil carbon instead of soil organic carbon.

2.3 Calculations and statistical analyses

All mixed linear models were fitted using the lme function from the nlme package (version 3.1 - 152) (Pinheiro et al., 2021 in R (version 4.0.3) (R Core Team, 2020).

2.3.1 Grain Yield

Normality and homogeneity of variance assumptions were met for yield statistical analyses, so data transformation was unnecessary. Quadratic terms for nitrogen rate were added to the linear model (Equation 1) since this variable exhibited a quadratic relationship with yield. The relationship between crop type (annual, perennial), tillage (no-tillage, tillage), year, nitrogen fertilization and grain yield were modeled with the equation:

Equation 1: Grain yield

$Y = A + A^2 + B + B^2 + C$;

Where:

Y = Grain yield

A = Nitrogen rate nested within year nested within crop type

B = Nitrogen rate nested within year nested within tillage

C = Random intercept of nitrogen rate nested within year nested within block

For grain yields, the interaction between till and no-till wheat was not significant, so the effects were combined when generating EMMs and standard errors. The r.squaredGLMM function in the MuMIn package (version 1.43.17) (Bartoń, 2020) was used to calculate Pseudo R^2 for the mixed linear models. The marginal R^2 (R^2m) generated with this function represents the variation explained by only the fixed effects in the model and the conditional R^2 (R^2c) represents the variation explained by the entire model (fixed plus random effects). The effects of crop, tillage, year, and nitrogen on grain yield were tested using a type three ANOVA in the car package (Fox & Weisberg, 2019).

While there are direct quantitative measures of temporal yield stability, such as the mean yield value (μ) for a time period divided by the temporal standard deviation over the same interval (**σ**) (Tilman et al., 2006), we simply discuss grain yield and yield stability more broadly by contrasting the mean yield values derived from our model. We chose this approach because the extreme variation in IWG yields is easily apparent within our presented table.

2.3.2 NUE as measured by N-Fertilizer Recovery Efficiency (Congreves et al., 2021; Ladha et al., 2005)

Harvested grain and biomass was analyzed for total N and N fertilizer recovery efficiency was calculated with the equation:

Equation 2: Aboveground and grain NUE

a. $RE_{NT} = (U_T - U_0)/ F_N$ (kg kg^{-1})

Where RE_{NT} is the N fertilizer recovery efficiency based on total aboveground plant N, U_t is the plant nitrogen uptake measured in total aboveground biomass (kg ha^{-1}) in a plot that received N at the rate of F_N (112 kg N ha^{-1}), and U_0 is the plant nitrogen uptake measured in total aboveground biomass that received 0 kg N ha^{-1}.

b. $RE_{NG}= (U_T - U_0)/ F_N$ (kg kg^{-1})

Where RE_{NG} is the N fertilizer recovery efficiency based on grain N, U_t is the plant nitrogen uptake measured in the grain (kg ha^{-1}) in a plot that received N at the rate of F_N (112 kg N ha^{-1}), and U_0 is the plant nitrogen uptake measured in grain that received 0 kg N ha^{-1} .

2.3.3 Soil carbon mass as determined by Equivalent Soil Mass (ESM) approach

Soil carbon concentration, oven dry soil mass, and soil depth were used to calculate soil carbon mass for each reference soil mass layer using the equivalent soil mass (ESM) approach described by von Haden et al. (2020). Reference soil mass layers were selected such that: (a) each average calculated depth to reference mass (cm) was close to the fixed soil depth that was sampled (30,60,90 cm) and (b) depth to reference mass (cm) did not extrapolate past the depth that was sampled (90 cm). Soil carbon mass values and ESM soil mass/layers were calculated by fitting a cubic spline to the data generated by the depth-based sampling. While all reported soil carbon mass data align with a specific ESM soil mass/layer, data will be reported on a fixed depth layer (0-30,30-60,60-90 cm) basis for ease of interpretation.

Once soil carbon mass was calculated for each plot, the relationship between cropping system type (IWG, tilled wheat, no-till wheat), nitrogen fertilization (0, 112 kg N ha^{-1}), soil depth (0-30, 30-60, 60-90 cm) and soil carbon mass was modeled with the equation:

Equation 3: Soil carbon mass

Z = A + B;

Where:

Z = Soil carbon mass

A = Soil depth nested within nitrogen rate, nested within crop type (tilled annual wheat, no-till annual wheat, IWG)

B= Random intercept of soil depth nested within nitrogen rate, nested within block

To investigate how crop type (IWG, no-till wheat, tilled wheat) affected soil carbon mass since 2017, mean values for baseline samples were calculated using equation 3. A single mean baseline value for each soil depth was then used to calculate a change (Δ) in soil carbon mass for each sample:

Equation 4: Δ Total soil carbon mass at each soil depth and N rate

Z = A + B;

Where:

Z = Δ Soil carbon mass for each individual soil depth (0-30,30-60,60-90 cm).

A = Soil depth nested within nitrogen rate, nested within crop type (tilled annual wheat, no-till annual wheat, IWG)

B= Random intercept of soil depth nested within block

Equation 4.1: Δ Total soil carbon mass for the whole soil profile at each N rate

Z = A + B;

Where:

Z = Δ Soil carbon mass for the whole soil profile (0-90 cm)

A = Nitrogen rate nested within crop type (tilled annual wheat, no-till annual wheat, IWG)

B= Random intercept of block

Equation 4.2: Δ Total soil carbon mass for the whole soil profile across N rates

Z = A + B;

Where:

Z = Δ Soil carbon mass for the whole soil profile (0-90 cm)

A = Crop type nested within year (baseline or 2020)

3. Results

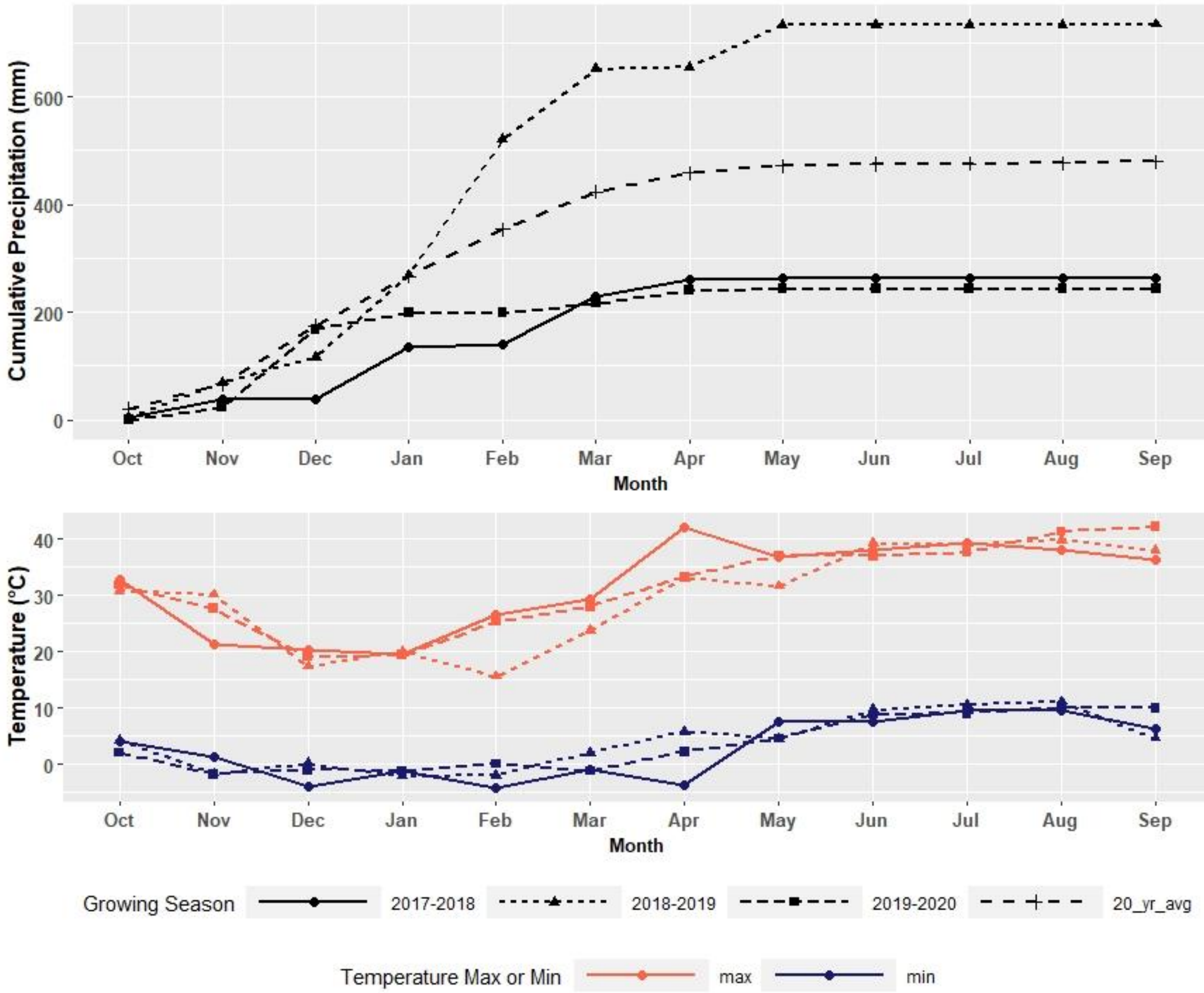

Figure 2.1 Cumulative precipitation (mm), minimum temperature (°C), and maximum temperature (°C) for the three experimental growing seasons between 2017- 2020 and cumulative precipitation (mm) average for 2000-2020 in Yolo County.

3.1. Temperature and precipitation

The cumulative precipitation totals for the first (2017-2018) and third (2019-2020) experimental seasons were 55% and 51% of the 20-year average annual precipitation of 479 mm (2000-2020) for this experimental location, respectively (Fig. 2.1). Conversely, the second (2018-2019) experimental season had 65% more precipitation than the 20-year average, with cumulative totals approximately triple that observed in the first or third year of the experiment.

Additionally, recorded maximum temperatures during the 2018-2019 growing season were 40%, 15%, and 14% lower in February, March, and May, respectively, than maximum temperatures during the same periods for the first and third year of the experiment.

3.2. Grain yield stability

No-till and tilled annual wheat did not have significantly different grain yields ($p = 0.78$) thus grain yield estimates for the two systems were combined. The mean annual wheat grain yield for all years and N inputs combined was approximately 3,000 kg ha^{-1}. While IWG grain yield was negligible in 2018 and 2020, it yielded 36% (± 423 kg ha^{-1}) and 33% (± 322 kg ha^{-1}) of the grain yield of annual wheat in 2019 at N rates of 0 and 112 kg N ha^{-1}, respectively (Table 2.1). The 2019 harvest index was 0.39 for annual wheat and 0.1 for IWG (Table 2.1). The IWG grain was not dehulled so the reported yield also includes the lemma and palea; thus, yields of grain alone could be up to 30% lower (Altendorf et al., 2021) than reported. Across years and N fertilization rates, annual wheat grain yields varied by less than 45%. The largest source of variation in annual wheat yields was largely driven by 2020 yields, such that yield was significantly lower in 2020 than 2018 and 2019 at both N rates ($p \leq 0.05$).

Year	Grain Yield	
	IWG	Annual Wheat
	kg ha^{-1}	
2018		
0 kg N ha^{-1}	0	2986 ± 237
112 kg N ha^{-1}	0	4344 ± 190
2019		
0 kg N ha^{-1}	840 ± 400	2341 ± 254
112 kg N ha^{-1}	1324 ± 310	4061 ± 203
2020		
0 kg N ha^{-1}	0	1130 ± 253
112 kg N ha^{-1}	0	2918 ± 202

Table 2.1. Mean grain yield (kg ha^{-1}) +/- one SE reported for annual wheat (tilled and no-till) and intermediate wheatgrass for each experimental year (2018, 2019, 2020) and nitrogen rate (0, 112 kg N ha^{-1}) combination. Mean values derived from linear model in Eqn. 1. Grain yields reported for IWG with lemma and palea intact.

3.3. Nitrogen use efficiency (NUE) in aboveground biomass and grain

The NUE was not significantly different in no-till and tilled annual wheat, so NUE estimates and standard errors for the two systems were combined. Aboveground and grain NUE of IWG varied more than 60%, while annual wheat NUE varied by less than 20% across years (Table 2.2). IWG had approximately 30%, 100%, and 40% of the aboveground NUE of annual wheat in 2018, 2019 and 2020, respectively. Only in 2019 did IWG produce enough grain to calculate grain NUE, when it was approximately 30% of the NUE of annual wheat.

Year	Aboveground NUE		Grain NUE	
	IWG	Annual Wheat	IWG	Annual Wheat
	—— kg N taken up kg^{-1} N applied ——		—— kg N grain kg^{-1} N applied ——	
2018	0.16 ± 0.03	0.51 ± 0.05	0	0.32 ± 0.04
2019	0.49 ± 0.06	0.49 ± 0.03	0.10 ± 0.01	0.37 ± 0.02
2020	0.19 ± 0.04	0.48 ± 0.05	0	0.3 ± 0.02

Table 2.2. Nitrogen fertilizer recovery efficiency (NUE) based on total aboveground plant nitrogen (grain + straw; defined as RE_{NT}) and grain nitrogen (grain only; defined as RE_{NG}) for IWG and annual wheat (tilled and no-till wheat) +/- one SE from mean value for the 2018, 2019, and 2020 growing season. Values based on Eqn. 2.

3.4. Soil carbon mass as affected by crop type and nitrogen fertilization

In the 0-30 cm depth, the two conservation systems (IWG and no-till annual wheat) when fertilized had 17% higher mean soil carbon mass than tilled annual wheat by year three (Fig. 2.2). No-till wheat had the highest soil carbon mass and was the most different from tilled annual wheat ($p = 0.13$). Deeper in the soil profile, at 30-60 cm, the relationship between systems reversed, such that tilled annual wheat trended higher in soil carbon mass than both conservation systems when fertilized with 112 kg N ha^{-1} . At the bottom soil depth (60-90 cm), tilled annual wheat had 40-48% greater soil carbon mass than IWG ($p= 0.12$) and no-till wheat ($p=0.08$) across N fertilization rates. However, the difference between tilled wheat and IWG at this depth was greatest when fertilized ($p = 0.2$), whereas the difference between tilled and no-till annual wheat was greatest when no fertilizer was applied ($p = 0.2$).

Looking at changes in soil C from 2017 baseline (i.e., Δ soil carbon mass; Fig. 2.3) to year 3, we found that gains in soil carbon mass in the two conservation systems occurred primarily in the top layer of soil, whereas gains in soil carbon mass in the tilled wheat system occurred at the 60-90 cm soil depth. The two conservation systems had the greatest increases in soil carbon mass (approximately 6.3 Mg ha^{-1}) relative to tilled annual wheat in the top 30 cm, but only when fertilized with 112 kg N ha^{-1}. No-till wheat had the greatest increase in soil carbon mass relative to tilled wheat in the surface layer at this N fertilization rate (Table 2.3; $p = 0.09$). At 30-60 cm in the absence of fertilizer, the tilled and two conservation systems showed similar gains in soil carbon mass. However, when the conservation systems were fertilized with 112 kg N ha^{-1}, soil carbon mass increases trended smaller than those observed in tilled wheat. At the 60-90 cm depth, greater increases in soil carbon mass were measured in tilled wheat than the conservation systems at either N rate. The greatest differences between tilled and no-till wheat were detected at the 0 kg N ha^{-1} ($p = 0.15$; Table 2.3), whereas the greatest difference between tilled wheat and IWG occurred in the fertilized plots ($p = 0.15$; Table 2.3). It is notable that the deepest soil depth aligns closely with the soil depth at which increases in clay and organic matter percentage have been observed in Yolo silt loam soil profiles (~100 cm; Fig. S2.2)

Across N rates and the whole soil profile (0-8600 Mg ha^{-1}), all cropping systems generally gained soil carbon mass relative to baseline samples ($\Delta > 0$), but tilled annual wheat was the only cropping system that significantly gained soil carbon mass relative to the baseline ($p = 0.02$; Table 2.3). When observing trends across the whole profile at two different N rates (Fig. 2.3; Eq. 4.1), the amount gained within each system varied by nitrogen treatment.

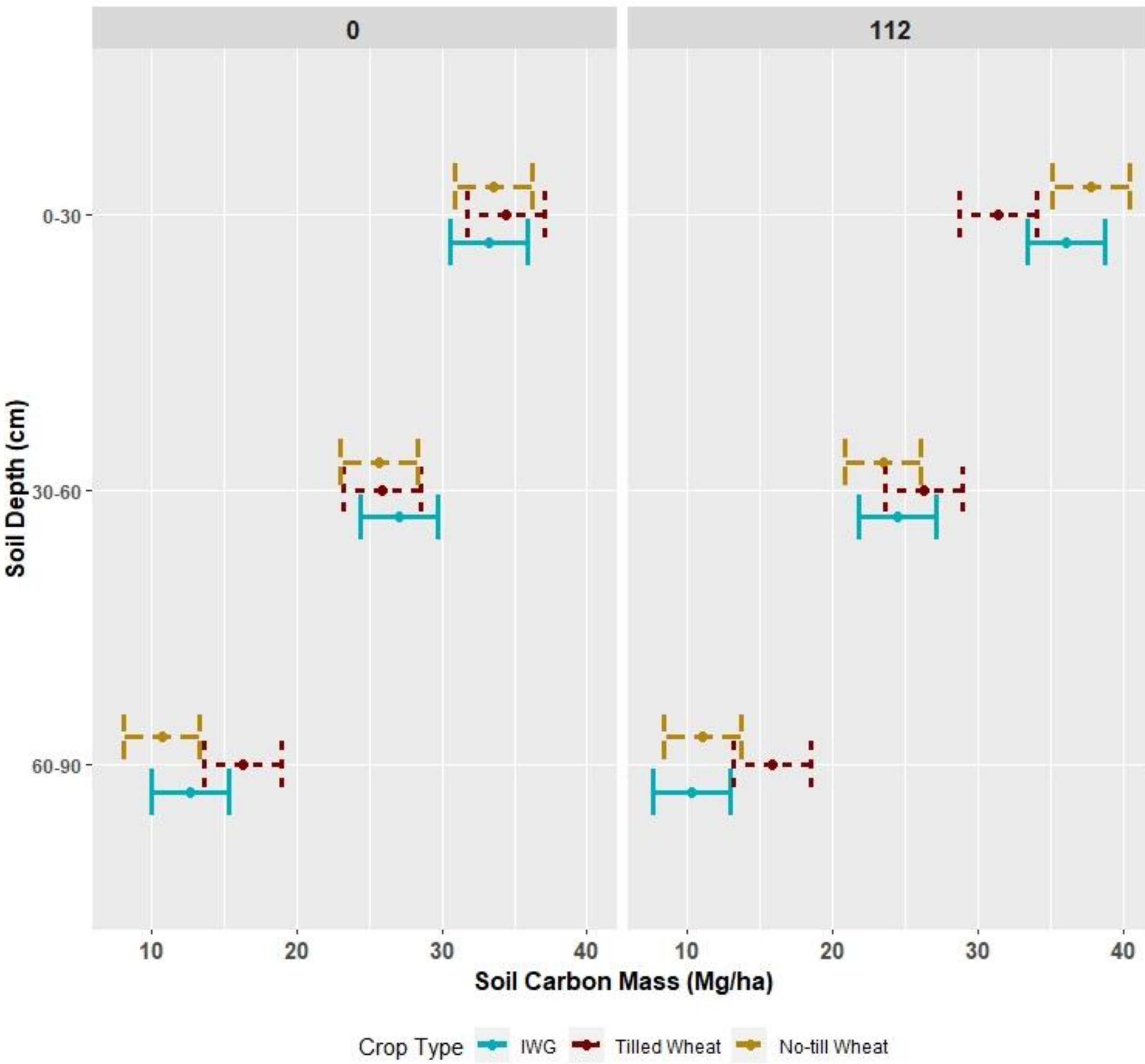

Figure 2.2 Soil carbon mass (as determined by ESM) as affected by crop type (intermediate wheatgrass, tilled annual wheat, no-till annual wheat) at two nitrogen rates (0, 112 kg N ha^{-1}) and three soil depths (0-30, 30-60, 60-90 cm) by the third experimental year (2020). Values based on Eqn. 3. Error bars represent ± one SE from the mean.

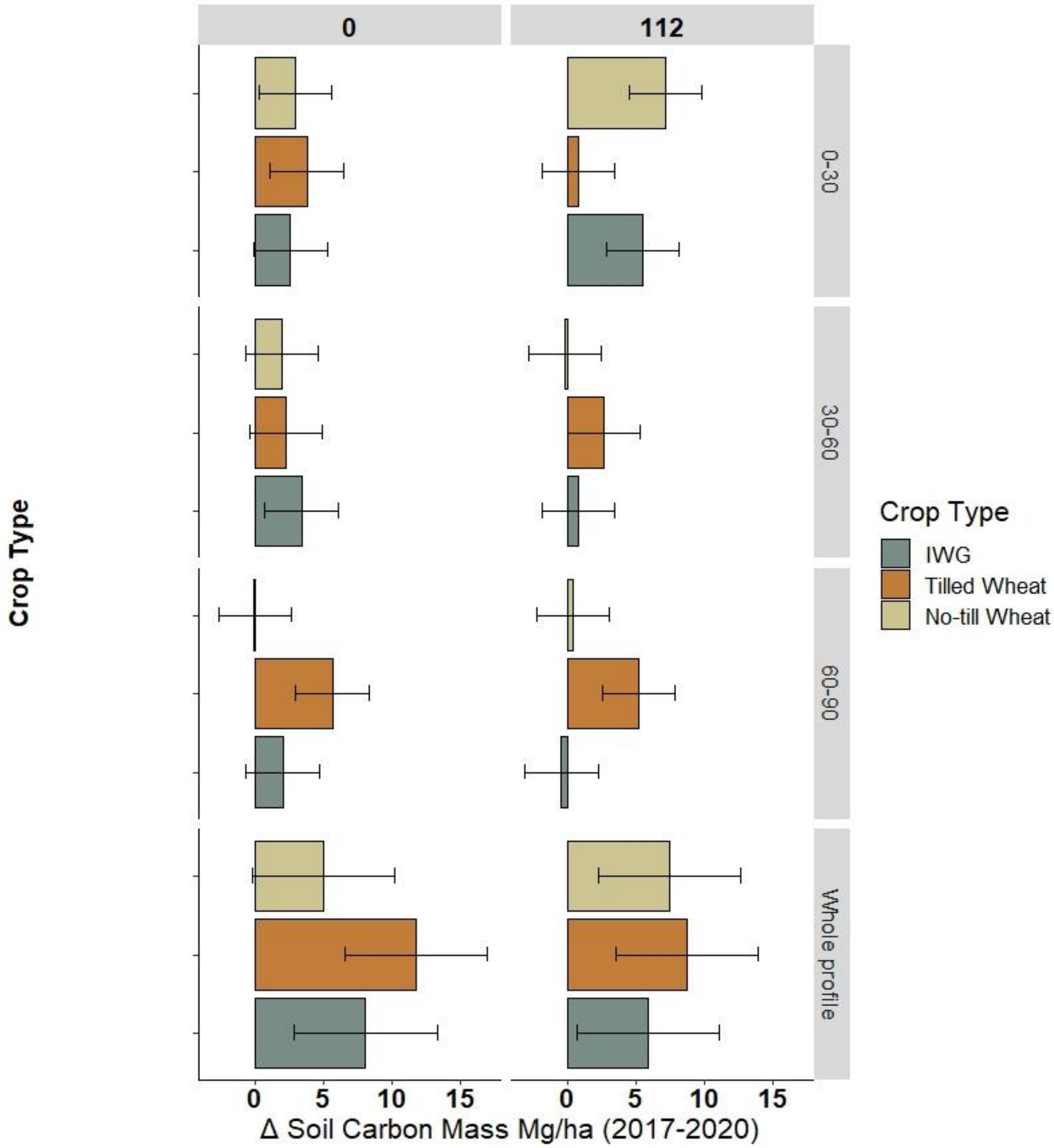

Figure 2.3 Soil carbon changes as determined by ESM in three crop types (IWG, tilled annual wheat, no-till annual wheat) at two nitrogen rates (0,112 kg N ha^{-1}) from 2017-2020, expressed as changes in soil carbon mass at four soil depths (0-30 cm, 30-60 cm, 60-90 cm, whole profile). Values based on Eq. 4 and 4.1. Error bars represent ± one SE from the mean.

N Fertilization Rate Kg ha $^{-1}$	Soil Depth (cm)	Hypothesis Test	Δ Carbon Mass (Mg ha^{-1}) 2017-2020	p-value
Across 0 and 112	Whole profile	IWG	6.99	0.11
	Whole profile	No-till Wheat	6.23	0.15
	Whole profile	Tilled Wheat	10.25	0.02
0	0-30	IWG - Tilled Wheat	-1.2	0.91
	0-30	IWG - (No-till Wheat)	-0.36	0.99
	0-30	Tilled Wheat - (No-till Wheat)	0.84	0.96
	30-60	IWG - Tilled Wheat	1.12	0.92
	30-60	IWG - (No-till Wheat)	1.41	0.88
	30-60	Tilled Wheat - (No-till Wheat)	0.29	0.99
	60-90	IWG - Tilled Wheat	-3.62	0.44
	60-90	IWG - (No-till Wheat)	2	0.77
	60-90	Tilled Wheat - (No-till Wheat)	5.62	0.15
	Whole profile	IWG - Tilled Wheat	-3.69	0.65
	Whole profile	IWG - (No-till Wheat)	3.06	0.74
	Whole profile	Tilled Wheat - (No-till Wheat)	6.75	0.28
112	0-30	IWG - Tilled Wheat	4.68	0.26
	0-30	IWG - (No-till Wheat)	-1.68	0.83
	0-30	Tilled Wheat - (No-till Wheat)	- 6.36	0.09
	30-60	IWG - Tilled Wheat	-1.89	0.80
	30-60	IWG - (No-till Wheat)	0.96	0.94
	30-60	Tilled Wheat - (No-till Wheat)	2.85	0.60
	60-90	IWG - Tilled Wheat	-5.63	0.15
	60-90	IWG - (No-till Wheat)	-0.82	0.96
	60-90	Tilled Wheat - (No-till Wheat)	4.8	0.25
	Whole profile	IWG - Tilled Wheat	-2.84	0.78
	Whole profile	IWG - (No-till Wheat)	-1.54	0.93
	Whole profile	Tilled Wheat - (No-till Wheat)	1.29	0.95

Table 2.3 Δ Soil carbon mass (Mg ha^{-1}) hypotheses tests for whole profile across N rates (0,112 kg N ha^{-1}) (Eq. 4.2) and pairwise comparisons for a given crop system (IWG, no-till wheat, tilled wheat) and soil depth (whole profile, 0-30,30-60,60-90 cm) combination (Eq. 4 and 4.1) at a given N rate. Δ values were calculated by subtracting 2017 baseline soil carbon mass values from a given soil depth for each crop type, nitrogen treatment and soil depth combination in 2020.

4. Discussion

4.1 Grain yield stability is greater in annual than perennial grain cropping systems

IWG demonstrated significant yield instability, with a viable grain yield in only one out of the three years of the experiment. In contrast to IWG, annual wheat (till and no-till) produced robust grain yields that varied by less than 45% across years and N rates. Although IWG yield varied greatly with respect to annual wheat, the 2019 IWG grain yields in a Mediterranean climate were comparable to yields reported from other regions. For instance, at an average grain yield of 1,080 kg ha^{-1} in 2019, IWG yield was either slightly higher (Law et al., 2020; Bajgain et al., 2020; Zimbric et al., 2020) or comparable (Jungers et al., 2017, Casamitjana et al., 2021, Culman et al., 2013, Hunter et al., 2020, Favre et al., 2019; Fernandez et al., 2020, Clément et al., 2022) to grain yields reported in regions of the U.S. Midwest, U.S. Northeast, and Denmark. Similarly, the harvest index (HI) for IWG in 2019 was 0.10, approximately 26% of the HI for annual wheat; this is similar to Culman et al. (2013) and higher than the HI reported by Hunter et al. (2020) and Dick et al. (2018).

The much greater interannual variation observed in IWG than annual wheat grain production over the experiment could have been caused by multiple factors. First, annual wheat has been bred more intensively and for many more years with attention to yield performance. Further, the variety grown in our experiment has been selected and developed for southwest U.S. regions and is particularly well suited for the Sacramento and Central Valley of California (Nelsen et al., 2022). Conversely, IWG is native to Eurasia and recent germplasms have primarily been bred and selected in the U.S. Midwest region over the past twenty years (the specific IWG germplasm used in this experiment was from a 2016 harvest at The Land Institute in Salina, Kansas) (Bajgain et al., 2020). Second, low IWG grain yields during the first year of production might be attributable to late planting and establishment due to climatic factors. For instance, nearly 50% of the 264 mm of seasonal precipitation had occurred prior to IWG planting in the 2017-18 season, which decreased the overall amount of precipitation IWG received within that growing season. Planting late may have also resulted in decreasing the number of days with cold temperatures and short daylengths necessary for inducing IWG reproductive growth, also known as vernalization (Fig. 2.1; Ivancic et al., 2021; Locatelli et al., 2021). Lastly, IWG stand age in tandem with dry conditions in 2020 may have led to negligible grain yields, as a decline in IWG grain number $spike^{-1}$ overtime has recently been reported (Hunter et al., 2020).

4.2 Aboveground nitrogen use efficiency trends higher in annual than perennial grain cropping systems

The NUE of annual wheat (tilled and no-till) aboveground and grain was relatively stable year to year and comparable to studies across various global regions (Ladha, et al., 2005). In contrast, the NUE of IWG showed significant interannual variation and was often less than half that of the annual wheat NUE and lower than values previously reported for annual wheat (Ladha et al., 2005) and IWG (Sprunger et al., 2018b). Conversely, in 2019, IWG had the same aboveground NUE and approximately one-third of the grain NUE as annual wheat. This latter finding agreed with our grain yield results, as IWG yielded approximately one third of the grain as annual wheat in 2019. That our IWG aboveground NUE values, especially during low precipitation years, were lower than those reported by Sprunger et al. (2018b) in SW Michigan might be due to large difference in climate and precipitation between the study sites. Our study site received less than half of the annual precipitation reported in certain experimental years in the southwestern Michigan study and had correspondingly lower biomass yields. It is plausible that IWG experienced stress associated with lower precipitation and warmer temperatures in 2018 and 2020, and thus, N uptake was limited. Lower N uptake could have led to both lower biomass yields (Fig. 1.2) and lower total plant N in harvested biomass. It is also plausible that differences in NUE between IWG and annual wheat would have been smaller if we had measured whole plant (root + shoot), versus only aboveground, NUE. For instance, Sprunger et al. (2018b) found aboveground NUE of IWG and annual wheat was similar, but IWG had a greater NUE when considering the whole-plant (roots + shoots) because of the importance of IWG's extensive root system in utilizing and soil N.

4.3 Cropping system and nitrogen effects on soil carbon storage

4.3.1 Tillage effects on soil carbon storage

In this study, we controlled for the effect of tillage on soil carbon throughout the soil profile in annual and perennial grain systems. A lack of soil disturbance increased soil carbon in the topsoil. Yet, soil carbon mass was not significantly higher in the no-till wheat system relative to IWG despite the fact its increased aboveground biomass production (Fig. 1.2) and lack of soil disturbance significantly increased soil carbon stabilization processes (Fig.1.6) in this system. Furthermore, we expected gains from a lack of tillage to be constrained to the tillage layer in no-till wheat but extend deeper (> 30 cm) in IWG based on previously reported rooting patterns. However, we found that across N rates, the conservation systems had similar trends in soil carbon across the soil profile, while the tilled system gained soil carbon at depth and gained the most soil carbon across the whole soil profile in four years.

Higher soil carbon accumulation is often observed in the top layer of soil in no-till systems (Bai et al., 2019; Aguilera et al., 2013; Syswerda et al., 2011; Dolan et al., 2006), but this does not necessarily translate to higher soil carbon deeper in the soil profile. There is growing consensus that increases in soil carbon under no-till relative to tilled systems is a result of altered depth distribution, where increases in soil carbon are largely concentrated near the surface (Powlson et al., 2014). Furthermore, gains in soil carbon deeper in the soil profile have been reported in tilled rather than no-till systems. For instance, Angers et al. (1997) found that soil carbon and nitrogen content was higher in no-till versus tilled systems in the top 10 cm of soil, but the trend was reversed in deeper soil layers (20-40 cm). Similarly, Dolan et al. (2006) found soil organic carbon (SOC) was greater under no-tillage than moldboard plow in the surface 0-20 cm of soil, but greater in tilled than no-tillage at 20-25 cm deep. A recent study of the Century Experiment, adjacent to our plots at Russell Ranch, found that a long-term tilled wheat-fallow system had similarly accumulated increased SOC concentrations and stocks at 100-200 cm soil depth (Tautges et al., 2019).

Primary mechanisms proposed to explain differences in soil carbon distribution in tillage versus no-tillage systems are differences in residue placement and movement in the soil profile (Angers et al., 1997). For instance, tillage incorporates residue deeper into the soil profile where residue-derived carbon may become embedded within the mineral matrix of the soil at depth (Gregorich et al., 2009). Furthermore, tillage activities may liberate occluded soil carbon within soil aggregates, which could then be translocated to deeper soil depths via percolation in the form of dissolved organic carbon and subsequently adsorbed to mineral surfaces. Thus, tillage induced movement of carbon to deeper soil layers, where decay rates and microbial activity is lower, may result in a net gain in soil carbon over time. This may be particularly true in our study soil, Yolo silt loam (Fig. S2.2), which has a buried A soil horizon with high clay content at 100 cm depth, where there may be a high potential for mineral adsorption of organic C compounds.

Contrary to our hypothesis, there was lower soil carbon mass accumulation in IWG than tilled annual wheat below the topsoil (> 60 cm) and summed up across the whole soil profile. In contrast, van der Pol et al. (2022), found IWG had approximately 4 Mg ha^{-1} more bulk SOC than annual sorghum-oat-wheat and wheat-sorghum-soy rotations across 0-100 cm of soil. Similarly, Audu et al. (2022) reported higher SOC in the subsoil (30-60 cm) of IWG than annual wheat rotations. Reasons for these differences may be that Audu et al. (2022) reported their SOC values normalized to soil bulk density, whereas we report SOC differences using equivalent soil masses (ESM). Using ESM accounts for differences in bulk density measurements that may arise over time as a result of SOM inputs and tillage (von Haden et al., 2020). Also, increased soil carbon at depth in IWG in the van der Pol et al. (2022) study was primarily in the form of particulate organic carbon (POM), not mineral associated organic matter (MAOM). POM can decompose readily under favorable conditions, whereas MAOM is older, more resistant to disturbance and accounts for the majority of SOC in annually crop agricultural ecosystems

(Cotrufo & Lavallee, 2022). Finally, the importance of the effect of climate contributing to these differences cannot be overlooked. Despite that IWG has an extensive deep rooting system (Duchene et al., 2020), in our water-limited Mediterranean climate it may not be possible to achieve the benefits observed for IWG in climates with more precipitation and lower annual temperatures, such as where these studies were conducted. This underscores the importance of conducting long-term studies in a variety of climates before new cropping systems are adopted in new regions.

4.3.2 The effects of N fertilization within two conservation agriculture systems

The positive impact of nitrogen fertilization on increases in soil carbon mass in both fertilized IWG and no-till wheat systems was consistent with conclusions of the meta-analysis by Bai & Cotrufo (2022), which states inorganic fertilizers stimulate biomass productivity and high-quality plant carbon inputs in the soil profile. These outcomes may result in increased microbial carbon use efficiency and thus formation and persistence of stabilized soil carbon (Bai & Cotrufo., 2022). We had expected to see greater soil carbon storage in IWG than no-till annual wheat at depths > 60 cm because of IWG's demonstrated higher percent of soil area colonized with root biomass than annual grains (Duchene et al., 2020). However, the trends between these two systems were generally similar at each soil depth and nitrogen combination, except for the effect of nitrogen fertilization on Δ soil carbon mass across the whole soil profile. This difference was largely an additive effect of smaller Δ carbon mass values in fertilized versus unfertilized IWG systems at depths below 30 cm. For instance, at the 30-60 cm soil depth in the IWG system, we found that only when not fertilized with N did the IWG system have increases (Δ > 0) in soil carbon mass since 2017. Perhaps carbon inputs increased at depth because N fertilization increases IWG fine root biomass in the subsoil (Sprunger et al., 2018b), but these soil carbon increases were not efficiently stabilized in microbial biomass when root inputs were not matched with adequate N inputs to satisfy microbial stoichiometry (Craine et al., 2007). In that case, priming of soil organic matter may have occurred in response to microbes satisfying their N requirement, leading to a net decrease in soil carbon storage in fertilized IWG systems.

4.4 Multifunctionality and ecosystem services: tradeoffs in tilled wheat, no-till wheat and IWG systems

When evaluating the provisioning services (Fig. S2.1) these three cropping systems provide through a multifunctionality framework (Huang et al., 2015), we observed that tilled and no-till wheat had both the highest absolute grain yields and grain yield stability over time (Table 2.1). With respect to the regulating services (Fig. S2.1), cropping systems differed comparatively by depth and nitrogen rate. Broadly, when the two conservation agriculture systems (IWG and no-till wheat) were fertilized with 112 kg N ha^{-1}, they had greater soil carbon mass in the top 0-30 cm of soil than tilled annual wheat by the third year of the experiment (Fig. 2.2). While soil carbon mass in the topsoil can improve other regulating services such as soil retention (i.e., erosion and flood control; Adhikari & Hartemink, 2016), it may fall short of providing climate regulating services since topsoil carbon can be subjected to future tillage activities that oxidize SOC (Tautges et al., 2019). Below 60 cm and at both N rates, tilled annual wheat had the greatest soil carbon mass and was the only system that significantly gained soil carbon mass across the whole profile in four years (Fig. 2.2, Fig. 2.3, Table 2.3). Subsoil carbon accumulation in the tilled wheat system may be more protected from losses because deep soil carbon may be more resistant to decomposition (Chabbi, Kögel-Knabner, & Rumpel, 2009) and is deeper than the till zone so not as vulnerable to loss by oxidation from tillage disturbance. Thus, the tilled wheat system may better address climate regulation services. With respect to NUE, tilled and no-till annual wheat had the greatest and most consistent aboveground and grain NUE (Table 2.2). This suggests that till and no-till annual wheat potentially decreased disservices, as the most N was captured (and thus less potentially lost to ground or surface water) in these two systems relative to IWG. However, an important caveat to this is that a considerable amount of N could have been stored in the roots of IWG (Sprunger et al., 2018b), thus N losses to the environment could have been minimal in the IWG system despite low aboveground NUE. Our results imply sampling the subsoil (> 30 cm) is crucial when comparing system-level soil carbon storage potential of annual and perennial grain systems and that plant adaption plays a major role in providing provisioning services (i.e., grain yield stability and NUE) and decreasing agricultural disservices (via increased aboveground NUE).

5. Conclusion

Tilled and no-till annual wheat exhibited the highest and most stable grain yields, aboveground NUE and grain NUE, while IWG had unstable grain yields and a NUE that varied more than 60% between years. When fertilized, the two conservation systems (IWG and no-till wheat) had greater soil carbon relative to tilled annual wheat in the topsoil, while tilled annual wheat had greater soil carbon at depth and was the only system that significantly gained carbon across the whole soil profile within four years. Thus, plant productivity, likely as a function of plant adaptation, lead to increased provisioning services and decreased agricultural disservices as measured by increased grain yield stability and NUE in annual wheat, respectively. Furthermore, our results highlight that the two conservation systems only partially addressed regulating services considering their soil carbon gains were contingent upon nitrogen fertilization and constrained to the top of 30 cm of soil. Conversely, the more productive tilled annual wheat system, in the Mediterranean climate of CA, may contribute greater climate regulation by storing carbon in the subsoil and across the whole soil profile, across N fertilization rates. Our results underscore the importance of measuring subsoil carbon and conducting long-term studies in a variety of climates before novel cropping systems are adopted in new region.

6. Appendices

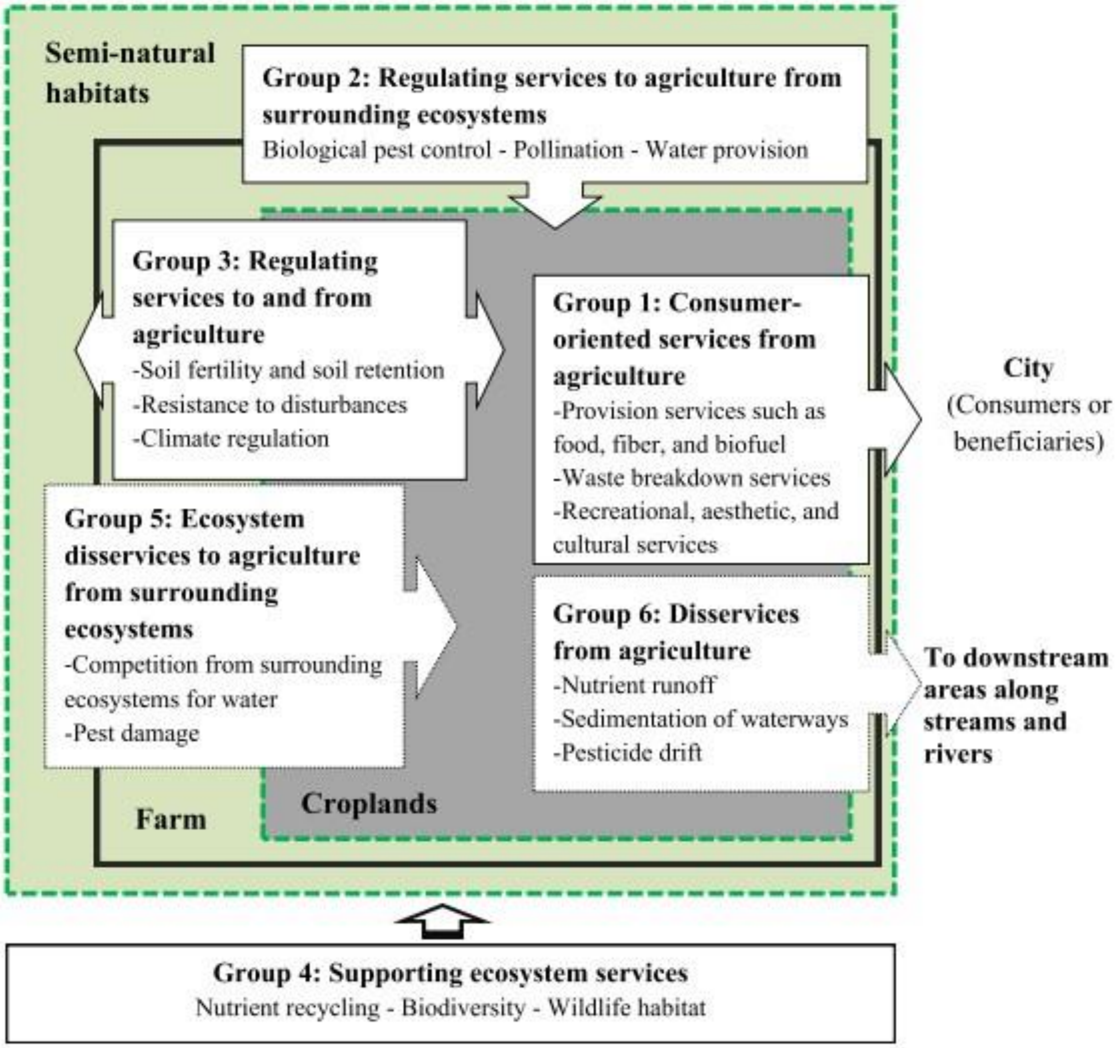

Figure S2.1 Integrated framework of multifunctional agriculture and ecosystem services proposed by Huang et al. (2015). The framework distinguishes six groups of ecosystem services and disservices, assuming that croplands (grey) are surrounded by semi-natural habitats (green), farm is the principle decision unit, and farm may encompass some surrounding semi-natural habitats.

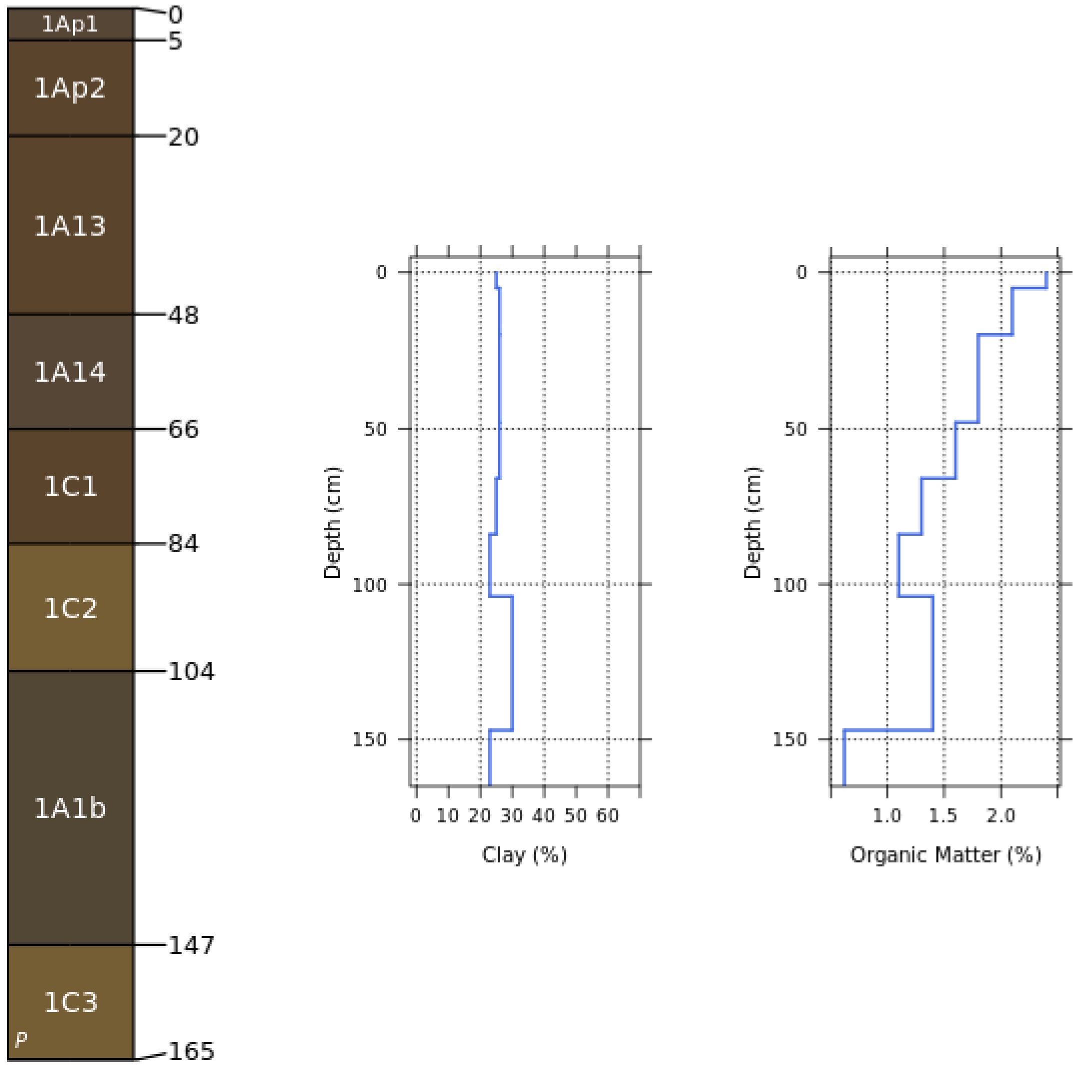

Figure S2.2. Yolo silt loam soil profile, clay content (%) and organic matter content (%). Data obtained from https://casoilresource.lawr.ucdavis.edu/gmap/ .

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Chapter 3: Soil carbon storage and compositional responses of soil microbial communities under perennial grain IWG vs. annual wheat

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Abstract

The introduction of novel perennial grains into annual row crop rotations is proposed to increase soil ecosystem services and enhance plant-soil-microbial linkages because perennials provide deeper root systems and more continuous ground cover than annuals. While it is known that soil microbial communities underpin many ecosystem services, we know little about how soil microbial composition and diversity, and soil carbon storage, differ between soils of annual vs. perennial grain crops. We measured soil fungi: bacteria (F/B) ratios and soil carbon within the novel perennial intermediate wheatgrass (IWG; trademarked Kernza®) and tilled annual wheat system and compared soil microbial diversity and community composition within their rhizosphere, shallow bulk soil (0-15 cm) and total bulk soil (0-90 cm). After three years, soil depth explained 30-40% and 12-22% of the variance in bacterial and fungal community composition, respectively, while crop type explained 10% and 9-16% of the variance, respectively. Fungal communities were most impacted by crop type in the rhizosphere and shallow bulk soil and less sensitive to differences in soil depth. In contrast, crop type had a smaller effect on bacterial communities which were more influenced by soil depth. IWG had a higher (F/B) ratio than tilled annual wheat at depths below 15 cm, but tilled annual wheat had higher soil carbon concentration (p = 0.01) and soil carbon mass (p=0.15) at the 60-90 cm soil depths. Our results indicate that fungi were more responsive than bacterial communities to crop type and that IWG may select a fungal community composition different from that in annual wheat and increase fungal biomass at depth. Changes in microbial communities, however, did not translate into increased soil carbon mass at depth. Knowledge from our research about how perennial grain crops interact with soil microbiomes provides a foundation to design new cropping systems that maximize ecosystem services in combination with food production.

1. Introduction

While annual grain crops are currently grown on nearly 70% of earth's cultivated land and provide the majority of the world's food (Pimentel et al., 2012), market interest in perennial grains has increased (Broom, 2019) as some of the negative impacts of annual grain agriculture become evident. These environmental consequences stem partly from intensive tillage, which has shown to increase soil erosion (Pimentel et al., 1995; Capello et al. 2019; Novara et al., 2019), reduce microbial biomass (Follett and Schimel, 1989), and degrade soil structure (Lal et al., 2007). Even under best management practices, conversion of perennial grassland to annual never-tilled soybean and wheat cropland rotations was found to reduce active soil carbon fractions and impact soil microorganisms within three years after conversion (Dupont et al., 2010). Deep-rooted perennial grains could potentially provide an alternative to intensive annual systems, such as annual wheat, by producing forage and a modest grain yield (Culman et al., 2013; Jungers et al., 2017), as well as increasing labile soil carbon (Culman et al., 2013) and decreasing tillage frequency (Lanker et al., 2020).

A domesticated wild perennial wheatgrass known as intermediate wheatgrass or IWG (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey; trademarked Kernza®), is a dual-use perennial grain (Favre et al., 2019; Lanker et al. 2020) that has gained international attention and is currently being cultivated on approximately 1,400 hectares of cropland in the United States (The Land Institute, 2022). IWG produces a high-quality forage (Favre et al., 2019), palatable grain (DeHaan & Ismail, 2017), and, relative to annual wheat results in less nitrate leaching (Culman et al., 2013) and higher whole-crop nitrogen use efficiency (Sprunger et al., 2018). Recent studies also show that IWG has a greater root/shoot ratio and greater coarse and fine root biomass (Sprunger et al., 2019), as well as more labile soil carbon relative to annual wheat (Culman et al., 2013; Sprunger et al., 2019; Audu et al., 2022). While perennial grains such as IWG have been identified as a potential pathway to long-term global food security (Glover et al., 2010; DeHaan & Van Tassel, 2022), the grain yield of IWG is only approximately only one-third that of annual wheat (Culman et al., 2013; Cassman et al., 2022). Recent studies have demonstrated differences between IWG and annual wheat regarding biomass (Fig 1.2; Culman et al., 2013; Sprunger et al., 2018) and grain yield (Fig. 2.1 ; Favre et al., 2019; Hunter et al., 2020), soil health (Culman et al., 2013, Sprunger et al., 2019, Pugliese et al., 2019), and nutrient cycling (Sprunger et al., 2018), though few studies have yet explored if and how soil microbial (i.e., fungal and bacterial) community composition, soil microbial taxonomy, and soil carbon mass are differentially influenced by IWG and annual wheat systems.

Annual row crop systems, including annual wheat, typically prioritize maximizing aboveground biomass yields (Tilman, 1999) and achieve this through the utilization of tillage and cultivar selection. Though these are effective means for maximizing production, they potentially deemphasize the importance of extensive root systems in close relationship with bacteria and fungi (Bender et al., 2016). In contrast to annual wheat rotations, perennial grain systems have demonstrated the potential to boost plant and soil microbial linkages and increase soil organic matter in agroecosystems (Audu et al., 2022). This could be because perennial plant systems, such as IWG, lack soil disturbance and have denser, deeper root systems than annual grain crops (Duchene et al., 2020). The lack of soil disturbance inherent in IWG systems could increase soil fungal to bacterial (F/B) ratios (Bailey et al., 2002), while the rooting characteristics could extend the zone of root influence (Duchene et al., 2020) in the soil, potentially increasing the total soil volume capable of microbial activity and soil organic carbon storage. Although there is not consensus on whether soil fungal or bacterial community members most accurately reflect shifts in soil carbon and soil health, it has been postulated that F/B ratios are linked to higher soil carbon storage potential (Malik et al., 2016) and that soil fungi are reliable indicators of increased soil carbon storage due to changes in cropping system (Rodgers et al., 2021). Therefore, fungi may be a more accurate indicator of soil health change than soil bacteria (Rodgers et al., 2021). This may have implications for IWG considering bacterial lipid markers were not found to not be significantly affected by annual vs. perennial grain crop treatments, but fungal and arbuscular mycorrhiza fungi (AMF) markers were (Duchene et al., 2020). Such differential responses to annual vs. perennial grain production among fungi and bacteria may result from different habitat or environmental filters for the two groups. Even still, the effect of perennial vs. annual grain production on soil microbial communities and its implications for soil carbon remains largely unexplored. In addition, little is known regarding how soil communities in IWG and annual wheat differ between rhizosphere and bulk soil, and with depth.

Plant rhizospheres are hotspots for many biogeochemical transformations and processes and are strongly influenced by environmental filters such as plant genotype (Patel et al., 2015). This genotypic influence is largely driven by the copious amounts of root exudates, where an estimated half of a plant’s photosynthates are released into the soil (Jones et al., 2009) via root exudation. The chemical composition of root exudate varies by plant type, and, in conjunction with microbial substrate uptake traits, may yield unique microbial community assembly in the rhizosphere (Zhalnina et al., 2018). Given that roots are potentially rhizosphere ambassadors (Bais et al., 2006), and that IWG has three to twelve times greater coarse and fine root biomass than annual wheat (Sprunger et al., 2018), it would be expected that IWG recruits rhizosphere communities that are distinctly different from annual wheat. Furthermore, because IWG's deep rooting pattern exploits a greater and deeper volume of soil than annual wheat, we expect differences in labile carbon, F/B ratio, and soil carbon mass between these crops to extend to deeper soil depths (i.e., > 60 cm). To date, little is known about how IWG and annual wheat soil microbiomes (i.e., soil fungal and bacterial communities) and their impacts on soil carbon mass differ, not only between bulk soil and rhizosphere, but at soil depths exceeding 60 cm. Given that soil carbon is more likely to be protected from abiotic and biotic degradation in the subsoil (Jobbagy & Jackson, 2000; Pries et al., 2018), investigating soil carbon deeper than 30 cm is critical for understanding if either crop type can promote soil carbon sequestration (Tautges et al., 2019).

Our objectives were to: 1) measure if and how perennial and annual grains affect soil microbial diversity and community composition within the rhizosphere and bulk soil; 2) determine the relationship between soil chemical and biological properties and soil microbial community composition; 3) compare F/B ratio and soil carbon mass across soil depths in annual wheat versus IWG. We hypothesized that: 1) soil microbial diversity would be higher in IWG than annual wheat. Furthermore, we expected fungal communities to be most affected by crop type, especially in the rhizosphere and shallow bulk soil; 2) pH and labile soil carbon would drive soil microbial community composition; 3) F/B ratio and soil carbon mass would be higher in IWG than annual wheat across depths.

2. Materials and methods

2.1. Soil sampling and processing

The experiment was conducted at the Russell Ranch Sustainable Agriculture Facility near the University of California, Davis (38°32’27.30" N, 121°52’7.39” W). Rhizosphere and bulk soil samples were collected in select treatments from a field trial established in 2017. Briefly, rhizosphere samples are defined as soil clinging to the roots in the 0-15 cm soil depth, where bulk soil is defined as either i) shallow bulk soil (i.e., soil not clinging to roots in the 0-15 soil depth) or ii) total bulk soil (i.e., soil not clinging to roots in the 0-90 cm soil depth). Three replications of two treatments were measured. These treatments were: (a) a rainfed and tilled annual wheat (Hard Red Spring wheat, var. WB 9229) cropping system where with annual tillage and planting and mineral fertilizer applications of 112 kg ha^{-1} y^{-1}; (b) a rainfed perennial grain (IWG; Kernza ®) cropping system where tillage and planting activities occurred during the first season but in no subsequent season and the crop received 112 kg ha^{-1} y^{-1} of nitrogen mineral fertilizer annually. All plots were disked to a depth of approximately 20-30 cm when establishing the experiment. In subsequent years, only the tilled annual wheat plots were disked and rototilled each fall to a depth of 15-25 cm prior to soil sampling and planting operations.

Soil samples were collected prior to harvest in early May of 2020 (the third year of the experiment), when annual wheat was at soft dough stage and IWG was at a late vegetative stage. In each plot, two samples were taken from a representative area with a Geoprobe® machine (Salina, Kansas) using the MC7 Core Sampler Soil Sampling System with an inner diameter of 70 mm. Samples were taken directly on top of the given crop (annual wheat or IWG) to a depth of approximately one meter in effort to capture the root crown and associated bulk soil. Samples were immediately placed on ice in the field and processed the same day in the lab.

In the laboratory, cores for a given plot were opened and the root crowns were removed and immediately shaken by hand to remove excess soil, such that approximately 1 mm of soil remained attached to the roots. The roots were then processed as described in Samaddar et al. (2021). Briefly, the roots were clipped from the root crowns into small pieces and placed inside a sterile 250 mL Erlenmeyer flask containing 50 mL of 1X phosphate buffer solution and shaken in a mechanical shaker at 150 rpm for 60 minutes to extract the soil that was strongly adhered to the roots. The extract was placed in 50 mL falcon tubes and centrifuged at 7000 rpm for 10 minutes. The pellets obtained from this process were considered rhizosphere soil and frozen at -20℃ degrees until DNA extraction. For the bulk soil samples, the two cores for a given plot were separated into 0-15, 15-30, 30-60, and 60-90 cm depth increments and the respective depth increments from the two cores were homogenized. Subsamples from each bulk soil depth increment were taken (~ 40 grams), roots were removed/handpicked from the subsample, and the sample was then stored in a 50 mL falcon tube and immediately frozen for DNA extraction.

To investigate the effect of soil compartments (shallow bulk soil vs. rhizosphere) and discrete depths within the total bulk soil (0-15, 15-30, 30-60, 60-90 cm), we analyzed them separately (Fig. S3.1): i) shallow bulk soil in the 0-15 cm depth interval vs. rhizosphere (representing roots collected from 0-15 cm depth) and ii) discrete total bulk soil depths (0-15, 15-30, 30-60, 60-90 cm).

2.2. DNA extraction

Soil DNA was extracted from 0.25 grams of soil by the MoBio PowerSoil DNA Isolation Kit (MoBio 136 Laboratories Inc., Carlsbad, CA, USA) following manufacturer instructions. Extracted DNA with low concentrations were then concentrated using Savant DNA 120 SpeedVac (Thermo Fisher Scientific, USA). Finally, the concentrated DNA extract was quantified using the Qubit® dsDNA HS Assay kit with a Qubit®138 fluorometer (Life Technologies, Carlsbad, CA, USA).

2.3. Amplicon sequencing

Microbial community profiling was performed at Integrated Microbiome Resource (IMR) sequencing facility at Dalhousie, Canada. The V3-V4 region of 16S rRNA gene was targeted for bacteria and the ITS2 region was targeted for fungi on an Illumina Miseq platform. The V3-V4 region of 16S rRNA gene was amplified using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') (Herlemann et al., 2011) and the ITS2 region of fungi were amplified using the primers ITS86F (5'-GTGAATCATCGAATCTTTGAA-3') and ITS4R (5'-146 TCCTCCGCTTATTGATATGC-3') (Op De Beeck et al., 2014). Once raw sequences were obtained from IMR, cutadapt (Martin, 2011) was used to remove primers and dada2 (Callahan et al., 2016) was used for further processing of sequences. For analysis, 16S rRNA gene forward reads were truncated to 250 bp and reverse reads to 200bp based on quality profile plots; quality trimming and filtering included maxEE = 2 and truncQ =2. The fungal ITS sequences did not have their forward and reverse reads truncated to a specific length since the ITS region is known to be highly variable in length; trimming and filtering were conducted using the same parameters as 16S. After quality processing, dereplication of paired sequences occurred and the 'dada' function was used to denoise the unique reads based on the error rates calculated with the 'learnErrors' function. Once paired sequences were merged and chimera were removed, we used the SILVA database release 138 (Quast et al., 2013) and UNITE (Nilsson et al., 2019) database to assign taxonomy to 16S and ITS sequences, respectively. All 16S rRNA sequences that were assigned to chloroplast, eukaryota, and mitochondria were removed prior to further analysis. A total of 2064 bacterial/archaeal amplicon sequence variants (ASVs) for bulk and rhizosphere samples and 3595 ASVS for samples at different depths were obtained. Raw sequences were deposited at the Sequence Read Archive at NCBI under accession number PRJNA801357.

2.4. Bioinformatic analysis

Data analyses were performed in R version 4.1.0 (R Core Team, 2021) using multiple packages. Soil microbial diversity estimates for the samples were calculated using the package phyloseq (McMurdie & Holmes, 2013) and pairwise differences between crop types or soil depth/soil compartment (i.e., shallow bulk soil vs. rhizosphere) were analyzed using Tukey's HSD within the package agricolae (de Mendiburu, 2021). Soil microbial diversity means and standard errors for each crop type and soil depth/soil compartment combinations were calculated with the package dplyr (Wickham et al., 2014) and graphed with the package ggplot2 (Wickham, 2016). For calculating microbial community composition, normalization across samples was performed with the deseq2 (Love et al., 2014) package using the variance stabilizing transformation method. The normalized data was used to compute multidimensional scaling using the Euclidean distance in the phyloseq package. Permutational analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersions (BETADISP) were used to test the effect of crop type, soil compartment (shallow bulk soil vs. rhizosphere) and total bulk soil (0-90 cm) on microbial community using the functions adonis and betadisp, respectively, in the vegan package (Oksanen et al., 2013). A p-value threshold of 0.05 was used to determine statistical significance for all analyses. Canonical Correspondence Analysis (CCA) was used to analyze community composition by soil depth and crop type, constrained by soil physicochemical parameters and plotted using the vegan () package. Relative abundance of ASV of interest was calculated and multiple group comparisons among crop type and depths were calculated using STAMP v.2 (Statistical Analysis of Metagenomic Profiles) package (Parks et al 2014).

2.5 Soil microbial biomass and carbon sampling, measurements, and analyses

Soils were sampled for soil chemical and biological parameters with a Geoprobe® machine (Salina, Kansas) using the MC5 Core Sampler Soil Sampling System with an inner diameter of 4.3 cm in October of 2020. Samples were stored on ice and sub-samples from each core increment were either freeze-dried and sent to Microbial ID Inc. for phospholipid fatty acid analyses (PLFA) or they were air dried, sieved to 2mm, and sent to Ohio State University Soil Fertility laboratory for soil chemical analysis (POXC, phosphorus, calcium, potassium, pH, organic matter, magnesium). POXC measurements were carried out with a weak oxidizing solution as described by Culman et al. (2012) and Weil (2003). PLFA analysis was performed following the methods described by Buyer & Sasser (2012). PLFA fatty acids associated with fungal biomass that had concentrations below instrument detection limit were assigned one-half the fatty acid detection limit (0.1 nmol $gram^{-1}$) for the purpose of further statistical analysis. For soil carbon sample processing, each soil depth increment was air-dried, sieved <2mm, and archived in plastic bags at room temperature. Subsamples were then collected from each air-dried and sieved soil depth increment, all visible plant material was removed, and it was oven dried at 60°C for 72 hours and ball mill ground for a minimum of 24 hours. Total C and N were then determined for each subsample by dry combustion with a Costech ESC 4010 Elemental Analyzer (Valencia, CA, USA). Total carbon and nitrogen for each soil depth increment was then calculated on concentration basis. Soil carbon concentration, oven dry soil mass, and soil depth were then used to calculate soil carbon mass for each reference soil mass layer using the equivalent soil mass (ESM) approach described by von Haden et al. (2020). Reference soil mass layers were selected such that: (a) each average calculated depth to reference mass (cm) was close to the fixed soil depth that was sampled (30,60,90 cm) and (b) depth to reference mass (cm) did not extrapolate past the depth that was sampled (90 cm). Soil carbon mass values and ESM soil mass/layers were calculated by fitting a cubic spline to the data generated by the depth-based sampling. While all reported soil carbon mass data align with a specific ESM soil mass/layer, data will be reported on a fixed depth layer (0-30,30-60,60-90) basis for ease of interpretation.

Once soil carbon mass was calculated for each plot, the relationship between cropping system type (IWG, tilled wheat), soil depth (0-30,30-60,60-90 cm) and soil carbon mass (Mg ha^{-1}) were modeled with the equation:

Equation 1: Soil carbon mass

Z = A + B + C;

Where:

Z = Soil carbon mass

A = Soil depth nested within crop type (tilled annual wheat, IWG)

B= Random intercept of soil depth nested within block

A mixed linear model was fitted using the lme function from the nlme package (version 3.1 – 152) (Pinheiro et al., 2021 in R version 4.0.3) (R Core Team, 2020).

3. Results

3.1. Effect of crop type, soil compartment and soil depth on diversity and composition of soil bacterial and fungal communities

We compared Shannon biodiversity indices and species richness in annual wheat and IWG soil communities, analyzing all samples, including the rhizosphere and soil depth intervals together. Crop type had no significant impacts on fungal community Shannon diversity or richness except at 60-90 cm where significantly higher fungal richness was measured in IWG than annual wheat (Fig. S3.2a,b ; Table 3.1). Observed richness and Shannon diversity within annual wheat fungal communities were the same across depths (Table 3.1), whereas bacterial communities had lower richness and diversity values in wheat at at 60-90 cm than any other depth. In comparison to IWG at the 60-90 cm depth, bacterial and fungal community Shannon diversity and observed richness values were numerically, but not statistically, greater in IWG than annual wheat at 60-90 cm (Fig. S3.2a,b ; Table 3.1).

Soil Depth/ Comp.	16S				ITS			
	Shannon		Richness		Shannon		Richness	
	IWG	Wheat	IWG	Wheat	IWG	Wheat	IWG	Wheat
Rhizosphere	5.9 B a	6.0 AB a	744 ABC a	724 AB a	3.8 A a	3.6 A a	309.3 A a	321.7 A a
0-15 cm	6.2 A a	6.2 A a	817.7 AB a	805.7 AB a	4.1 A a	4.0 A a	263.7 ABC a	284.7 A a
15-30 cm	6.2 A a	6.2 A a	846 A a	830.3 A a	4.2 A a	3.9 A a	288.7 AB a	229.3 A a
30-60 cm	5.9 B a	6.1 A a	667 BC a	780.3 AB a	3.5 A a	3.7 A a	95.7 C a	117.7 A a
60-90 cm	5.7 B a	5.7 B a	617 C a	578 B a	3.4 A a	3.3 A a	111.3 BC a	62 A b

Table 3.1. Effects of crop type and soil compartment (comp.) /soil depth on observed species richness and Shannon diversity index. Values with the same uppercase letters in each column indicate no significant ($p > 0.05$) differences among the total bulk soil depth (0-90 cm) or soil compartment (0-90 cm total bulk soil vs. rhizosphere) within a given crop type according to Tukey's HSD test. Values with the same lowercase letters in each row indicate no significant ($p > 0.05$) differences among crop type within a given soil depth or soil compartment according to Tukey's HSD test.

Soil compartment (rhizosphere vs. shallow bulk soil), not crop type (IWG vs. annual wheat) significantly influenced soil bacterial community composition, as seen in the principal co-ordinate analysis (Fig. 3.1a) and the corresponding PERMANOVA test (Table 3.2). Soil compartments differed along the first axis, explaining approximately 29% of the variance, whereas IWG and annual wheat systems varied along the second axis, explaining 10% of the variance. Conversely, crop type (IWG vs. annual wheat) and soil compartment (rhizosphere vs. shallow bulk soil) both significantly (Table 3.2) influenced soil fungal community composition (Fig. 3.1b). The IWG and annual wheat systems differed along the first axis, which explained approximately 16% of the variance in the soil fungal community composition. Soil compartment differed along the second axis and explained approximately 12% of the variance.

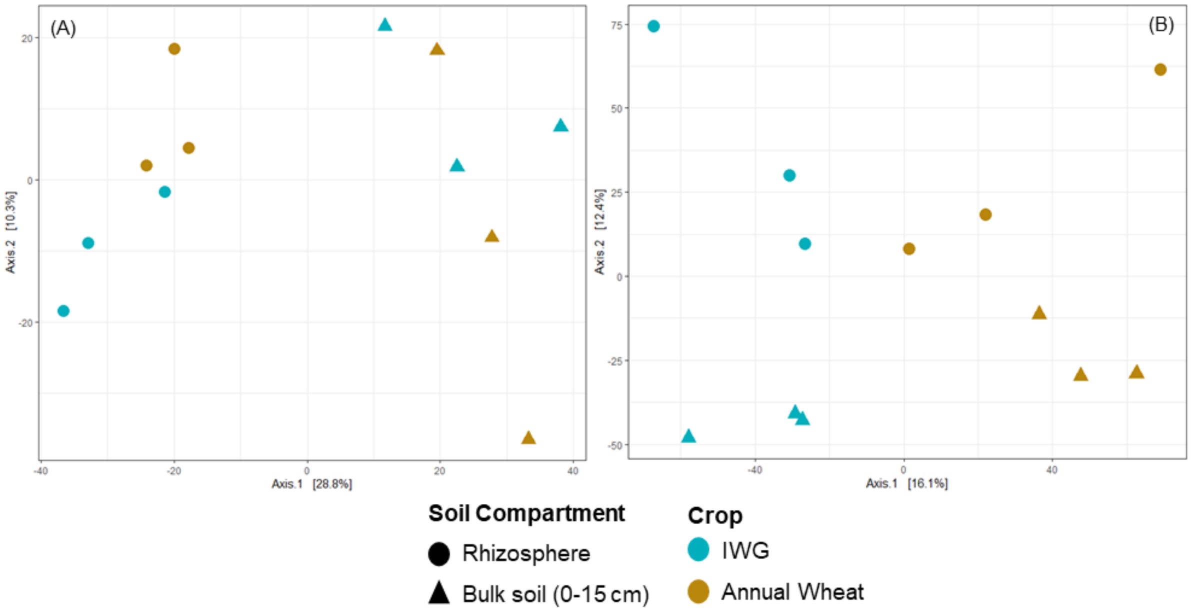


Figure 3.1. Ordination plots showing separation of soil bacterial (A) and fungal (B) communities according to crop type (annual wheat, IWG) and soil compartment (rhizosphere, shallow bulk soil 0-15 cm). All ordinations were performed using ASV level data.

Statistical tests				
	Bacterial community		Fungal community	
BETADISPER	P value = 0.44		P value = 0.88	
PERMANOVA	Pseudo F	R^2	Pseudo F	R^2
Crop Type	1.18	0.08	1.72***	0.14
Soil Compartment	3.77***	0.27	1.37*	0.11
Crop * Compartment	1.10	0.08	0.89	0.07

Table 3.2. Statistical tests analyzing the effect of crop type and soil compartment (rhizosphere vs. shallow bulk soil), and the interaction of crop type and soil compartment on bacterial and fungal community composition. Asterisks indicate statistical differences where * p < 0.05, ** p <0.01 and *** p <0.001.

Similar to the results displayed in Figure 3.1(a), only total bulk soil depth (0-15,15-30,30-60,60-90), not crop type (IWG vs. annual wheat) significantly influenced soil bacterial community composition (Fig. 3.2a, Table 3.3). Soil depth increments clearly differed along the first axis, explaining approximately 40% variance in soil bacterial community composition. IWG and annual wheat systems varied along the second axis, however it explained only ~10% of the variance in soil bacterial community composition. As in Figure 3.1(b), crop type (IWG vs. annual wheat) and total bulk soil depths (0-15,15-30,30-60,60-90) significantly influenced soil fungal community composition (Table 3.3). Soil depth was differentiated along the first axis and explained approximately 22% of the variance in soil fungal community composition (Fig. 3.2b). Crop type, which differed along the second axis, explained approximately only 9% of the variance.

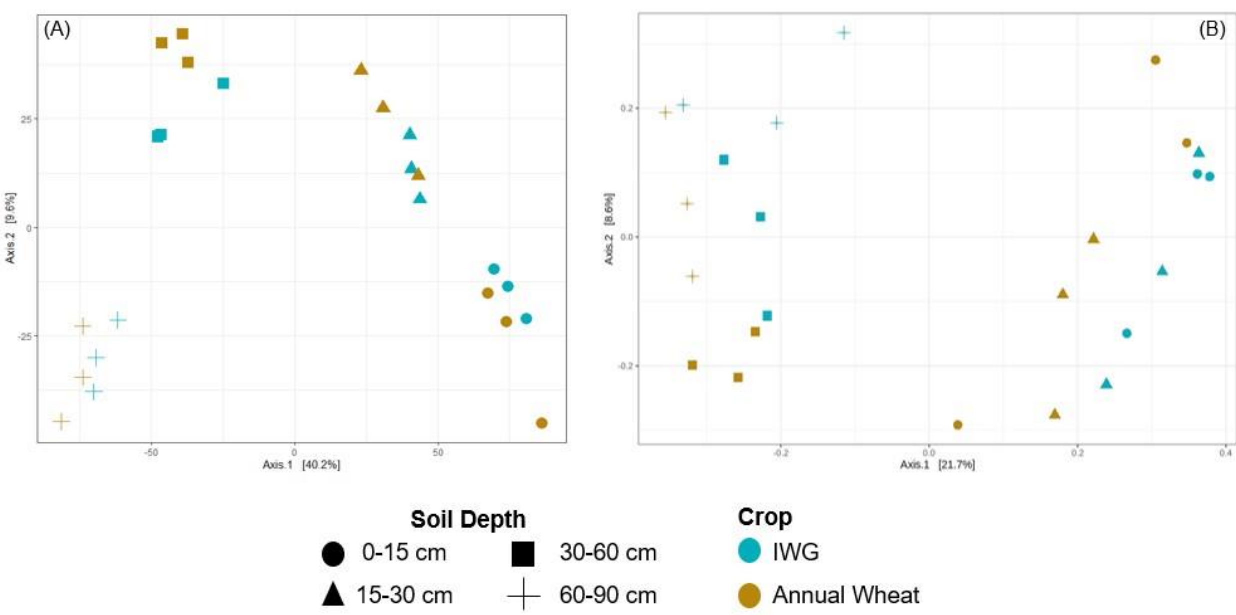


Figure 3.2. Ordination plots showing separation of soil bacterial (A) and fungal (B) communities according to crop type (annual wheat, IWG) and total bulk soil depth (0-15,15-30,30-60,60-90 cm). All ordinations were performed using ASV level data.

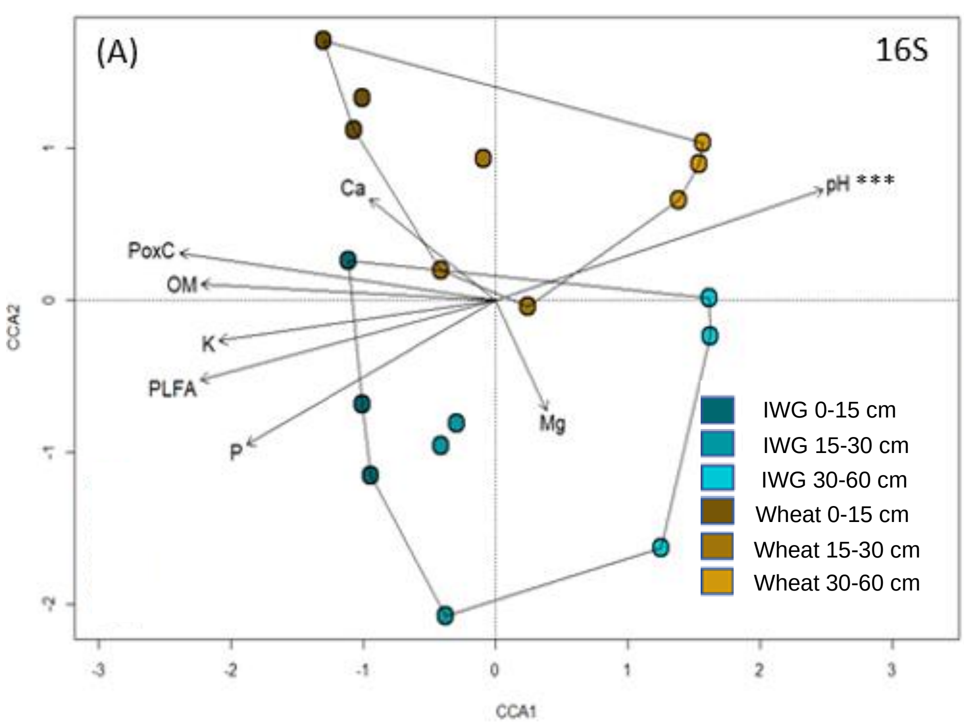
Statistical tests				
	Bacterial community		Fungal community	
BETADISPER	P value = 0.33		P value = 0.77	
PERMANOVA	Pseudo F	R^2	Pseudo F	R^2
Crop Type	1.37	0.03	1.96*	0.06
Soil Depth	7.24***	0.51	2.93***	0.30
Crop * Depth	1.10	0.07	1.11	0.11

Table 3.3. Statistical tests analyzing the effect of crop type, total bulk soil depth (0-90 cm), and the interaction of crop type and total bulk soil depth on bacterial and fungal community composition. Asterisks indicate statistical differences where * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

We tested whether soil fungal and bacterial community composition differences observed in this study are a result of differences in community dispersion within or between crop types and soil depths/soil compartments. The parameter β-diversity accounts for differences in community composition within microbial groups from the same crop type or soil compartment and between different crop types and soil compartments. BETADISP was performed to differentiate between true biological differences and differences in group dispersion. This test indicated a nonsignificant value ($p > 0.05$), meaning differences between groups were driven by true biological differences rather than differences of within-group dispersion (Table 3.2, Table 3.3). This result implies differences were largely the effects of different cropping systems (IWG, annual wheat), total bulk soil depths (0-15,15-30,30-60,60-90 cm), or soil compartment (rhizosphere, shallow bulk soil).

3.2 Soil chemical and biological properties and soil microbial community composition

To investigate the relationship between soil chemical and biological properties and microbial community composition, we conducted a canonical correlation analysis (CCA) of soil bacterial and fungal community composition data constrained by soil properties. Here, the CCA demonstrates that the IWG fungal community (Fig. 3.3b) appears to differentiate from annual wheat more clearly than the bacterial community (Fig. 3.3a) at every soil depth. The only significant relationship between soil fungal and bacterial community composition and measured soil properties was observed in annual wheat, where high pH values in annual wheat had a significant relationship with soil microbial communities at the 30-60 cm soil depth ($p<0.001$; Fig. 3.3, Fig. S3.3). Though not significant, the soil fungal and bacterial community in the top 0-15 cm of IWG related to high values of total PLFA biomass, soil potassium (K) and soil phosphorus (P) (Fig. 3.3, Fig. S3.3). Additionally, higher soil calcium (Ca) values in the top depth of annual wheat appear to be slightly related to soil bacterial and fungal communities (Fig. 3.3, Fig. S3.3). Given that the orientation of the arrows for POXC and O.M. lie between the top depths of IWG and annual wheat, it is inferred that values of these soil measurements were similar between the two crop types and largely driven by higher values occurring in the topsoil depths (Fig. S3.3).



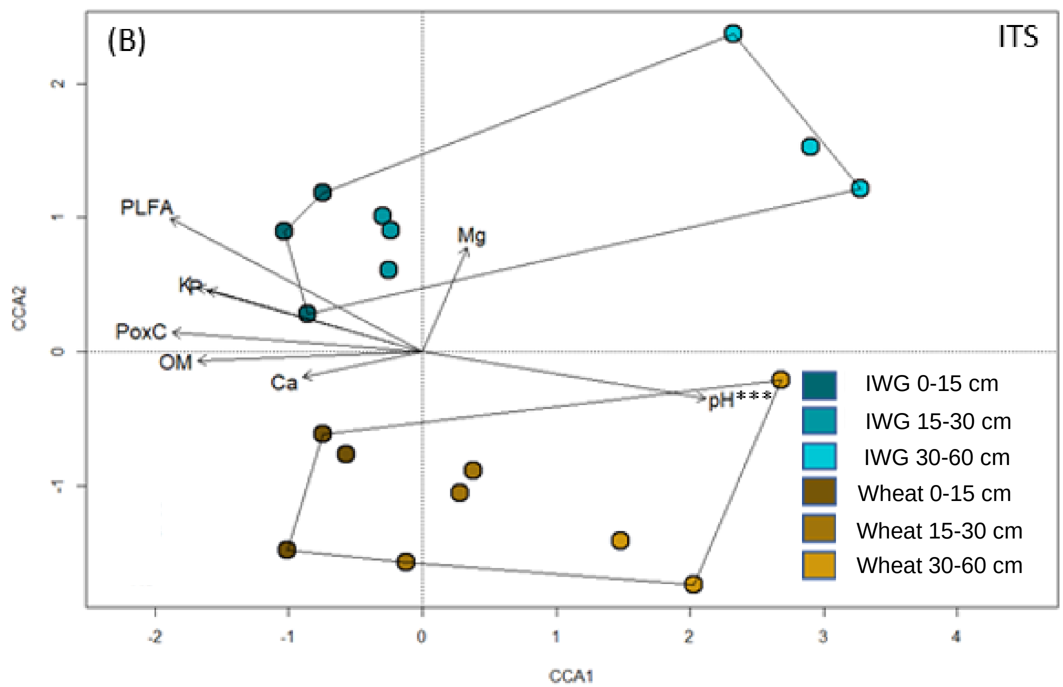


Figure 3.3 Bacterial 16S (A) and fungal ITS (B) sequence data. Canonical correlation analysis (CCA) of fungal and bacterial community data constrained by measured soil physicochemical characteristics. K0-15cm, K15-30, K30-60 cm indicates microbial community associated with IWG at the 0-15,15-30,and 30-60 cm depth, respectively. W0-15cm, W15-30, W30-60 cm indicates microbial community associated with tilled annual wheat at the 0-15,15-30,and 30-60 cm depth, respectively. Asterisks indicate statistical differences where * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

3.3. PLFA fungi : bacteria ratio and soil carbon mass

While the PLFA fungi: bacteria (F/B) ratio was the same for IWG and annual wheat at the 0-15 cm depth, IWG had a significantly higher F/B ratio at soil depths greater than 15 cm (Table 3.4). The most significant difference in F/B ratio between IWG and annual wheat occurred at the 30-60 cm depth, where the F/B ratio of IWG was 33% greater than annual wheat.

Greater F/B ratios in IWG at depth did not to translate to significantly greater soil carbon concentrations or soil carbon mass at soil depths below 15 cm (Fig. 3.4, Fig. 3.5). More specifically, soil carbon concentrations ($p = 0.29$) and soil carbon mass ($p = 0.22$) trended higher than annual wheat in the top 0-15 cm of soil but trended less than annual wheat at depths below 15 cm. Furthermore, while annual had a significantly lower F/B ratio than IWG at soil depths below 15 cm (Table 3.4), the annual wheat system had significantly greater soil carbon concentration ($p = 0.01$) and great soil carbon mass ($p = 0.15$) on average than IWG at the 60-90 cm soil depth.

	Fungi: bacteria	
	IWG	Annual Wheat
	nmol g^{-1} soil	
Soil depth (cm)		
0-15	0.12 ± 0.006	0.12 ± 0.005
15-30	0.1 ± 0.003	0.08 ± 0.010
30-60	0.08 ± 0.012	0.06 ± 0.005

Table 3.4. Mean fungi: bacteria ratio ± one SE as determined by phospholipid fatty acid analysis (PLFA) for IWG and annual wheat crop types at each soil depth (0-15, 15- 30, 30-60 cm).

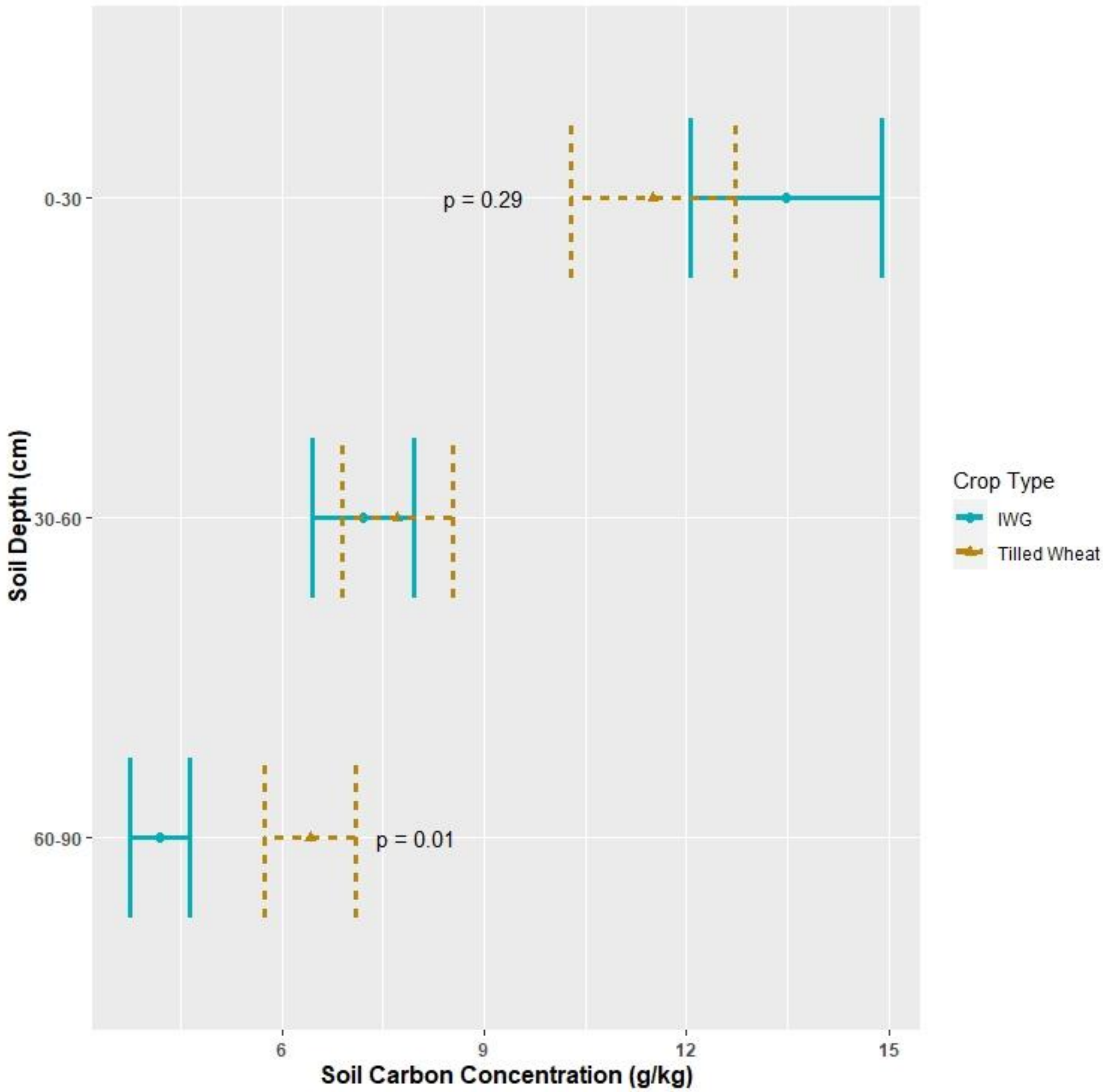


Figure 3.4 Soil carbon concentration (g kg^{-1}) for the effect of crop type (IWG, tilled annual wheat) and three different soil depths (0-30,30-60,60-90 cm). Error bars represent ± one SE from the mean. P-values indicate significant differences between crop types at a given soil depth.

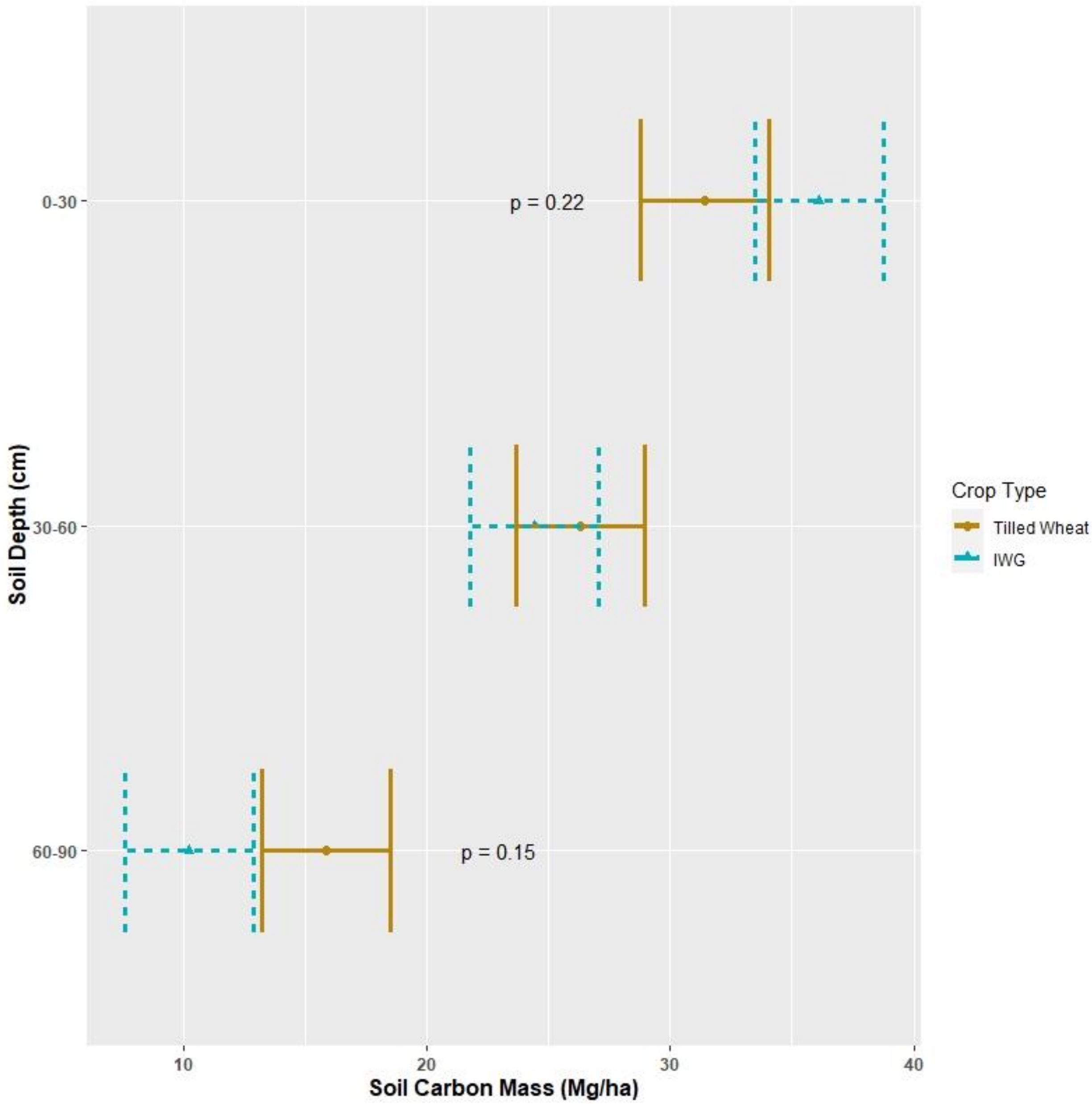


Figure 3.5 Soil carbon mass (Mg ha^{-1}) for the effect of crop type (IWG, tilled annual wheat) and three different soil depths (0-30,30-60,60-90 cm). Error bars represent ± one SE from the mean. P-values indicate significant differences between crop types at a given soil depth.

3.4 Fungal taxonomy in tilled annual wheat and IWG at depth

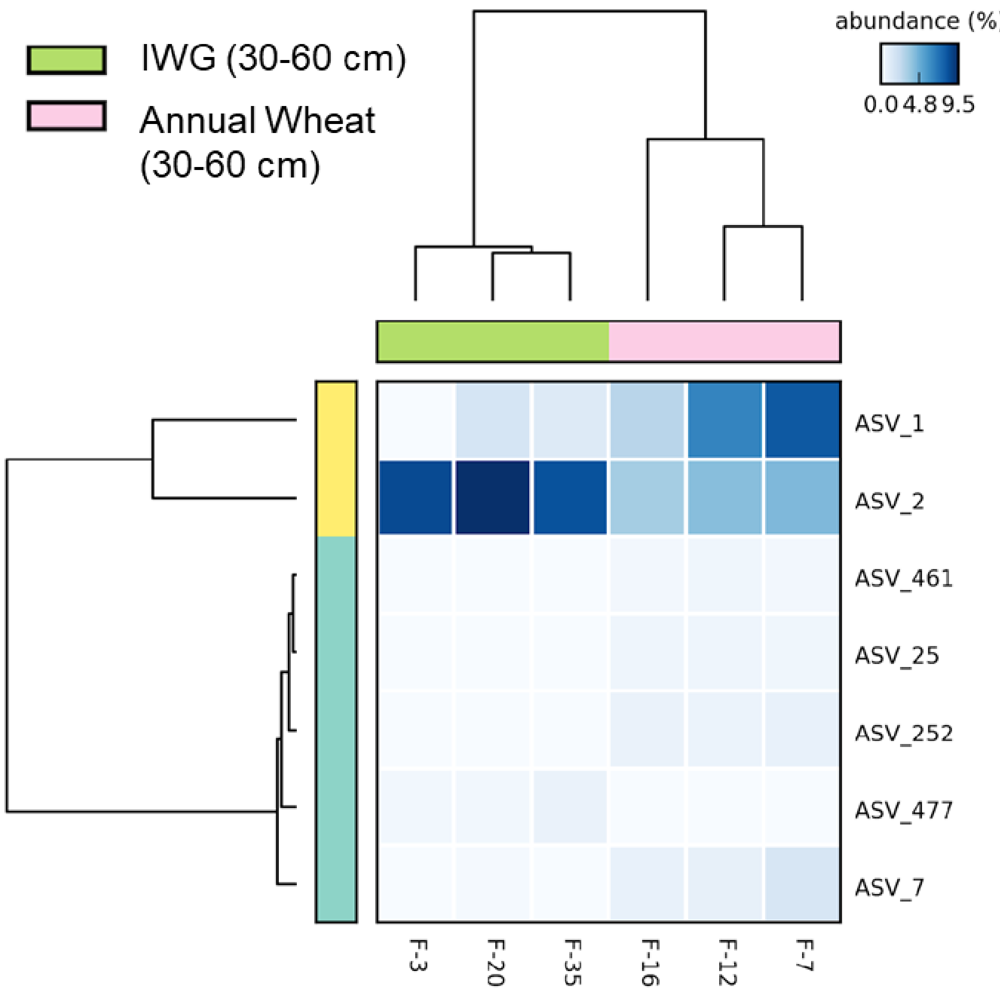


Figure 3.6 Heatmap showing relative abundance of fungal groups among IWG and tilled annual wheat at the 30-60 cm soil depth. Rows indicate specific ASVs for a given sample and the shade of blue indicates percent of relative abundance of that ASV. ASV_1 represents the genus *Mycosphaerella* and ASV_2 represents the genus *Solicoccozyma*.

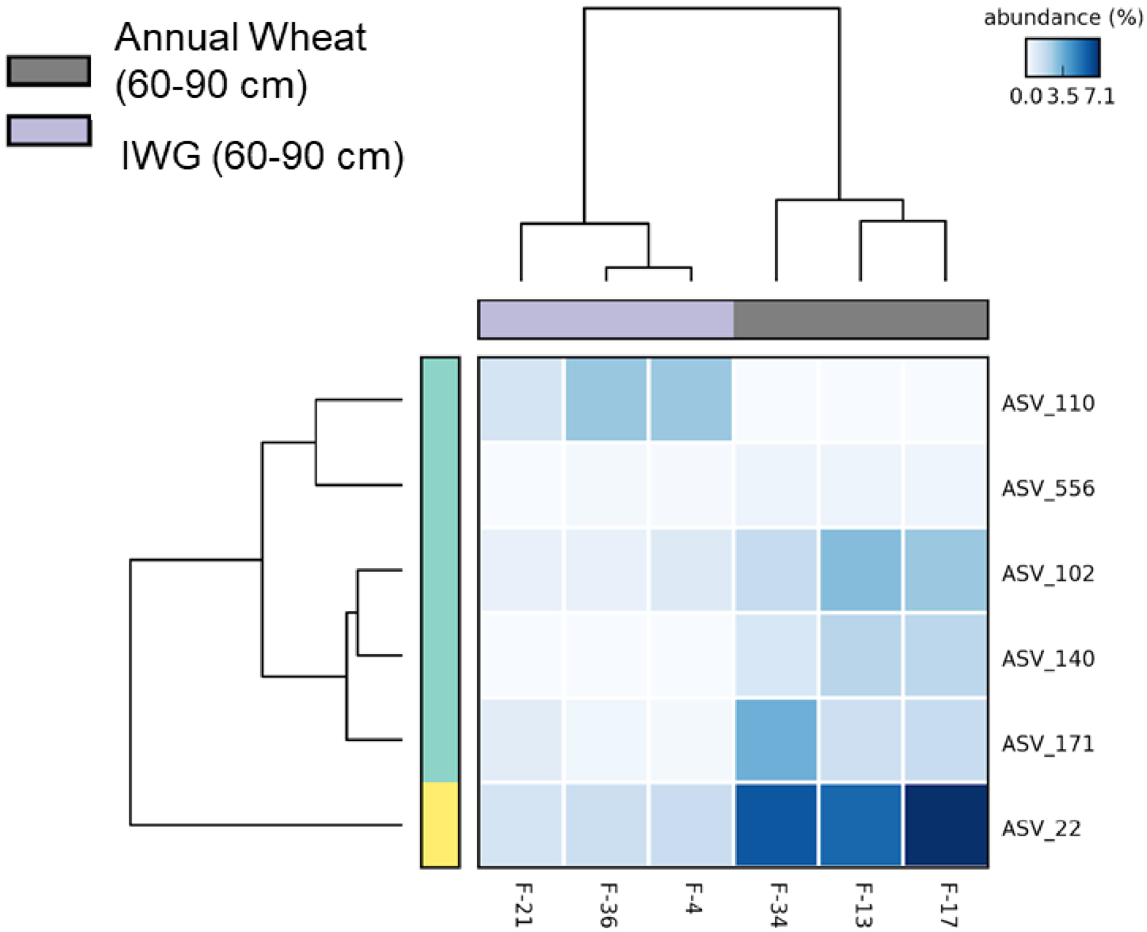


Figure 3.7 Heatmap showing relative abundance of fungal groups among IWG and annual wheat at the 60-90 cm soil depth. Rows indicate specific ASVs for a given sample and the shade of blue indicates percent of relative abundance of that ASV. ASV_22 represents the genus *Cistella.*

Given the differences in F/B ratio and soil carbon mass between annual wheat and IWG at depth, we compared fungal taxonomy at soil depths below 30 cm in the two cropping systems. Where IWG had a significantly higher F/B ratio than annual wheat, the 30-60 cm soil depth (Table 3.4), we found that IWG had significantly higher relative abundance of the genus *Solicoccozyma* while annual wheat had significantly higher relative abundance of the genus *Mycosphaerella* (Fig. 3.6). The soil depth at which tilled wheat had higher soil carbon concentration and soil carbon mass, the 60-90 cm soil depth, annual wheat had significantly higher abundance of the genus C*istella.*

4. Discussion

Differences in soil fungal and bacterial diversity indices between IWG and annual wheat were evident in bulk soil, but not within the rhizosphere. There were significant differences in soil fungal, but not bacterial community composition between IWG and annual wheat (Fig. 3.1, Fig.3.2 ; Table 3.2, Table 3.3). Only soil depth explained significant variation in bacterial community composition, whereas crop type and soil depth explained significant variation in fungal community composition, with the effect of crop type on the fungal community decreasing with increasing soil depth (Fig. 3.2, Table 3.3). Soil pH was the only soil variable that had a significant relationship with microbial community composition. IWG had a higher fungal richness and F/B ratio at lower soil depths, but lower soil carbon concentration and mass than annual wheat at the 60-90 cm depth. Our results reveal that while a perennial grain crop may select a fungal community composition different from that in annual wheat, higher fungal richness at depth and a higher F/B ratio at depth as compared to annual wheat, this did not result in higher soil carbon at depth. Furthermore, our results demonstrate that three years was adequate for crop type to significantly influence fungal community composition across depths, but not for significant shifts in soil microbial diversity across depths and bacterial community composition to occur. These findings are consistent with another recent study that demonstrated longer periods (>3 years) of time may be necessary for significant shifts in bacterial community composition to occur in perennial vs. annual grain cropping systems (Sprunger et al., 2019).

4.1 The effects of crop type on soil microbial diversity metrics at various depths

Approximately 35% (Fierer et al., 2003) to 50% (Schütz et al., 2010) of the total microbial biomass in soil usually resides in the sub-surface horizons, where many taxa are directly involved in carbon biogeochemistry (Lavahun et al., 1996) and nutrient cycling (Eilers et al., 2012). We found that bacterial and fungal community Shannon diversity and observed richness values were numerically, but not statistically, greater in IWG than annual wheat at 60-90 cm depth with significantly higher fungal richness values in IWG than annual wheat at this depth (Fig. S3.2, Table 3.1).

The increased diversity values at depth in IWG may reflect the impact of the deeper rooting system of IWG than annual wheat. The IWG rooting system exceeding that of annual wheat was recently confirmed by Sprunger et al. (2018), where fine root biomass was significantly higher in IWG than annual wheat at 40-70 cm and coarse root biomass was slightly higher at 70-100 cm soil depth. Despite our results demonstrating higher diversity values than annual wheat at depth and previous work reporting that IWG has greater root biomass than annual wheat both at the surface and at depth (Sprunger et al., 2018; Duchene et al., 2020), we observed no consistent differences in rhizosphere fungal and bacterial diversity values between IWG and annual wheat. While this was contrary to our hypothesis, a similar trend was observed by Yin et al. (2017) who found that when reducing tillage in semiarid annual wheat, rhizosphere communities were buffered and/or protected against tillage effects and thus affected less than the bulk soil communities. It is critical to note that microbial diversity calculations are simply a starting point for further inquiry and that microbial diversity does not necessarily correlate with the health or stability of soil microbial communities (Shade, 2016).

4.2 Fungal and bacterial community composition cluster differently among crop types

After three seasons, IWG shallow bulk and rhizosphere soil fungal community composition was distinct from annual wheat, but soil bacterial community composition was not (Fig. 3.1, Table 3.2). Such differences in responses of bacterial and fungal community composition were similar to the recent findings of Duchene et al. (2020), which found that fungal and AMF fatty acid markers responded to annual vs. perennial grain crop treatments after two years, while bacterial fatty acids did not. A similar lack in bacterial response to annual vs. perennial production was found by Mackelprang et al. (2018), where corn and three-year-old switchgrass stands had similar bacterial community composition. The weak response of bacterial community composition to crop type is also consistent with the findings of Sprunger et al. (2019), where variation in bacterial community structure was not significantly influenced by plant type (annual wheat vs. IWG) until the fourth year of the experiment. This suggests that perennials may need to be fully established (>3 years) before changes in bacterial community structure are detectable. Given that samples from this experiment were collected early in the third year of production, it is plausible that the plants were not yet established to the point where changes in bacterial community composition were observable.

Conversely, our results demonstrate that three years is ample time for distinct fungal communities to form between annual wheat and IWG. McKenna et al. (2020) also found clear differentiation in fungal community (specifically the saprotroph and symbiotroph community members) between IWG and a winter wheat-sorghum-soybean rotation. This differential response among bacterial and fungal community composition to crop type could be due to fungi and bacteria having different environmental filters. For example, a recent review conducted by Rodgers et al. (2021) presents studies from dryland wheat, irrigated wheat, and semiarid grassland systems that demonstrate N fertilization may be a strong environmental filter of soil bacterial communities where fungi may be more sensitive to plant growth, plant community and soil organic carbon. Sprunger et al. (2019) corroborates this with their finding that nitrogen additions were a stronger environmental filter than plant type on bacterial communities. The environmental filter hypothesis is consistent with our results, given that both annual wheat and IWG received an equal amount of nitrogen fertilization while perenniality and associated tillage differences (i.e., crop type) were the only environmental filters applied. More specifically, the contrasting chemical composition of IWG and annual wheat's root exudates could have directly influenced the differences in soil fungal community.

4.3 Fungal and bacterial community composition cluster differently among soil depths

Within the soil fungal community, crop type explained more variation in community composition when comparing soil compartments (rhizosphere vs. shallow bulk soil), but soil depth explained more variation than crop type when considering the entire bulk soil depth (0-90 cm) (Table 3.2, Table 3.3). This decrease in crop type effect in soil fungal community composition with increasing soil depth aligns with our hypothesis and with a study that found there were differences in microbial community structure between cropland and grassland sites, but the differences generally diminished with increasing soil depth (Culman et al., 2010).

In contrast to the soil fungal community, soil bacterial community composition was not significantly clustered by crop type, but only by soil depth or soil compartment (Fig. 3.1, Fig.3.2, Table 3.2, Table 3.3). Soil depth can serve as a proxy for soil biogeochemical and physical gradients and has also been shown in other studies to be a strong factor in shaping soil microbial communities (Fierer et al., 2003) within or across different crop types (Zhang et al., 2017; Schlatter et al., 2018; McKenna et al., 2020). While both fungal and bacterial community composition was significantly influenced by soil depth, we observed that bacterial communities clustered into four distinct depth intervals while soil fungi communities only clustered into two intervals (Fig. 3.2; Table 3.3). Regardless of clustering differences between bacterial and fungal communities, soil depth consistently explained more variation in community composition than crop type across depths (Table 3.3). This is particularly relevant for the communities present in the depth of bulk soil that is tilled annually in the annual wheat system (i.e., 0-30 cm). Namely, significant variation in community composition between the two crop types at soil depths where tillage occurred (i.e., rhizosphere, shallow bulk soil, and 15-30 cm) would be expected if tillage were the primary or only driver of microbial community composition in this study.

The large effect of depth on soil communities can be attributed, in part, to differences in impacts of tillage at depth, but may also reflect differences in how fungi vs bacteria respond to soil resource availability (i.e., C, pH, N, Ca, pH, Mg, etc.) and root morphology which vary with increasing soil depth. Decreases in root exudation and changes in root morphology (decreased specific root area, increased fine root diameter, and increased tissue density) with increasing soil depth has been recently observed in European beech trees (Tückmantel et al., 2017) and IWG (Duchene et al., 2020) and this may have implications for soil carbon availability throughout the soil profile. For the fungal community specifically, stratification of carbon derived from organic residues associated with above and belowground biomass throughout the soil profile could have led to the observed differences in fungal community composition in the first 30 cm vs. 30-90 cm depths (Fig. 3.2). Changes in fungal community composition has long been known to follow a succession of decomposing residues (Sadasivan, 1939), where stratification of fungal communities reflect the quantity and composition of available carbon sources throughout the soil profile (Schlatter et al., 2018). For instance, carbon at the surface, derived primarily from plant residues, consists of more labile organic compounds whereas carbon deeper in the profile, associated with plant roots and soil organic matter, is more recalcitrant (Schlatter et al., 2018). In no-till wheat fields, Schlatter et al. (2018) found that fungal communities were highly stratified by soil depth and distinct fungal taxa inferred to have saprotrophic lifestyles were harbored at deeper depths (Schlatter et al, 2018). A similar phenomenon may have been going on in our study where fungal taxa most competitive at decomposing the simple and labile C substances associated with rhizodeposition (Derrien et al., 2004) and plant litter were present above 30 cm. Conversely, taxa more competitive at degrading more recalcitrant forms of carbon, such as the particulate organic carbon fraction composed in part by belowground roots (Villarino et al., 2021), were present below 30 cm. The fungal communities detected deep in the soil profile in this study (i.e., > 60 cm) may also include inactive spores or relic DNA (Carini et al., 2016).

4.4 pH, labile soil carbon and active microbial biomass relationship to soil microbial community composition

High pH values in the annual wheat system at the 30-60 cm soil depth were the only measured soil property that had a significant relationship with soil fungal and bacterial community composition. Numerous studies have also found that soil pH strongly influences soil microbial community composition and assembly (Dumbrell et al., 2010; Fan et al., 2018 ; Qi, Daihua, et al., 2018; Wang et al., 2019 ; Tan et. al., 2020). Total active microbial biomass (total PLFAs) was higher in IWG than annual wheat at all depths and total PLFA biomass appeared to have the most apparent relationship with soil microbial community composition in the top depth of IWG. However, a recent study found that increased values of active microbial biomass in the topsoil is largely a function of both decreased soil disturbance and plant vigor, not just perennial root presence (Table S1.3). Tilled annual wheat and IWG had similar values of POXC and organic matter across depths, thus the relationship between these soil measurements and soil microbial community composition was not crop type specific, but rather a function of higher values of O.M. and POXC in the topsoil. Tian et al. (2017) also found that labile soil carbon was related to microbial community composition and other studies have reported no significant differences in POXC values between tilled annual wheat and IWG systems (Table S1.3; Culman et al., 2013).

4.5 Differences in fungal community composition and F/B ratio in IWG vs. annual wheat does not correspond to greater soil carbon at depth in IWG

IWG selected a fungal community composition different from that in annual wheat in the rhizosphere, shallow bulk soil, and across soil depths (0-90cm). It also had a higher F/B ratio than tilled annual wheat at the 15-30 and 30-60 cm soil depths and higher fungal richness at soil depths greater than 60 cm. We found that at the 30-60 cm soil depth, IWG had a high abundance of the fungal genus *Solicoccozyma.* Part the fungal phylum known as

Basidiomycota, which is a known fungal decomposer (Osono & Takeda, 2006), *Solicoccozyma* is an ectomycorrhizopheric yeast that is adapted to wide range of pH (Grządziel & Gałązka, 2019) and may positively influence plant growth (Wang et al., 2021). However, while IWG did select a fungal community composition different than annual wheat across soil depths, have high abundance of a known fungal decomposer at 30-60 cm, and increase the F/B ratio below the 15 cm soil depth relative to annual wheat, this did not translate to significantly greater soil carbon concentration or mass at any soil depth relative to annual wheat.

While an increased F/B ratio in IWG did align with our hypothesis and the findings of Frey et al. (1999), which reported reduced tillage increased the proportion of total biomass composed of fungi, this did not translate to significant increases in soil carbon mass in IWG. Moreover, IWG had lower soil carbon concentration and carbon mass than tilled annual wheat at depths where the F/B ratio was higher than tilled annual wheat. This result partially contrasts with the study by Malik et al. (2016) and Rogers et al. (2021), which proposed higher fungal dominance could lead to higher soil carbon storage potential. However, Malik et al. (2016) and Bailey et al. (2002) both highlight the importance of microbial activity (i.e., respiratory losses) for soil carbon storage, such that Bailey et al. (2002) found fungal activities were associated with increased soil carbon more so than F/B ratios. Considering IWG has demonstrated higher PLFA biomass and mineralizable soil carbon than tilled annual wheat at depths below 15 cm (Fig. S3.3, Table S1.3) and greater soil carbon mineralization processes than tilled annual wheat at 30-60 cm soil depths (Fig. 1.6), perhaps IWG is losing soil carbon via microbial processes at depth within the three-year period of this experiment. This would align with Shahzad et al. (2018) and Bernal et al. (2016) who suggest exploitation of deep soil layers and additions of organic carbon in deep soils, which may be achieved by the deep roots of IWG, may result in a priming effect where soil carbon is lost in the subsoil.

4.6 Annual wheat has high abundance of fungal taxa associated with total soil organic carbon and higher soil carbon at depth

While annual wheat had high relative abundance of the fungal pathogen *Mycosphaerella tassiana* (Fareed Mohamed Wahdan et al., 2020) at the 30-60 cm depth, it had greater soil carbon and relative abundance of the fungal genus *Cistella* than IWG at the 60-90 cm soil depth (Fig. 3.7). *Cistella* is part of the fungal phylum *Ascomycota*, which like *Basidiomycota,* has previously identified as a major fungal decomposer (Štursová et al., 2012). Furthermore, *Cistella* was recently shown to positively correlate with total soil organic carbon in rice systems treated with biochar from wheat straw feedstock (Wang et al., 2021). Greater soil carbon concentration and mass (Fig. 3.4, Fig. 3.5) at deeper soil depths align with a meta-analysis that found conversion of conventional tillage to no-tillage increased soil carbon in the top 10 cm but resulted in a decline of soil carbon relative to conventional tillage at soil depths of 20-40 cm (Luo et al., 2010). Additionally, a recent study by Vilakazi et al. (2022) found that when fertilized at nitrogen rates above 60 kg N ha^{-1}, conventionally tilled systems had higher total soil carbon than a no-till system at a 10–20 cm soil depth. Angers et al. (1997) proposed that conventional tillage vertically distributes soil carbon, such that the concentration of soil carbon increases with tillage at depth. Once this carbon is redistributed to the subsoil, it may be protected from biotic and abiotic losses (Jobbagy & Jackson, 2000; Pries et al., 2018). Furthermore, Luo et al. (2010) proposes that in addition to physically distributing the carbon into deeper soil layers, tillage loosens the soil to depths as deep as 50 cm and changes soil physical conditions. These changes could increase downward movement of soil carbon into the subsoil, whereas no-till systems with high soil strength could reduce downward movement of surface soil carbon (Luo et al., 2010).

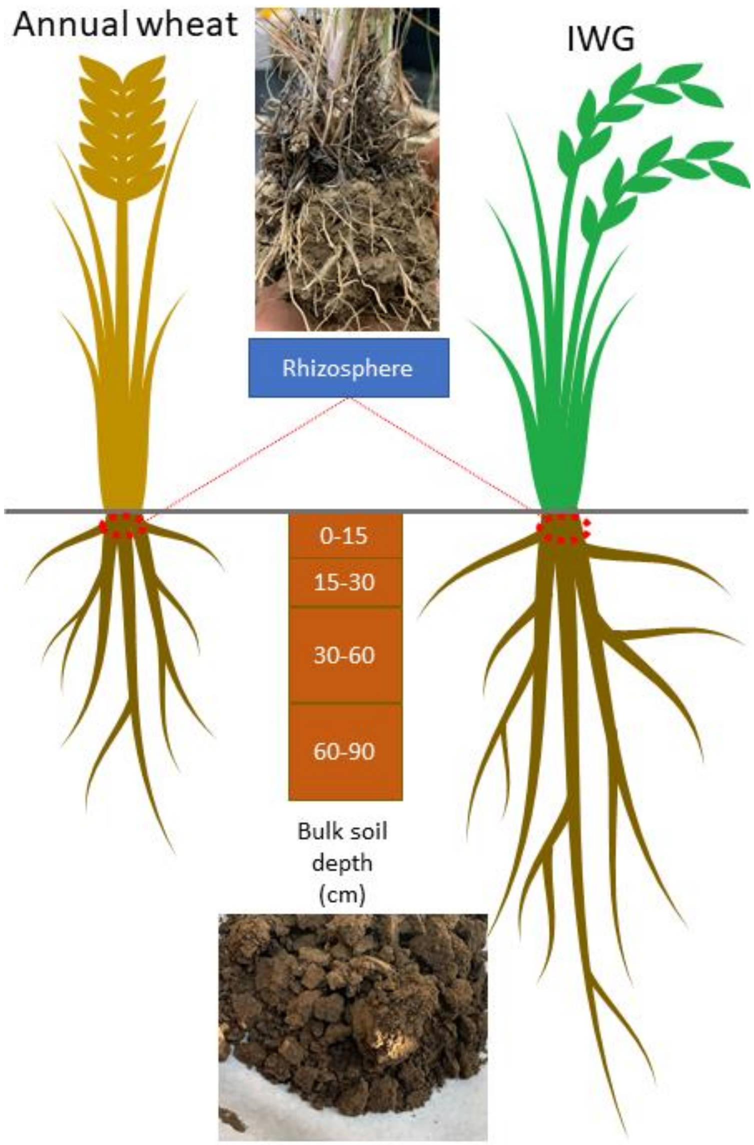
5. Conclusion

We found differences in responses of bacterial versus fungal communities to the presence of annual versus perennial grain crops. Bacterial community composition was far more sensitive to soil depth than crop type. In contrast, fungal community composition was most influenced by annual versus perennial crop types and differences were more evident in rhizosphere and shallow bulk soil (0-15 cm) than total bulk soil (0-90 cm). In IWG, fungal richness was higher at soil depths greater than 60 cm and a higher F/B ratio was detected at greater than 15 cm depths in IWG than annual wheat. These trends did not, however, correspond to higher soil carbon mass in IWG at depth, as carbon mass was higher in annual wheat than IWG at the 60-90 cm depth. Differences in labile soil carbon and organic matter were minimal between IWG and annual wheat, and pH was the only measured soil variable that was significantly associated with microbial community composition. We know soil microbial communities are the drivers of many ecosystem services; however, these linkages are not always easy to demonstrate. Understanding the functional significance of differences in microbial community composition requires other methods, such as metagenomics, that may help explain the soil carbon trends we observed in IWG and annual wheat. Even still, our study contributes novel information about how soil carbon mass and microbiomes differ between annual and perennial grains, particularly at depths where deep-rooted perennials are proposed to support large and active soil communities.

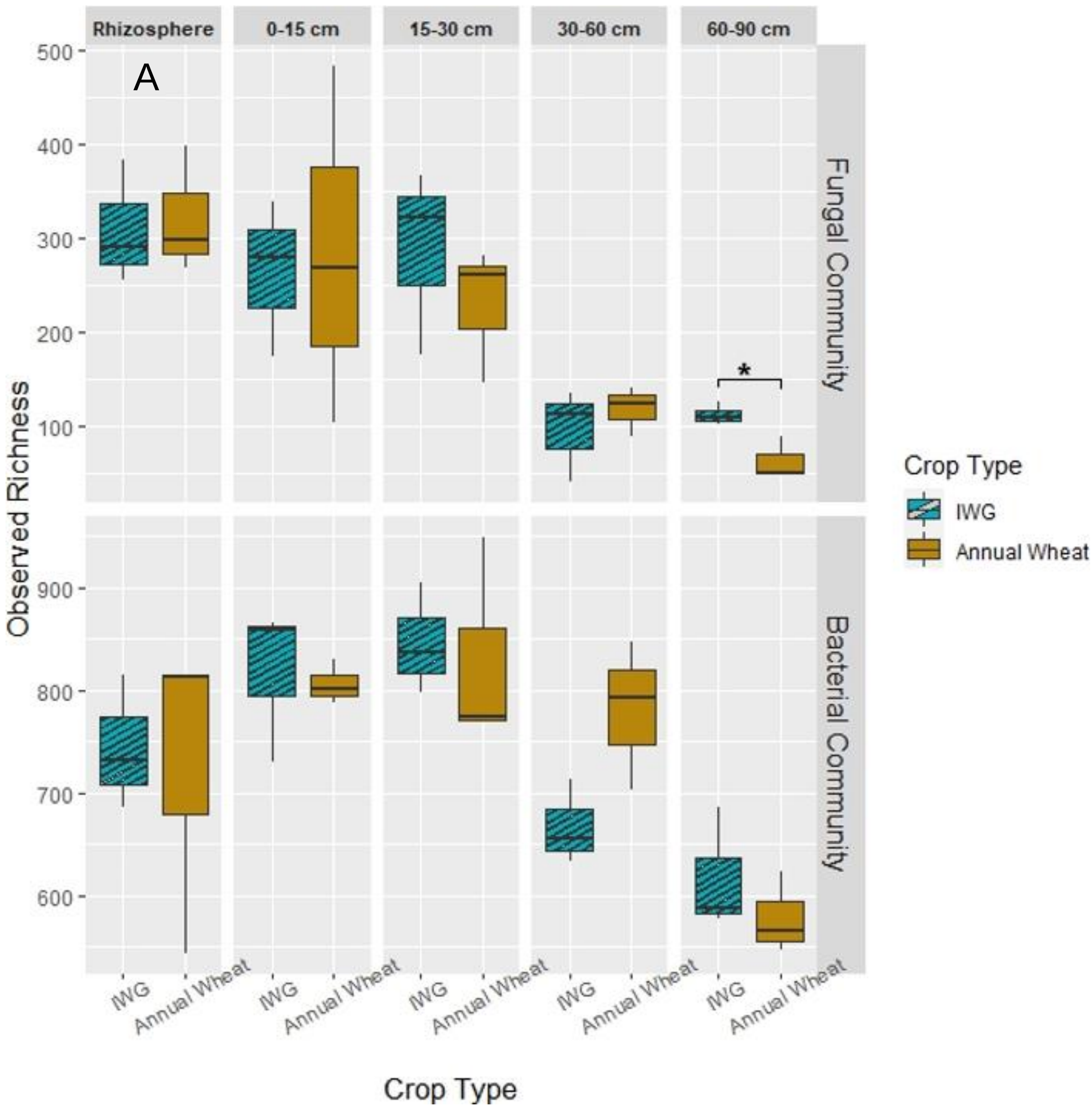
6. Data accessibility statement

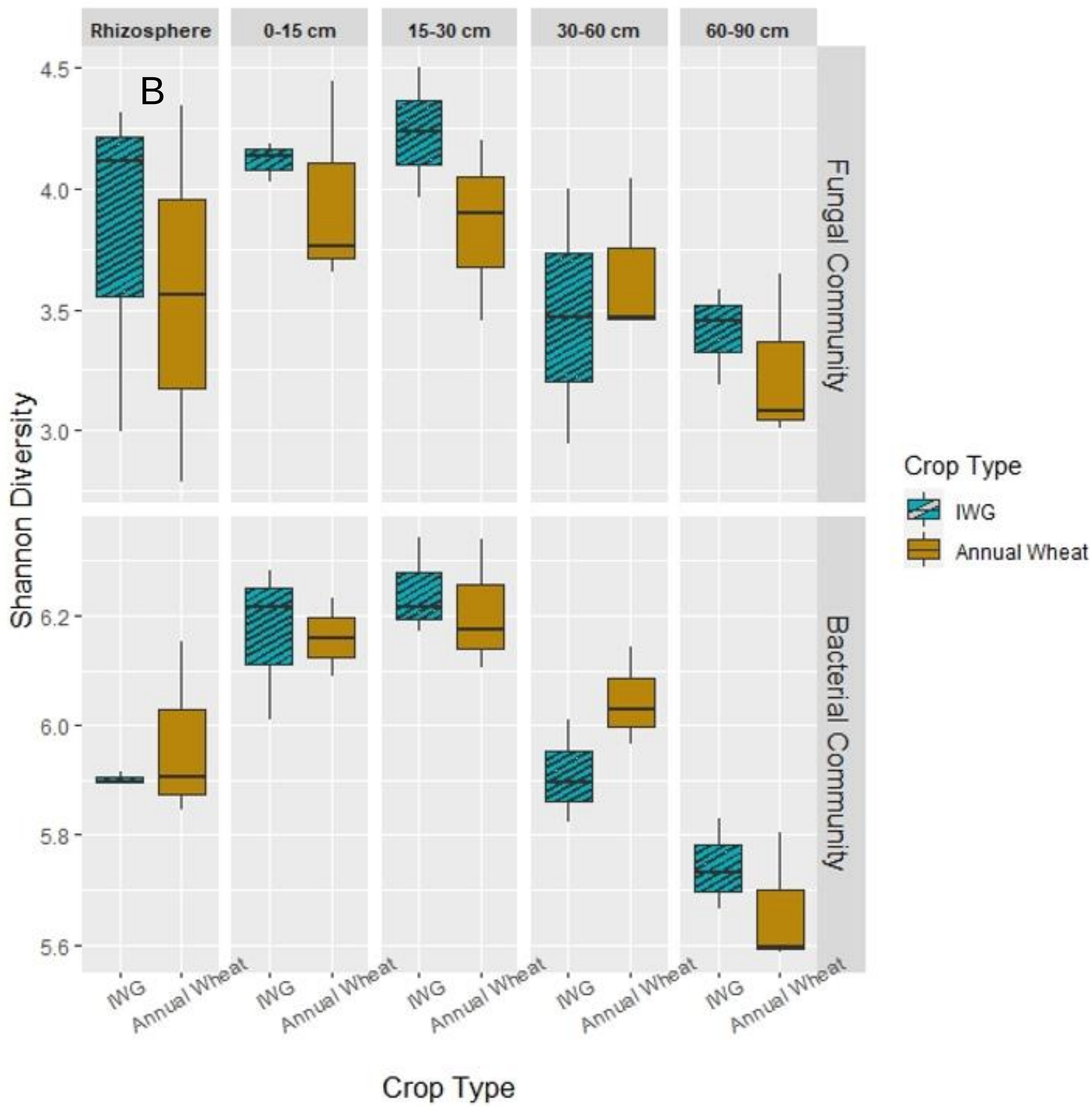
Raw sequence files are available on the NCBI SRA (BioProject PRJNA801357).

7. Appendices

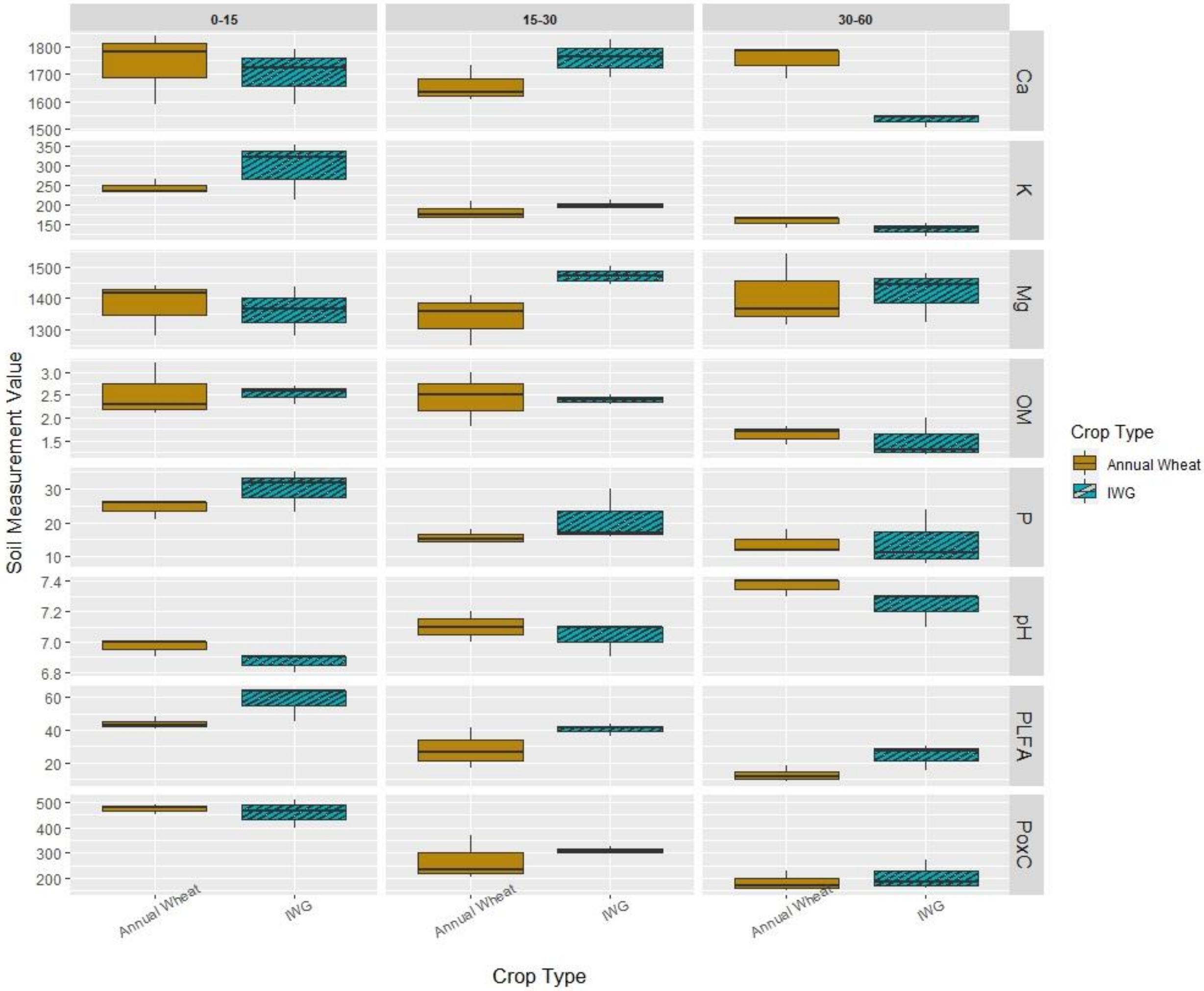


Supplementary Figure 3.1. Graphical representation of how samples were collected from the rhizosphere and bulk soil (0-15,15-30,30-60,60-90) depths in annual wheat and IWG.


Rhizosphere
0-15 cm
15-30 cm
30-60 cm
60-90 cm
A
500
400
300
200
100
900
800
700
600
*
Fungal Community
Bacterial Community
Observed Richness
IWG
Annual Wheat
Crop Type
Crop Type
IWG
Annual Wheat



Supplementary Figure 3.2. Effects of crop type (IWG vs. annual wheat), soil depth (rhizosphere, 0-15, 15-30, 30-60, 60-90cm) on observed species richness (a) and Shannon diversity (b) metrics. Asterisks indicate statistical differences * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.



Supplementary Figure 3.3. Soil calcium (Ca, ppm), potassium (K, ppm), magnesium (Mg, ppm), organic matter (OM, %), phosphorus (P, ppm), pH, total phospholipid fatty acid biomass (PLFA, nmol g^{-1}), and permanganate oxidizable carbon (PoxC, mg kg^{-1}) in each crop type (tilled annual wheat, IWG) at each soil depth (0-15, 15-30, 30-60 cm).

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Conclusions

In chapter 1, we showed that IWG yielded 28% less AGB than annual wheat (tilled and no-till annual wheat) over the course of three years. Reduction in soil disturbance (comparing no-tillage vs. tillage) contributed foremost to greater microbial biomass abundance in the topsoil by the final season of the experiment, while crop type (annual vs. perennial) had the greatest influence on whether microbial communities tended toward stabilizing versus mineralizing soil carbon. This was evidenced by higher stabilization processes being primarily driven by the increased plant productivity of annual wheat compared to IWG, while lack of tillage contributed secondarily. Overall, no-till wheat was associated with higher microbial biomass and higher soil carbon stabilization in topsoil than IWG and tilled annual wheat systems, presumably by matching tilled annual wheat in plant productivity and IWG in lack of soil disturbance.

In chapter two, we found IWG produced 35% of the grain yield of annual wheat systems in the single year it produced grain. The aboveground and grain NUE of IWG varied more than 60% between experimental years, while annual wheat varied by less than 20%. In the top 30 cm of soil and only when fertilized with 112 kg N ha^{-1}, IWG and no-till annual wheat had an average of 17% greater soil carbon mass by the third year of the experiment and an average of 5.5 Mg ha^{-1} more in soil carbon gains (Δ soil carbon mass) within 4 years relative to tilled wheat. However, below the 60 cm soil depth and across N rates, the tilled annual wheat system had 40-48% more soil carbon mass than the conservation systems and was the only system that significantly gained soil carbon mass within four years. Our results imply plant productivity led to increased provisioning services and decreased agricultural disservices as measured by increased grain yield stability and NUE in annual wheat, respectively. Furthermore, our results highlighted that two conservation agriculture systems, no-till wheat and IWG, only partially addressed regulating services considering their soil carbon gains were contingent upon nitrogen fertilization and constrained to the top of 30 cm of soil. Conversely, the more productive tilled annual wheat systems, in the Mediterranean climate of CA, may contribute to greater climate regulation by storing carbon in the subsoil, regardless of N fertilization.

In chapter three, bacterial community composition was far more sensitive to soil depth than crop type, whereas fungal community composition was more influenced by crop type with larger differences more evident in rhizosphere and shallow bulk soil (0-15 cm) than throughout the bulk soil (0-90 cm). Fungal community richness was greater in IWG than annual wheat at depth (≥ 60 cm). While IWG had a different fungal community composition, higher fungal richness, and higher F/B ratio at soil depths exceeding 15 cm, soil carbon concentration and mass were either similar or lower than annual wheat across soil depths. Despite a lower F/B ratio, tilled annual wheat had a higher abundance of fungal taxa that have been shown to positively correlate with soil carbon, higher soil carbon concentration, and higher soil carbon mass than IWG at 60-90 cm soil depth. This suggests that differences in fungal community composition did not translate to significantly higher soil carbon mass in the topsoil or at depth.

A broader impact finding of our study was plant adaptation and subsequently plant productivity, was critical for providing consistent provisioning services, in our case in the form of aboveground yield and nitrogen-use efficiency. Furthermore, we discovered that a combination of plant productivity and lack of tillage within the topsoil increases active soil microbial biomass, carbon stabilization processes, and ultimately increases soil carbon storage when fertilizer N is applied. Conversely, when tilled, even treatments with higher aboveground biomass were associated with decreases in active microbial biomass and soil F/B ratios. And in those cases, soil carbon mineralization was higher in surface layers and more soil carbon was stored in the subsoil. While the novel perennial grain IWG selected a fungal community composition different from that in annual wheat, with higher fungal richness and F/B ratios at depth, it was unable to sustain stable yields and aboveground NUE or store soil carbon at depth.

To conclude, two major takeaways from our three-year field experiment are as follows. First, sampling the subsoil (> 30 cm) is crucial when comparing system-level soil carbon storage potential of annual and perennial grain systems. Second, plant productivity underpins numerous components of agricultural multifunctionality, and this highlighted the importance of regional plant adaption when designing and implementing future food systems. An on-going challenge for novel crops like IWG is that breeding and adaptation activities have been concentrated in the Midwest U.S. and not considered other, such as Mediterranean, climates. As California is expected to experience more frequent heatwaves, a decline in winter chill hours, and be 15 to 35% drier by the year 2100 (Pathak et al., 2018), it is increasingly important that new alternatives evolve cropping systems better adapted for these conditions. Based on our results, the TLI-C5 germplasm of IWG used in this experiment is not well adapted to the drought conditions in northern California and more research will be needed to see if other management practices and future IWG germplasms show greater potential in our region.