Biomass yield and soil microbial response to management of perennial intermediate wheatgrass (*Thinopyrum intermedium*) as grain crop and carbon sink

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Dedication

To all of my family and loved ones.

Abstract

IWG intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey; IWG) is a perennial grain crop with an extensive root system that could prevent erosion and nutrient leaching, build soil fertility, and potentially sequester atmospheric carbon. Two field experiments were established in southeastern Minnesota, USA to 1) address management strategies for preventing IWG grain yield decline, 2) quantify plant biomass production of IWG relative to other conventional field crops, 3) determine how this perennial grass may alter soil microbial activity and composition to the benefit of soil organic carbon (SOC) accumulation, and 4) investigate the potential for carbon sequestration. Inter-row cultivation using rotary-zone tillage (RZT) as well as herbicide, burning, and mowing at different times were employed for two years, but none effectively prevented grain yield decline. However, the application of herbicide in spring and fall cultivation had positive impacts on grain, straw, and forage yield (relative to other management treatments). IWG can produce significantly more root biomass than annual grain crops, and even more than alfalfa (*Medicago sativa).* These large root systems, left undisturbed, were likely responsible for an increase in soil fungal biomarkers and overall microbial biomass after three years of growth. Soil respiration rates and microbial biomass-carbon were greater under IWG in the spring and fall seasons when soybean and wheat had either not emerged yet or were already senesced, providing evidence that perennial grasses may alter soil nutrient cycling by expanding and prolonging soil microbial activity. Particularly, changes in nutrient cycling that increase labile

carbon pool sizes and promote SOC formation may, in addition to storage in root biomass, support carbon sequestration in agricultural soils.

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Introduction

Agricultural production in the United States (U.S.A.) has shifted to much larger farming operations (Macdonald et al., 2018) that are more reliant on chemicals for nutrient and pest management (Hellerstein et al., 2019), and more mechanized (NAE, 2000). This has increased food production but also environmental risks of soil and nutrient erosion, contamination of water resources (Hellerstein et al., 2019), and production of greenhouse gases (Melillo et al., 2014). One strategy to address this challenge is to look for new food crops that can enhance natural resources in addition to producing marketable food. Kernza® intermediate wheatgrass (*Thinopyrum intermedium*; IWG) is a perennial cool-season grass being developed by The Land Institute (TLI) for use as a perennial grain crop (L. DeHaan et al., 2018) that can provide multiple ecological services (Cox et al., 2006; Culman et al., 2013). The University of Minnesota has partnered with TLI to expand the breeding effort for improved grain yield. Continuous living cover, reduced soil disturbance, extensive root biomass production, and reduced input costs are all advantages that IWG can have over other annual grain crops, three of which are considered as management solutions to soil quality problems (Karlen et al., 2001).

Long-term sustainability of food crop production is dependent on the availability of fertile soil and clean water, and subsequently on the capacity for crop fields to retain these resources. The rich natural resources of northern latitudes like the upper Midwest USA are mostly attributed to the benefits of thousands of years of perennial vegetative growth following the end of the most

recent glaciation of the northern hemisphere more than 11,000 years B.P. The formation of rich wetlands, prairie, savannah, and forest ecosystems that built this wealth of fertility were promptly liquidated by European colonists and converted to crop land, leading to the losses of 25-60% of soil organic carbon (SOC) (Lal, 2003, 2011). This loss of SOC from the fracturing of ecosystems continues today in other parts of the world such as the draining of peatlands that are converted to cropland, resulting in the oxidation of long stored SOC (IPCC, 2014).

The conversion of a natural ecosystem (grassland, peatland, forest, etc.) into cropland removes biomass and disrupts soil structure, leading to rapid oxidation and loss of older carbon through microbial respiration (Abraha et al., 2018). After this initial phase of conversion, which might persist for a year or more, net losses of SOC slow by orders of magnitude and soil respiration responds to a new equilibrium of frequent disturbance that is typical of cropland, and in which annual precipitation and temperature more strongly influence net gains or losses of SOC (Abraha et al., 2018; Lal, 2004; Stockmann et al., 2013). However, the carbon lost is still lost to the atmosphere, and a new equilibrium for the annual soil respiration of one location does not change the damages done to the soil or the atmosphere. Future agriculture must establish new strategies that reverse the trend of soil "mining" and instead rebuild resources that are critical to longevity of the food system.

Rapid accumulation of greenhouse gases (GHGs), particularly carbon dioxide (CO_2), but also nitrous oxide (N_2O) and methane (CH_4) in the atmosphere has resulted in global change including warming annual temperatures, especially

winter temperatures of the northern latitudes (Angel et al., 2018; Melillo et al., 2014). Unprecedented changes in weather patterns have led to severe flooding, drought, rising sea levels, wildfires, and other environmental disasters that can have devastating impacts on agriculture, society, and the global economy. In the Midwest U.S., increasing annual precipitation and warmer winter temperatures are creating greater risks of spring nutrient runoff, erosion, flash frosts, and pest proliferation (Angel et al., 2018), which also result in negative consequences for producers and consumers.

The employment of diverse strategies can strengthen agriculture systems to simultaneously provide food while protecting and even building natural resources. The rapidly growing human population, shrinking wildlife habitat, and emergent threats of global climate change such as rising sea levels, pollinator decline, and increased flooding and drought are revealing weaknesses in the management of natural resources and food production (Benckiser, 2010; Easterling & Apps, 2005). In the upper Midwest U.S., increasing annual precipitation, especially of higher intensity that produce flash flooding, over the last several decades has resulted in significant economic losses (Melillo et al., 2014). This increased erosion risk of soil and nutrients combined with rising winter temperatures and prolonged, wet spring weather have compounded farmer's already challenging task (Angel et al., 2018). To combat these challenges, cover crops, continuous living covers (CLC), reduced tillage, agroforestry, and alternative food crops are all examples of methods that are being used today to improve agricultural land and build its resilience against

unforgiving weather. However, the livelihood of farmers is not immediately supported by these measures and often they are employed as more of a dedicated commitment to the future. Everyone wants to protect their crops and soil from damage, but farmers must make a living at the end of the day and financial incentives are not widely available to make big changes to an operation feasible.

IWG has generated interest in the food industry, has many potential environmental benefits, but still faces obstacles to commercialization (Culman et al., 2013; L. DeHaan et al., 2018; Jungers et al., 2019). Perennials like IWG contain many traits that limit grain yield and do not offer the dependability of crops like corn and wheat that have been domesticated for thousands of years. The challenge is to increase seed yield of IWG and maintain its perenniality by artificial selection in an agricultural environment (DeHaan et al., 2005). Lower grain yield presents a market challenge to this strategy of mitigating climate impacts, and thus a viable market and economic potential must be considered in parallel with goals of environmental quality (Howden et al., 2007). IWG has drawn publicity as a great candidate for sustainable food, already appearing in limited release beer products, snacks, and bread. The idea of sustainable food—implying that its production costs are balanced by ecological provisioning such as increased soil protection or nutrient availability—has become a successful marketing strategy in response to consumer demand for healthier and more environmentally beneficial food production systems (Crews et al., 2014).

IWG may struggle from intraspecific competition for soil nutrients, water, and sunlight, and is otherwise disadvantaged by its long-stemmed morphology

that can easily lodge with heavy seed heads. Different management practices, including plant growth regulators (Frahm et al., 2018), different nitrogen fertilization rates (Jungers et al., 2017), and intercropping (Tautges et al., 2018) have been investigated to help improve and maintain IWG grain yields. Consistent with other field crops, an optimum range for nitrogen fertilization rates has been established, however, this may change as new germplasm is released that exhibits more tolerance or sensitivity to fertilization. Intercropping with a legume was successful in moderating grain yield declines, possibly due to a reduced need for mineral fertilizer inputs that can increase lodging (Frahm et al., 2018; Tautges et al., 2018) or because of physical separation of IWG plants. Precision termination, suppression, or other inter-row management, including tillage, herbicide, and mowing, should be investigated to prevent root competition among plants and reduce plant lodging.

The improved management of perennial IWG may lead to increased grain yields and the potential for a viable commodity crop that could enhance natural resources. This thesis reports results of implementing new management strategies in an established IWG stand, determine the relative changes IWG has on soil microbial activity and its capacity for carbon retention, as well as a novel method of measuring soil respiration in perennial grass systems.

Chapter 1

Managing perennial intermediate wheatgrass (*Thinopyrum intermedium*) stands to prevent grain yield decline and retain soil microbial activity

Summary

IWG intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey; IWG) is a perennial cool-season grass that is a candidate dual-use grain and forage crop with the potential to improve environmental sustainability in field crop agriculture. New management strategies were tested in a field experiment located in southeastern Minnesota, USA, for IWG maintenance of biomass and grain yield persistence, in addition to the effects these management practices would have on soil microbial activity. Inter-row cultivation, glyphosate herbicide, burning, and mowing were applied at different physiological stages during the growing season for two years on an established IWG stand to evaluate their effects on grain and biomass yield, weed production, and soil microbial properties. Grain and straw yields were highest in the year preceding any application of management treatments, however, root biomass and spring forage biomass increased in the 3rd and 4th production years, respectively. In the 4th production year (2019), after two years of application, fall-applied herbicide resulted in the lowest grain yield and highest weed pressure, and both spring-applied herbicide and fall cultivation had the highest grain yield. IWG plots that received the greatest levels of soil disturbance (fall + spring cultivation) and that were burned had the highest soil microbial biomass in 2019 for three separate lipid biomarkers.

Introduction

Intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey; IWG) is a sod-forming perennial grass that is being domesticated to serve as a perennial grain crop (cite). One agronomic characteristic that limits the long-term viability of this perennial crop is that grain yields can decline with stand age, and the rate of grain yield decline has been shown to vary across environments (cite Hunter and Jungers). For example, Jungers et al. (2017) found that grain yields declined during the third production year after fall seeding while spring seeded stands declined during the second production year. (L. DeHaan et al., 2018; Tautges et al., 2018). Once IWG is established it begins to spread vegetatively, producing tillers that may crowd neighboring plants and increase competition for light or other resources. This growth habit, along with seedlings emerging from shattered seed, can also create a thick mat of roots in the soil surface (0-10 cm) that may exacerbate the competition for water and nutrients among plants.

Mechanical disturbance or chemical suppression (glyphosate herbicide) between planted rows may limit plant competition and result in sustained grain yields of IWG as stands age. Glyphosate has been used with success to manage invasions of Kentucky bluegrass, a perennial grass species, especially in areas that were densely populated with the grass (Ereth et al., 2017). Because IWG stands can become overpopulated in a similar way, banded herbicide may be effective in reducing plant population density. Two studies testing the effects of different

tillage and herbicide applications on precision termination of a living mulch were conducted on Kura clover (*Trifolium ambiguum*) as a perennial living mulch under corn (*Zea maize*). These studies found that rotary-zone tillage (RZT), meaning precision strip tillage with a rotovator implement, was more effective than herbicide and strip tillage with shanks in supporting corn emergence and yield within the rows where Kura clover had been terminated (Dobbratz et al., 2019; Ginakes et al., 2018). Because these studies illustrated how RZT was an effective method for precision termination of an established perennial plant, it is possible this method could also be effective in IWG systems to reduce competition between planted rows.

However, these precision termination practices could also have negative effects on soil microbial communities such as decreased bacterial diversity from tillage (Lupwayi et al., 1998) or suppression of arbuscular mycorrhizal fungi from glyphosate (Druille et al., 2013). Soil tillage can have varying effects on soil characteristics depending on tillage method, soil texture, parent material, climate, and timing of application (Strudley et al., 2008), but in general can have effects of reducing soil aggregate stability (Pagliai et al., 2004), increasing the volume of macropores in the topsoil, and decreasing pore connectivity between topsoil and subsoil (Abdollahi et al., 2013; Goss et al., 1984). The repeated disturbance of soil from tillage (usually occurring twice per year at minimum) can also result in significant changes to the soil biology, particularly in suppressing fungal mycelial growth (Beare et al., 1997; Daigh et al., 2019) and reducing bacterial community diversity (Lupwayi et al., 1998). Dobbratz et al. (2019) found no significant

impact of RZT on soil microbial biomass, but Ginakes et al. (2018) observed, in a similar experiment with RZT, an increase in microbial biomass and concurrent decrease in permanganate oxidizable carbon (POXC) over time that may have been linked to the incorporation of Kura clover biomass. Overall microbial activity has also been observed to increase in annual no-till systems compared to conventionally tilled systems using soil respiration (Franzluebbers et al., 1995) and soil dehydrogenase activity as proxy (Nivelle et al., 2016).

Cultivation and herbicide can be effective strategies for maintaining desired plant densities, while mowing and burning have been employed in managed perennial grasslands to simulate the natural ecosystem drivers of grazing and seasonal fire that initially suppress plant growth but can lead to enhanced productivity of perennial grasses (Hulbert, 1969; Towne & Kemp, 2003). In the case of this study, IWG was planted in rows for grain harvest. This is typical of small grain production, but as a perennial cool-season grass undergoing domestication, IWG grows a dense crown of roots, producing rhizomes and propagules that may compete with each other. The competitive ability of IWG was observed in a study comparing mixed and pure stands of several perennial grain crops where IWG was not outcompeted by, nor did it overwhelm, any of the companion crops tested (Weik et al., 2002). A competitive growth habit is important for stand establishment, but in IWG this competitive growth does not always apply to the establishment phase and, in fact, is often difficult to establish against weed competition. Once established, IWG may be overly competitive, causing a decline in grain yield for pure stands due to overcrowding of rhizomes.

The introduction of different types of disturbance may create conditions that would selectively suppress rhizome propagation or otherwise improve productivity within planted rows of IWG.

The goals of this study were to 1) distinguish the impacts of cultivation and herbicide timing and frequency as methods to reduce intraspecific plant competition, 2) determine if burning or mowing was effective in bolstering plant productivity over time, and 3) measure changes in soil microbial abundance and composition in response to these management practices. We hypothesized that inter-row cultivation and/or herbicide applications would improve plant vigor within planted rows by suppressing growth between rows. We also hypothesized that the introduction of burning would be more likely to improve plant productivity than mowing within our 3-yr time frame.

Materials and Methods

Study Area

This experiment was conducted in Rosemount, MN (44°71'N, 93°7'W) at the University of Minnesota Southeast Agricultural Research Station from 2016 to 2019. Soil type is Tallula silt loam, 6 % slope, eroded. Coarse-silty, mixed, superactive, mesic Typic Hapludolls. IWG ((Host) Barkworth & D.R. Dewey) was established in fall 2015 following soybean. IWG seed was sown using a no-till drill (Truax Company, Inc.) with 20.3 cm between rows. Openers were

blocked to achieve a planting configuration resulting in alternating 40.6 cm and 61 cm spacing between rows.

Experimental design

Treatments were applied in a split-plot, randomized complete-block design with four replications of management treatments. The main plot, or inter-row termination, treatments were inter-row cultivation (zone tillage; RZT) and glyphosate herbicide applications, all at three different timings (spring, fall, or spring + fall) and a control. The split plot, or suppression, treatments were spring mow, fall burn, or control. Plots had 6 seeded rows, each 3 m wide and 3.2 m long. All biomass and grain yield samples were collected from the two central rows. Plots were fertilized each spring with 80 kg N ha^{-1} as urea (Table 1.1 has management details).

The RZT implement for cultivation was a PTO-driven tool bar with three rotary units, each (Multivator®), in which each set of rotary units tilled a zone 40 cm wide and approximately 10 cm deep. Glyphosate herbicide was applied with a CO2 backpack sprayer and custom boom measuring 165 cm wide to cover two inter-row spaces at a time. Burning was performed on individual plots in November of each year using a butane fueled flame-weeder. Aluminum siding pieces measuring 1 m x 1.5 m were used as flame barriers to contain the fire within the treatment area. A Carter forage harvester was used to mow and removal all aboveground biomass within plots to a stubble height of 8 cm. Management details are listed in Table 1.1.

Plant biomass sampling

IWG grain and biomass were collected in August annually. Biomass and grain were collected from plants from sown seed within rows and volunteer plants between rows and weighed separately. Within row samples were collected by cutting two 103 cm lengths of parallel rows, equivalent to an area of 0.314 m^2. Between row (inter-row) samples were collected from the 61 cm space between planted rows. Weeds were also separated from each sample and weighed. A hydraulic Gidding's soil probe (ID = 3.8 cm) was used to extract root samples within and between rows. One core to a depth of 60 cm was extracted from each space and separated into four 15-cm depth increments. Root samples were washed of soil using a hydropneumatic elutriation system (Smucker et al., 1982), cleaned of sand and other non-root debris, and then dried and weighed.

Soil sampling

Post-harvest surface soil samples were collected using a hand probe. Five cores (ID = 1.8 cm) to a depth of 15 cm were taken in a diamond pattern (to capture inter-row and in-row space) and homogenized for each plot. All other samples were collected using a hydraulic Gidding's soil probe using the same protocol as described for roots. Soil was stored in a cooler during sampling before being returned to the lab where subsamples were stored at -20° C for lipid analysis, 4° C for microbial C and N analysis.

Microbial biomass C and N

Microbial biomass C was determined using a modified technique based on (Gregorich et al., 1990). Fresh soil sampled from 0-15 cm was sieved to 2 mm and 10 g ± .05 of soil weighed into acid-washed and muffled 70 mL test tubes.

Duplicates of each sample were made for a baseline and fumigated set of samples. Chloroform was added in the amount of 50 µl to each of the "fumigated" samples. Then 40 mL of 0.5 M K_2SO4 was added to both baseline and fumigated samples, tubes were capped and placed in a oscillator at 150 rpm for 4 h. After shaking, sample tubes were left to settle for 1 h before pouring the supernatant through 589/1 Whatman carbonless filters into 40 mL plastic acid-washed specimen cups. The filtered extracts were inspected for contamination before transferring the liquid into a labelled 20 mL plastic scintillation vial. Vials were frozen at -20° C before final trace carbon and nitrogen analysis. On day of analysis, fumigated samples were sparged to remove trace chloroform using a modified vacuum chamber. All thawed samples were subsampled into muffled 24 mL glass vials and diluted in water to a ratio of 20:3 and 1 mL of 2 M HCl was added before covering tops in aluminum foil in preparation for trace carbon and nitrogen analysis in a Shimadzu TOC-L. Carbon and nitrogen standards were diluted to 50 mg C or N L^{-1} using stock potassium hydrogen phthalate (1000 mg C/L KHP) and potassium nitrate (1000 mg N/L KNO_3). Microbial biomass carbon was calculated by the difference in mg C kg dry $soil^{-1}$ between a fumigated and a baseline set of the same samples. Microbial C in both sets was calculated by dividing the concentration of C in the extract solution by soil dry weight and then multiplying by a dilution factor (Eq. 2, A.4).

Lipid analysis

Extraction of lipids from soil was performed as described in (Oates et al., 2017). Briefly, 3 g of freeze-dried soil was weighed into labelled 40 mL

centrifuge tubes followed by an extraction with chloroform, methanol, and citric acid buffer. The extraction was repeated 3 times followed by overnight phase separation. The bottom (chloroform) liquid phase was transferred to 15 mL glass test tubes, followed by acid methylation to convert fatty-acids to fatty-acid methyl esters (FAME) before analysis on an Agilent 7890 gas chromatograph. Internal standards were used to convert peak areas of each fatty acid to nmol fatty acid g soil^{-1}. Individual lipids were used as biomarkers to indicate broad groups within the microbial community: 16 : 1 ω5c for arbuscular mycorrhizal fungi (AMF; Balser et al., 2005; Gutknecht et al., 2012); 18 : 1 ω9c and 18 : 2 ω6,9c for general fungi excluding AMF (general fungi, GF; Balser et al., 2005; Gutknecht et al., 2012); 16 : 1 ω7c and 18 : 1 ω9t for Gram-negative bacteria (Wilkinson et al., 2002); and 15 : 0 iso and 17 : 0 iso for Gram-positive bacteria (Wilkinson et al., 2002). Lipid biomass (nmol g soil^{-1}) was calculated by dividing the peak area by an internal standard constant (K value) and this result was divided by a ratio of fatty acid molecular weight to soil dry weight.

Statistical Analysis

All analyses were conducted with R version 3.4.4 (R Core Team 2018). Although 3-way interactions between main and split plot treatments and sample date were not always observed, interactions between date and both treatments individually were always observed, with the exception of microbial biomass data. Thus, for plant biomass and grain production, data were analyzed for each year separately using linear mixed-effects models (LME) to conduct two-way analysis

of variance (ANOVA) tests with block as a random effect (main plot treatment nested) and management treatments, main and split as fixed main effects. Degrees of freedom were lower for root biomass data because root data was only collected from a subset of main treatments across control split treatments. Means were compared by obtaining least-square means (package, code: emmeans, *emmeans*; Lenth 2019) and adjusted for Tukey's HSD test. Soil microbial data were analyzed using the same LME approach because sampling date was significant and, due to losing control samples from 2018, we were unable to combine all dates into a three-way ANOVA using date as a main effect. Means of microbial biomass were compared over the levels of treatment and were obtained using the same method described above. Non-significant ($P > 0.05$) interaction terms were removed from LME models and coefficient estimates were generated using a simplified model without interaction terms. Results from ANOVA tests are listed in Table 1.2. Assumptions of normally distributed residuals, independence of error and constant variance were checked for each linear model using qqplots, histograms of residuals, and plots of the residuals against fitted values.

Results

For all variable measured, interactions among inter-row termination and suppression treatments were only significant for grain and straw yield. Because nearly all variables measured also interacted strongly with year, data was analyzed by each year separately and the interactions among termination and

suppression treatments mentioned previously occurred only in 2019, the final treatment year. Grain, straw, and spring forage yields decreased from 2017 to 2018 and increased slightly in 2019. Root biomass increased slightly from 2017 to 2018 (although variance was high) and decreased in 2019. Weed pressure was measured in 2017 and 2019, with negligible pressure in 2017. Gram-negative bacterial lipid biomass was the only soil microbial variable that responded to any treatment; to burning (suppression) treatments in 2018 and 2019.

Grain

Grain yields were highest in 2017, lowest in 2018, and did not respond to management treatments until 2019 (Table 1.2, 1.3). In 2019, there was a significant interaction between termination and suppression treatments because plots that had not been mowed were more productive by this year (Figure 1.3). In 2019, spring herbicide and fall cultivation (in plots that did not receive mowing) resulted in the highest grain yields, averaging 220 ± 38 kg ha^{-1} and 213 ± 26 kg ha^{-1}, respectively (Table 1.3). However, the highest yields in 2019 were not significantly greater than the control, but spring herbicide was significantly greater than fall herbicide (for burned plots, $P = 0.049$, for plots not burned or mowed, $P = 0.019$). Fall herbicide applications, across all suppression treatments, resulted in the lowest grain yields of 2019.

Straw

A significant effect of suppression treatments was observed in 2017, before the initiation of these treatments, indicating that spatial variability (related to plant establishment, disease, or soil properties) and not the treatment had

introduced bias to these plots. A significant interaction between termination and suppression treatments was observed in 2019 (Table 1.2). At the time of grain harvest in 2019, spring mowed plots (that received fall cultivation, spring herbicide, and spring + fall herbicide) yielded significantly less straw than plots that were burned or that received no suppression treatment (Table 1.3, Figure 1.5). Because spring mowed plots in 2017 that were designated for spring herbicide and fall cultivation also yielded significantly less than burned, and less than both burned and control plots, respectively, the effects of these suppression treatments observed in 2019 may be confounded by other environmental variables not measured. Overall straw production was greatest in 2017 (Table 1.4).

Spring forage

Forage biomass was analyzed for only termination treatments, because all spring forage was "mowed" in the spring and no burning treatments were applied to plots where spring forage was harvested. A termination treatment effect was only observed in 2019 when spring herbicide, followed by fall cultivation, yielded the most forage; significantly more than fall herbicide ($P = 0.015$, $P = 0.034$, respectively), but not more than a control (Figure 1.3).

Weeds

The final treatment year (2019) was assessed for weed pressure to determine any cumulative impact of management practices over time. Weed biomass collected in 2017 was negligible in comparison to weed biomass from 2019. No interaction was found between termination and suppression treatments. Total annual weeds, including biomass collected from inter-row and in-row

spaces, at the time of 2019 grain harvest was greatest in the fall + spring and fall herbicide treatment plots (Figure 1.2). The control had significantly less weed biomass than plots which received spring ($P = 0.015$), fall ($P < 0.001$), and spring + fall ($P < 0.001$) applications of herbicide (Figure 1.2). The only difference in weed biomass between in-row and inter-row was that in-row weeds responded to the split treatment while inter-row and total weeds did not. For in-row weeds, the spring mowed plots had significantly more weed biomass than plots that were burned in fall ($P = 0.011$) and no differences from the control were observed (Table 1.6).

Roots

Root biomass, to a depth of 60 cm, was only collected from a subset of the termination treatments (all inter-row cultivations and the control), across only the controls of suppression treatments. Year was significant for both in-row and inter-row roots, and cultivation treatment was only significant for inter-row roots. No interaction was found between year and cultivation treatment, however, when years were analyzed separately, cultivation treatment for inter-row roots was only slightly significant in 2019 ($P = 0.04$). A post-hoc mean comparison using Tukey's HSD did not support this significance. However, spring + fall cultivation resulted in almost 2 Mg ha^{-1} less inter-row roots than both the control and fall cultivation in 2019 (Table 1.5). Root biomass in-rows was greatest in 2018 and lowest in 2019 across all treatments (Table 1.5). **Microbial Biomass Carbon and Nitrogen**

Neither inter-row termination nor suppression treatments significantly influenced soil microbial biomass C or N in our experiment. Soil MBC did not vary greatly over three years, however, MBN increased in 2018 and then decreased again in 2018 back to levels similar to 2017 (Figure 1.6).

Microbial lipid biomass

Gram-negative bacteria was the only microbial functional group that responded to a treatment, in which suppression treatment was significant in 2018 and 2019. Significance in mean comparison was greatest in 2018 ($P = 0.04$), but for both years the fall burning of plots, averaged over all termination treatments, resulted in higher Gram-negative lipid biomass in the soil (Figure 1.8).

Discussion

Neither inter-row termination nor IWG suppression prevented grain yield decline. However, inter-row herbicide, applied in spring, improved spring forage production, and inter-row cultivation, applied in spring + fall, decreased inter-row root biomass. Mowing in the spring, as a suppression treatment, lead to decreased grain and straw yields. Soil microbial biomass did not change except for lipid biomass of Gram-negative bacteria, which responded to fall burning of plant residues in 2018 and 2019. The effects of management on nearly all response variables were not observed until 2019 (after two years of management applications).

No management treatments resulted in significantly higher grain yields than from 2017 yields, however, mean total grain yields increased 140% from

2018 to 2019. This increase cannot be attributed solely to management because of some exceptionally hot days (≥36° C) in late May 2018 that lead to atrophy of many IWG flowers and thus unusually poor grain yields. Part of the reason our management strategies were not successful in bolstering grain yields above 2017 yields may be due to the timing of treatment applications—applications began in fall of 2017, 1.5 years after planting. Grain yields in 2017 and 2018 followed a trend of progressive decline from 2016 yields, as we expected. This trend of declining grain yields over time has been observed in other studies (Hunter et al., 2019).

Fall-applied herbicide and spring mowing were detrimental to grain, straw, and spring forage yields, while spring-applied herbicide, and fall cultivation to some extent, resulted in higher yields of all three. Mowing in the spring decreased grain yields in 2019 (Table 1.3, Figure 1.4), but fall cultivation and spring herbicide improved spring forage yields in the same year (Figure 1.3). Straw biomass yields appeared to also decline with spring mowing, except that a significant difference also occurred in the 2017 harvest, before spring mowing treatments began, indicating that the spring mowing was not responsible for the difference (Table 1.3, 1.4). This is important when IWG has been labelled as a candidate for dual-use; spring forage could be grazed or harvested for hay without damaging grain yield potential in late summer (Ryan et al., 2018, Hunter et al., 2020).

Fall and spring applications of herbicide in 2019 resulted in the lowest (fall application) and highest (spring application) grain yields of that year,

indicating the importance of timing for herbicide applications (Table 1.3). It is possible that this difference in herbicide response was because glyphosate, a systemic herbicide, may have been translocated, along with carbohydrates, to IWG roots in the fall, similar to other perennial grasses like *Miscanthus* (Purdy et al., 2015), leading to tissue death, whereas phloem transport of carbohydrates in perennial grasses moves primarily from roots to shoots in the spring (Purdy et al., 2015; Williams, 1964). Thus, a foliar glyphosate application in the spring may not have been translocated throughout the IWG plants to the extent that it would be in the fall.

We observed a reduction in root biomass from inter-row cultivation over time (Table 1.4) that we hypothesized would help stimulate more growth above ground (in-rows) by preventing resource competition below ground (J. B. Wilson, 1988; S. D. Wilson & Tilman, 1993), but this was only reflected with significance in 2018 straw production when fall cultivation resulted in greater straw yield that year (Table 1.4). By 2019, inter-row root biomass was greatly reduced from spring + fall cultivation (Table 1.5). Higher yields of grain, straw, and spring forage were observed under inter-row termination treatments, but they were not significantly greater than any other treatments, indicating that inter-row cultivation was partially effective in reducing belowground root competition--but not enough to significantly alter aboveground yields.

The consequence of inter-row termination treatments was that soil in that space became available to colonize by other annual weeds, however, the effects of herbicide and cultivation were different. Plots that received any herbicide

application, but particularly when applied in the fall, had significantly more weed pressure in 2019. This was an interesting result because; 1) areas that did not receive herbicide or cultivation had very low to negligible weed pressure in 2019, indicating that IWG in this experiment established well and outcompeted weeds on its own, and 2) cultivated treatments that we expected to stimulate inter-row weed abundance had less weed pressure than herbicide treatments. It is possible that weeds were more successful in plots that received herbicide for the same reason mentioned previously regarding the translocation of glyphosate through IWG that could result in further die back of IWG than from superficial cultivation that would only inflict minor mechanical damage to rhizomes.

The effects of soil disturbance on IWG is important because the motivation behind the development of this new crop is to improve environmental quality, including retention of soil microbial life and soil nutrients like carbon (Cox et al., 2006; Hargreaves & Hofmockel, 2014). With the exception of burning in the fall, we observed no significant response of soil microbial biomass C, N, or lipids to our management treatments. Microbial biomass extracted as trace C and N or as lipids provides an estimation for the density of active soil microorganisms at the time and location of sampling, but their functional structure or abundance may be resistant to rapid changes in soil environmental properties (Bowles et al., 2014). It can take three or more years, following a shift in vegetation or soil/crop management, before changes in soil microbial C or N are observed (Rosenzweig et al., 2016), as was the case for a recent study on IWG affecting soil food webs (Sprunger et al., 2019). The short time frame of this experiment (two years of

management application) may not have been enough time to observe changes in the microbial community.

Management treatments in this experiment did not begin their application until fall of the post-establishment year and further research should implement similar management strategies immediately upon establishment. Additionally, studies that employ a range of different row spacing and use the same inter-row management strategies would be very helpful in determining the level of influence on intraspecific competition for soil resources, as well as the management's potential effects on soil microbial activity.

Summary and Conclusions

This study provides valuable insight on the management of perennial IWG in an upper Midwestern climate, particularly regarding the ongoing challenge of preventing grain yield decline and for IWG's potential as a dual-use crop. Inter-row termination may reduce plant biomass between IWG rows, but this did not consistently improve plant productivity of IWG in rows. Glyphosate herbicide applied in the spring resulted in higher grain and forage yields, but when applied in the fall this herbicide caused significant yield reductions and IWG die back that allowed annual weeds to proliferate. Mowing in the spring resulted in decreased plant productivity after two years. Burning of fall residues was the only management treatment that had a significant effect on soil microbial biomass after two years.

Table 1.1. Management and harvest details from 2017-2019.

	2017	**2018**	**2019**
Spring cultivation	--	17 Apr	14 May
Spring herbicide	--	18 Apr	15 May
Urea (80 kg N/ha)	24 Apr	15 Apr	26 Apr
Spring forage harvest/mow	30 May	23 May	28 May
Grain and straw harvest	22 Aug	10 Aug	14 Aug
Fall herbicide	20 Oct	18 Oct	--
Fall cultivation	26 Oct	22 Oct	--
Fall mow-burn	17 Nov	15 Nov	--

Table 1.2. Analysis of variance (ANOVA) test results for total grain, straw, and spring forage yield, weeds, roots (IR = inter-row, INR = in-row; 0-60 cm depth), microbial biomass 0-15 cm carbon (MBC) and nitrogen (MBN), and lipid biomass of 3 major microbial functional groups (actinomycetes, fungi, and gram-negative bacteria;0-15 cm depth).

		Plant biomass						Microbial biomass		Microbial lipid biomass		
Source of variation		Grain yield	Straw	Spring forage	Weeds	Roots (INR)	Roots (IR)	Carbon	Nitrogen	Actino	Fungi	Gram -
2017												
	Main	NS	NS	NS	*NA*	NS	NS	NS	NS	NS	NS	NS
	Split	NS	***	*NA*	*NA*	*NA*	*NA*	NS	NS	NS	NS	NS
	Main X Split	NS	NS	*NA*	*NA*	*NA*	*NA*	NS	NS	NS	NS	NS
2018												
	Main	NS	.	NS	*NA*	NS	NS	NS	NS	NS	NS	NS
	Split	NS	***	*NA*	*NA*	*NA*	*NA*	NS	NS	NS	NS	*
	Main X Split	NS	NS	*NA*	*NA*	*NA*	*NA*	NS	NS	NS	NS	NS
2019												
	Main	*	NS	*	***	NS	*	NS	NS	NS	NS	NS
	Split	***	***	*NA*	**	*NA*	*NA*	NS	NS	NS	NS	.
	Main X Split	*	***	*NA*	NS	*NA*	*NA*	NS	NS	NS	NS	NS

. = 0.05 < P < 0.10; * = 0.01<P<0.05; **=0.001<P<0.01; *** P<0.001
NS = P > 0.05

Table 1.3. Mean grain and straw biomass yield (± SE, n = 4) for 2019. For grain and straw yield of this year, there was a significant interaction between inter-row termination and suppression treatments. Different lower-case letters denote statistical significance between termination treatments, under each suppression, at $P < 0.05$. No differences were found between termination treatments that were mowed in spring.

		2019 Dry biomass yield (kg ha^{-1})	
Inter-row termination	Suppression	Grain	Straw
Control	Control	208 (32)^{ab}	3024 (356)^{ab}
Fall cultivation	Control	231 (47)^{ab}	3360 (280)^{ab}
Fall + spring cultivation	Control	235 (33)^{ab}	3384 (237)^{ab}
Spring cultivation	Control	141 (22)^{ab}	2908 (420)^{ab}
Fall herbicide	Control	80 (23)^{a}	2001 (395)^{a}
Fall + spring herbicide	Control	128 (42)^{ab}	1673 (582)^{a}
Spring herbicide	Control	265 (58)^{b}	4547 (770)^{b}
Control	Fall burn	185 (35)^{ab}	3005 (349)^{ab}
Fall cultivation	Fall burn	272 (44)^{ab}	3850 (651)^{ab}
Fall + spring cultivation	Fall burn	177 (51)^{ab}	3088 (393)^{ab}
Spring cultivation	Fall burn	177 (37)^{ab}	3040 (352)^{ab}
Fall herbicide	Fall burn	150 (30)^{a}	2483 (654)^{a}
Fall + spring herbicide	Fall burn	218 (34)^{ab}	3749 (545)^{ab}
Spring herbicide	Fall burn	312 (48)^{b}	4574 (484)^{b}
Control	Spring mow	134 (33)	2246 (328)
Fall cultivation	Spring mow	136 (13)	1699 (207)
Fall + spring cultivation	Spring mow	133 (14)	2454 (204)
Spring cultivation	Spring mow	115 (15)	2532 (284)
Fall herbicide	Spring mow	50 (14)	1718 (610)
Fall + spring herbicide	Spring mow	94 (46)	1460 (462)
Spring herbicide	Spring mow	82 (12)	1600 (193)

Table 1.4. Mean plant biomass production (± SE, n = 12 for grain and straw, n = 4 for spring forage) for 2017 and 2018. Biomass values for inter-row termination treatments (top) represent the average over all suppression treatments, and vice-versa for suppression treatment values reported (bottom). Spring forage biomass was averaged over only "mowed" suppression treatments. Different lower-case letters denote statistical significance between treatments within years at $P < 0.05$.

	Dry biomass kg ha^{-1}					
	Total Grain		Total Straw		Spring Forage	
	2017	**2018**	**2017**	**2018**	**2017**	**2018**
Control	338 (31)	47 (5)	4719 (514)	2209 (229)^{a}	700 (90)	669 (66)
Fall cultivation	404 (24)	85 (14)	4931 (306)	3017 (181)^{b}	833 (28)	718 (75)
Fall + spring cultivation	392 (30)	79 (8)	4268 (235)	2243 (118)^{ab}	745 (28)	700 (37)
Spring cultivation	426 (20)	71 (7)	4285 (277)	2752 (197)^{ab}	757 (74)	558 (94)
Fall herbicide	392 (37)	49 (6)	4818 (314)	2100 (196)^{a}	783 (168)	613 (107)
Fall + spring herbicide	404 (13)	82 (12)	4486 (188)	2658 (150)^{ab}	832 (10)	765 (68)
Spring herbicide	381 (28)	74 (13)	4566 (368)	2819 (215)^{ab}	907 (49)	711 (92)
Fall burn	*394 (17)*	*73 (6)*	*4946 (216)^{b}*	*2642 (140)^{b}*		
Control	*390 (18)*	*68 (6)*	*4808 (213)^{b}*	*2815 (125)^{ab}*		
Spring mow	*389 (19)*	*68 (8)*	*3992 (165)^{a}*	*2171 (107)^{a}*		

Table 1.5. Mean root biomass (± SE, n = 4) to a depth of 60 cm, averaged over control suppression treatments, for 2017-2019.

	Dry biomass Mg ha^{-1}		
Total Roots	**2017**	**2018**	**2019**
In-row			
Control	7.24 (2.80)	10.45 (2.90)	5.28 (1.42)
Fall cultivation	9.87 (2.33)	10.87 (3.78)	6.19 (0.56)
Fall + Spring cultivation	11.19 (2.87)	12.97 (2.68)	5.63 (0.75)
Spring cultivation	8.38 (1.97)	5.41 (0.44)	7.87 (1.73)
Inter-row			
Control	4.83 (1.82)	2.81 (0.43)	3.01 (0.81)
Fall cultivation	3.54 (0.48)	2.33 (0.39)	3.13 (0.55)
Fall + Spring cultivation	3.17 (0.25)	1.53 (0.39)	0.98 (0.17)
Spring cultivation	3.03 (0.52)	2.44 (0.33)	1.70 (0.12)

Table 1.6. Weed biomass (± SE, n = 12) from time of grain harvest in 2017 and 2019. Values are averaged over all termination treatments. Different lower-case letters denote statistical significance at $P < 0.05$.

	Dry biomass g plot^{-1}	
Suppression	**2017**	**2019**
In-row		
Control	--	38 (11)^{ab}
Fall burn	--	16 (5)^{a}
Spring mow	--	56 (13)^{b}
Inter-row		
Control	5 (2)	80 (18)
Fall burn	3 (1)	78 (18)
Spring mow	5 (2)	118 (20)

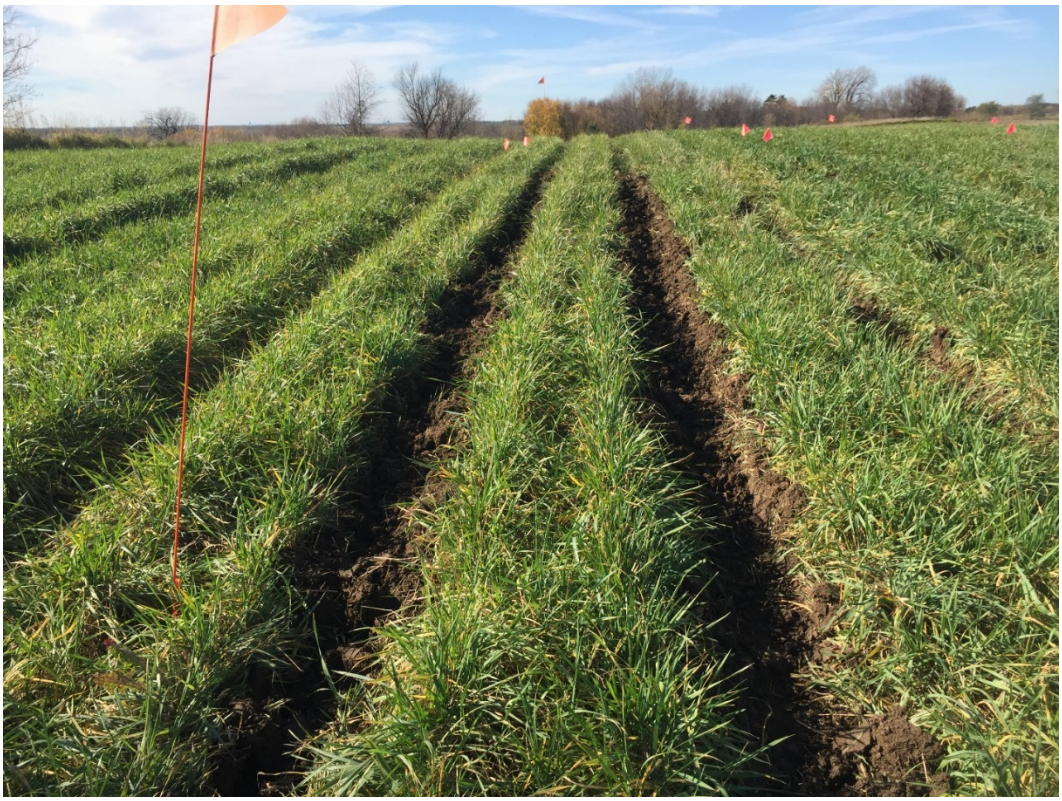


Figure 1.1. Image of RZT between IWG rows.

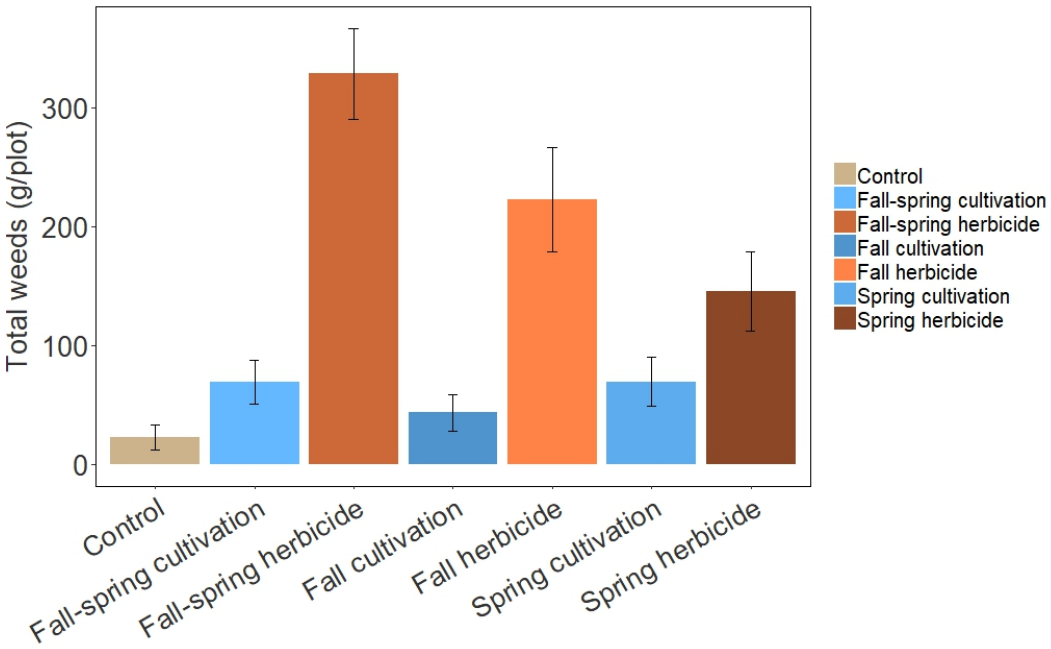


Figure 1.2. Mean dry biomass of total annual weeds per plot (sum of inter-row and in-row samples, ±SE, n = 12) at the time of 2019 grain harvest, by termination treatment (values are averaged over all suppression treatments).

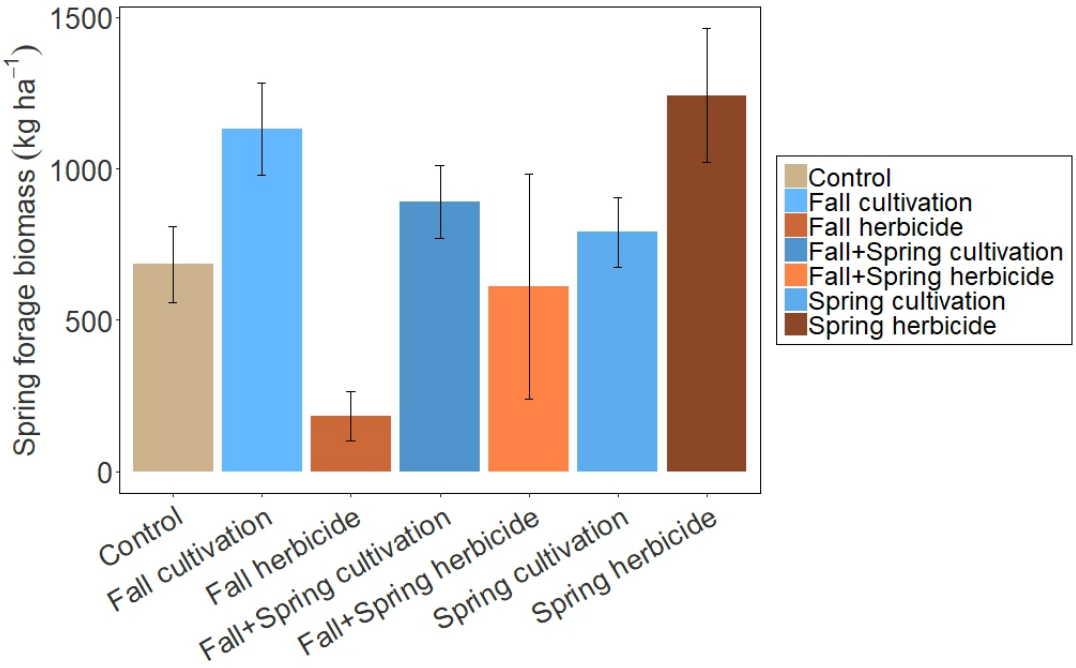


Figure 1.3. Mean dry biomass of spring forage (±SE, n = 12) in 2019, by termination treatment (values are averaged over all suppression treatments).

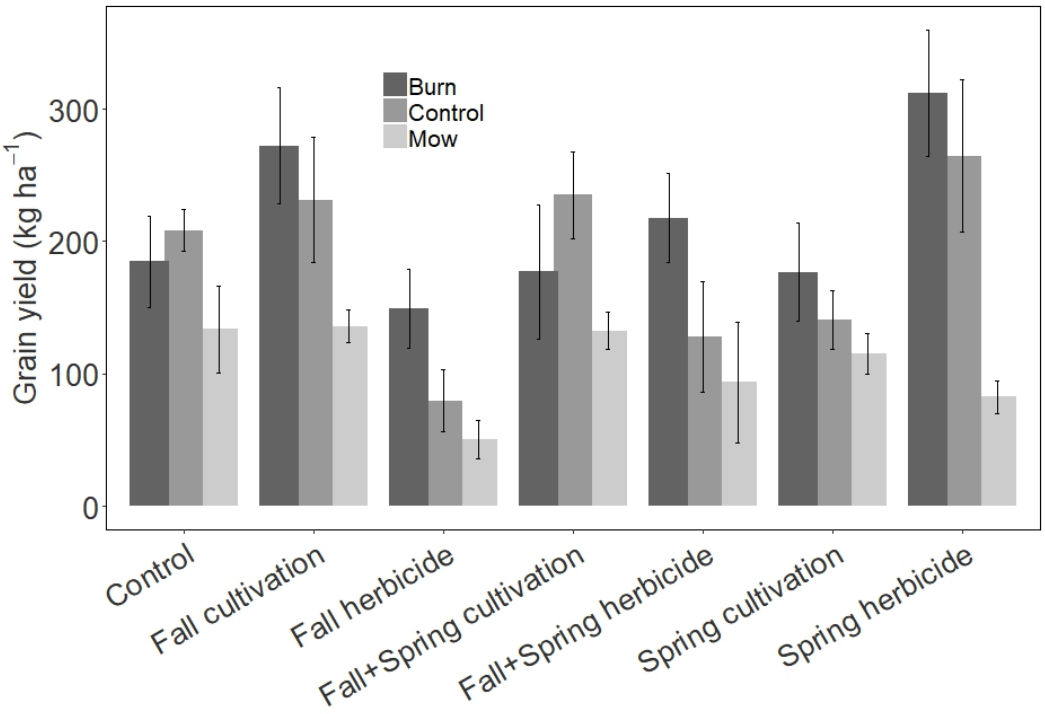


Figure 1.4. Mean grain yields (±SE, n = 4) in 2019. Mean values are separated by suppression treatment over each termination treatment due to a significant interaction.

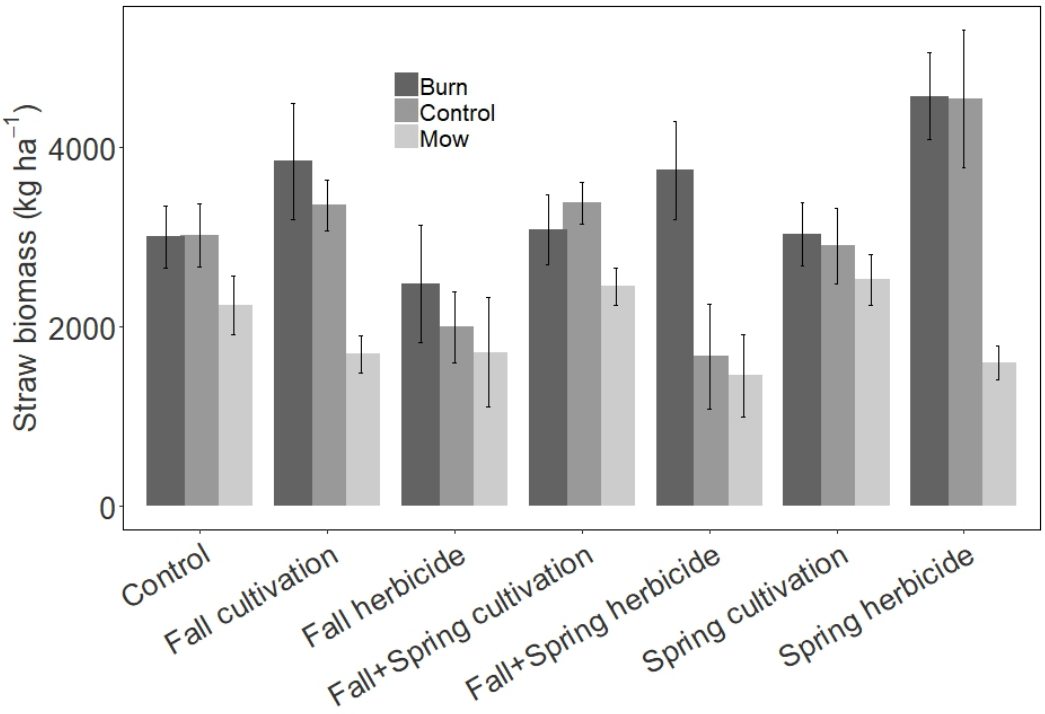


Figure 1.5. Mean straw yields (±SE, n = 4) in 2019. Mean values are separated by suppression treatment over each termination treatment due to a significant interaction.

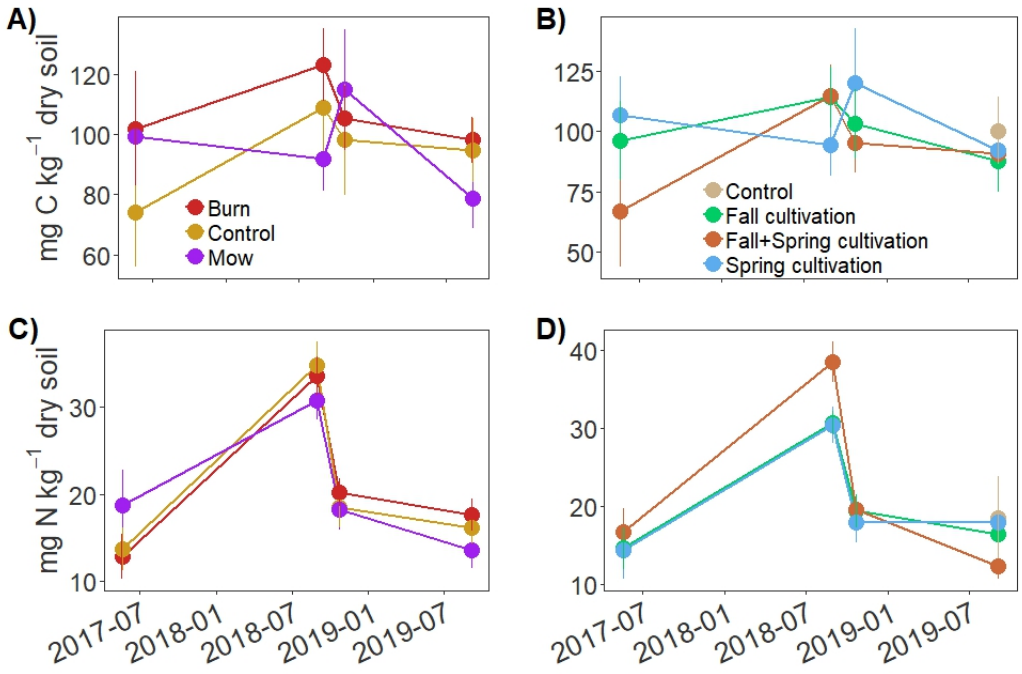


Figure 1.6. Microbial biomass (± SE, n = 12) C and N of termination and suppression treatments over 3 years. A) MBC by suppression treatment, averaged over all termination treatments. B) MBC by termination treatment, averaged over all suppression treatments. C) MBN by suppression treatment, averaged over all termination treatments. D) MBN by termination treatment, averaged over all suppression treatments. Dates in order from left to right; 2017 May 20, 2018 Aug 30, 2018 Oct 23, 2019 Sep 05.

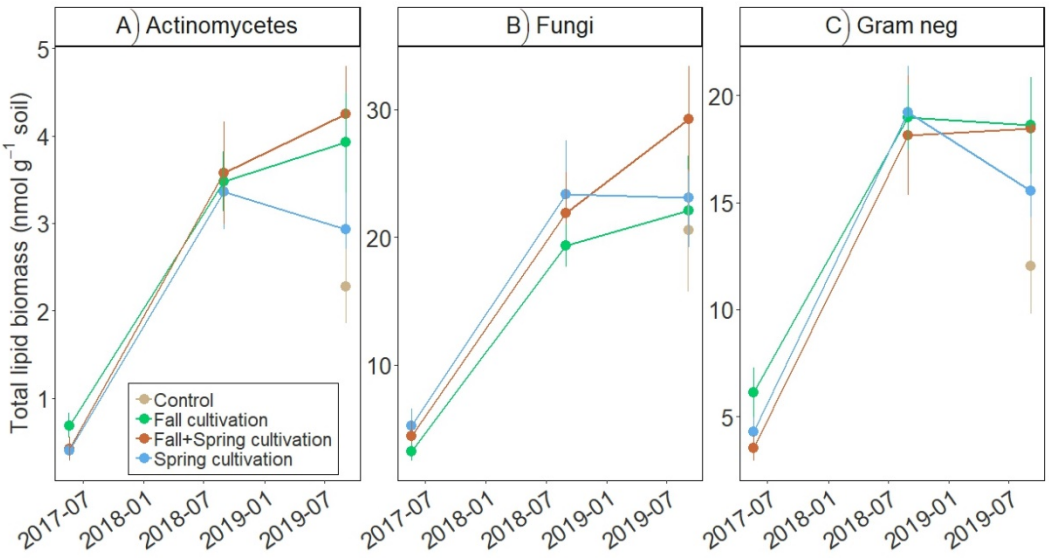


Figure 1.7. Mean total biomass (± SE, n = 12) of lipids corresponding with specific soil microbial functional groups. Mean values are from termination treatments and averaged over all suppression treatments, over three years. Lipid biomass of A) actinomycetes, B) fungi (including arbuscular mycorrhizal fungi and free-living saprophytes), and C) Gram-negative bacteria. Dates in order from left to right; 2017 May 20, 2018 Aug 30, 2019 Sep 05.

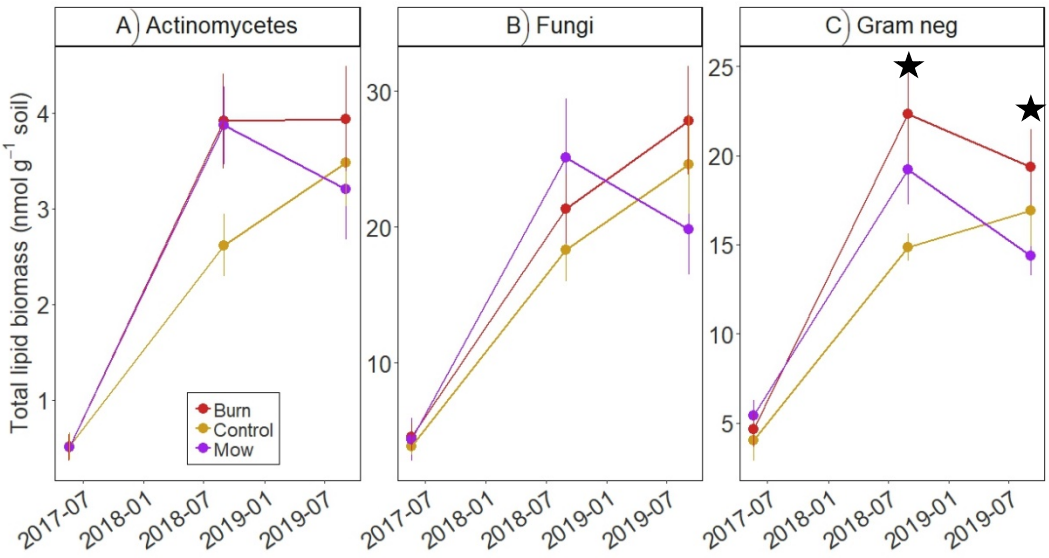


Figure 1.8. Mean total biomass (± SE, n=4) of lipids corresponding to specific soil microbial functional groups. Mean values are from suppression treatments and averaged over all termination treatments, over three years. Lipid biomass of A) actinomycetes, B) fungi (including arbuscular mycorrhizal fungi and free-living saprophytes), and C) Gram-negative bacteria. Dates in order from left to right; 2017 May 20, 2018 Aug 30, 2019 Sep 05. Stars indicate where significant differences between treatments occur ($P < 0.05$).

Chapter 2

Change in soil biomarkers and carbon cycling of intermediate wheatgrass (*Thinopyrum intermedium*) and annual cropping systems

Summary

Perennial crops can mitigate climate impacts on agriculture by protecting soil from increased flooding or drought, and by improving soil quality for enhanced retention of soil carbon (C). Extensive root biomass and continuous living cover of perennials have the potential to store carbon and increase soil microbial abundance. Soil microbial activity and carbon cycling were compared among three intermediate wheatgrass (*Thinopyrum intermedium;* IWG*)* cropping systems with different nitrogen sources, alfalfa, and two annual crop rotations in southeastern Minnesota. Over three years (2017 – 2019), perennial IWG produced significantly more root biomass than annual wheat, soybean, corn, and alfalfa. IWG had greater soil respiration and canopy density in spring and fall months compared to annual crop systems, thus influencing the soil environment. A significant increase in total microbial lipid biomass and in fungal lipid biomarkers, indicated that three years of IWG growth changed the soil microbial composition. Interestingly, the opposite effect was observed in alfalfa and other annual crop rotations which had reduced microbial biomass of fungi and other biomarkers after 3 years. There was also strong inter-annual variation in microbial growth, where we observed the strongest treatment effects in the highest growth year, implying that microbial community development is influenced both by ambient conditions and by specific cropping systems over time

Introduction

Rapid accumulation of greenhouse gases (GHGs) such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) in the atmosphere has resulted in warming annual temperatures around the world, especially winter temperatures of the northern latitudes (Melillo et al., 2014). In the Upper Midwest U.S., increasing annual precipitation and warmer winter temperatures are creating greater risks of flash floods, erosion, and pest proliferation that could lead to yield declines of up to 25% for corn and soybean by mid-century (Angel et al., 2018). One strategy to mitigate these climate impacts in agriculture is through the integration of perennial crops that can reduce the GHG contribution of cropland and produce significant quantities of root biomass that reduce erosion and retain carbon (C) (Crews et al., 2014; Gelfand et al., 2013; Glover et al., 2010). Intermediate wheatgrass (*Thinopyrum intermedium;* IWG) is a promising candidate among perennial crops to provide grain, forage, and extensive roots to increase ecosystem security and climate adaptation (Cox et al., 2006; Glover et al., 2010; Ryan et al., 2018). IWG could also reduce fossil fuel emissions since fewer management inputs are needed in perennial compared to annual crops (Glover et al., 2010; Jungers et al., 2017).

Because soil (not including vegetation) contains twice the amount of C as the atmosphere, it is critically important to the global C budget and thus uniquely equipped to act as a carbon sink (Mielnick & Dugas, 2000). Managing the

physical, chemical, and biological characteristics of agricultural soils is critical to limiting GHG emissions from agriculture. The contribution of GHG emissions from agriculture, forestry, and other land use was estimated at 24% of global GHG emissions as of 2014 (IPCC, 2014). Reduction of emissions from agricultural production can be achieved by implementing new management practices that minimize soil disturbance, fossil fuel combustion, and excess fertilization. Farming practices like soil tillage, crop rotation, fallow, etc. that affect soil temperature, moisture, and physical structure can have significant influence over gas emissions (Fortin et al., 2010; Jabro et al., 2008) and erosion of soil (Daigh et al., 2019). For example, conventional tillage for malt barley in a North Dakota climate resulted in a 34% increase of carbon flux from soil compared to no-till barley (Jabro et al., 2008). The rate of loss of soil carbon may be increased by frequent soil disturbance (Gaiser et al., 2008) and by erosion-induced mineralization and preferential transport of runoff (Polyakov & Lal, 2004). No-till and biennial tillage strategies have shown that this reduction of soil disturbance can increase SOC accumulation (Halvorson et al., 2002; Venterea et al., 2006), however, variability still occurs for the effects of tillage reduction in other systems (Johnson et al., 2005).

Carbon sequestration involves the capture and retention of atmospheric C and can be achieved in biological systems by maximizing plant growth and soil organic carbon (SOC) retention. Furthermore, up to 60% of carbon stored in soil and soil organic matter may be lost from conversion of grassland or forest to agriculture in temperate regions (Lal, 2004) and while net C loss may persist after

this conversion for a decade or more (Abraha et al., 2018), restoring net C accrual by conversion from annual cropland to perennial grassland can be achieved within a year or two (Abraha et al., 2018; Beniston et al., 2014; Rosenzweig et al., 2017). As part of an agricultural system, and in comparison to annual crop rotations, the accounting of C export from the field via grain and biomass harvest, and inputs of root biomass are important in the determination of net ecosystem exchange (Jans et al., 2010). Jungers et al. (2017) described how perennial bioenergy crop systems like switchgrass (*Panicum virgatum*) could sequester up to 7.6 Mg C ha^{-1} yr^{-1} from root biomass (0-90 cm) alone. However, no study like this has been done on IWG systems, which have potential to increase C sequestration in their roots and in more stabilized soil C pools (Cox et al., 2006; Sprunger et al., 2019).

The effects of undisturbed, living roots in the soil for time periods greater than a typical annual grain harvest period in the Midwest USA (June to September) must also be considered as a benefit of perennials to soil fertility and SOC development. This is supported by studies indicating that the rhizospheres of perennial grasses had greater total C, N, microbial biomass, and acid phosphatases than of annual grasses in the same ecosystem (Yé et al., 2017), that perennial switchgrass had higher microbial lipid abundances in their rhizospheres compared to bulk soil (Liang et al., 2016), and that conversion of agricultural land to perennial grassland resulted in a 5-fold increase in microbial carbon and nitrogen (Rosenzweig et al., 2017). Furthermore, an increase in soil fungi specifically, which has been observed in no-till systems (Mbuthia et al., 2015), may improve soil carbon storage potential from increased soil aggregation via fungal hyphal

interactions (Beare et al., 1997) and from greater microbial biomass contribution to SOC (Malik et al., 2016).

This study compared agronomic outputs, soil biomarkers, and carbon cycling in two annual crop rotations of soybean-corn-soybean and wheat-soybean-wheat, alfalfa, and three IWG systems varying in fertility management in a northern temperate climate. Our objectives were to 1) quantify grain and total biomass production, 2) compare soil microbial properties (lipids, enzymes, microbial biomass-C, soil respiration) that might be related to carbon cycling, and 3) determine how different cropping systems alter the soil environment for greater carbon retention or loss. We hypothesized that IWG root growth would stimulate more microbial growth and biomass accumulation, particularly fungi that might alter soil carbon dynamics.

Materials and Methods

Study area and Experimental design

The experiment was conducted at the University of Minnesota Research and Outreach Center in Rosemount, MN (44.684658, -93.069299) from 2017 to 2019. The soil type was characterized as Tallula silt loam, a coarse-silty, mixed, superactive, mesic Typic Hapludoll with 6 % slope, and eroded. The entire study area was seeded on 29 September, 2016 with IWG on alternating 40 and 61 cm rows using a no-till drill following corn harvested for silage. In March of 2017, plots 7.5 x 15 m were delineated in a completely randomized block design with

four replications. All treatment plots that did not include IWG were rototilled to a depth of 20 cm before planting. (Table 2.1).

The cropping systems included in this trial consisted of 1) IWG monoculture fertilized with 80 kg N ha^{-1} as urea, 2) IWG monoculture fertilized with 80 kg N ha^{-1} as composted poultry manure, 3) IWG intercropped with alfalfa (*Medicago sativa* L.), 4) alfalfa monoculture, 5) spring wheat-soybean (*Glycine max* and authority)-spring wheat (*Triticum aestivum* authority) rotation, and 6) soybean-corn (*Zea maize* and authority)-soybean rotation (Table 2.1). Nitrogen (N) fertilizers were applied annually between April and May, with the exception of alfalfa, IWG + alfalfa, and soybean that did not receive nitrogen amendments. Spring wheat and corn received Chilean sodium nitrate as a source of N. On 3 May 2018, and 9 April 2019, 67 kg ha^{-1} bone meal was added to all treatments to maintain phosphorous levels based on soil tests.

Soil Sampling

Soil samples were collected each spring from 2017-2019 using a step-in hand probe (ID = 3.1 cm) to a depth of 15 cm, homogenized from 6 subsamples taken in an "X" pattern from each plot. Soil samples were also collected each fall from 2017-2019 with a hydraulic Gidding's probe to a depth of 60 cm, homogenized from 2 cores (ID = 3.8 cm) per plot (one from center of each crop row, one from the center of the inter-row space) and separated into four 15-cm depth intervals. All samples were transported from the field in coolers; and then separated the same day into fresh samples that were stored at 4° C and frozen samples that were stored at -20° C until analysis.

Plant Biomass Sampling

Grain yield and plant biomass were collected differently depending on crop type and row spacing. IWG plant biomass was collected from hand sampled quadrats measuring 45.7 cm x 50.8 cm for "row" and 45.7 cm x 61 cm for "inter-row". The difference in these quadrat sizes accounted for the IWG row spacing that alternated 61 cm and 40 cm, and the two samples were summed together to account for the total area. Per hectare theoretical grain and biomass yields were calculated based on Eq.1 (A.2). Spring wheat biomass was collected from quadrats measuring 61 cm x 61 cm. The seed heads were removed by hand and bagged separately for wheat and IWG. Seed heads were dried and threshed with a laboratory thresher (Wintersteiger LD-50). For soybean, total biomass (grain plus stem) was collected from two 3-m row lengths of the center row of each plot while grain yield was calculated by small plot combine harvester. For corn, grain and stover yield was determined by hand harvesting mature plants from two 3-m row lengths in the center of each plot, weighing the wet ears, then taking a subsample of 20 ears and separating kernels from these ears for drying and shelling. Crop residues of corn, wheat, and IWG were mowed after manual harvesting of yield subsamples were completed. Alfalfa was harvested three times each year using from a 1 by 6 m area of the center of each plot using a small forage harvester (Carter, Inc.). For all crops, dry matter content of sampled and harvested plant materials was determined by drying a subsample at 95° C. Yields were then adjusted to a dry matter basis.

A hydraulic Gidding's soil probe (ID = 3.8 cm) was used to extract soil cores to determine root biomass from the in-row and inter-row spaces. One core to a depth of 60 cm was extracted from each space and separated into four 15-cm depth increments. Cores were washed of soil using a hydropneumatic elutriation system (Smucker et al. 1982), manually cleaned to remove sand and organic debris, then dried and weighed.

Plant canopy density

Leaf-area index (LAI; m^2 m^{-2}) was measured in each plot throughout the growing season using a Li-Cor 2200 plant canopy analyzer. Samples per plot were obtained from an average of 5 measurements taken (in diffuse light conditions only) in an "X" pattern through the center of each plot. Samples were taken at points within and between planted crop rows, at angles parallel and perpendicular to the orientation of the rows. A 45° lens cover was also used to remove bias of the measurer's light attenuation.

Microbial biomass C

Microbial biomass C was determined using a modified technique based on (Gregorich et al., 1990). Field wet soil sampled from 0-15 cm was sieved to 2 mm and 10 g ± .05 of soil weighed into acid-washed and muffled 70 mL test tubes. Duplicates of each sample were made for a baseline and fumigated set of samples. Chloroform was added in the amount of 50 µl to each of the "fumigated" samples. Then 40 mL of 0.5 M K_2SO4 was added to both baseline and fumigated samples, tubes were capped and placed in a oscillator at 150 rpm for 4 h. After shaking, sample tubes were left to settle for 1 h before pouring the supernatant through

589/1 Whatman carbonless filters into 40 mL plastic acid-washed specimen cups. The filtered extracts were inspected for contamination before transferring the liquid into a labelled 20 mL plastic scintillation vial. Vials were frozen at -20° C before final trace carbon and nitrogen analysis. On day of analysis, fumigated samples were sparged to remove trace chloroform using a modified vacuum chamber. All thawed samples were subsampled into muffled 24 mL glass vials and diluted in water to a ratio of 20:3 and 1 mL of 2 M HCl was added before covering tops in aluminum foil in preparation for trace carbon and nitrogen analysis in a Shimadzu TOC-L. Carbon standards were diluted to 50 mg C or N L^{-1} using stock potassium hydrogen phthalate (1000 mg C/L KHP). Microbial biomass C was calculated by the difference in mg C kg dry $soil^{-1}$ between a fumigated and a baseline set of the same samples. Microbial C in both sets was calculated by dividing the concentration of C in the extract solution by soil dry weight and then multiplying by a dilution factor (Eq. 2, A.4).

Lipid analysis

Extraction of lipids from soil was performed as described in (Oates et al., 2017; Balser et al., 2019). Briefly, 3 g of freeze-dried soil was weighed into labelled 40 mL centrifuge tubes followed by an extraction with chloroform, methanol, and citric acid buffer. The extraction was repeated 3 times followed by overnight phase separation. The bottom (chloroform) liquid phase was transferred to 15 mL glass test tubes, followed by acid methylation to convert fatty-acids to fatty-acid methyl esters (FAME) before analysis on an Agilent 7890 gas chromatograph. Internal standards were used to convert peak areas of each fatty

acid to nmol fatty acid g soil^{-1}. Individual lipids were used as biomarkers to indicate broad groups within the microbial community: 16 : 1 ω5c for arbuscular mycorrhizal fungi (AMF; Balser et al., 2005; Gutknecht et al., 2012); 18 : 1 ω9c and 18 : 2 ω6,9c for general fungi excluding AMF (general fungi, GF; Balser et al., 2005; Gutknecht et al., 2012); 16 : 1 ω7c and 18 : 1 ω9t for Gram-negative bacteria (Wilkinson et al., 2002); and 15 : 0 iso and 17 : 0 iso for Gram-positive bacteria (Wilkinson et al., 2002). Lipid biomass (nmol g soil^{-1}) was calculated by dividing the peak area by an internal standard constant (K value) and this result was divided by a ratio of fatty acid molecular weight to soil dry weight.

After batch processing of the GC data, FAME standards and representative sample chromatograms were examined to determine the identity of consistently observable peaks that corresponded to known fatty-acids based on their retention times through the GC. The area underneath these peaks, representing volume of the fatty acid in a sample, was used to calculate nmol of fatty-acid g soil^{-1} using Eq. 5 (A.5). The K-value (total peak area ng^{-1}) was computed by dividing the peak area of a standard by the concentration of sample the GC extracts from each sample.

Extra-cellular enzyme assay (EEA)

Soil enzyme incubation assays were performed as described by Sinsabaugh et al. 2003 and based on pre-optimization (German et al., 2011; R. L. Sinsabaugh et al., 2003). Briefly, frozen soil was weighed into 50 mL glass beakers and then mixed with 50 mL of 2.5 M Trizma base buffer. This soil slurry was sonicated for 5 minutes and added to 96-well plates that contained 4 different

substrates for hydrolytic enzymes; β-glucosidase and cellobiohydrolase for carbon degradation, N-acetylglucosaminidase for nitrogen acquisition, and phosphatase for phosphorous acquisition. Separate 96-well plates were made for 2 oxidative enzymes; phenol oxidase and peroxidase. Hydrolytic enzyme plates were incubated in the dark for 1 hour, oxidative enzymes for 10 min, before adding sodium hydroxide and 1.8 M sulfurous acid respectively, and measuring fluorescence intensity on a BioTek® Synergy HT microplate reader.

Trace gas flux measurements

Soil CO_2 flux was measured using an Gasmet® DX4040 portable FTIR gas analyzer connected to a closed chamber system following the GRACEnet protocol for chamber measurements (Parkin et al., 2003; Parkin and Venterea, 2010). The chamber was a 16.2 x 52.7 x 10.2 cm tray constructed of 18-gauge stainless steel, with a threaded PVC coupler (ID = 8 cm) and 70 cm PVC extension attached to the top of the chamber for accommodating vegetation (see chapter 3 for method development and detailed information on GHG emissions measurements; Bergquist 2019). The chamber anchors were similar steel trays with the bottom removed. The anchors were inserted into the soil (~10 cm deep) so that the top rim protruded < 5 cm above the soil surface and soil was tamped around the anchor to ensure a good seal. Anchors were installed at least 24 h prior to sampling and left *in situ*. A foam weather seal was attached to the rim of the chamber to ensure a tight seal prior to fastening the chamber to the anchor using alligator clips. The sample area volume was approximately 6250 cm^3 without the extension and 9550 cm^3 with the extension. Our chamber design was specifically

intended to capture CO2 flux from the whole plant-soil system and thus our reported flux values represent the sum of both heterotrophic (microbial) and plant (primarily root) respiration at the time of sampling. Sampling was always performed between 10:00 and 16:00 hours to align with the time of greatest activity within diurnal gas flux patterns.

Flux Calculations

After data quality control was performed (A.3), flux of CO_2 was calculated using eq. 3 (Collier et al., 2014) with the assumption that change in concentration within the chamber increased linearly over time:

$$F = S * V * A^{-1} \qquad \textit{Eq. 3}$$

Where F = flux, S = slope of regression ($\frac{\Delta conc}{\Delta t}$), V = chamber volume, A = chamber area. Thus:

$$\frac{\left(\text{mol L}^{-1}\ \text{hr}^{-1}\right) * \text{L}}{m^2} \qquad \textit{Eq. 4}$$

Statistical Analysis

All analyses were conducted with R version 3.4.4 (R Core Team 2018). For plant biomass production, each year was modeled separately due to interactions with year for all biomass variables. A linear mixed-effects model (LME) (package, code: lme4, *lme*; Bates et al. 2015) was used to conduct one-way analysis of variance (ANOVA) tests with block as a random effect and crop system as a fixed main effect. Means were compared by obtaining least-square means (package, code: emmeans, *emmeans*; Lenth 2019) and adjusted for Tukey's

HSD test. Because leaf-area index (LAI) data was collected at multiple dates throughout 2018 and 2019, day was added as a main effect to the LME (including block as random effect). However, the final model for LAI included day as a factor instead of as a continuous variable due to a lower Aikake information criterion (AIC) estimate for the former model. Soil respiration and microbial data, including lipids, MBC, and enzyme activity, were analyzed using a repeated measures LME approach, with crop system and sample date (as a continuous variable) treated as main effects to conduct two-way ANOVA tests (block included as random effect). These models, which used a first-order autocorrelation structure (package, code: nlme, *lme*; Pinheiro et al., 2019), performed better than traditional two-way ANOVAs (using date as a factor) based on lower AIC estimates. Due to interactions between crop system and date, MBC, lipid, and soil respiration data were separated by sample date and each date was analyzed with one-way ANOVAs using crop system as the main effect and block as a random effect. Mean comparisons among crop systems were made for each sample date using Tukey's HSD test, as described earlier. Enzyme activity did not have significant interactions between crop system and date, and so activity between crop systems was compared over all dates. Relationships between soil moisture content and soil microbial biomass, lipids, and enzymes were tested using linear regression. Non-significant ($P > 0.05$) interaction terms were removed from LME models. Results from ANOVA tests are listed in Table 2.3. Assumptions of normally distributed residuals, independence of error and

constant variance were checked for each linear model using qqplots, histograms of residuals, and plots of the residuals against fitted values.

Results

Perennial and annual cropping systems performed differently in terms of biomass allocation, with the most root production observed in IWG, the most shoot production from alfalfa, and the most grain production from corn. Plant canopies of perennial crops established quickly in the spring, weeks before annuals, and IWG canopies also grew back (after grain harvest) to a lesser extent in the fall. Soil respiration mirrored plant canopy data, showing greater C flux of IWG systems in the spring and fall. MBC and lipids increased across all crop systems in 2019 with no differences among crop systems for MBC, but a few differences among crop systems for fungal and Gram-negative bacterial lipids. Soil enzyme activity among crop systems was only slightly different for cellobiohydrolase and oxidative enzymes.

Soil respiration (CO_2 flux)

There was a significant interaction between crop system and date across all years, and so each date was analyzed separately to determine any difference in soil respiration between crop systems on a specific date. In 2017, CO_2 flux from IWG + manure was significantly greater than soybean on 13-June (means of 17.6 and 3.8 g CO_2-C m^{-2} d^{-1}, respectively; $P = 0.008$) and, on 28-September, CO_2 flux from both IWG + urea and IWG + alfalfa was greater than soybean (means of 6.5, 6.9, and 1.5 g CO_2-C m^{-2} d^{-1}, respectively; $P = 0.028$, $P = 0.025$, respectively). In

the post-establishment year (2018), IWG + urea, IWG + alfalfa, and alfalfa all had greater CO2 flux than soybean on 6-May (means of 4.8, 4.0, 2.8, and 1.3 g CO_2-C m^{-2} d^{-1}, respectively; P = 0.004, P = 0.006, P = 0.02), and all perennials (three IWG crop systems and alfalfa) had greater fluxes than both soybean and corn on 22-May (mean P-value of all comparisons = 0.001). In 2019, fluxes of IWG + alfalfa were significantly greater than wheat on 16-September (means of 8.9 and 4.1 g CO_2-C m^{-2} d^{-1}, respectively; P = 0.03), and flux from all three IWG systems (mean = 6.1 g CO_2-C m^{-2} d^{-1}) were greater than wheat on 25-September (mean P-value = 0.02). IWG + alfalfa CO_2 flux (mean = 6.6 g CO_2-C m^{-2} d^{-1}) was also greater than from soybean on 25-September (P = 0.02).

Plant Cover

Leaf-area index (LAI; m^2 m^{-2}) was measured in 2018 and 2019 as a proxy for canopy density and plant productivity throughout the growing season (Figure 2.2). Significant differences in LAI among crop systems were observed on every day sampled in 2018 and 2019 based on pairwise t-test (Tukey's HSD) results. In 2018, all perennial crop systems (annual mean = 3.2) had greater LAI than soybean (annual mean = 1.1) and corn (annual mean = 2.1) from May until August (mean P-value < 0.001), at which point the mature corn canopy grew to be denser than all crop systems (except for IWG + alfalfa) until it was harvested in October (mean P-value = 0.01). The regrowth of IWG + alfalfa recorded 6-October 2018 produced higher LAI (mean = 3.0) than soybean (mean = 0.8; P < 0.001), IWG + manure (mean = 2.0; P < 0.001), and IWG + urea (mean = 1.9; P < 0.001). In May 2019, all perennial crop systems had greater LAI (May mean =

2.6; annual mean = 3.0) than soybean (mean P-value < 0.001) and wheat (mean P-value < 0.001). In June 2019, all three IWG systems had greater LAI (June mean = 5.5) than soybean (mean P-value < 0.001) and wheat (mean P-value = 0.01). From June 2019 until IWG grain harvest in August, alfalfa had lower LAI (annual mean = 2.6) than all other crop systems (mean P-value < 0.001). On 26-August 2019, IWG + alfalfa had greater LAI (mean = 3.9) than soybean (mean = 2.8; P = 0.006) and wheat (mean = 3.0; P = 0.049), and those were the only significant differences among crop systems observed on that date. By 10-September 2019, alfalfa had greater LAI (September mean = 2.9) than all other crop systems (mean P-value < 0.001), and soybean also had greater LAI (mean = 1.6) than wheat (mean = 0.5; P-value = 0.01). On 28-October 2019, regrowth of all three IWG crop systems resulted in greater LAI (mean = 2.2) than soybean (mean = 0.1; P < 0.001) and wheat (mean = 0.03; P < 0.001) while alfalfa had lower LAI (mean = 0.6) than all three IWG crop systems (mean P-value < 0.001).

Plant Biomass

Biomass production varied between crop systems and year (significant interactions were observed for all biomass types). Total biomass production of crop systems in 2017, including grain, straw (or stover), and roots, was highest in alfalfa and lowest in soybean, averaging 11.2 Mg ha^{-1} and 4.6 Mg ha^{-1} respectively (P < 0.001; Figure 2.3). Grain production from soybean, wheat, and IWG + urea (produced the most grain among IWG systems for 2017) accounted for approx. 38%, 41%, and 0.05% of its total biomass production in 2017, respectively (Table 2.4). Total biomass production in 2018 was highest in corn

and lowest in soybean, averaging 17.5 Mg ha^{-1} and 4.2 Mg ha^{-1} respectively ($P < 0.001$; Figure 2.3). Grain production from corn, soybean, and IWG + manure accounted for approx. 56%, 26%, and 0.04% of its total biomass production in 2018, respectively (Table 2.4). In 2019, total biomass production was greatest from IWG + manure and lowest from soybean, averaging 16.34 Mg ha^{-1} and 5.59 Mg ha^{-1} respectively ($P < 0.001$; Figure 2.3). Root biomass production for IWG + manure, soybean, wheat, and alfalfa accounted for approx. 57%, 41%, 29%, and 21% of its total biomass production in 2019, respectively (Table 2.4). Over three years (2017 – 2019), alfalfa roots accounted on average for 36% of its total biomass production, while the mean roots of all three IWG systems accounted for 61% of their average total biomass production and roots of annual crop rotations accounted for 27% and 33% of their total biomass production for soy-corn-soy and wheat-soy-wheat, respectively.

Grain

Over all crops and years, grain yield was highest in corn in 2018, averaging 9.86 ± 0.72 Mg ha^{-1}, and lowest in IWG + alfalfa in 2019, averaging 0.19 ± 0.08 Mg ha^{-1} (Table 2.4). Grain yields in IWG systems were highest in 2018, with the most from IWG + manure in 2018, averaging 0.764 ± 0.13 Mg ha^{-1}. This is compared to annual wheat, which yielded an average of 3.32 ± 1.13 Mg ha^{-1} in 2017 and 1.90 ± 0.33 Mg ha^{-1} in 2019 (Table 2.4). Soybean yields were relatively low across all years compared to state average organic soybean yields, with the highest yields in 2019 averaging 2.06 ± 0.11 Mg ha^{-1}, and the lowest in 2018, 1.14 ± 0.51 Mg ha^{-1} respectively.

Straw/Stover/Herbage

Straw refers to the shoot biomass of IWG and wheat while stover refers to the stems and leaves of corn and soybean, both harvested at the same time as grain for all of these crops. Herbage, the shoot biomass of alfalfa, represents the cumulative total of three cuttings per year that is consistent with typical hay/forage harvest schedules in the Midwest US. Aboveground biomass production (to include all three categories mentioned above) on average was highest in alfalfa across all years, because annual yields included the sum of three biomass harvests, with the highest yield in 2019, averaging 7.23 ± 0.50 Mg ha^{-1} (Table 2.4). Excluding alfalfa, straw/stover yield in 2017 was highest from IWG + urea and lowest from soybean ($P < 0.001$), averaging 3.28 ± 0.26 Mg ha^{-1} and 0.63 ± 0.23 Mg ha^{-1} respectively (Table 2.4). Straw/stover yield in 2018 was highest from IWG + manure and lowest from soybean ($P < 0.001$), averaging 4.93 ± 0.54 Mg ha^{-1} and 0.57 ± 0.09 Mg ha^{-1} respectively (Table 2.4). Corn stover yield in 2018 was very similar to IWG + manure and also greater than soybean ($P < 0.001$), with mean of 4.73 ± 0.13 Mg ha^{-1} (Table 2.4). In 2019, straw/stover yield was highest from IWG + manure and lowest from soybean ($P < 0.001$), averaging 6.71 ± 0.46 Mg ha^{-1} and 1.23 ± 0.08 Mg ha^{-1} respectively (Table 2.4). Stover production from all three IWG systems increased from 2017 to 2019 by 56% on average, while alfalfa herbage production remained relatively consistent over this time period (reduced by about 3%), and corn and wheat consistently produced more stover/straw than soybean (Table 2.4).

Roots

Root production varied across crop systems and years, but perennial cropping systems generally had higher root biomass. In 2017, IWG + alfalfa produced significantly more root biomass (mean = 6.08 ± 1.72 Mg ha^{-1}) than soybean (mean = 2.22 ± 0.31 Mg ha^{-1}; P = 0.03; Table 2.4). In 2018, IWG + manure produced significantly more root biomass (mean = 8.80 ± 3.44 Mg ha^{-1}) than corn and soybean (means = 2.93 ± 0.41 Mg ha^{-1} and 2.50 ± 0.20 Mg ha^{-1}; P = 0.01 and 0.008, respectively). Root production from IWG + urea (mean = 7.32 ± 0.63) in 2018 was also very close to IWG + manure but was not statistically greater than roots of corn and soybean. Mean root production from alfalfa in 2018 was 5.85 ± 2.94 Mg ha^{-1}, but decreased to 1.91 ± 0.51 Mg ha^{-1} in 2019. In 2019, all three IWG systems produced significantly more root biomass than wheat, soybean, and alfalfa (mean P-value = 0.001) with the highest mean production of 9.57 ± 2.37 Mg ha^{-1} from IWG + urea. However, IWG + manure had only slightly different mean production, 9.38 Mg ha^{-1}, with a much lower standard error of 0.6 (Table 2.4).

Microbial Biomass Carbon

Soil microbial biomass C (MBC) varied in magnitude across time, but no significant differences were found between crop systems on any given date. There was a significant interaction between cropping system and time (P = 0.049). Over three years of spring and fall samples, spring soil samples had an average of 162 mg C kg^{-1} dry soil and fall soil samples had an average of 159 mg C kg^{-1} dry soil. Mean total MBC (including spring and fall samples from all crop systems) of each year was 113, 164, and 201 mg C kg^{-1} dry soil for 2017, 2018, and 2019,

respectively. The soybean-corn-soybean rotation experienced the greatest decrease in MBC from spring 2017 to spring 2018. Appendix A.6 provides additional information on soil water content.

Enzymes

Activity of cellobiohydrolase and oxidative enzymes was found to be significant among cropping systems over all sampling dates, and no interactions between cropping system and date were found for any of the enzymes tested. Cellobiohydrolase activity for IWG + manure (3-yr mean = 13.4 nmol h^{-1} g^{-1} soil) was greater than for IWG + urea (3-yr mean = 8.7 nmol h^{-1} g^{-1} soil; P = 0.02; Figure 2.5). Oxidative enzyme activity (including peroxidase and phenol oxidase) for alfalfa (3-yr mean = 337 nmol h^{-1} g^{-1} soil) was greater than both IWG + manure (3-yr mean = 197 nmol h^{-1} g^{-1} soil; P = 0.02) and IWG + urea (3-yr mean = 159 nmol h^{-1} g^{-1} soil; P = 0.02; Figure 2.6).

Lipids

Significant interactions between cropping system and date were found for soil fungal (P = 0.001) and Gram-negative bacterial lipid biomass (P < 0.001), but cropping system was not significant for any other lipid group measured (Table 2.3). On 24-September 2019, fungal lipid biomass of IWG + urea (mean = 63.4 nmol g^{-1} soil) was greater than both alfalfa (mean = 37.3 nmol g^{-1} soil; P = 0.03) and IWG + alfalfa (mean = 38.0 nmol g^{-1} soil; P = 0.04; Figure 2.8, Table 2.5). Also on 24-September 2019, Gram-negative bacterial lipid biomass of IWG + urea (mean = 39.4 nmol g^{-1} soil) was greater than both alfalfa (mean = 20.5 nmol

g^{-1} soil; P = 0.01) and IWG + alfalfa (mean = 23.8 nmol g^{-1} soil; P = 0.047; Figure 2.8, Table 2.5).

Discussion

The results of this study showed that perennial and annual cropping systems can have different impacts on the soil and carbon cycle because of the unique phenology, physiology, or management of each cropping system. Annual cropping systems, especially corn, yielded large amounts of grain--to the immediate economic and caloric benefit of producers and consumers, respectively, while perennial cropping systems yielded large amounts of biomass in the form of shoots and roots that might have some immediate value as shoots (hay/forage), but roots can only provide long-term value as carbon sinks or promoters of soil quality. In this study, greater root production, observed in perennials IWG and alfalfa, may have been related to increased spring and fall soil respiration rates, increased microbial biomarkers in IWG, and increased soil cover throughout the year. However, plant biomass allocation as well as overall crop function (end-use) was different between these perennial systems and thus carbon balance of each system was unique. Furthermore, the effects of perennial roots on soil microbial communities diverged in this experiment, indicating that other attributes of the plant, beyond the presence of continuous living roots, are responsible for influencing soil microbiology.

Among all cropping systems in this study, IWG systems allocated the most biomass belowground as roots and thus may be good candidates for increasing carbon sequestration in soil. However, plant biomass is not adequate in

representing total carbon assimilation or export from crop systems. Harvested shoot biomass and root biomass may account for most of the annual carbon assimilation, however, it excludes perennial regrowth in the fall, as well as root decomposition. Root biomass that is sampled once a year (the case for this study) provides only a snapshot of root productivity and mortality that is dynamic throughout the growing season and that varies between plant species (Vivanco & Austin, 2006). Straw biomass of IWG was only harvested at peak (summer) biomass when in practice it may be cut or grazed in the spring and/or fall, much like alfalfa (where herbage was harvested 3 times each year), thus our results are an underestimation of total potential above ground biomass production from IWG (Pugliese et al., 2019). To determine net ecosystem exchange, total carbon assimilation must be measured in addition to carbon export (soil respiration and biomass harvest). Furthermore, net ecosystem exchange is subject to change annually and if a perennial grass like IWG is used in a crop rotation and terminated/tilled after several years of growth, it may lose a significant portion of any soil carbon it gained through mineralization (Abraha et al., 2018).

The LAI and soil respiration data reported in this study provide additional insight to patterns of crop growth (Figure 2.2) and evidence for seasonal differences in soil respiration and soil cover between the perennial and annual crop systems. Clear differences can be seen between canopies of perennials v. annual crops in the spring and fall months because of their phenological disparity. The extended growing season of IWG is a critical factor in evaluating net ecosystem exchange and thus capacity to retain carbon (Oliveira et al., 2018).

Furthermore, in early spring and late fall plant growth can protect soil from nutrient runoff that is common in the upper Midwest US. Higher soil respiration rates of IWG in the spring and fall seasons imply some carbon loss where annuals are neutral, but primarily it is from root growth that can trap carbon underground. Oliveira et al. (2018) reported that IWG can act as a carbon sink in the Central Great Plains region using a micrometeorological approach with eddy covariance, but more research is needed for other climates and using different approaches (Oliveira et al., 2018).

The management of each crop, especially how it is fertilized, can influence the carbon cycle. The soil enzyme, cellobiohydrolase (involved in breakdown of cellulose), had greater activity in IWG + manure compared to IWG + urea (Figure 2.5) across all three years. This could be a result of the composted poultry manure input that likely contained either microbial biomass, C substrate, or both. Similar studies have found that manure additions in crop systems can lead to greater short-term C availability based on higher levels of mineralizable-C (Hurisso et al., 2016; Sprunger et al., 2019).

Soil lipid biomarkers among all cropping systems, remained unchanged until the third year of production when an increase of soil fungal and Gram-negative bacterial biomarkers was observed in IWG + urea. This was consistent with a similar study on IWG and soil food webs that did not observe changes in soil microbial communities until three years after initialization of the study (Sprunger et al., 2019). This change in the soil microbial community under IWG may be a result of perennial root systems' capacity for facilitating microbial

growth observed in other studies comparing soil lipid abundance in the rhizosphere (Jesus et al., 2016; Liang et al., 2016). Compared to annual crops, the continuous living roots in the IWG cropping system are also likely to foster and conserve arbuscular mycorrhizal fungi because of their potential for mutualistic symbiosis (Bever et al., 2009). A comparison of restored tallgrass prairie and agricultural cropland (including no-till systems) found that the restored prairie had increased fungal activity and total soil carbon (Bailey et al., 2002), indicating that perennial grasses more than tillage regime were responsible for the carbon gains. The connection between fungal activity and higher soil carbon storage has been tied to the capacity for fungal mycelia to improve soil aggregate stability and thus protect SOC from mineralization (Beare et al., 1997). However, this apparent connection may also be related to the ability of fungal mycelia to breakdown lignin and other larger and more complex organic residues that, from their decomposition, subsequently produce SOC by-products which are retained for longer in the SOC pool (Malik et al., 2016). Fungi are also equipped to initiate further decomposition of these recalcitrant by-products if soil resources, especially carbon and nitrogen, are limited (Fontaine et al., 2011).

Importantly, the change in soil lipid biomarkers we observed was specifically a difference between only IWG + urea, alfalfa, and IWG + alfalfa, in which the latter two crop systems contained the smallest amount of fungal and Gram-negative bacterial lipids in 2019 (Figure 2.8; Table 2.5). This opposite response, where a perennial grass was apparently facilitating soil microbial growth while a perennial legume appeared to suppress microbial growth, was

unexpected. The distinction in soil microbiological influence could potentially be related to differences in plant root architecture or exudate chemistry. For example, alfalfa typically invests more energy into fewer tap roots compared to other legumes like white clover that produce more fine roots and, in fact, tap roots can account for more than 90% of alfalfa's total root system (Louarn et al., 2015). Unlike alfalfa, intermediate wheatgrass roots have a higher proportion of fine roots to coarse roots, while the majority of alfalfa root biomass has been categorized as tap roots. This difference in root architecture can affect soil microbial communities in the rhizosphere, especially in the surface soil (0-15 cm) where IWG produces many fine lateral and crown roots. With a more fibrous root structure than alfalfa (Figure 2.3, Table 2.4), IWG may have the capacity to retain greater microbial biomass in mycorrhizal associations and in proximity to the root system where exudates and root tissue death can provide additional energy and substrate. It is also possible that the difference in soil microbial composition we observed between alfalfa and IWG is related to alfalfa's production of secondary metabolites (Read & Jensen, 1989) which is partly supported by our observation of higher oxidative enzyme activity in alfalfa (Figure 2.6) because oxidative enzyme activity has been found to be associated with the presence of plant secondary compounds in soil (Sinsabaugh, 2010). Because lipid biomass of the IWG + alfalfa was also significantly lower than IWG + urea in 2019, this may indicate a potential negative effect the alfalfa roots are having on the soil microbiome. Despite this unexpected result, alfalfa has been shown to increase SOC in other studies looking at inter-seeding in rangeland ecosystems

(Mortenson, Schuman, & Ingram, 2004) and in pure stands of a northern temperate climate (Angers, 1992), as well as support nitrogen needs of a companion grass such as IWG (Tautges et al., 2018).

This study prompts additional questions about how perennial plants, despite their similarity in root productivity, influence the soil carbon cycle in diverse ways. Further research should consider how different perennial root systems and climates interact, especially in terms of decomposition rates and dynamics of fast and slow cycling pools of soil carbon (Ye & Hall, 2019). The influence of soil disturbance and plant species on soil microbial activity can be variable, and more research is also needed to determine the relative interactions of plant root architecture, soil legacy, or soil amendments in transforming microbial composition and SOC accumulation in different management regimes.

Summary and conclusions

Intermediate wheatgrass grain production systems show potential to retain carbon in their roots and alter soil microbial composition. However, alfalfa did not exhibit these same trends and therefore qualities of the plant species itself, instead of its perenniality may be responsible for its effects on the soil.

Table 2.1. Nitrogen treatment and crop rotation schedule from 2017 – 2019. (--) = no fertilizer added or N/A.

2017	2018	2019
IWG urea (80 kg N ha^{-1}) IWG poultry manure (80 kg N ha^{-1}) IWG intercropped with alfalfa (--) Alfalfa (--)		
Spring wheat (80 kg N ha^{-1}) Soybean (--)	Soybean (--) Corn (120 kg N ha^{-1})	Spring wheat (80 kg N ha^{-1}) Soybean (--)
Fertilizer applied: 26-April	3-May	9-April

Table 2.2. Row spacing and harvest details for each crop system from 2017 – 2019. IWG row spacing was alternating 40 and 61 cm.

Crop system	Row spacing	Grain/stover harvest date		
		2017	**2018**	**2019**
IWG + urea	40, 61 cm	23 Aug	9 Aug	12 Aug
IWG + manure	40, 61 cm	23 Aug	9 Aug	12 Aug
IWG + alfalfa	40, 61 cm	23 Aug	9 Aug	12 Aug
+ alfalfa	15.2 cm			
Spring wheat	15.2 cm	29 July	*NA*	12 Aug
Alfalfa	15.2 cm	*NA*	*NA*	*NA*
Soybean	76.2 cm	20 Oct	18 Oct	30 Oct
Corn	76.2 cm	*NA*	22 Oct	*NA*

**NA = not applicable*

Table 2.3. Analysis of variance (ANOVA) results for total grain, straw/stover, roots (0-60 cm), and leaf-area index (LAI) separated by year. Soil respiration (Rs), microbial biomass carbon (0-15 cm soil depth), activity of 4 soil hydrolytic and 2 oxidative enzymes (0-15 cm), and lipid biomass of 4 major soil microbial functional groups (0-15 cm).

		Plant biomass					Canopy
Source of variation		Grain	Straw/stover	Roots		Source of variation	LAI
2017					2018		
	Crop system	***	***	*		Crop system	***
2018						Day	***
	Crop system	***	***	**		Crop system X Day	***
2019					2019		
	Crop system	***	***	***		Crop system	***
						Day	***
						Crop system X Day	***

		Microbial biomass	Microbial lipids				Enzyme activity					Soil respiration
		Carbon	Actino	Fungi	Gram-neg	Gram-pos	B	C	N	P	O	Rs
2017 - 2019	Crop system	NS	NS	**	***	NS	NS	*	.	NS	*	**
	Date	***	**	***	***	NS	**	NS	***	***	NS	***
	Crop system X Date	*	NS	**	***	NS	NS	NS	NS	NS	NS	*

. = 0.05 < P < 0.10; * = 0.01<P<0.05; ** = 0.001<P<0.01; *** = P<0.001; NS = P > 0.05
Actino = Actinomycetes; Gram-neg/pos = Gram-negative/positive bacteria
Enzymes: B = β-glucosidase; C = cellobiohydrolase; N = N-acetylglucosaminidase; P = phosphatase; O = oxidative (peroxidase + phenol oxidase)

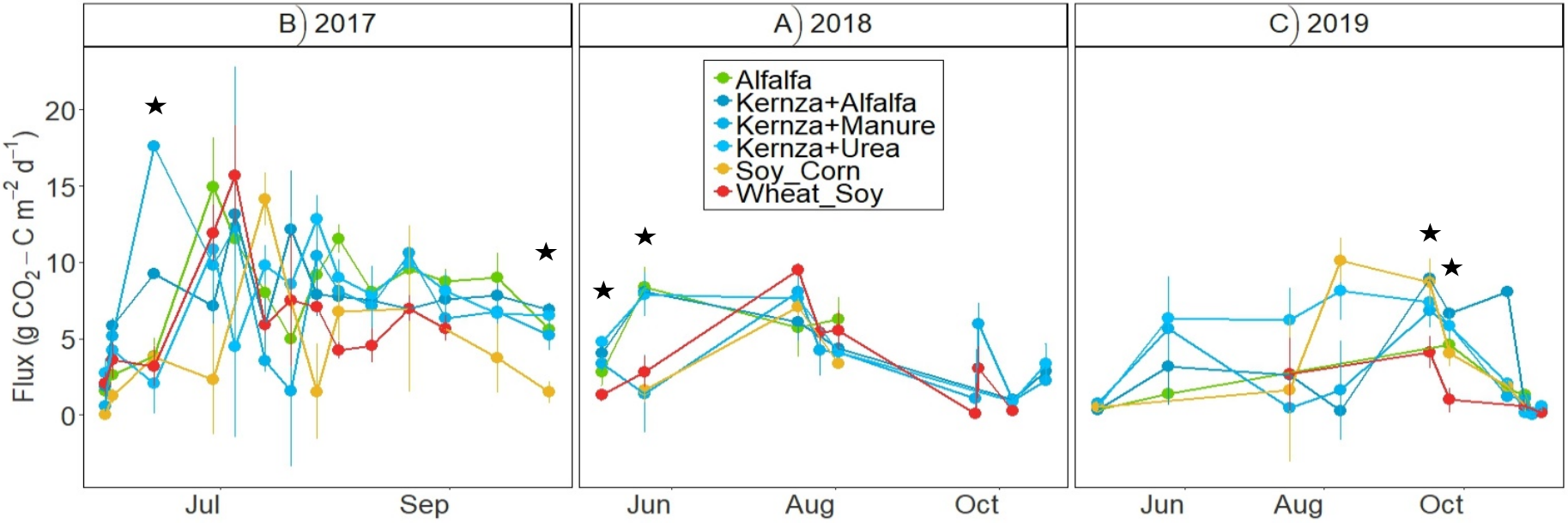


Figure 2.1. Fluxes of carbon dioxide-carbon (± SE, n = 4) over three years (A) 2017, B) 2018, C) 2019). Stars indicate where significant differences between crop systems occur ($P < 0.05$).

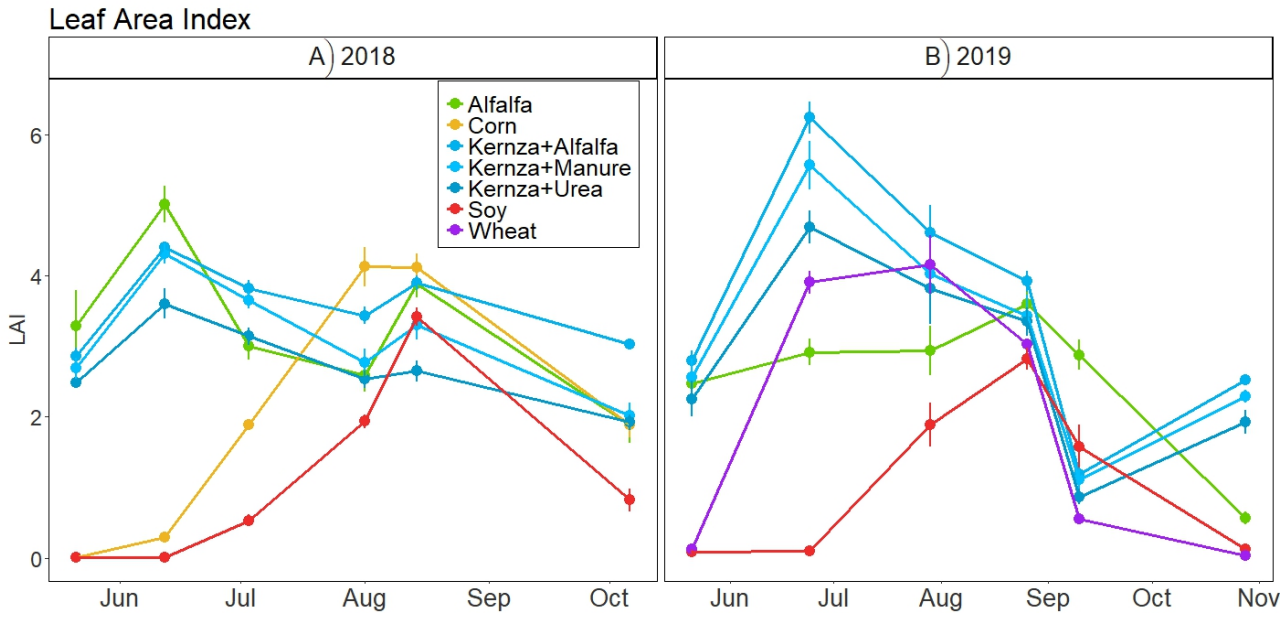


Figure 2.2. Leaf area index (LAI; ± SE, n = 4) from 2018 and 2019, LAI is reported in dimensionless units (m^2 m^{-2}).

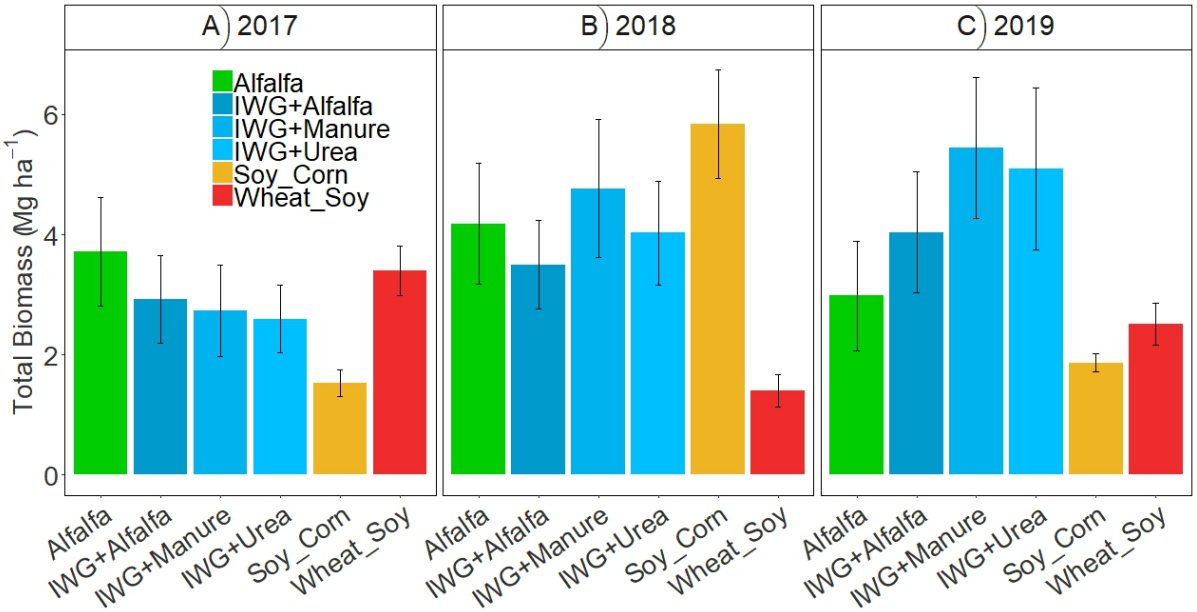


Figure 2.3. Total plant biomass production (sum of grain, straw/stover, and roots; ± SE, n = 4) by type and separated by year.

Table 2.4. Mean plant biomass production (± SE, n=4) across years and treatments. Shaded rows indicate annual crop rotations. Different lower-case letters denote statistical significance at $P < 0.05$.

		---------Dry biomass Mg ha^{-1}----------	
Treatment/Crop	—2017—	—2018—	—2019—
	Grain		
IWG + urea	0.41 (0.09)^{a}	0.53 (0.03)^{a}	0.34 (0.08)^{a}
IWG + manure	0.24 (0.08)^{a}	0.57 (0.07)^{a}	0.26 (0.07)^{a}
IWG + alfalfa	0.41 (0.06)^{a}	0.53 (0.04)^{a}	0.19 (0.08)^{a}
Spring wheat	3.81 (0.79)^{c}	--	1.90 (0.33)^{b}
Soybean	--	1.14 (0.25)^{b}	--
Soybean	1.73 (0.12)^{b}	--	2.06 (0.11)^{b}
Corn	--	9.86 (0.36)^{c}	--
	Stover		
IWG + urea	3.28 (0.26)^{b}	4.23 (0.30)^{b}	5.42 (0.34)^{bc}
IWG + manure	2.31 (0.29)^{b}	4.92 (0.54)^{b}	6.71 (0.46)^{c}
IWG + alfalfa	2.27 (0.28)^{b}	4.23 (0.46)^{b}	3.94 (0.87)^{b}
Alfalfa	*7.23 (0.50)^{c}	*6.69 (0.42)^{c}	*7.03 (0.41)^{c}
Spring wheat	2.22 (0.08)^{b}	--	3.46 (0.57)^{b}
Soybean	--	0.57 (0.09)^{a}	--
Soybean	0.63 (0.12)^{a}	--	1.23 (0.08)^{a}
Corn	--	4.74 (0.13)^{b}	--
	Roots (0-60 cm depth)		
IWG + urea	4.10 (0.95)^{ab}	7.32 (0.63)^{ab}	9.51 (2.37)^{b}
IWG + manure	5.66 (1.14)^{bc}	8.80 (1.72)^{b}	9.38 (0.60)^{b}
IWG + alfalfa	6.08 (0.43)^{c}	5.73 (1.01)^{ab}	7.98 (0.47)^{b}
Alfalfa	3.93 (0.18)^{a}	5.85 (1.50)^{ab}	1.91 (0.51)^{a}
Spring wheat	3.25 (0.79)^{a}	--	2.17 (0.68)^{a}
Soybean	--	2.50 (0.20)^{a}	--
Soybean	2.22 (0.31)^{a}	--	2.30 (0.13)^{a}
Corn	--	2.93 (0.41)^{a}	--

**Alfalfa herbage biomass was determined from the sum of three separate harvests each year.*

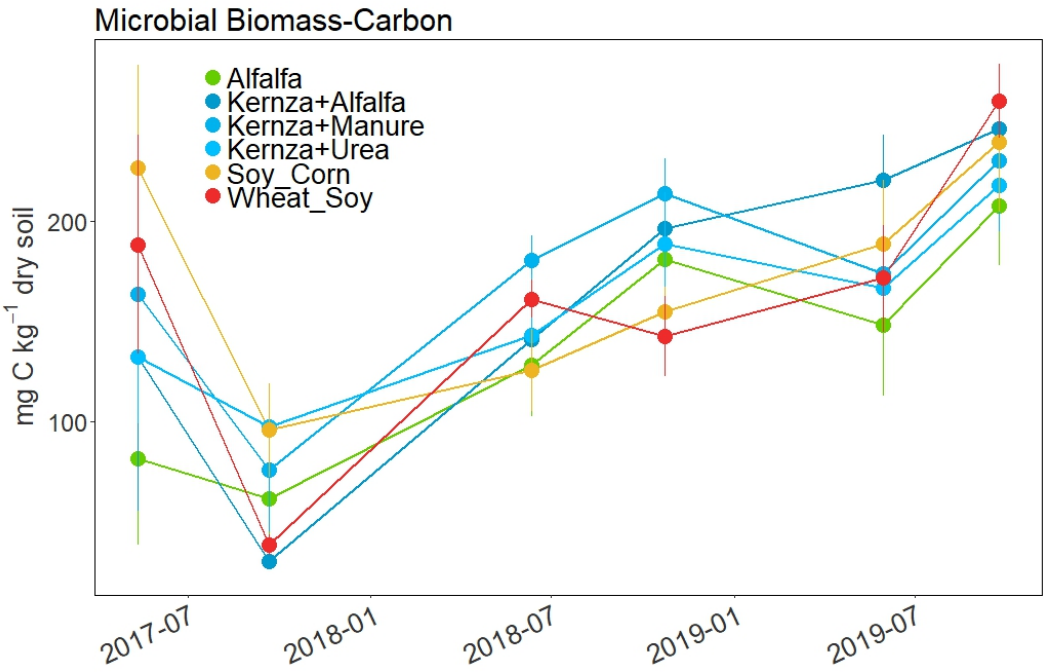


Figure 2.4 Soil microbial biomass-carbon to 15 cm depth, over 3 years (2017 – 2019). Dates in order from left to right; 2017 May 12, 2017 Sep 21, 2018 Jun 11, 2018 Oct 23, 2019 May 31, 2019 Sep 24.

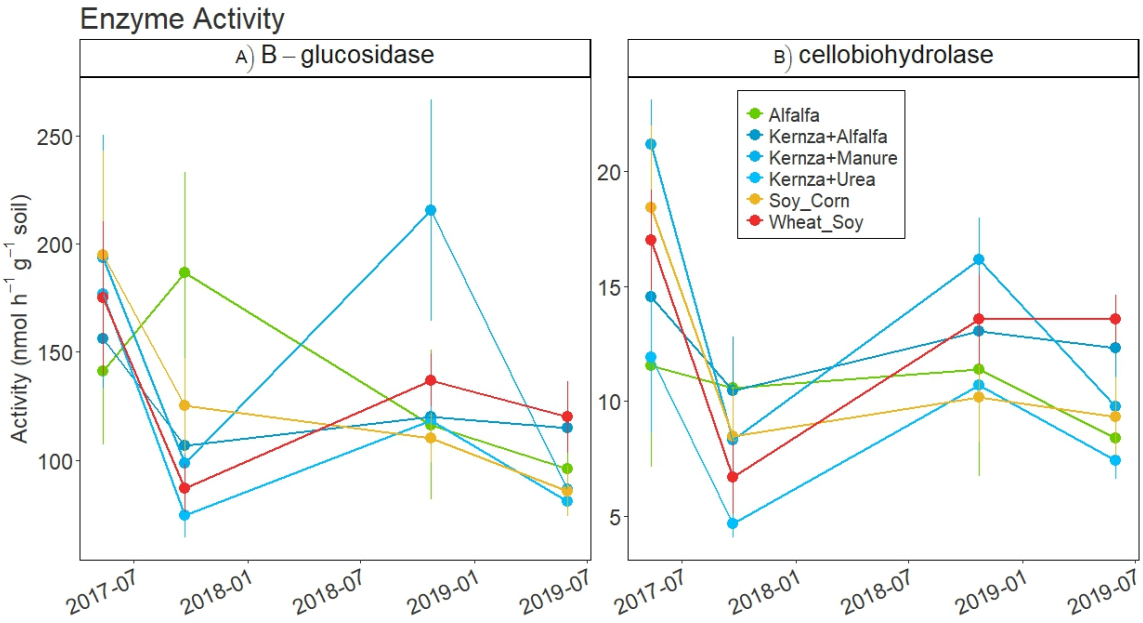


Figure 2.5. Soil enzyme activity (0-15 cm; ± SE, n=4) for two enzymes involved in the breakdown of cellulose, β-glucosidase and cellobiohydrolase. Dates in order from left to right; 2017 May 12, 2017 Sep 21, 2018 Oct 23, 2019 May 31.

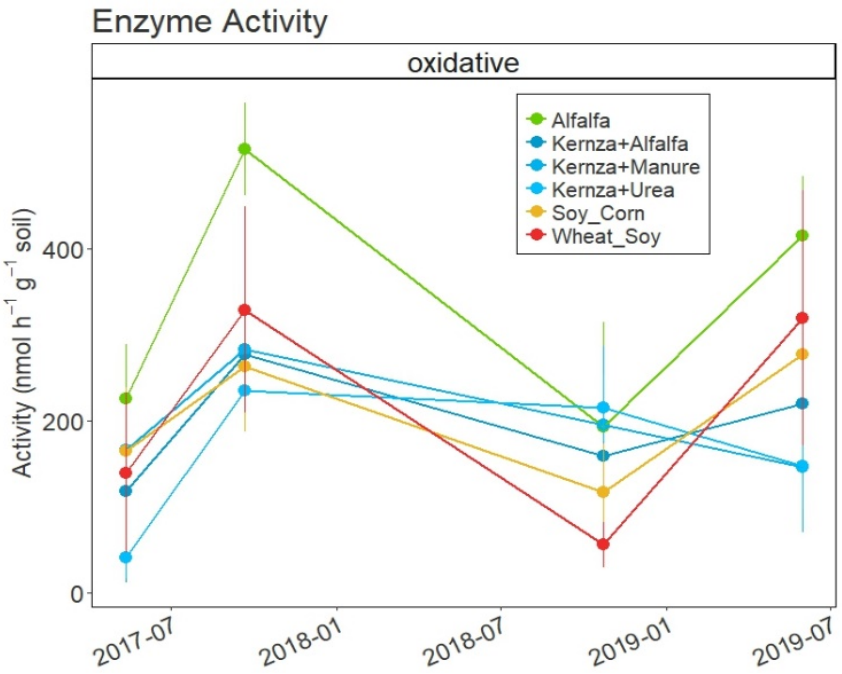


Figure 2.6. Soil enzyme activity (0-15 cm; ± SE, n=4) from the cumulative activities of oxidative enzymes, peroxidase and phenol oxidase. Dates in order from left to right; 2017 May 12, 2017 Sep 21, 2018 Oct 23, 2019 May 31. Stars denote statistical significance at $P < 0.05$.

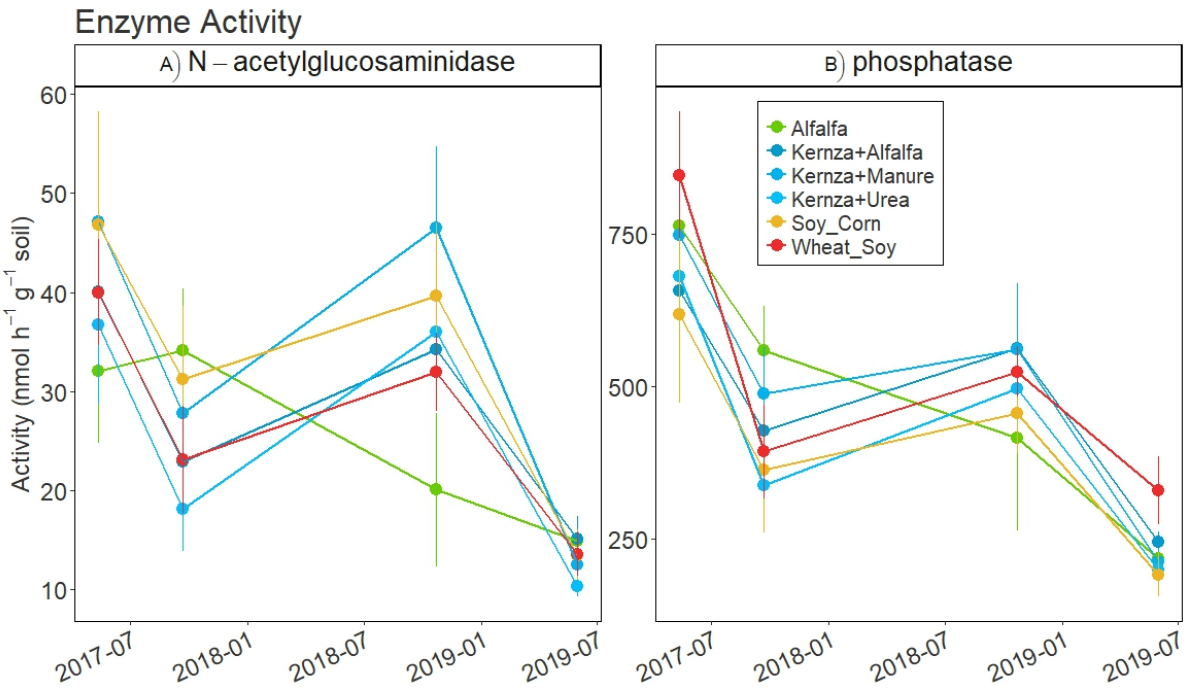


Figure 2.7. Soil enzyme activity (0-15 cm; ± SE, n=4) for the acquisition of mineral nitrogen (N-acetylglucosaminidase) and phosphorous (phosphatase).

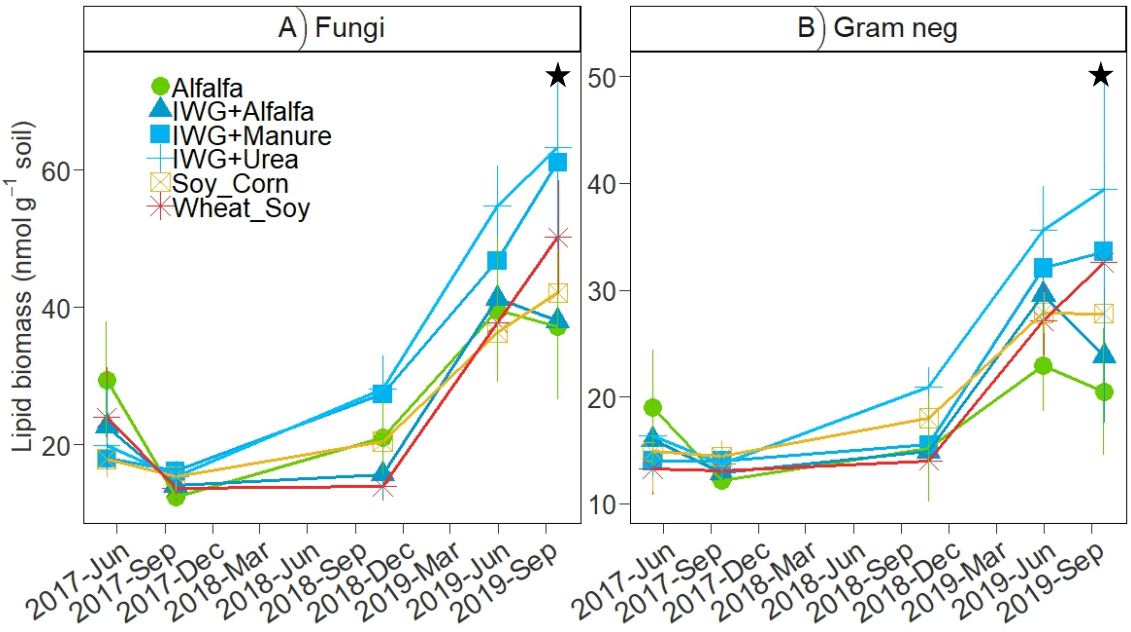


Figure 2.8. Lipid biomass (0-15 cm; ± SE, n=4) of two functional groups A) fungi, and B) Gram-negative bacteria from three years of fall samples and two years of spring samples. Dates in order from left to right; 2017 May 12, 2017 Sep 21, 2018 Oct 23, 2019 May 31, 2019 Sep 24. Stars denote statistical significance at $P < 0.05$.

Table 2.5. Lipid biomass, averaged over treatment for each year (2017 – 2019), of different soil microbial functional groups. Letters denote statistical difference for 31 May 2019 and 24 Sep 2019 at $P < 0.05$, standard error in parentheses beside means (n=4).

	Mean lipid biomass (nmol g-1 soil)		
	2017	**2018**	**2019**
Fungi	18.3 (1.2)	21.1 (1.8)	45.4 (2.6)
			IWG + urea[a]
			IWG + manure[ab]
			Soybean[ab]
			IWG + alfalfa[c]
			Alfalfa[c]
			Wheat[ab]
Gram (-) bacteria	14.6 (0.7)	16.4 (1.5)	29.3 (1.8)
			IWG + urea[a]
			IWG + alfalfa[b]
			Alfalfa[b]
Gram (+) bacteria	7.1 (1.1)	6.0 (0.6)	14.3 (0.7)
Actinomycetes	2.2 (0.2)	2.6 (0.5)	5.0 (0.3)
Anaerobic bacteria	4.6 (0.9)	5.3 (0.9)	4.2 (0.3)
Eukaryotes	14.5 (2.5)	2.7 (1.2)	10.3 (0.8)

Chapter 3

Non-destructive method for closed-chamber gas flux measurements

Summary

Managing the physical, chemical, and biological characteristics of agricultural soils is critical to limiting GHG emissions from agriculture. The contribution of GHG emissions from agriculture, forestry, and other land use was estimated at 24% of global GHG emissions as of 2014 (IPCC, 2014). Researchers and stakeholders are looking for more cost-effective and accurate ways to quantify these emissions as they address new strategies to mitigate emissions to the atmosphere. In this study, we evaluated a novel chamber design for plot scale measurements of three key greenhouse gases: carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). The chamber was designed to accommodate the biomass of intermediate wheatgrass (*Thinopyrum intermedium;* IWG), a perennial small grain, while also retaining adequate detection sensitivity. Soil respiration was measured with and without an IWG or soybean plant inside the chamber and dynamics within the chamber were evaluated by taking gas syringe samples pulled from four loci of the chamber. On average, CO_2 flux was greater from intermediate wheatgrass and soybean by 34% and 7%, respectively, when the chamber was directly covering a plant. A sensitivity analysis revealed minor or negligible effects of plant biomass volume on gas flux calculations using this chamber. The very top of the chamber extension was the only location with significantly different concentration of CO_2 over both experiments. This chamber

system represents a novel method for sampling trace gas flux from the plant-soil interface that minimizes disturbance and captures activity from the whole system.

Introduction

The measurement of greenhouse gases (GHGs) from agricultural systems is critical to understanding the impacts and climate mitigation potential of different crops and management practices. The most abundant GHGs; H_2O, CO_2, CH_4, and N_2O, are produced from natural cycles of climate, geography, growth and decomposition in the biosphere, however, they may be enhanced or reduced by human activities. For example, agricultural practices such as soil tillage may facilitate CO_2 production compared to reduced or no-till (NT) strategies (Almaraz et al., 2009; Silva et al., 2019). However, CO_2 production may also be higher for NT at certain times of the year because it often retains soil moisture for longer (Regina & Alakukku, 2010). Other GHG fluxes such as N_2O can be more difficult to predict, with negative, positive, or null responses all being reported depending on the length of time of data collection and on the soil moisture regime (Pelster et al., 2011; van Kessel et al., 2013). Furthermore, management strategies such as manure injection into the soil may reduce NH_3 volatilization but increase soil N_2O emissions relative to surface broadcasting manure slurries as plant fertilizer (Duncan et al., 2017).

From 1750 to 2017, the concentrations of atmospheric CO_2, CH_4, and N_2O have increased by 40%, 150%, and 20%, respectively (NOAA/ESRL, 2017). This dramatic shift in atmospheric composition over the last two and a half centuries has largely been attributed to anthropogenic influences on the global environment. Further knowledge of source and sink dynamics within agricultural systems can

provide insight towards future mitigation and sequestration strategies (IPCC, 2006).

Chamber measurements of soil respiration in the field were conducted as early as the 1920s (Lundegardh, 1926), using the method of a chamber attached to a collar that was pounded into the soil. However, for longer-term studies, and especially at the ecosystem scale, modern micrometeorological techniques (e.g. eddy covariance (EC)) have been used in quantifying CO_2 exchange between the biosphere and atmosphere (Baldocchi, 2014). An important limitation of EC and other micrometeorological techniques (e.g. flux gradient) are that large sections of uniformly managed field are required, thus making replicated field studies and examining effects of imposed treatments very difficult. With chambers, many replications are easier to establish and manage. The EC technique is also costly to establish and operate, thus chambers are still widely used as a cost-effective method for estimating soil gas exchange in a variety of contexts. This is especially true for measurement of N_2O and CH_4, for which chamber methods remain the most common measurement technique, because fast and precise analyzers for these gas species have only recently been developed and are more expensive than fast CO_2 sensors (Denmead, 2008). Furthermore, chamber measurements can be useful to capture spatial variability of GHG fluxes, and using many replications can be employed in a wide range of field environments to fill gaps in situations where EC technique cannot be applied (Pavelka et al., 2018).

Therefore, for measuring small plots with many replications, ground-level chambers are an effective tool and are relatively easy to deploy. However, a high

coefficient of variation is often observed when averaging emission values over the space and time represented by the study site (Dugas, 1993). The size of chambers can be variable and are more subject to problems including altering the temperature, pressure, and humidity conditions inside the chamber relative to outside (Denmead, 1984; Leuning & Foster, 1990).

Another limitation of chamber measurements includes the loss of detection sensitivity with an increase in chamber height, which presents a challenge for taller vegetation (Parkin et al., 2003). The inclusion of plants inside the chamber is often avoided because it implies the chamber height be increased to accommodate this volume which would subsequently diminish the detection sensitivity of trace gas emissions (Parkin and Venterea, 2010). Furthermore, living vegetation inside the chamber presents a challenge of determining what portions of the CO_2 emissions measured should be attributed to heterotrophic soil respiration or mitochondrial respiration of the roots and leaves, and what amount is assimilated by the plant through photosynthesis. According to the USDA-ARS GRACEnet protocol for chamber-based trace gas flux measurements, the production of CO_2 by living plant tissue (both above and below ground) contained within the chamber cannot be considered in estimates of ecosystem scale GHG production unless annual photosynthetic CO_2 uptake is also measured (Parkin and Venterea, 2010). Soil respiration represents both plant and soil microbial contributions and, because the chamber is opaque, we make the assumption that photosynthetic uptake of CO_2 halts in < 1 minute from the absence of light (Taiz and Zeiger, 2002).

The exclusion of living plants within the chamber may underestimate the soil biological activity if it is not situated in a location close enough to a plant's rhizosphere, where the exchange of nutrients attracts microbes. Greater substrate availability from root exudation and tissue death, along with plant-derived signaling for the purpose of nutrient acquisition and pathogen defense, among other functions, provide support for a larger microbial habitat under the influence of the rhizosphere (Berendsen et al., 2012; Cheng et al., 1996; Raaijmakers et al., 2009). Clipping vegetation from above the soil surface before deploying a chamber may also introduce bias with increased root respiration or exudate production. A study looking at N_2O emissions from switchgrass (*Panicum virgatum*) and alfalfa (*Medicago sativa*) grown in a productive prairie soil found that the day-to-day effects of biomass manipulation (including clipping and folding) on emissions were at first unobservable, but over time amounted to significant trends in most of their replications (Collier et al., 2016).

This study presents a novel chamber-based method to trace gas flux measurement with the intention of both preserving living vegetation within the chamber and maintaining adequate detection of gas exchange. We specifically focus our design to accommodate the large biomass of perennial intermediate wheatgrass (*Thinopyrum intermedium*; IWG), but imagine a similar design could be used for other novel or large biomass crops. Our objectives in evaluating this method were to (i) test the effect of different plant volumes on chamber flux calculations, (ii) compare measurements of gas concentration during deployment extracted at different locations throughout the chamber that would provide insight

to air flow and diffusion dynamics inside the chamber, and (iii) determine how the presence or absence of a living plant within the chamber would influence measured flux rates of CO_2.

Materials and Methods

Experimental site and design

Experiments were conducted on two different field sites at the same location; the University of Minnesota Southeast Agricultural Outreach and Research Station in Rosemount, MN. Soil type was Tallula silt loam, 6 to 12% slopes, eroded. Coarse-silty, mixed, superactive, mesic Typic Hapludolls. Depth to restrictive layer or water table >2 m.

Field plots of IWG, established in fall 2016, and soybean (*Glycine max*), established in spring of 2017, were part of a randomized complete-block design with four replications. Plot sizes were 5 m x 15 m.

Chamber design and operation

Trace gas flux of CO_2 was determined from data collected using an FTIR Gasmet® DX4040 portable gas analyzer connected to a closed chamber system. The chamber (Figures 3.1 and 3.2) consists of a stainless steel tray with a threaded PVC coupler (diameter = 8 cm) positioned and glued into a hole that was cut in the bottom of the tray. A PVC column 70 cm in length was threaded into this coupler and sealed with a threaded cap, acting as the extension for accommodating vegetation. For anchors, another steel tray with the bottom cut out was hammered into the soil (~10 cm deep) so that the top rim protruded < 5

cm above the soil surface and soil was tamped around the anchor to ensure a good seal. Anchors were installed at least 24 h prior to sampling and left *in situ*. Alligator clips were used to close the chamber to the anchor and a foam weather seal was also duct taped to the rim of the chamber to prevent leaks. The resulting sample area was approximately equal to the dimensions of the steel tray opening (14 cm width by 50 cm length).

Because the DX4040 contained a pump that circulated air continuously through the chamber and sample cell, a mixing fan was not used in the chamber to avoid disturbances to the pressure equilibrium (Madsen et al., 2010). Instead, an air vent proportional to the chamber size was used to stabilize the chamber pressure (W. A. Dugas et al., 1997; Hutchinson & Mosier, 1981; Parkin and Venterea, 2010). A manifold drawing air from opposite points inside the chamber was installed to ensure mixing of sample air.

The chamber was deployed for six minute sample runs and the DX4040 analyzer was programmed to record CO_2 concentration from the chamber every 20 s. The chamber was connected directly to the gas analyzer with two 6 mm (diameter) teflon hoses, one inlet and outlet, allowing the air that accumulated in the chamber to be pumped continuously through the gas analyzer at a rate of 1.5 L min^{-1} (Figure 3.1). Sample runs were performed over a period of about 4 hours during midday sun (typically between 11:00 am - 3:00 pm) where plot replications 1 - 4 were sampled in sequence in the first two hours and then resampled in the last two hours.

Pairs of chamber anchors were installed in soybean and IWG plots; one designed to accommodate a single plant within the chamber (to achieve greatest proximity to the rhizosphere) and the other to capture activity from the inter-row space (Figure 3.3). In soybean plots, interrow cultivation was applied three times over the growing season to prevent weed growth. To ensure a "no-plant" representation in the perennial IWG plots, tillers and weeds were avoided or removed in the area of anchor installation at least 24 hour prior to sampling.

Syringe Gas Sampling Procedure

Concentrations of CO_2 at different loci within the gas chamber were sampled using 15 mL plastic syringes with 22 gauge needles from multiple points on the chamber (Figure 3.1). Gas samples were stored temporarily in glass vials with grey butyl rubber stoppers before being analyzed with gas chromatography (Parkin and Venterea 2010). Sample ports on the chamber were made from black rubber stoppers cut to a thickness of approx. 1 cm. Each location on the chamber was sampled 8 times, giving 8 replicates from each sample run (Figure 3.1(b)). Syringe sampling was performed on 26-July and 22-September of 2018.

Plant volumes

To calculate plant volume, biomass was clipped (as close to the soil surface as possible) from an area equivalent to the chamber footprint. Most of this sampling occurred through August of 2018 when soybean and intermediate wheatgrass had reached peak biomass. Fresh biomass was briefly stored at 4 C° if necessary. To measure plant volume, a simple water displacement technique was applied. First, the stalks (biomass) were consolidated and held in a bundle by

wrapping pieces of masking tape around them at several points. The bundle was then cut into smaller bundles (each one held together by a single piece of tape) that fit into a 250 mL graduated cylinder filled with water. The volume of each bundle was determined from the difference of water level in the graduated cylinder before and after submerging the bundle and tapping the cylinder to remove air bubbles. These values were then summed together to achieve a volume for the total biomass.

Flux Calculations

Flux of CO_2 was estimated using equation (1) (Collier et al., 2014) with the assumption that change in concentration within the chamber increased linearly over time (linearity assumption was made based on Aikake information criterion (AIC) estimates of linear and non-linear models):

$$F = S \cdot V \cdot A^{-1} \qquad \text{Eq. 1}$$

Where F = flux, S = slope of regression ($\Delta conc \,/\, \Delta t$), V = chamber volume, A = chamber area. Thus:

$$\frac{(S\ mol\ L^{-1}\ hr^{-1}) \cdot V\ L}{A\ m^2} \qquad \text{Eq. 2}$$

Because the DX4040 records concentration measurements every 20 seconds as the air circulates from chamber to sample cell and back again, this

method can provide a sufficient slope term for flux calculation of CO_2 within 5 minutes. Longer sampling times may be needed in cases where flux rates are very low, or for other gas species like N_2O that are more episodic in production, generated as a response to volatile fertilizer and manure applications or from natural events such as seasonal flooding that can increase denitrification processes (Norman et al., 2012).

This is could be and advantage over sample collection from static chambers by yielding more concentration values in a shorter period, thus reducing the time that the chamber is deployed. However, this is assuming that changes in gas concentration can be detected accurately in that short period of time.

Data analysis and statistics

Syringe/GC gas samples were analyzed with linear mixed-effects models (LME), using gas concentration as a response to chamber loci (fixed main-effect) and block as a random effect for one-way ANOVAs. Because of an interaction between sample date and chamber loci, sample dates were analyzed separately. Gas flux data from the Gasmet FTIR was analyzed using an LME with crop treatment and date as main effects and block as a random effect. Two-way ANOVAs were performed on each crop type separately, and no interaction between crop treatment and date was found.

Results

Living Plant in the Chamber

Results for both plant types indicated that soil respiration is greater when a living plant resides within the chamber, however, this trend disappeared when measurements were taken after plant senescence had begun (for IWG and soybean). On average, CO_2 flux was greater from intermediate wheatgrass and soybean by 34% and 7%, respectively, when the chamber was directly covering a plant (Figure 3.4, 3.5). Flux of N_2O followed an opposite trend, indicating that N_2O production is reduced when a plant is present in the chamber (this was observed for both IWG and soy). The syringe samples supported these trends.

Gas concentrations within the chamber (Syringe/ gas chromatography)

Results from all of the syringe samples revealed more inconsistencies in the concentrations of CO_2 within the chamber compared to N_2O. Date of sampling had a strong interaction with chamber loci for CO_2 concentration (Figure 3.7). The concentration of CO_2 was significantly greater in the top of the chamber on July 26, but the opposite occurred (greater concentration in the bottom of the chamber) on September 22.

Plant Volume Effect on Gas Flux Calculations

Using average plant volumes (near and at peak biomass) of IWG and soybean, a sensitivity analysis to determine their influence on trace gas flux calculations when present inside the chamber revealed little effect of even the largest plant volumes (Table 3.1). This analysis only makes consideration towards the physical effect of air volume displacement within the chamber and does not

account for any influence the living plant may have on humidity, air temperature, or chemical composition and reactivity of air within the chamber environment. Furthermore, the complex architecture of a plant canopy, combined with the uncertainties of how the plant arranges itself upon closing the chamber over it, present additional challenges towards interpretation of these results.

Discussion

Closed chamber effects on canopy environment

Wagner and Reicosky (1992) found that plant leaf temperatures (of maize grown in southern Minnesota, USA) responded nearly instantaneously to the altered environment of a closed chamber, reporting leaf and air temperatures from inside the chamber increased from 2° to 4° C during the 60 s measurement period when ambient air temperatures ranged from 28° to 32° C (Wagner & Reicosky, 1992). However, Wagner and Reicosky used a chamber built with transparent plastic to allow light through. The chamber described in this paper was not transparent and was also covered in a white reflective tape to reduce warming of the chamber. Therefore, it is possible that leaf and air temperatures inside this chamber did not experience as significant of an increase over the same measurement period.

Living Biomass Inside the Chamber

Based on our results indicating higher CO_2 flux rates when a living plant is present in the sampling chamber, the assumption of a plant's rapid light

response and shutdown of photosynthesis in darkness is well supported. The opposite trend would be expected if photosynthesis (carbon assimilation) had persisted for several minutes into the relatively short 6 minute sample run. The production of carbohydrates from photosynthesis is closely associated with the rate of rhizosphere respiration as a substrate supply to the roots and rhizo-microbial life (Kuzyakov & Gavrichkova, 2010). Uptake and assimilation of carbon in photosynthesis can occur in seconds, but it is a light-dependent function of the plant that would just as quickly be halted in the absence of light energy (Taiz and Zeiger, 2002). Transport of sugars through phloem tissue from leaves to roots would continue in the absence of light and this transport typically moves at 0.5 to 1 m h^{-1} for most plants, indicating that sugars may continue to fuel rhizosphere activity and soil respiration for the entire 6 minutes of sample time in darkness (Kuzyakov and Gavrichkova, 2010).

It is possible the increased respiration for a plant-inclusive chamber can be directly attributed to the exact position of the chamber above the plant rhizosphere, more effectively capturing this CO_2 source relative to a chamber over the inter-row space. Respiration from the rhizosphere, including root respiration and rhizo-microbial respiration (e.g. oxidation of fine root litter and exudates) may contribute to 35-45% of total soil respiration in organic soils during the active growth period (Silvola et al., 1996) and up to 90% of soil derived CO_2 in forested and other densely vegetated ecosystems (Hanson et al., 2000). For an agroecosystem, this percentage of soil derived CO_2 from respiration will be less than 90%, but the rhizosphere is still the predominant contributor of soil CO_2. The

chamber's effect of blocking out sunlight may also have triggered dark respiration rates of the plant leaves to the effect of increasing mitochondrial respiration of CO_2 (Taiz and Zeiger, 2002). Further experimentation is needed to confirm this trend in chamber measurements that can be replicated across a longer time scale and with different plants.

Chamber Loci and Dynamics Inside the Chamber

Concentrations of N_2O within the chamber had less variation than CO_2, and in general had opposite trends from CO_2 in variability (Figure 3.8). High concentrations of CO_2 measured from the top of the chamber extension on 26 July, which was not observed in the second experiment on 22 September, is potentially related to both the phenology of the plant (IWG was within two weeks of grain harvest) and to the mechanics of positioning the chamber over the plant. Respiration of the seed spikes and flag leaves may have been higher than average during this time, or the disturbance of the seed spikes in positioning the chamber may have elicited stress in the plant that induced higher respiration rates from the seed spikes and/or flag leaves. However, due to abnormally low CO_2 concentrations (below ambient) of other chamber loci on 26 July, it is not clear whether this trend can be explained by the plant, chamber design, or by experimental error. Further experimentation is needed to determine the effects of the chamber at different times of the year.

Summary and Conclusions

This study provided evidence that chamber-based gas flux measurements, especially for CO_2, are sensitive to positioning directly over a plant v. directly next to it. The specific design of the chamber in our experiment, to maintain detection sensitivity while also including a living perennial plant inside, needs additional tests to verify that sample air is representative of emissions from the plant-soil system. However, CO_2 was the only gas that varied slightly within the chamber and there was no indication that plant volume had a significant effect on gas flux.

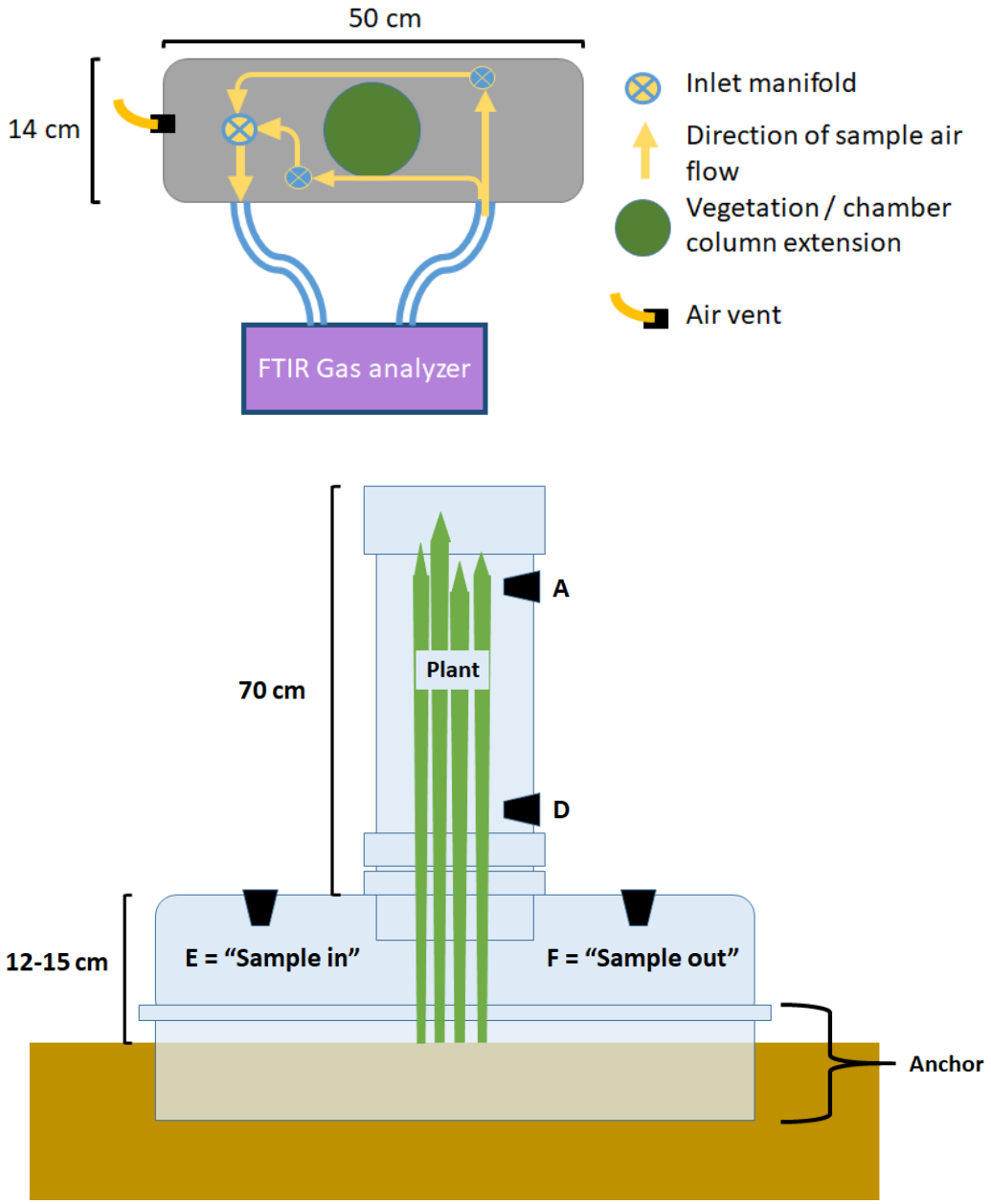


Figure 3.1 (a) Schematic diagram of air flow through the sampling chamber as seen from above. **(b)** Chamber diagram with anchor, including height dimensions and gas sample port loci. Sample port E was directly beside the sample inlet manifold for FTIR gas analysis.

Figure 3.2 Images of the inside of the chamber (top) and the chamber deployed in the field (bottom).

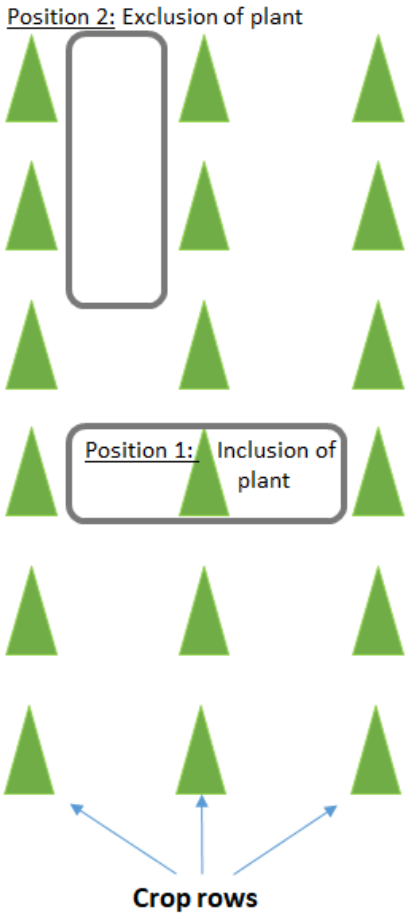


Figure 3.3. Chamber anchors were installed in two ways to compare inclusion and exclusion of the plant.

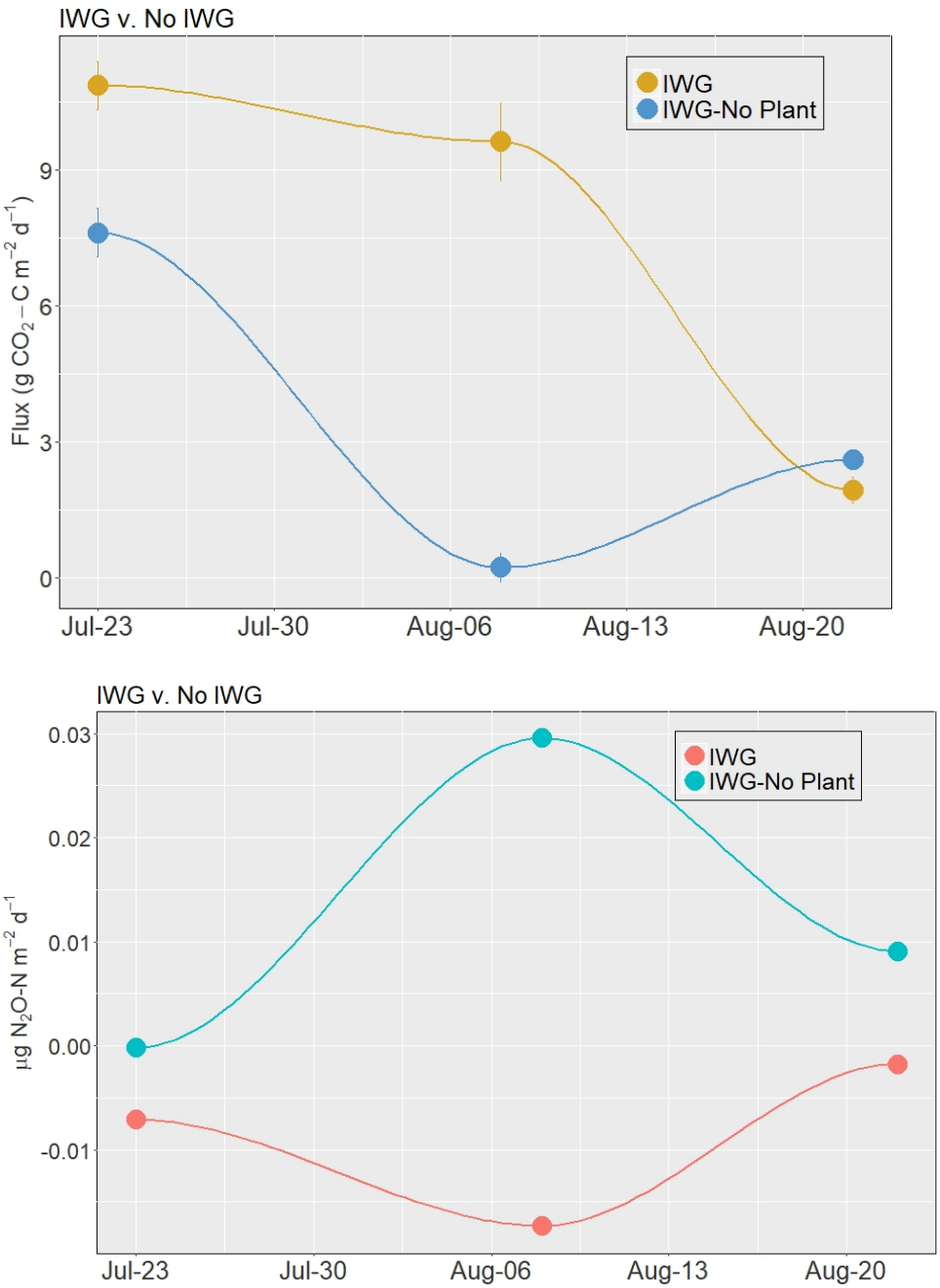


Figure 3.4. Soil respiration (top) and N_2O flux (bottom) with and without an IWG plant inside the chamber. Respiration rates were higher on average while N_2O was

reduced when IWG was included. The last sampling date was taken after grain harvest.

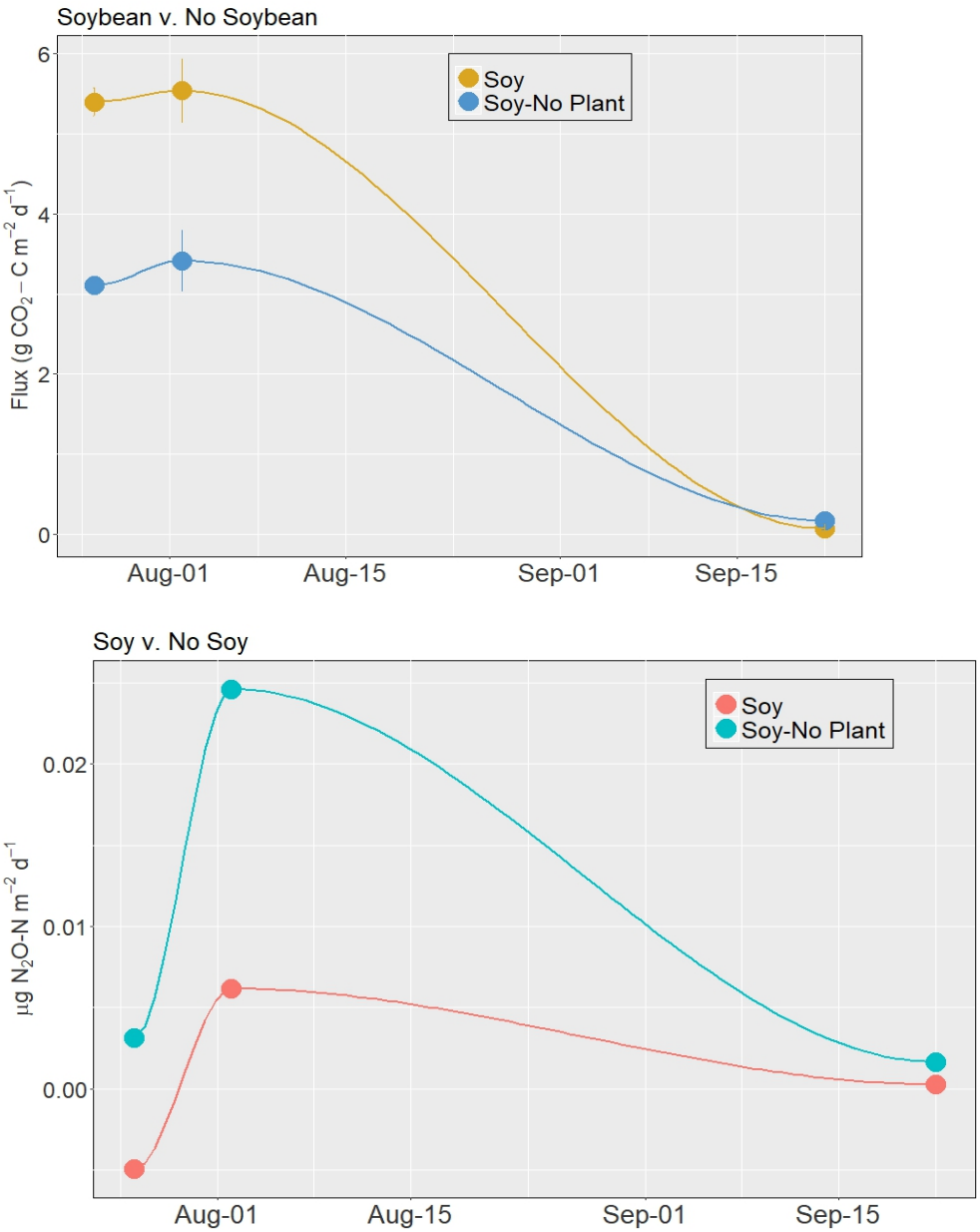


Figure 3.5. Soil respiration (top) and N_2O flux (bottom) with and without a soybean plant inside the chamber. Respiration rates were higher on average and N_2O was reduced soy was included, except for the last sampling date in September that coincides with soybean senescence.

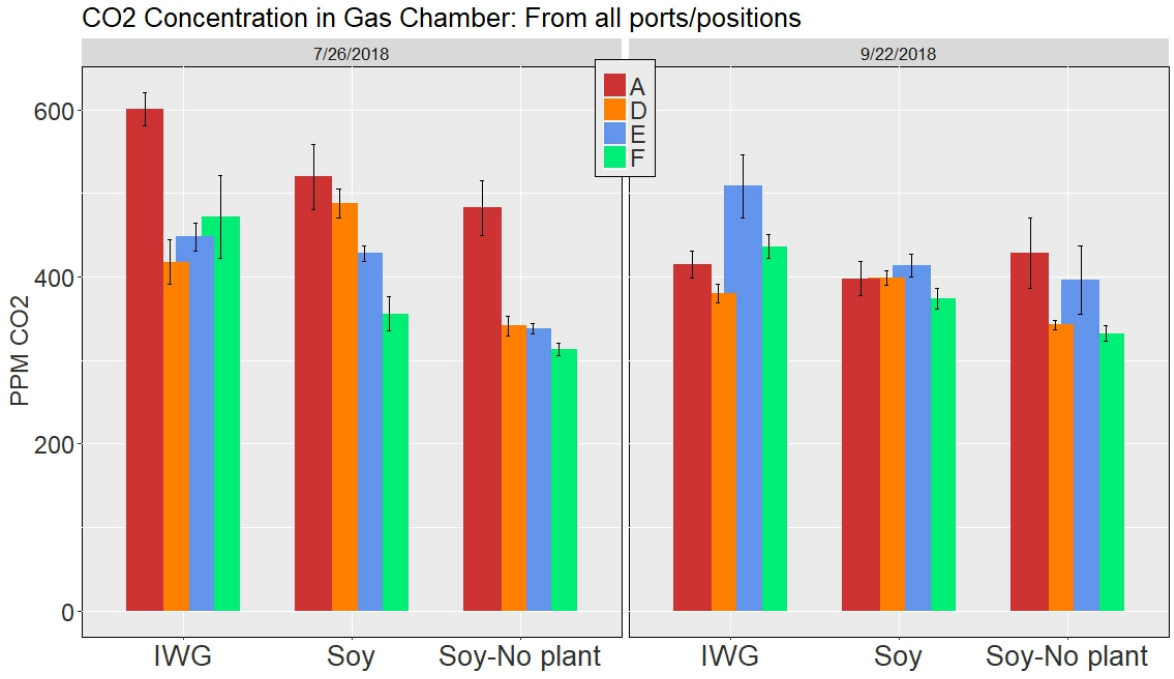
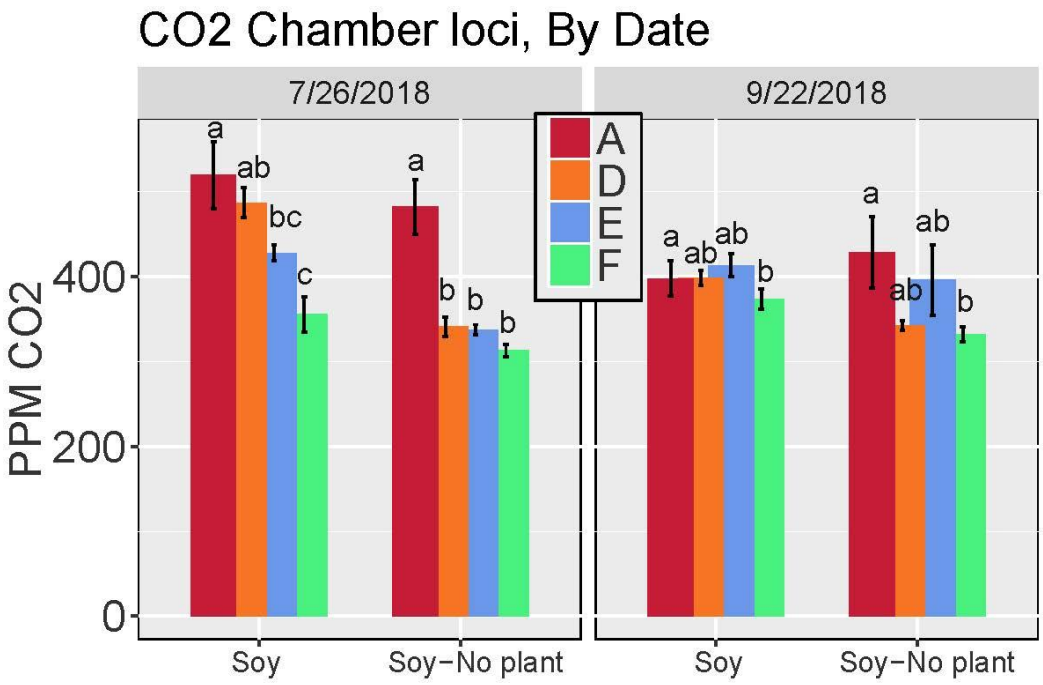


Figure 3.6. The concentrations of CO_2 measured from all chamber loci inside the chamber, across different treatments. Separated by dates, 26 July (left) and 22 Sep (right).


CO2 Chamber loci, By Date
7/26/2018
9/22/2018
A
D
E
F
PPM CO2
400
200
0
Soy
Soy−No plant
Soy
Soy−No plant

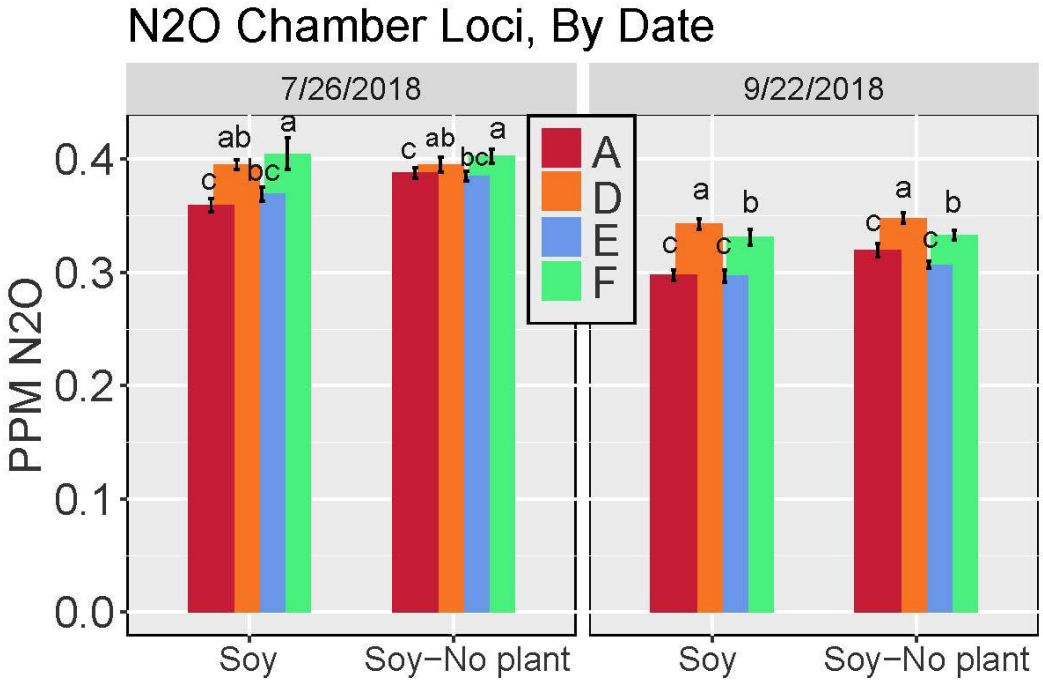

N2O Chamber Loci, By Date
7/26/2018
9/22/2018
A
D
E
F
PPM N2O
0.4
0.3
0.2
0.1
0.0
Soy
Soy−No plant
Soy
Soy−No plant

Figure 3.7. Concentrations of CO_2 (top) and N_2O (bottom) with and without a soy plant, separated by day. Letters denote statistical significance at $P < 0.05$.

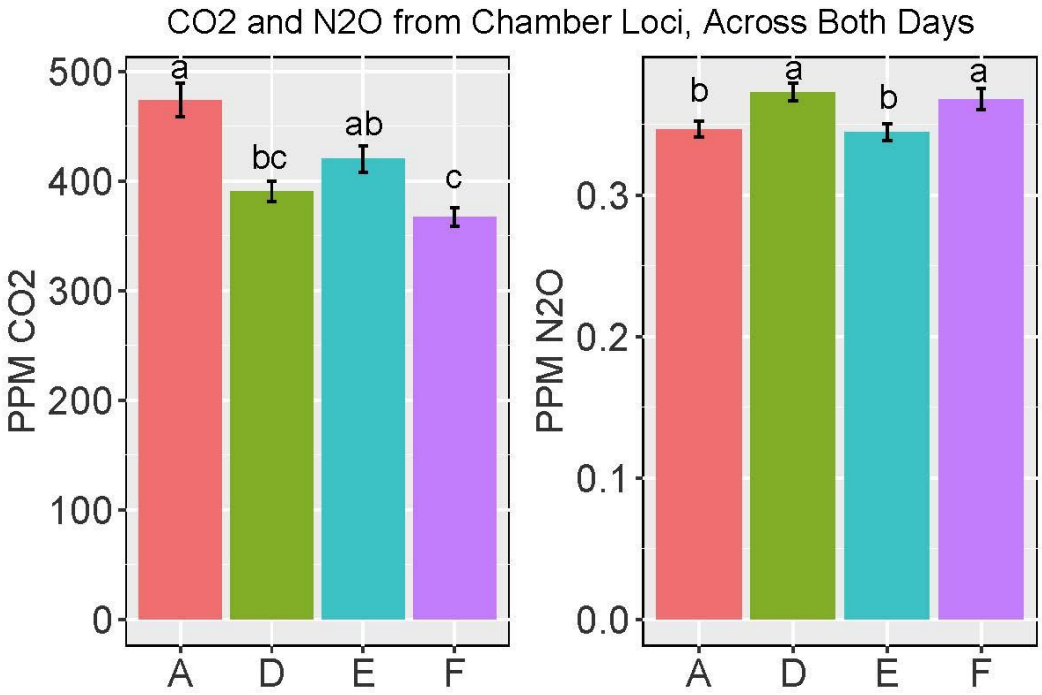


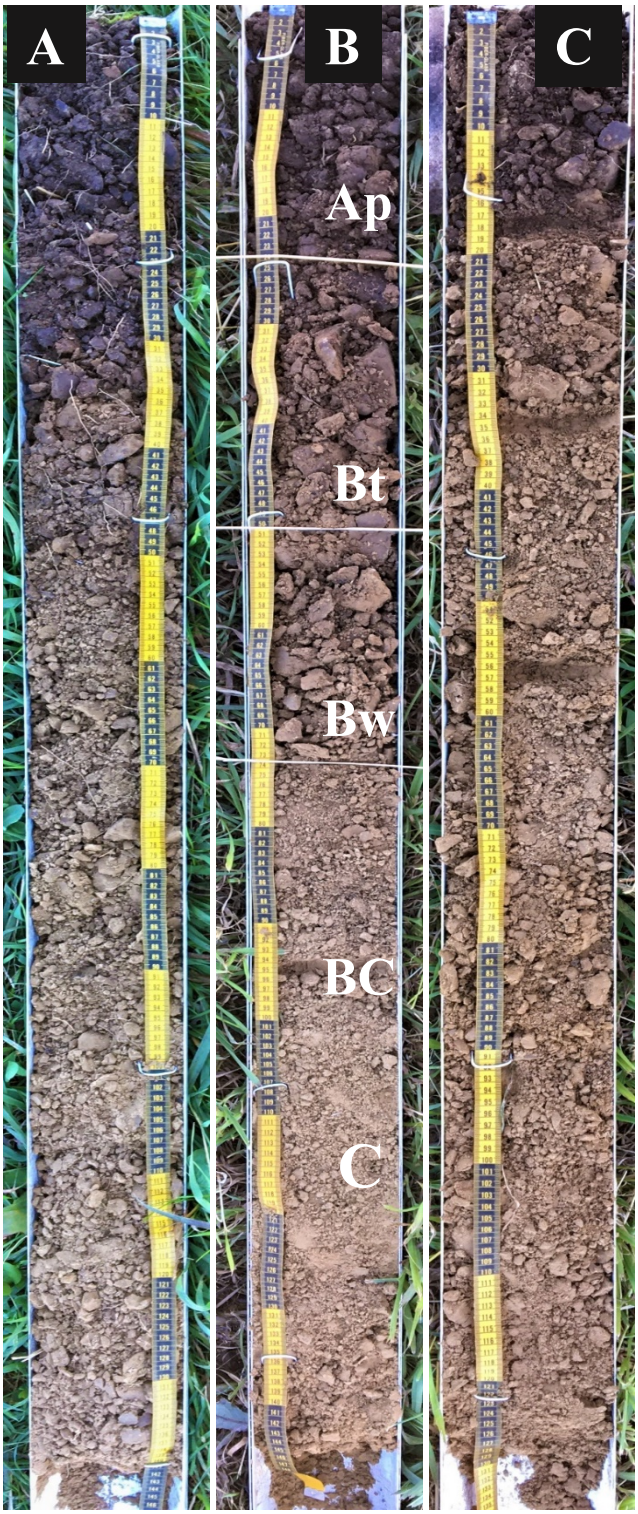
Figure 3.8. Concentrations of CO_2 (left) and N_2O (right) from syringe samples across both sample days (26 July and 22 September). This data combines samples from when both IWG and soybean were inside the chamber during sampling. Letters denote statistical significance at $P < 0.05$.

Table 3.1. Reduction in flux values based on calculation with three volume displacement scenarios. The average plant volumes of IWG and soybean were estimated leading up to and during time of peak biomass (for IWG; 12 June and 1, 3, 8, 15 August, for soybean; 14 August). The highest estimated plant volume displaced < 3% of the total chamber volume that would result in a reduction of flux value < 0.259 g C - CO_2 m^{-2} d^{-1}.

	Chamber volume w/extension	Volume displacement of 3.14%	Volume displacement of 5.24%	Volume displacement of 10.47%	Average plant volumes (cm^3)
"Free air" Volume (cm^3)	9550	9250	9050	8550	IWG: 176 ± 65.8
Delta flux (g C – CO_2 m^{-2} d^{-1})	--	-0.259	-0.432	-0.864	Soybean: 92 ± 19.8

Appendix

A.1. Soil profile images from study site of Ch. 2, augered to a depth of 120 cm. A) Block 1, northeast facing shoulder slope. B) Block 3, crest of hill. C) Block 4, southeast facing shoulder slope.



A.2. Calculations for theoretical grain and biomass yield.

A x B x C x D ***Eq. A1***

A = conversion "plot_grain_yld"g to kg,

B = number of row feet per length of 1 hectare side (i.e. 328.1ft/9ft=36.4)

C = # of rows per foot,

D = length (ft) of side of 1 hectare

A.3. Gas flux data quality control

Text files containing the concentrations of gases (CO2, N2O, MH4, in ppm) were read and organized in RStudio Version 1.0.143 – © 2009-2016 RStudio, Inc. R-script "2019 Gas flux analysis" was modified from "Annual gas analyzer" (Jungers, 2019) and designed for the purpose of visualizing the gas flux data, creating figures, and performing statistical analysis. Concentrations of gases measured every 20 s over a period of 5 to 7 min for each plot in the field were truncated (initial 100 s removed) to account for transitional disturbance during establishment of a chamber. Once a state of equilibrium within the chamber was reached, a continuous measurement was initiated (see Ch. 3 for details). The text files from each date were converted to a data frame in RStudio and merged with treatment information to create a time series data set. Concentration values (PPM) were converted to units of micrograms of element (C or N from CO2, N2O) per

cm^{-3} of air. A linear regression model (function "dlply" from plyr package) was used to determine slope:

Mass of element from gas (concentration) ~ time

Covariates = date, block, treatment ***Eq. A2***

The slope was used to calculate flux (eq. 3).

A.4. Microbial biomass carbon calculations.

$$\frac{baseline\ corrected\ conc.\left(\frac{mg}{L}\right)*.04\ L}{soil\ dry\ weight\ (g)} * \frac{20\ mL}{3\ mL} * \frac{1000\ g}{1\ kg} \qquad \textit{Eq. A3}$$

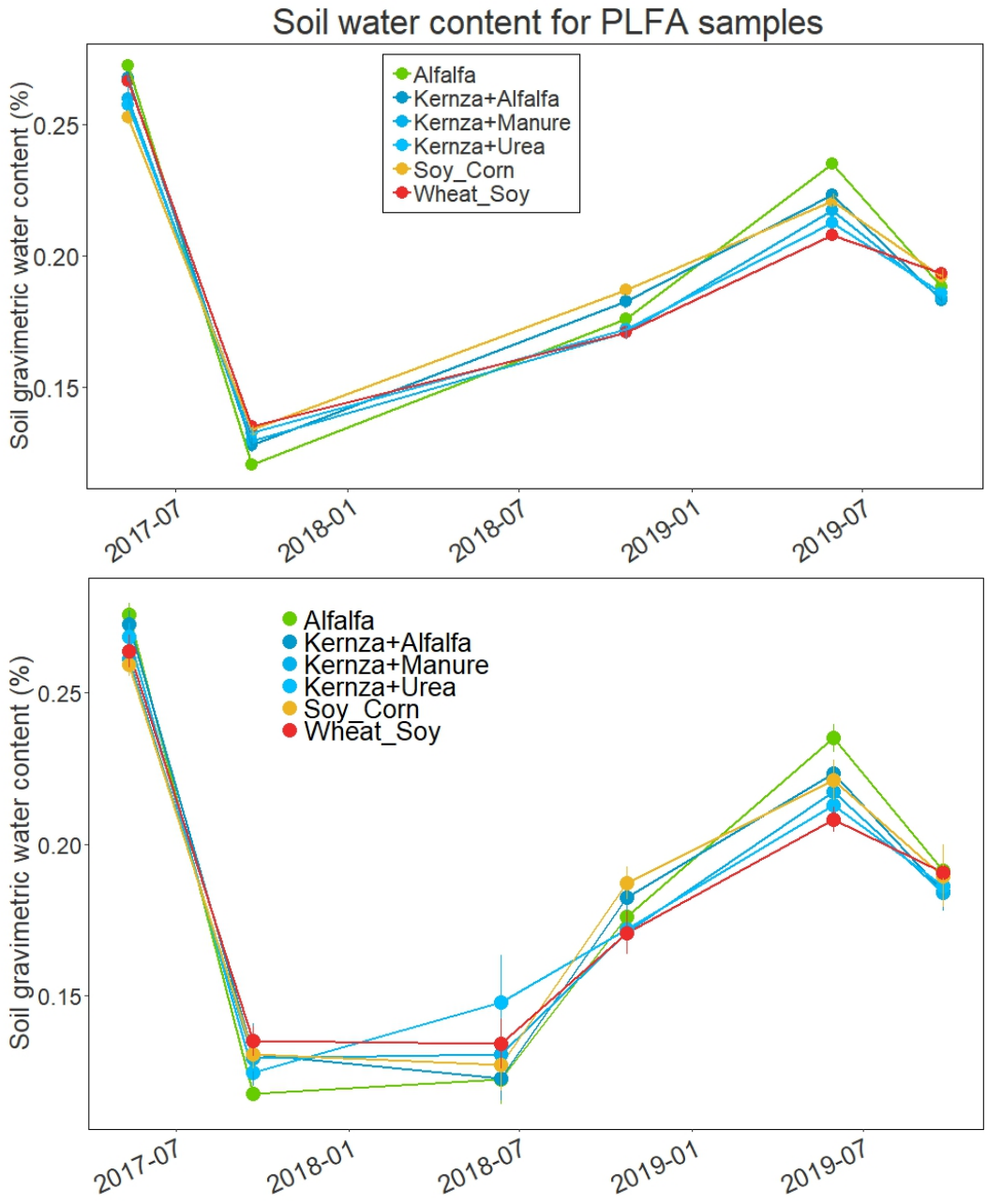
$$= mg\ C\ kg^{-1} dry\ soil$$

$$\text{MBC} = \frac{mg\ C\ kg^{-1} dry\ soil\ (fumigated\ soil) - mg\ C\ kg^{-1} dry\ soil\ (baseline\ control\ soil)}{0.45}$$

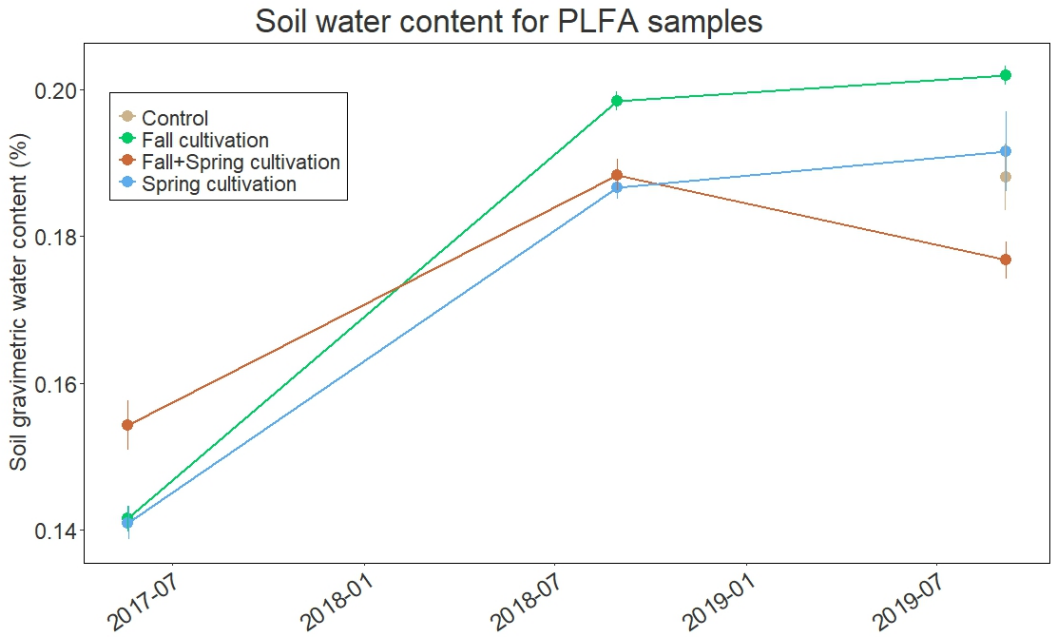
A.5. Calculations for lipid biomass (PLFA).

$$\text{nmol g soil}^{-1} = \frac{\text{(Peak area/K value)*(total volume in GC vial (µl)/2)}}{\text{(molecular weight of fatty-acid * soil mass (g))}} \qquad \textit{Eq. A4}$$

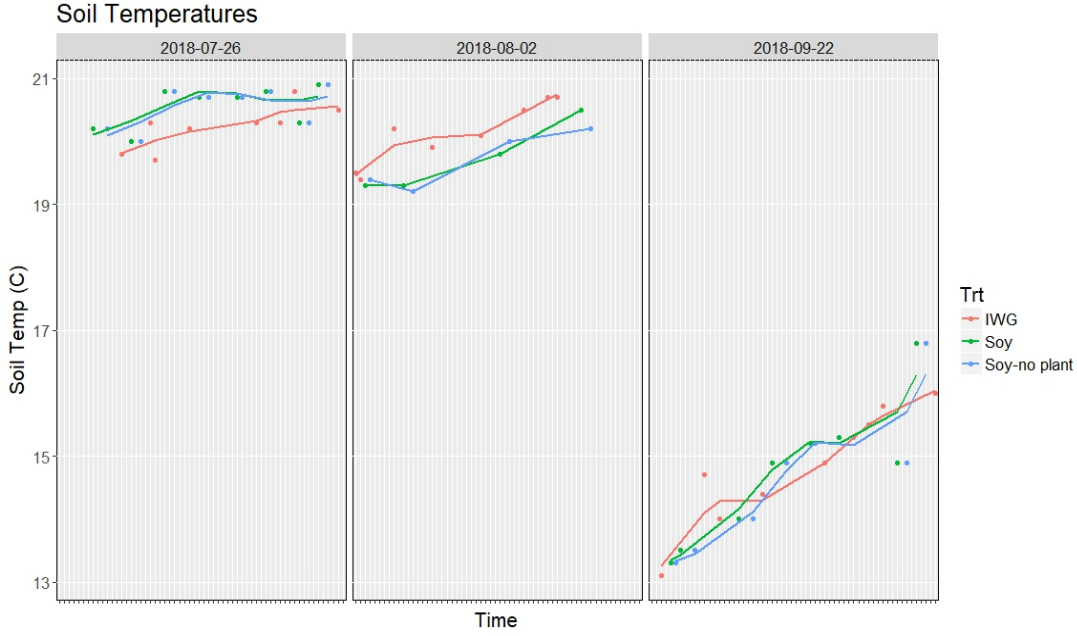
A.6. Soil gravimetric water content for each sampling date of PLFA (top) and MBC (bottom) from Ch. 2, both to a depth of 15 cm.

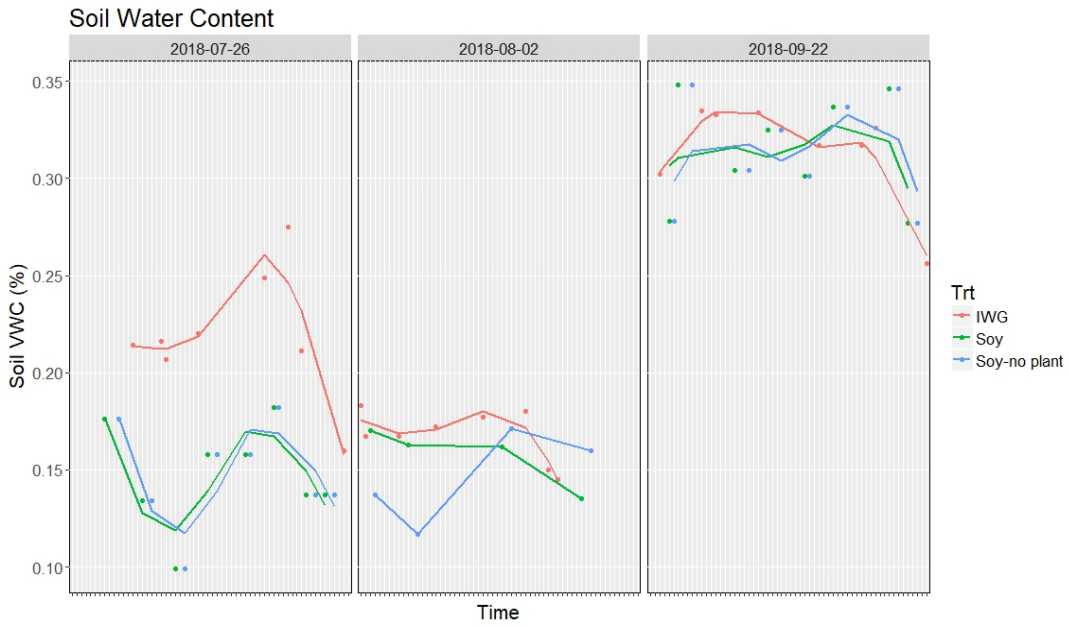


A.7. Soil gravimetric water content for PLFA of Ch.1.



A.8. Soil temperatures (top) and volumetric water content (bottom) for Ch. 3.




Soil Water Content
2018-07-26
2018-08-02
2018-09-22
0.35
0.30
0.25
0.20
0.15
0.10
Soil VWC (%)
Time
Trt
IWG
Soy
Soy-no plant

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