Above- and Belowground Response to Managing Kernza (*Thinopyrum intermedium*) as a Dual-Use Crop for Forage and Grain

THESIS

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By

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Abstract

Kernza is a novel perennial grain bred from lines of intermediate wheatgrass (*Thinopyrum intermedium*). Though recent developments in breeding have increased seed yields, Kernza still produces less grain than most annual cereals creating an economic barrier to farmer adoption. Managing Kernza as a dual-use crop for grain and forage would add another source of revenue thereby increasing the economic feasibility of Kernza and potentially alleviating the barrier to adoption. However, due to Kernza's novelty, little research has been conducted on this specific perennial grain let alone its performance as a dual-use crop. Therefore, the goal for this thesis was to assess the agronomic performance and soil health outcomes of Kernza when managed as a dual-use crop.

The first chapter of this thesis addressed whether managing Kernza as a dual-use crop would affect the crop's ability to positively influence soil health, a key selling point for this perennial grain. Specifically, this chapter determined aboveground biomass, root biomass and three soil health indicators under three defoliation treatments: summer cut, summer and fall cut, and no cut (control). Defoliation was carried out using mechanical harvesting equipment. Plant and soil measurements were taken in one month intervals throughout the first and second growing season at the OARDC in Wooster, Ohio. Aboveground measurements included plant height, forage biomass and grain yield which were determined with dry weights of hand harvested quadrat samples. Roots and soils

were sampled down to 20 cm using a 4-cm soil probe and separation of roots was carried out using a hydropneumatics root elutriator. Changes in soil health were evaluated using mineralizable carbon (C-min) and permanganate oxidizable carbon (POXC) as measures of active C and soil protein as a measure of organic N. Repeated measures mixed linear model analysis revealed that all plant and soil measurements were significantly affected by date, but only plant height, forage yield, grain yield, root biomass, and mineralizable C (C-min) were significantly affected by defoliation. Root biomass and C-min were significantly greater under defoliation treatments. Plant and soil dynamics varied significantly throughout the season and were closely related. Overall, the seasonal dynamics of soil C and N are primarily driven by plant dynamics and secondarily by weather. The findings of this study reveal that dual-use management had a positive effect on Kernza root biomass and stimulated greater short-term nutrient availability and cycling as opposed to carbon accumulation and stabilization in the soil. Overall, managing Kernza as a dual-use crop did not have negative effects on soil health.

The second chapter of this thesis evaluated Kernza's performance across a wide range of regions and environments both in terms of general productivity and dual-use management. Specifically, this chapter measured overall grain and forage yields in the first two years of growth across eleven sites in the United States and Canada to establish production potentials for regions and climates. This chapter also evaluated Kernza's performance as a dual-use crop in the first and second year of growth under four defoliation treatments: spring and summer cut, summer cut, summer and fall cut, and no cut (control). Forage and

grain yields were determined using a combination of hand-harvested samples and whole plot mechanical harvests. Mixed linear model analyses were carried out using repeated measures for individual sites. Overall, forage and grain yield were significantly affected by site, year, and a site by year interaction. Grain yields ranged from 526 to 1043 kg ha^{-1} in the first year of production and 3 to 655 kg ha^{-1} in the second year. Total annual forage yields ranged from 4.1 to 13.1 Mg ha^{-1} in the first year and 2.5 to 9.0 Mg ha^{-1} in the second year. Kernza produced more grain on average under dual-use management compared to managing Kernza for grain only and in general, Kernza grain yields were not affected by defoliation frequency or timing. Overall, Kernza crop performance varied between sites, with greater Kernza grain production on average in the Northeast region and greater Kernza forage production in the Great Plains and Upper Midwest regions. These results suggest that a summer forage harvest strategy or a summer and fall forage harvest strategy will maximum total Kernza grain and forage yields.

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1.1 Introduction

1.1.1. Perennials

There is a need in agriculture to adopt practices that increase sustainability due to the degradation of the environment and the increasing demand for food. The degradation of air, soil, and water is largely due to traditional annual cropping system's inability to provide the ecosystem services that are the foundation of natural systems (Tilman,1999; Syswerda and Robertson, 2014). Much attention has been devoted to practices that help offset the detrimental soil and water outcomes of annual production, such as no-till or conservation tillage, cover crops, and reduced inputs and more specified timing of fertilizer applications. However, a possible alternative to this patchwork of remedial practices exists in the perennialization of modern annual agriculture.

Perennial systems in nature, such as prairies and grasslands, maintain ecosystem services such as nutrient cycling (Crews, 2005), carbon sequestration (Beniston et al., 2014), microbial biodiversity (Culman et al., 2010, DuPont et al., 2010), and water retention and cycling (McIsaac et al., 2010) without regular external inputs as are required in annual grain systems (Glover et al., 2010; Crews et al., 2016). Perennial systems can efficiently maintain soil nutrients through belowground processes, as a study by Culman et al. (2010) showed that perennial grasslands that were annually harvested for 75 years and received no fertilizer inputs, maintained much greater soil fertility than adjacent annual croplands. Perennial systems excel at providing such services because their year-round ground cover and expansive and pervasive root systems influence the efficiency of nutrient and energy cycling (Kell et al., 2011; DuPont et al., 2014).

1.1.2. Kernza

Intermediate wheatgrass (IWG; *Thinopyrum intermedium*) has been the focus of breeding efforts and production research within the United States (Wagoner, 1990; DeHaan et al., 2005; DeHaan et al., 2013; Zhang et al., 2016). IWG is a cool-season, rhizomatous, perennial grass that belongs to the family Gramineae. Generally, the plant reaches 1-1.5 m in height and produces a grain visually similar to wheat but significantly smaller in size (Wagoner, 1995).

Intermediate wheatgrass possesses many attributes that make it suited for domestication relative to other perennial grass crops. Physiologically, IWG seed head matures relatively synchronously and exhibit less seed shattering than many other perennials, both important characteristics for achieving high yields (Wagoner, 1990). Intermediate wheatgrass varieties have been planted in the Great Plains and Intermountain West regions of the U. S. and are readily capable of mechanical production, including planting, harvesting, and threshing (Atkins and Smith, 1967).

Intermediate wheatgrass has been bred for increased grain production over the past decade at The Land Institute; they have trademarked this bred line of IWG “Kernza.” Though progress has been made in increasing the seed yields of “Kernza” relative to IWG forage varieties, Kernza still fails to produce grain yields that are comparable to annual wheat (Wagoner, 1995; Culman et al., 2013; DeHaan et al., 2013); a substantial barrier to producer adoption. To compensate for this lack in yield, alternative crop management ideas are being explored. One production alternative to make Kernza more economically feasible is to manage it as a dual-use crop: for both forage and grain. By managing Kernza for both forage yield and grain yield, it might meet the economic demands of crop production. However, the effects of managing Kernza as a dual-use crop on plant properties such as grain yield, forage yield, and root biomass have not been comprehensively studied.

Research conducted on IWG at the Rodale Research Center in the 1980s showed that post-grain harvest grazing of biomass increased grain yield in subsequent years (Wagoner, 1990). However, no research was conducted on the belowground effects of post-grain harvest grazing. In addition, no research has been conducted on the effects of multiple defoliation events per season on the belowground biomass of IWG, let alone

Kernza. Lack of such knowledge is significant because the maintenance of belowground biomass is critical to sustaining important soil processes that provide numerous benefits to the crop, soil, and overall ecosystem.

1.1.3. Roots

Roots heavily influence ecosystem services and overall soil health. For example, root biomass and length are positively correlated with chemical and biological properties of soils such as soil organic carbon (SOC) (Gill et al., 1999; Nadelhoffer and Raich, 1992), soil nitrogen (Dell and Rice, 2005), and microbial biomass (Farrar et al., 2003; DuPont et al., 2014).

An key aspect of soil health is soil carbon. Soil carbon and soil organic matter are important sources for plant nutrients and also influence other properties of the soil such as structure, CEC, and water infiltration (Doran and Parkin, 1994). Soil organic carbon pools are regulated primarily by root residues, as root residues supply significantly more carbon to the soil than shoot residues (Balesdent and Balabane, 1996; Rasse, Rumpel, and Dignac, 2005). Roots of perennials have shown 2.3 times greater root C in the surface 50 cm and 4 Mg ha^{-1} more root C in the surface 1 m than annual crops (Buyanovsky, Kucera, and Wagner, 1987; Glover et al., 2010). This greater transfer of carbon to the soil under perennials through root turnover and root exudation has created significantly greater soil carbon pools in comparison to annual cropping systems (Glover et al., 2010; DuPont et al., 2014).

Nitrogen management in soils has important implications for soil health. Nutrient runoff and leaching of N from agricultural systems decreases system fertility. Therefore, the ability of the cropping system to maintain and regulate the N dynamics of soils is important both economically (e.g., need to reapply N fertilizer every year), agronomically (e.g., soil fertility), and environmentally (e.g., water degradation). One way of maintaining N in soils is to increase the synchronicity between plant demand of N and soils supply of N. A study by Crews (2005) found that perennial roots have a variety of mechanisms by which they significantly influence the N pools of soil by increasing this synchronicity. Another way in which N is maintained within a system is through water retention. Because of a perennials greater root biomass, they are better able to access water and use it more efficiently than an annual, which can result in less water drainage and nitrogen leaching from the system (Glover et al., 2010; Culman et al., 2013). Regardless of the acting mechanism, perennial roots are generally more efficient at retaining and cycling N within the soil system than annual roots (Glover et al., 2010; Jenkinson et al., 2004; Syswerda et al., 2012).

While we can speculate on the dynamics of Kernza roots and their effect on associated soil processes based on studies of forage, prairie, and other perennial cereal crops, the structure, characteristics, and dynamics of the Kernza root system will inevitably differ in one aspect or another, as even the most similar crops do (Weaver, 1926). Therefore, a comprehensive knowledge of seasonal Kernza plant dynamics is first necessary in order to understand its interactions and effects on soil processes that can ultimately influence soil health. Once we understand the general dynamics between Kernza root biomass and soil health indicators we can begin to explore how changes caused by defoliation would affect overall soil health. To date, no study has been conducted to determine the seasonal dynamics of Kernza, let alone the seasonal dynamics of the entire Kernza plant and soil system.

1.1.4. Defoliation and Roots

The impact of aboveground defoliation on root characteristics has long been studied. Many field studies have examined the effects of aboveground defoliation on the belowground biomass of perennials but produced opposing results (Milchunas and Lauenroth, 1993). In a study by Christiansen and Svejcar (1988) root biomass was negatively affected by heavy defoliation as compared to light defoliation, while results by Pearson (1965) and Smoliak et al. (1972) concluded that root biomass was greater under grazed grasslands than non-grazed. Other studies (Lorenz and Rogler, 1967; Bartos and Sims 1974) reported no root biomass differences between grazing treatments, which was further supported by a meta-analysis conducted by Ferraro and Oesterheld (2002) in which they found that overall root biomass was not significantly affected by defoliation. Even within the same study, opposing results have been found (Turner et al., 1993;

Mapfumo et al., 2002). These differences in belowground response to defoliation can be attributed to a wide range of experimental conditions. The quantitative review conducted by Ferraro and Oesterheld (2002) revealed that some of the variability in the effects of defoliation were dependent on the frequency and recovery time between defoliations, concluding that overall greater negative effects were experienced when recovery times were short.

Many of these studies focused on mixed stands of perennials in established, longstanding grasslands or prairies (Biondini et al., 1998; Gao et al., 2008; DuPont et al., 2014) as opposed to relatively short-lived perennial monocultures that would typically be associated with Kernza management. Studies also applied defoliation in terms of grazing intensities either by various herbivores (Mapfumo et al., 2002) or mechanical manipulation to simulate grazing (Turner et al., 1993). While findings of grazing studies on grasslands may offer insight on the effects of defoliation on root biomass they are difficult to transfer to a Kernza crop that is managed for grain and forage because treatments in grassland grazing studies are usually imposed continuously over time as opposed to prescribed mechanical harvests at only specified points during the season. The presence of herbivores also provides an additional confounding factor into the root response dynamics (Matches, 1992). Therefore, due to the vast variability between production systems and defoliation methods and the conflicting results that exist in the literature it is necessary to independently determine the effects of defoliation on Kernza roots in the context of dual-use management.

Because Kernza is a relatively new crop being considered for dual-use production, most studies have focused on forage yields (Wagoner, 1990; Liebig at al., 2008; Wang et al., 2014), grain yields (Lee et al., 2009), grain quality (Zhang el al., 2015), and forage quality (Karn et al., 2006). Those studies that are focused on belowground aspects of Kernza have evaluated Kernza has a perennial in comparison to an annual (Culman et al., 2013). To date, no studies have tried to link the effect of aboveground defoliation on Kernza's belowground biomass to soil health characteristics.

1.2 Objectives and Hypotheses

The objectives of this study were to i) determine the seasonal dynamics of Kernza plant biomass and C and N soil health indictors, ii) establish general relationships between plant and soil dynamics in a Kernza production system and iii) evaluate the effects of defoliation on Kernza root biomass and soil health.

We hypothesize that Kernza forage and root dynamics will be synchronized with soil carbon and nitrogen dynamics and above- and belowground production will be inversely related to soil labile C and N pools. We further hypothesize that above- and belowground growth will drive soil C and N seasonal dynamics in the soil and that roots will heavily influence C and N levels and cycling. We hypothesize that, overall, defoliation of Kernza forage biomass will not have a significant effect on root biomass and therefore will not affect soil health.

1.3 Materials and Methods

1.3.1 Site Description

The experiment was carried out at the Schaffter farm site in Wooster, Ohio (40°45’27.79”N, 89°53’56.71”W). The soil at this site is of the Wooster-Riddles silt loam soil series (fine-loamy, mixed, mesic Typic Fragiudalfs). The mean annual precipitation is 95.1 cm and the mean annual temperature is 9.8° C. Prior to this study the field was in wheat (*Triticum aestivum*).

1.3.2 Site Management

On August 27, 2014 Kernza was seeded at a rate of 16.8 kg ha^{-1} using a Great Plains no-till drill adjusted with the drops completely open. However, because of problems encountered with the machinery used to seed, the seeding was performed twice in a checkboard pattern to reach the desired seeding rate. During the following spring of 2015, a Barber drop spreader was used to broadcast monoammonium phosphate fertilizer (MAP, 52% P_2O_5) at 15 kg P ha^{-1} and muriate of potash (MOP, 60% K_2O) at 33 kg K ha^{-1}. Urea was applied to the field at 45 kg N ha^{-1}. Detailed site management can be found in Table 1.1.

Table 1. 1.Site management history of activities and measurements taken.

Date	Activity	Measurements Taken†
August 27, 2014	Kernza seeded with a Great Plains no-till drill at Schaffter Farm, Field 29 at 16.8 kg ha^{-1}.	-
April 24, 2015	Fertilizer application broadcast MAP at 67 kg ha^{-1}, MOP at 67 kg ha^{-1}, and urea at 45 kg N ha^{-1}	-
August 5, 2015	Baseline quadrat biomass sampling	Soil, Roots, Forage Biomass
August 12, 2015	Grain Harvest	-
August 13, 2015	Summer Harvest	-
August 19, 2015	Fertilizer application broadcast at 45 kg N ha^{-1}	-
September 3, 2015	Second biomass sampling	Soil, Roots, Forage Biomass
October 13, 2015	Fall Harvest and third biomass sampling	Soil, Roots, Forage Biomass, Plant Height
November 12, 2015	Fourth biomass sampling	Soil, Roots, Forage Biomass
March 30, 2016	Fertilizer application broadcast at 45 kg N ha^{-1}	-
April 25, 2016	Fifth biomass sampling	Soil, Roots, Forage Biomass, Plant Height
May 26, 2016	Sixth biomass sampling	Soil, Roots, Forage Biomass, Plant Height
June 28, 2016	Seventh biomass sampling	Soil, Roots, Forage Biomass, Plant Height
July 26, 2016	Eighth biomass sampling	Soil, Roots, Forage Biomass, Plant Height, Seed Heads
August 2, 2016	Grain Harvest	-

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Table 1.1 continued

August 3, 2016	Summer Harvest	-
August 15, 2016	Fertilizer application broadcast at 45 kg N ha^{-1}	-
August 30, 2016	Ninth biomass sampling	Soil, Roots, Forage Biomass, Plant Height
October 6, 2016	Fall Harvest and tenth biomass sampling	Soil, Roots, Forage Biomass, Plant Height
November 12, 2015	Eleventh biomass sampling	Soil, Roots, Forage Biomass, Plant Height

†Soil measurements include permanganate oxidizable carbon, carbon mineralization, protein, and moisture

1.3.3 Experimental Design

In the summer of 2015 a randomized complete block design with four replications was established and overlaid onto a solid stand of planted Kernza. Twelve plots were measured to 1.8 by 4.5 m and flagged to the northwest portion of the field. The three treatments assigned to the plots are i) Summer Cut (Su), ii) Summer and Fall Cut (Fa + Su) and iii) No Cut, which serves as the control for the experiment.

Grain was harvested from all plots and all treatments. Grain was mechanically harvested on a plot basis using a Hege 140 plot combine. The combine harvested a 1.4 m wide cut down the full 4.5 m of each plot. The table of the combine was raised to cut 7.6 cm below the seed head. Grain harvested from this single pass was used to calculate whole plot grain yields. After grain was removed from all plots, forage biomass was harvested from plots prescribed to the Summer Cut and Summer and Fall Cut treatments. The forage was removed using a mechanical hay harvester that was adjusted to cut at 10 cm above the ground. The harvester cut a 1.2 m wide cut down the full length of the plots. Forage harvested from this single pass was then used to calculate total plot forage biomass. After all Summer Cut and Summer and Fall Cut plots had been harvested for forage, remaining grain and biomass (excluding the No Cut plots) was removed using a flail chopper. Remaining seedheads in the No Cut plots were removed using a Sheerlund tree shearer.

The fall forage harvests were conducted using a Cub Cadet push mower. Forage was only harvested from plots prescribed the Summer and Fall Cut treatment. For the fall 2015 harvest one swath (2.4 m^2) was cut from each plot to calculate total plot forage yield; total wet weights were taken in the field and random subsamples were collected and taken back to the lab for moisture and dry yield determination. Any remaining forage within the plots was then cut and discarded. The fall 2016 forage harvest was conducted by cutting all biomass within the plot with multiple passes and using this to determine total plot forage yield. After harvest, forage biomass was discarded outside of the plots.

1.3.4 Biomass Sampling

A single quadrat (0.25 m^2) was systematically placed in each plot, with the exception of the first sampling event, which took two 0.25 m^2 quadrats measurements per plot in order to obtain more accurate baseline measurements. The quadrat was placed on each plot in an assigned location that differed for every sampling event with the intent of avoiding legacy effects caused by defoliation of forage biomass and core sampling for soil and roots. Ten tillers were randomly selected from within the quadrat for height measurements. Heights were then determined by measuring from where the tiller met the soil to the tip of the uppermost leaf. Following plant height measurements, all biomass within the quadrat (living or dead) was cut to a height of 10 cm above the ground. The cut quadrat biomass was then placed in individual brown paper bags, dried at 50-70°C for 48-72 hours and weighed to determine dried biomass per quadrat.

The only exceptions to these procedures were on the dates that corresponded to the summer forage and grain harvest (8/5/2015 and 7/26/2016). On these dates, quadrat placement method was not changed. In keeping with normal protocol ten individual tillers per quadrat were randomly sampled for plant height. However, plant height was determined by measuring from the base of the tiller to the tip of the seed head. For the 2016 date seed heads within the quadrat were counted and clipped off slightly below the base of the seed head using hand pruners. Clipped seed heads from quadrats were placed in individual brown paper bags, weighed for wet weights, dried for one week, and weighed again to determine moisture content and dry weight per quadrat. Quadrat seed head samples were sent to The Land Institute for threshing. Once received, seed heads were dried, weighed, and threshed to determine total grain weight per quadrat. After seed heads had been threshed, percent of naked seed present in the threshed sample was determined and conversely the percent of seed still in-hull. Total grain values were then adjusted using the naked and in-hull seed percentages and a conversion factor of 0.7 with the following equation.

(total threshed grain weight x % naked seed in threshed sample) + (total sample threshed grain weight x % seed in-hull in threshed sample x 0.7)

The 0.7 conversion factor is based on the estimate that Kernza seeds in hull are typically 70% seed and 30% hull by mass (Lee DeHaan, Personal Communication). The adjusted sample grain weights were then used to calculate grain yields on a kg ha^{-1} basis. The seed head portions of this specialized protocol were not implemented for the 2015 date, instead, seed heads were not removed and were included in the overall biomass harvested from quadrats. Grain yields for 2015 were determined from plots that were being used in a concurrent Kernza study which was taking place in the same field as this study. Plots selected for grain yield determination were those that had been prescribed identical treatments and shared the same management histories.

1.3.5 Root and Soil Sampling

Soils were sampled for both roots and soils collectively. The area within the quadrat used to sample aboveground biomass was used to sample roots and soil. Using a Giddings 5 cm bore soil probe with a 4.4 cm liner, two cores were taken from areas absent of crowns and tillers within each quadrat to a depth of 20 cm. The two samples were then bulked and mixed in plastic bags until a homogenous composite was obtained. 200 mg subsamples were taken and stored at 4°C until root elutriation. Subsamples of soil were taken to determine moisture content gravimetrically. Remaining samples were air-dried for soil analyses.

1.3.6 Root Elutriation and Separation

Separation of roots from soil was carried out using a hydropneumatic root elutriator (Smucker et al., 1982). 200 mg subsamples were taken from each combined soil and root sample for elutriation. Each subsample of soil and roots were individually released into the elutriator where soil was removed from the roots using a gentle bubbling of the water for 5 min. After the soil had been removed, roots then floated onto a 1 mm sieve. Roots and any other residue were removed from sieves manually with tweezers. Due to the characteristic of perennial roots to display varying texture and color it was not possible to accurately distinguish between living and dead roots and therefore, they were not separated accordingly. Root biomass was then oven-dried for 48-72 hours at 50-60°C and weighed to determine root mass per area.

1.3.7 Soil Analysis

1.3.7.1 Mineralizable Carbon

Short-term (24 h) mineralizable carbon was performed to determine the metabolic activity of the soil microbial community. The determination of carbon mineralization was based on the methods of Franzluebbers et al. (2000) and Haney et al. (2001). Briefly, exactly 10 g of air-dried soil was measured into 50-mL polypropylene screw-top centrifuge tubes. Soils were rewetted with deionized water to 50% water-filled pore space which was previously determined gravimetrically (Haney and Haney, 2010). The tubes were then tightly caped and kept in the dark at 22°C for 24 h. CO_2 concentrations were determined with an LI-840A CO_2/H_20 infrared gas analyzer.

1.3.7.2 Permanganate Oxidizable Carbon

Permanganate oxidizable carbon was performed based on the methods of Weil et al. (2003) with slight modifications as detailed by Culman et al. (2012). Briefly, 20 ml of 0.02 mol L-1 $KMnO_4$ was added to 50-mL polypropylene screw-top centrifuge tubes containing 2.5 g air-dried soil. The tubes were shaken for exactly 2 min at 240 oscillations min-1 then allowed to settle for exactly 10 min. After settling, 0.5 mL of the supernatant was transferred into a second 50-mL centrifuge tube and mixed with 49.5 mL of deionized water. Sample absorbance was read with a spectrophotometer at 550 nm.

POXC (mg kg^{-1} soil) was calculated as

$$POXC = [0.02 \text{ ml } L^{-1} – (a + b\text{Abs})] \times (9000 \text{ mg C } mol^{-1}) \times (0.02 \text{ L solution } Wt^{-1})$$

where 0.02 mol L^{-1} is the initial concentration of the $KMnO_4$ solution, *a* is the intercept of the standard curve, *b* is the slope of the standard curve, Abs is the absorbance of the unknown soil sample, 9000 mg is the amount of C oxidized by 1 mol of MnO_4 with Mn^{7+} getting reduced to Mn^{4+}, 0.02 L is the volume of $KMnO_4$ solution reacted with the soil, and Wt is the amount of soil (kg) used in the reaction.

1.3.7.3 Soil Protein

Organically bound N was determined using the Autoclaved Citrate Extractable Protein method (Wright and Upadhyaya, 1996; Moebius-Clune, 2016). Exactly 3 g of soil was measured into heat and pressure resistant glass screw-top tubes. Then, 24 ml of sodium citrate buffer (20 mM, pH 7.0) was added to the tubes to disaggregate the soil. Tubes were then capped and shaken at 180 rpm for exactly 5 min. Samples were then autoclaved for 30 min at 121°C and 15 psi. After cooling, 2 ml of the samples were removed and deposited into microcentrifuge tubes where they were centrifuged at 10,000 gravity for 3 min. Ten µl of the clarified extract were transferred from the centrifuge tubes into a 96-well microplate for a standard colorimetric protein quantification assay (Thermo Pierce BCA Protein Assay). Two hundred µl of the working reagent were added to each well of the microplate. The plate was then sealed and incubated on a heating plate for 60 min at 60°C. After 60 min the plate was unsealed and read in a BioTek spectrophotometric plate reader. The extractable protein content of the soil was calculated using the following equation provided by

$$\frac{\text{protein concentration of the extract x volume of extractant used}}{\text{number of grams of soil used}}$$

1.3.8 Data Analysis

Analysis of variance was performed on plant and soil data with the PROC MIXED procedure in SAS v9.4 (SAS Institute, Cary, NC). Management and date were treated as fixed effects. Block was treated as a random effect with the significant differences determined at $\alpha = 0.05$. As plant and soil variables were measured multiple times throughout the growing seasons, sampling date was modeled as a repeated measure. Means were compared with an adjusted Tukey's pairwise comparison. Graphs were created using the ggplot2 (Wickham, 2009) package in R.

1.4 Results and Discussion

1.4.1 Weather

Total annual precipitation was below average (3.97 cm less) throughout the 2015 growing season (Table 1.2). Spring 2015 rainfall was average, while the summer and fall months experienced slightly below average precipitation. Total annual precipitation in 2016 was below both 2015 (6.65 cm less) and 20-year average (10.62 cm less) levels (Table 1.2). Below average rainfall was experienced during anthesis and grain fill (11.58 cm less) and this trend remained throughout the rest of the season (Figure 1.1, Table 1.2). The 2015 growing season had slightly below average temperatures during July and August compared to the 20-year average resulting in slightly less than average growing degree days for 2015 (Figure 1.2, Table 1.2). The 2015 spring and fall seasons were warmer than average. Overall, 2016 was a slightly warmer year than average (Table 1.2). The spring season temperatures were slightly below average while the summer and fall seasons were above average.

Overall, the 2016 growing season was a drier and warmer than the 2015 growing season. The spring and summer seasons in 2016 received much less rainfall compared to 2015, while the fall precipitation averages were comparable.

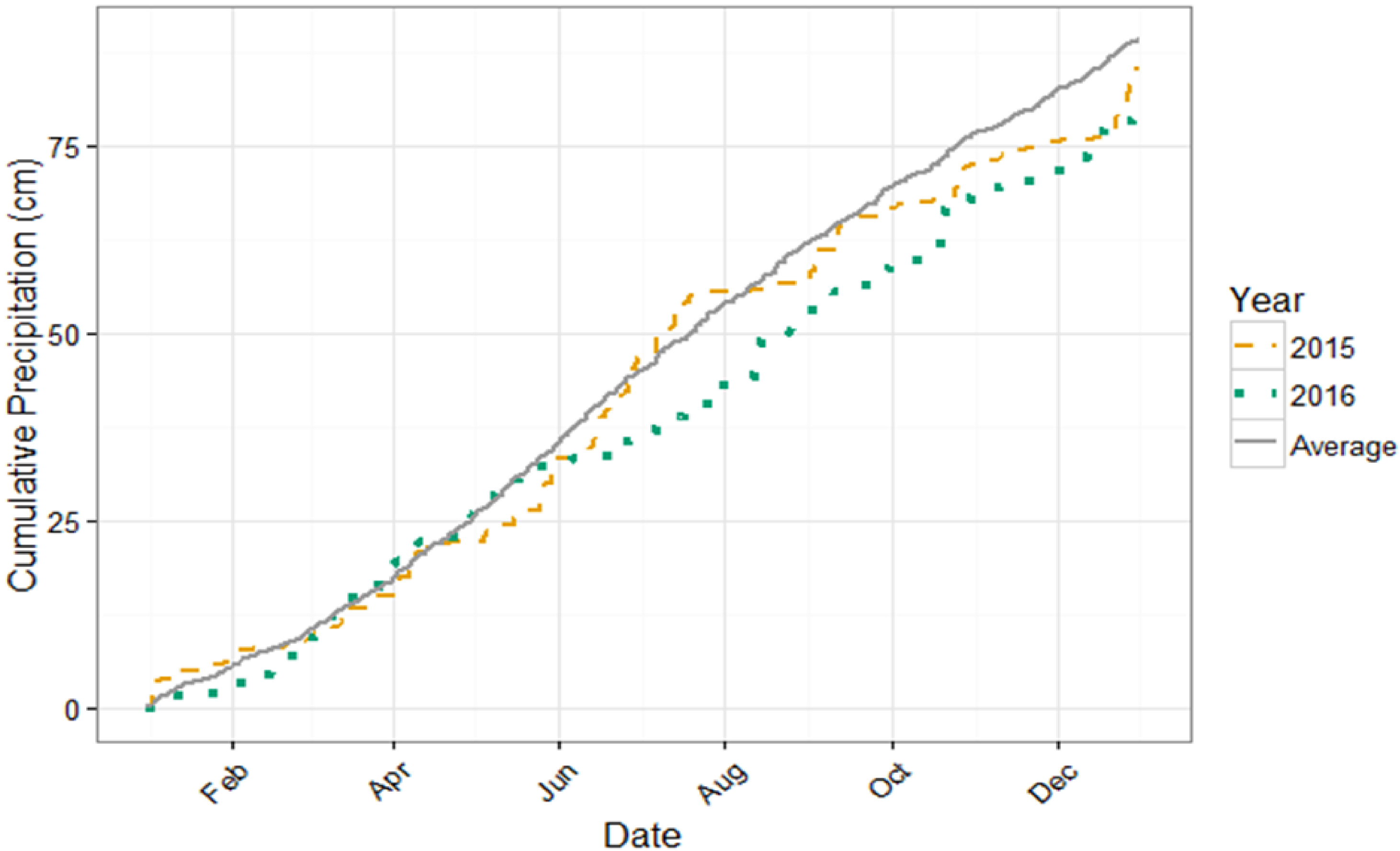

Figure 1. 1. Cumulative precipitation for the 2015 (gold dashed line) and 2016 (green dotted line) growing season and the 20-year average (grey solid line) at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH.

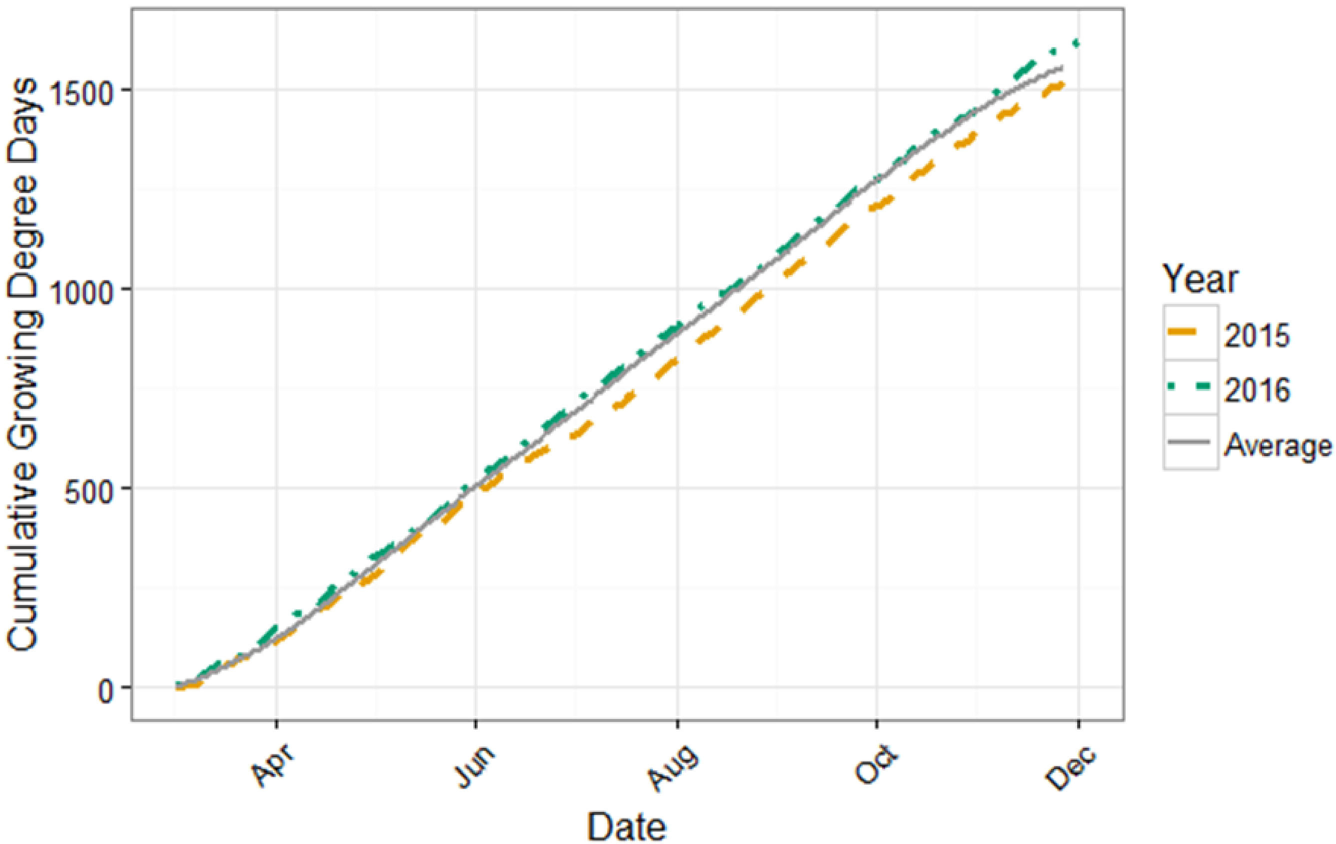

Figure 1. 2. Cumulative growing degree days for March through December for the 2015 (gold dashed line) and 2016 (green dotted line) growing seasons and the 20-year average (grey solid line) at the OARDC in Wooster,OH. Base = 0°C.

Table 1. 2. Total monthly, yearly and 20-year averages of precipitation and temperatures for the 2015 and 2016 growing seasons at the OARDC in Wooster, OH.

Year	Total Precipitation	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
	----------cm----------								
2015	85.1	7.1	11.0	13.8	8.5	2.0	9.1	5.9	3.2
2016	78.5	6.8	6.4	3.4	7.3	10.0	6.1	9.7	3.1
20-Year Avg	89.1	8.3	9.9	9.7	9.0	8.2	7.4	7.1	5.7

Year	Average Temperature	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
	----------°C----------								
2015	10.4	10.3	17.7	20.5	21.4	20.8	19.1	11.6	8.2
2016	11.4	8.7	15.1	21.2	23.3	23.6	19.5	13.3	7.0
20-Year Avg	10.4	10.1	15.8	20.5	22.2	21.4	17.6	11.3	5.6

1.4.2 Plant Measurements

1.4.2.1 Plant Height

Trends in Kernza plant height during the 2016 growing season reveal that in general all treatments follow a similar pattern of vertical growth (Figure 1.3), however significant differences in plant height between treatments were present throughout the entirety of the growing season (Table 1.3), but vary depending on the date (Table 1.4). Kernza plants grew steadily from spring to mid-summer, then experienced a rapid increase in height from 50 to 150 cm over a one month period, due to the elongation of seed-head bearing culms. During this period of growth, the control treatment heights were significantly less than the defoliated treatments. However, this trend is reversed following the grain harvest in August, where the control plant heights become significantly higher than those of the defoliated treatments for all remaining dates (Table 1.3). This reversal in trend is likely due the difference in post-grain harvest biomass removal, which was conducted in the defoliated plots but not in the control plots, therefore while plants seemingly grew at the same rate, plant height was always greater in the control plots due to a lack of defoliation at the summer harvest.

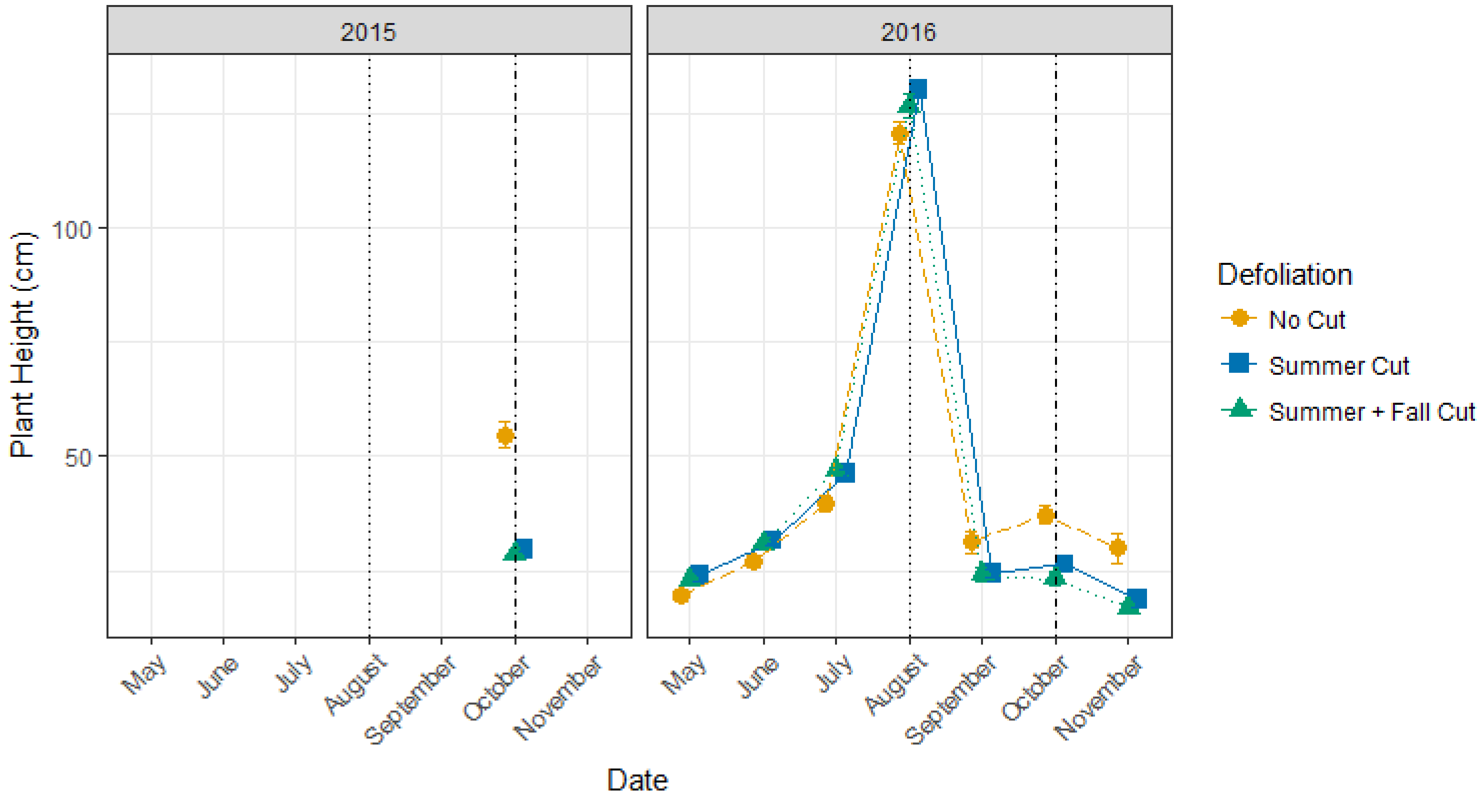

Figure 1. 3. Plant height for No Cut (gold circle dashed line), Summer Cut (blue square solid line), and Summer and Fall Cut (green triangle dotted line) treatments across two years. Error bars represent one standard error of the mean (n=40 for each sampling. The vertical dotted line represents the summer grain and forage harvest, and the dashed vertical line represents the fall forage harvest.

Table 1. 3 Plant and soil F-statistics and significance for defoliation differences analyzed for individual dates.

Source	8/5	9/3	10/13	11/12	4/25	5/26	6/28	7/26	8/30	10/6	11/2
	2015				2016						
Plant Height	-	-	69.63***	-	5.35*	8.61**	11.23**	4.62*	5.09*	36.71***	9.14***
Forage Yield	0.34	13.51**	14.65**	30.75***	9.79*	0.53	4.56*	3.99◊	25.47***	5.99*	2.03
Root Biomass	2.54◊	0.16	3.41◊	0.9	1.52	1.81	1.02	2.12	0.79	0.35	21.11**
Soil Moisture	-	1.78	2.12	0.04	0.45	1.55	0.12	1.25	1.51	1.15	2.92
POXC	1.22	0.1143	0.29	5.17*	0.21	0.11	0.44	0.57	0.06	1.5	2.4
C-Min	1.79	0.1	0.53	8.2*	1.38	1.72	0.64	0.25	0.55	0.2	4.52◊
Protein	0.66	1.05	1.14	1.11	1.35	0.09	0.91	1.1	0.35	0.6	0.11

* Significance level: P < 0.05

** Significance level: P < 0.01

*** Significance level: P < 0.001

◊ Significance level: P < 0.1

POXC = permanganate oxidizable carbon

C-Min = mineralizable carbon

Table 1. 4. Plant and soil F-statistics and significance from repeated measures ANOVA for all sampling dates, 2015 sampling dates, and 2016 sampling dates.

Source	**Plant Height**	**Forage Yield**	**Grain**	**Root Biomass**	**Soil Moisture**	**POXC**	**C-Min**	**Protein**
				2015 & 2016				
Defoliation (R)	8.63**	4.63*	18.31***	5.65	0.29	2.17	5.41**	1.94
Date (D)	1582.91***	52.07***	117.03***	7.53***	256.64***	2.2 *	3.35***	2.18*
R x D	20.71***	3.89***	0.27	1.21	1.61◊	0.56	0.94	0.43
				2015				
Defoliation (R)	69.63***	8.43*	7.66**	1.56	2.1	0.78	5.26*	1.09
Date (D)	-	68.09***	-	0.83	247.95***	2.49◊	9***	3.54*
R x D	-	2.99*	-	1.19	1.7	0.56	0.96	0.65
				2016				
Defoliation (R)	1.21	1.6	3.72◊	13.6**	0.45	1.79	6.8**	1.91
Date (D)	1906.67***	57.93***	-	6.92***	306.12***	2.44*	1.95◊	1.83
R x D	12.17***	4.62***	-	0.4	1.24	0.65	0.83	0.38

* Significance level: P < 0.05
** Significance level: P < 0.01
*** Significance level: P < 0.001
◊ Significance level: P < 0.1
POXC = permanganate oxidizable carbon
C-Min = mineralizable carbon

1.4.2.2 Forage Yield

Overall, for the period sampled in both years (August – November), trends in forage biomass are comparable (Figure 1.4), however there are some distinct differences between years that are most likely due to a combination of differences in weather (Table 1.2) and the carryover of the effects of treatments into year two. Seasonal trends of 2016 indicate, in general, all treatments undergo similar patterns of forage growth (Figure 1.4). Kernza forage undergoes a period of rapid and profound growth from May to June. The significant increase in biomass appears to be due to proliferation of leaves and tillers associated with vegetative growth, as plant height shows only a mild increase during this period (Figure 1.3). Over the next two months Kernza forage biomass remains constant, indicating vegetative growth has ceased and the plant has entered the reproductive stage where all energy is focused on grain development. In the two months following grain harvest, Kernza experiences adequate regrowth, evidenced by increases in yields in both years. While exact trends between October and November differ between years, overall it appears that Kernza forage growth does not take place after October.

In terms of individual treatments, defoliation had an overall significant effect on forage biomass, however the presence of a significant defoliation x date interaction across all analyses indicates that differences between treatments vary according to date (Table 1.4). Analysis of baseline measurements taken in August of 2015 reveal no significant differences between treatments (Table 1.3, 1.5), thus indicating there were no differences

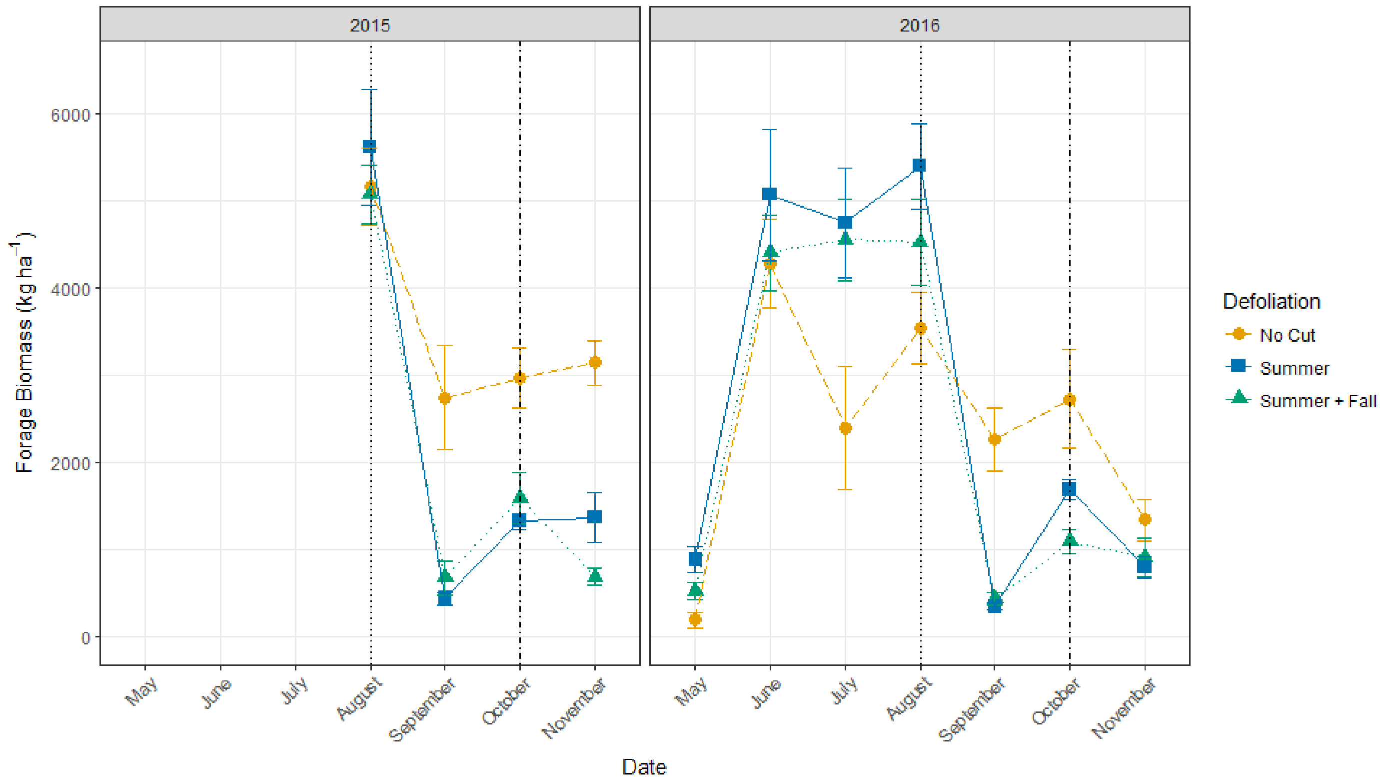

Figure 1. 4. Forage biomass for No Cut (gold circle dashed line), Summer Cut (blue square solid line), and Summer and Fall Cut (green triangle dotted line) across two years. Error bars represent one standard error of the mean (n=12 for each sampling). The vertical dotted line represents summer grain and forage harvest. The dashed vertical line represents the fall forage harvest.

Table 1. 5. 2015 and 2016 Kernza forage and root biomass defoliation treatment averages with standard error in parentheses. Different letters within the same row represent significantly different treatments a at α = .05. Asterisk indicates differences were significant at α = 0.1.

	Forage Biomass			**Root Biomass**		
Date	*No Cut*	*Su*	*Fa + Su*	*No Cut*	*Su*	*Fa + Su*
	-----------------------*kg ha*$^{-1}$-------------------------			-----------------------*kg ha*$^{-1}$-------------------------		
8/5/2015	5156 (*442*)a	5611 (*663*)a	5072 (*333*)a	1128 (*238*)a*	865 (*162*)ab	654 (*97*)b*
9/3/2015	2745 (*597*)a	443 (*76*)b	681 (*195*)b	872 (*244*)a	752 (*209*)a	857 (*260*)a
10/13/2015	2970 (*342*)a	1325 (*88*)b	1593 (*294*)b	932 (*140*)ab	1323 (*89*)a*	887 (*152*)b*
11/12/2015	3142 (*259*)a	1369 (*281*)b	694 (*102*)b	872 (*124*)a	1203 (*268*)a	887 (*173*)a
Mean 2015	**3503 (*410*)a**	**2187 (*277*)b**	**2010 (*231*)b**	**951 (*187*)a**	**1036 (*182*)a**	**821 (*170*)a**
4/25/2016	195 (*87*)a	890 (*142*)b	529 (*105*)ab	631 (*140*)a	797 (*513*)a	1654 (*636*)a
5/26/2016	4282 (*504*)a	5069 (*755*)a	4402 (*439*)a	1639 (*454*)a	2647 (*337*)a	3549 (*1094*)a
6/28/2016	2402 (*704*)a*	4747 (*635*)b*	4548 (*460*)b*	1579 (*271*)a	2331 (*549*)a	2692 (*755*)a
7/26/2016	3541 (*405*)a	5397 (*491*)b	4519 (*493*)ab	1353 (*288*)a	1624 (*323*)a	2436 (*528*)a
8/30/2016	2258 (*362*)a	346 (*36*)b	437 (*68*)b	1023 (*107*)a	1158 (*361*)a	1474 (*249*)a
10/6/2016	2730 (*557*)a	1691 (*122*)ab	1090 (*140*)b	842 (*141*)a	977 (*353*)a	1293 (*562*)a
11/2/2016	1340 (*234*)a	799 (*121*)a	914 (*225*)a	571 (*94*)a	692 (*58*)a	1218 (*140*)b
Mean 2016	**2393 (*408*)a**	**2705 (*329*)a**	**2349 (*276*)a**	**1091 (*214*)b**	**1461 (*356*)b**	**2045 (*566*)a**

in forage biomass amongst plots before treatments were implemented. After the summer grain and forage harvests, significant differences between the No Cut and the two defoliated treatments emerge for the remaining three dates of 2015, whereby the No Cut treatment yielded significantly more forage than the Su and Fa + Su treatments (Table 1.5). Overall, the differences in forage yield trends between treatments in 2015 were to be expected, as they are reflective of their respective defoliations. During the period from November 2015 to May 2016, all treatments experienced some decrease in forage biomass (Table 1.5), however the decrease experienced by the No Cut treatment was much more drastic than either of the defoliated treatments, decreasing by 94%. This dramatic decrease in the No Cut treatment may be the result of decomposition or the natural removal of dead biomass by snow melt, rain, or wind. Because of this dramatic decrease, the No Cut treatment started off the 2016 growing season with significantly less forage biomass than the defoliated treatments (Table 1.5), a trend that remained until after summer grain and forage harvest (Figure 1.4). Similar to 2015, the No Cut treatment yielded significantly more forage than the defoliated treatments in the months following the summer grain and forage harvest (Figure 1.4, Table 1.5), due to the lack of removal of remaining biomass after grain harvest.

Overall, these results indicate that in terms of forage, Kernza performs differently under dual-use and grain-only managements, whereby dual-use management appears to have a positive effect on forage production relative to single-use management for grain. As for differences in dual-use treatments, these results indicate that overall defoliation frequency does not affect forage yields, as the Su and Fa + Su treatments were comparable across the majority of dates (Table 1.5).

1.4.2.3 Grain Yield

Kernza grain yields averaged 642 kg ha^{-1}during the first year of production and 362 kg ha^{-1} in the second year of production (Table 1.6), an almost 50% decrease in grain yield between years. Grain yields in both years were lower than reported Kernza yields from Michigan (Culman et al., 2013) and Minnesota (Jungers et al., 2017). Compared to IWG commercial forage varieties, on average Kernza performed better than IWG under dryland conditions and as well as IWG under irrigated conditions (Ogle et al., 2011). The decrease in grain yield between years is not consistent with trends reported from both the Michigan and Minnesota studies (Culman et al., 2013, Jungers et al., 2017), where both studies reported an increase in grain yield between year one and two. As each individual treatment experienced declines in grain yield (Table 1.6) it is likely that this decrease is due to differences in weather. The period in which anthesis and grain-fill occurs was both drier and hotter in 2016 compared to 2015 (Table 1.2), likely effecting the grain yield.

Repeated measures analysis revealed that overall defoliation had significant effects on grain yields (Table 1.4). Differences in treatment grain yields in 2015 were due to inherent differences between plots, not treatments, as no defoliation treatments had been applied at the time of grain harvest. However, at the time grain data was collected in

2016, a year worth of treatments had been imposed, and differences in grain yield may be attributed to differences in treatments. Grain yields of the No Cut treatment were less than both defoliated treatments, but significantly less than the Su treatment (Table 1.6). These results indicate that not only does dual-use management not have a negative impact on grain yields, but that managing Kernza for dual-use purposes actually has a positive effect on grain yields relative to managing Kernza for grain only. As for differences between the defoliated treatments, grain yield, like forage biomass, does not appear to be affected by the frequency of forage harvest.

Table 1. 6. Yearly average grain yields and standard error for each defoliation treatment. Different letters within the same row represent significantly different treatments at α = 0.05. Asterisk indicates differences were significant at α = 0.1.

Date	*No Cut*	*Su*	*Fa + Su*	*Mean*
	------------------------------ *kg ha^{-1}*			--------------------------------
8/5/2015	581 (*17*)b	676 (*18*)a	669 (*22*)a	**642 (*19*)**
7/26/2016	279 (*41*)b*	421 (*56*)a*	387 (*39*)ab*	**362 (*45*)**

1.4.2.4 Root Biomass

Repeated measures analysis of all data revealed that, overall, Kernza root biomass, within the top 20 cm of soil, was significantly affected by date, but not defoliation or a defoliation and date interaction (Table 1.4, 1.5). However, analysis of individual years revealed very different results for year one and year two (Table 1.4), which are apparent in the very different seasonal trends of both years (Figure 1.5).

Kernza root biomass for the four dates sampled in 2015 was not significantly influenced by defoliation, date, or a defoliation x date interaction (Table 1.4). These results indicate that overall Kernza root biomass did not experience any significant growth or death from summer harvest to the end of the growing season, an interesting contrast to aboveground biomass, which experienced some growth during this period (Figure 1.4, Table 1.5). As for defoliation effects, the overall lack of significant differences in root biomass between treatments suggests defoliation may not illicit an immediate reaction in roots.

The greater number of sampling dates and overall longer sampling period during 2016 allows for better observation of the seasonal dynamics of Kernza root biomass. Date was a highly significant source of variation in root biomass during the 2016 season (Table 1.4) indicating that in the second year of growth Kernza root biomass does not remain constant, as was seen in the later part of 2015, but is actively changing through significant growth or death. Overall, the seasonal dynamics of Kernza root biomass are quite clear: roots experience a period of significant growth between May and June, then steadily decline throughout the rest of the season (Figure 1.5, Table 1.5). Similar patterns of root growth were reported by Stewart and Frank (2008).

In contrast to 2015, the 2016 season produced significant differences in root biomass between treatments (Table 1.4). For every date in 2016, the Fa + Su treatment yielded the greatest root biomass, followed by the Su treatment, then the No Cut treatment (Figure 1.5). From these results, it is clear that defoliation has a positive effect on Kernza root biomass. However, this effect is not immediate, as defoliation does not appear to trigger any unique response in root biomass in the immediate period following a defoliation event, in either year (Figure 1.5). Therefore, while defoliation of aboveground biomass may not illicit an immediate reaction in Kernza roots, it does appear to significantly affect root biomass over the long-term. Overall, managing Kernza for dual-use more positively effects total root biomass in the first two years of production compared to managing Kernza for grain only. Harvesting forage twice in a season compared to only once also produced overall greater root biomass in the subsequent year, indicating that increased defoliation frequency has a positive effect on Kernza roots.

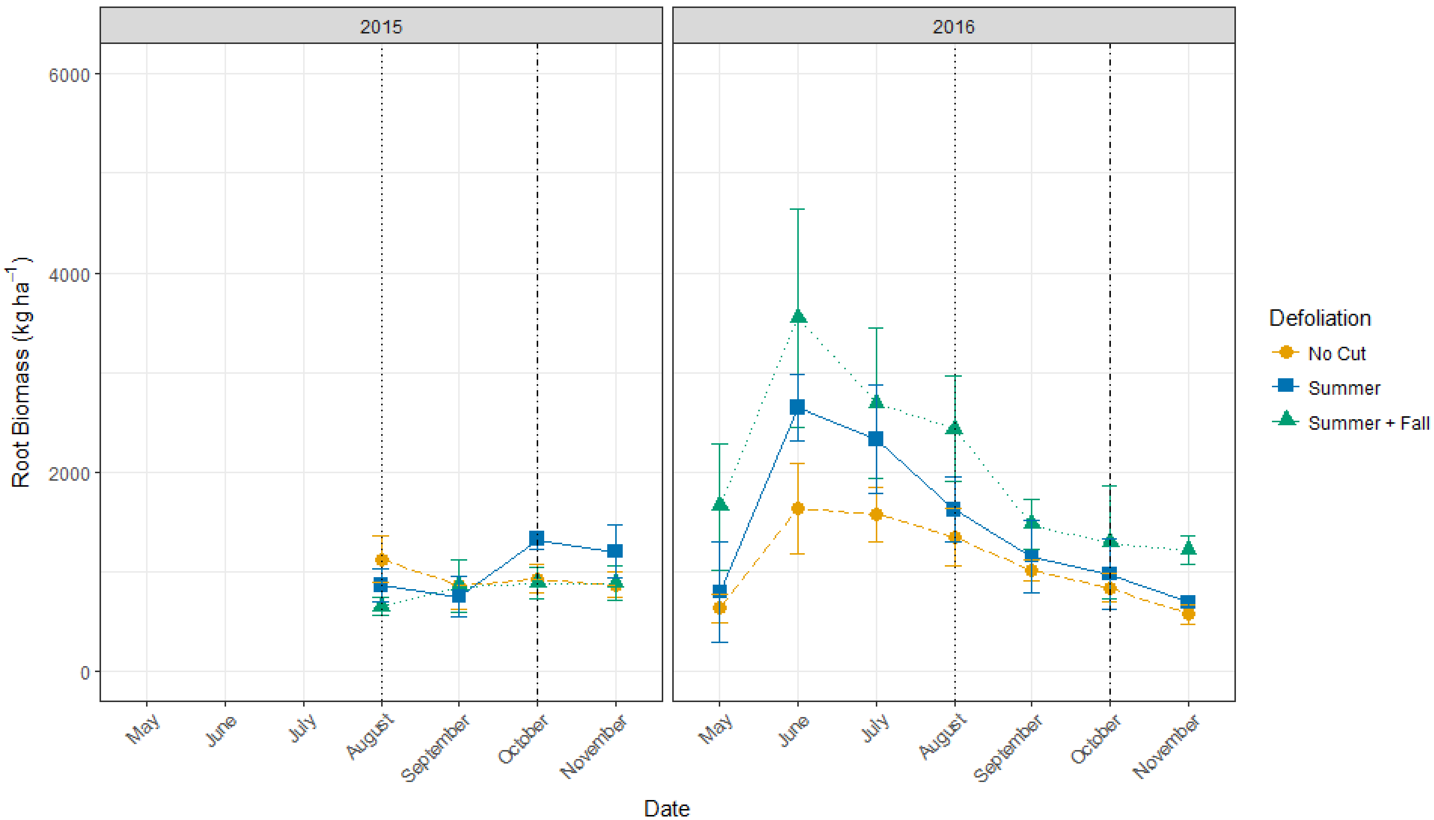

Figure 1. 5. Averaged root biomass for No Cut (gold circle dashed line), Summer Cut (blue square solid line), and Summer and Fall Cut (green triangle dotted line) over two years. Error bars represent one standard error of the mean (n=12 for each sampling). The vertical dotted line represents the summer grain and forage harvest. The dashed vertical line represents the fall forage harvest.

1.4.3 Soil Measurements

1.4.3.1 Soil Moisture

Our results reveal that under Kernza, soil moisture in the top 20 cm was largely dictated by time of the year and not influenced by the differences or changes in plant biomass caused by defoliation treatments, as moisture was not significantly affected by defoliation, but was significantly affected by date (Table 1.4).

The 2016 season provides a clear picture of the seasonal dynamics of soil moisture under Kernza. Soil moisture levels progressively decline from May until grain harvest, with the rate of decrease varying over months (Figure 1.6). After the summer harvests, soil moisture levels progressively increase until the end of the season, with the rate of increase varying over months (Figure 1.6). Soil moisture levels for 2016 range from as low as 5% at grain harvest to as high as 13% at both the beginning and end of the season. This seasonal pattern of soil moisture contrasts with the general patterns of Kernza plant growth (Figure 1.4, 1.5) and loosely follows the pattern of monthly precipitation in 2016 (Table 1.2). These relationships suggest that though soil moisture level is ultimately the result of a combination of both plant dynamics and weather, soil moisture may be primarily influenced by plant dynamics and secondarily by weather.

Overall seasonal differences between years is difficult to assess, as only three dates in the later part of 2015 were sampled for soil moisture. However, for the period sampled in both years, soil moisture values and trends between dates are very similar (Figure 1.6), despite differences in precipitation for this period (Table 1.2). This further supports the previous observation that soil moisture under Kernza is more heavily influenced by plant dynamics than precipitation.

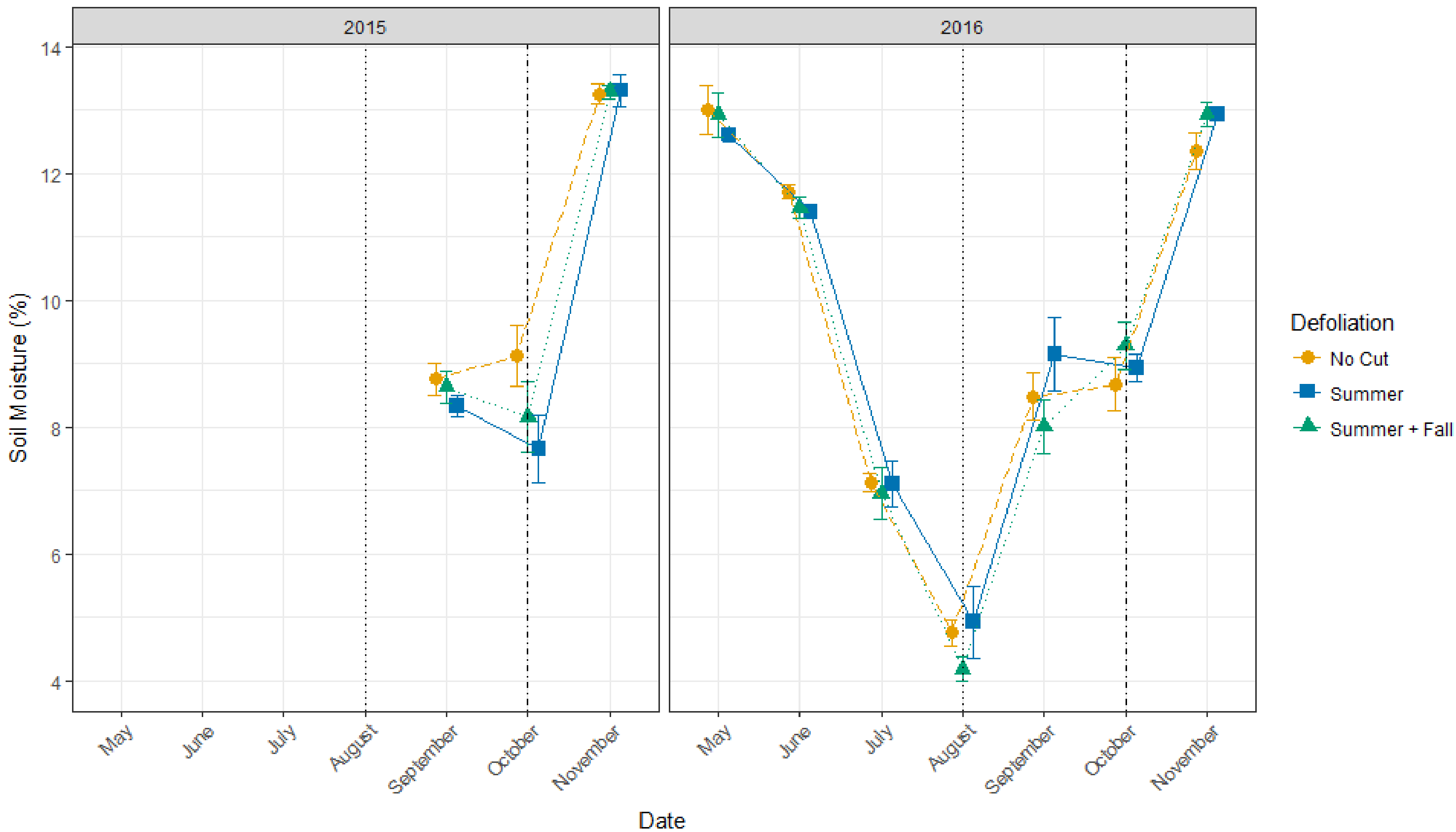

Figure 1. 6. Soil moisture for No Cut (gold circle dashed line), Summer Cut (blue square solid line), and Summer and Fall Cut (green triangle dotted line) over two years. Error bars represent one standard error of the mean (n=12 for each sampling). The vertical dotted line represents the application of the summer grain and forage harvest. The dashed vertical line represents the fall forage harvest.

1.4.3.2 Mineralizable Carbon

Measuring total soil organic carbon can take years before any changes become apparent (Wander, 2004), therefore because our study was only two years, we needed soil carbon measurements that would be sensitive to changes in this short-term period. Therefore, in order to assess changes in SOC, we used two measures of active organic C (mineralizable C and POXC) that are proven indicators of both short-term and long-term carbon dynamics in the soil (Culman et al. 2012a, 2012b; Franzluebbers et al., 2000; Haney et al., 2008). Mineralizable carbon (C-min) was significantly affected by defoliation and date across all analyses (Table 1.4) indicating that in general, trends between defoliation treatments remain constant even though exact values may change over time.

Analysis of individual dates in 2015 reveal that the November date was the only date with significant differences between treatments (Table 1.3, Table 1.7). Therefore, for the majority of the period in 2015 from summer harvest to the end of the season, C-min values were similar under all treatments. This similarity between treatments allows for a clear emergence of C-min trend for the later part of the 2015 season.

In general, mineralizable carbon decreased slightly in the month following the summer grain and forage harvests in 2015 then gradually increased till the end of the season (Figure 1.7). Overall, C-min values increased 27% from August to November in 2015. This increase could be attributed to a combination of increased substrate supply through defoliation triggered root exudation (Hamilton et al., 2008) and soil moisture increases

(Figure 1.6; Orchard and Cook, 1983; Curtin et al., 2012). While all treatments experienced an overall increase in carbon mineralization from August to November, the increases of the defoliated treatments (Su = 48%; Fa + Su = 44%) are almost four times that of the non-defoliated treatment (No Cut = 13%). These results suggest that defoliation of aboveground biomass more greatly increases the amount of mineralizable carbon occurring in soils in the months following grain harvest.

For the 2016 growing season, analysis of pairwise comparisons between treatments revealed that on average, C-min under the No Cut treatment was significantly less than the Fa + Su treatment (P = 0.0021) and when analyzed at the 0.1 level, was also significantly less than the Su treatment (P = 0.0971). Similar to 2015, individual analysis of sampling dates in 2016 revealed that only one of the seven dates produced significant differences between treatments (Table 1.3). Therefore, while the defoliated treatments were on average greater than the non-defoliated treatment, C-min values were for the most part comparable across all treatments. Our average C-min values during the second year of production were much less than those reported by Culman et al. (2013) as our values were only a fourth of the levels reported for the second year of Kernza production. As for 2016 general seasonal dynamics in mineralizable carbon, trends are not quite as clear cut as 2015.

Trends in mineralizable carbon during the period from May to July vary between treatments, however from July to the end of the season all treatments appear to follow a similar pattern of C-min fluctuations, similar to 2015 except for the period from October to November (Figure 1.7). On an individual basis, the Su and Fa + Su treatments increased by 25 and 19%, respectively, despite fluctuations, while the No Cut treatment experienced no overall change between May and November. From these results, it appears that defoliating Kernza aboveground biomass for forage harvest has an overall positive effect on carbon mineralization in the soil and therefore, managing Kernza as a dual-use crop stimulates carbon mineralization to a greater extent than does managing Kernza for grain only.

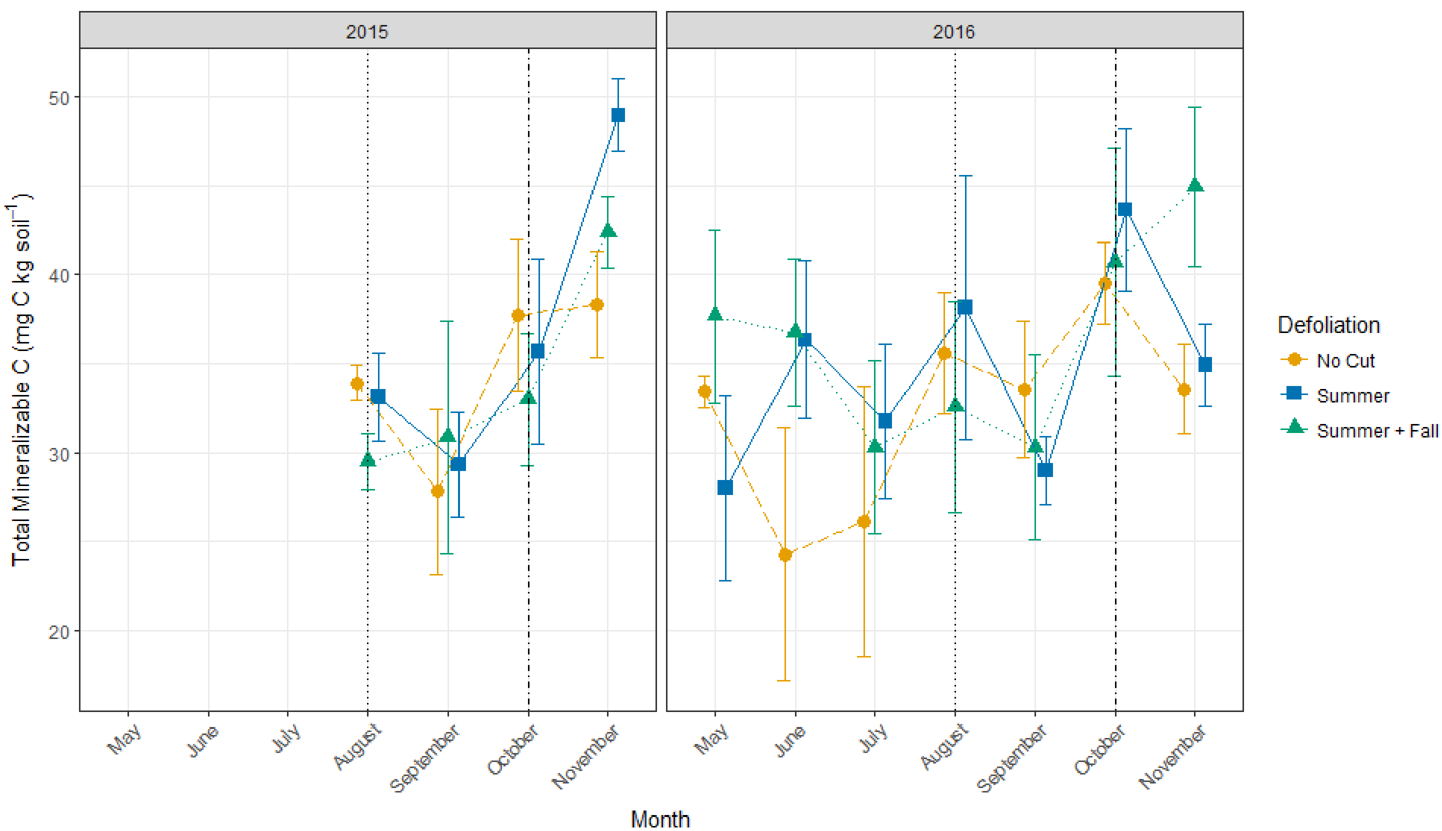

Figure 1. 7. Mineralizable carbon for No Cut (gold circle dashed line), Summer Cut (blue square solid line), and Summer and Fall Cut (green triangle dotted line). Error bars represent one standard error of the mean (n=12 for each sampling date). The dotted vertical line represents the summer grain and forage harvest. The dashed vertical line represents the fall forage harvest.

Table 1. 7. Soil health measurements averages and standard errors by date during the 2015 and 2016 growing seasons under Kernza. Different letters within the same row represent significantly different treatments at α = 0.05. Asterisk indicate significance at α = 0.1. POXC = permanganate oxidizable carbon, C-Min = mineralizable carbon.

	POXC			**Protein**			**C-Min**		
Date	*No Cut*	*Su*	*Fa + Su*	*No Cut*	*Su*	*Fa + Su*	*No Cut*	*Su*	*Fa + Su*
2015	**mg C kg soil^{-1}**			**mg g soil^{-1}**			**mg mineralizable C kg soil^{-1}**		
8/5	478 (*34*)a	405 (*33*)a	456 (*35*)a	4.68 (*0.12*)a	4.60 (*0.22*)a	4.80 (*0.16*)a	33.9 (*1.0*)a	33.1 (*2.5*)a	29.5 (*1.6*)a
9/3	402 (*17*)ab*	382 (*30*)a*	428 (*21*)b*	4.24 (*0.19*)a	4.17 (*0.22*)a	4.47 (*0.15*)a	27.8 (*4.6*)a	29.3 (*3.0*)a	30.8 (*6.5*)a
10/13	430 (*28*)a	437 (*53*)a	402 (*11*)a	4.51 (*0.30*)a	4.20 (*0.35*)a	4.12 (*0.17*)a	37.7 (*4.3*)a	35.7 (*5.2*)a	33.0 (*3.7*)a
11/12	503 (*13*)a*	469 (*20*)b*	468 (*18*)b*	4.52 (*0.17*)a	4.51 (*0.16*)a	4.27 (*0.12*)a	38.3 (*3.0*)a	48.9 (*2.0*)b*	42.4 (*2.0*)a*
Mean	**453 (*23*)a**	**423 (*34*)a**	**438 (*21*)a**	**4.49 (*0.20*)a**	**4.37 (*0.24*)a**	**4.42 (*0.15*)a**	**34.4 (*3.2*)b**	**36.8 (*3.2*)a**	**33.9 (*13.8*)b**
2016									
4/25	480 (*31*)a	451 (*19*)a	464 (*48*)a	4.60 (*0.05*)a	4.30 (*0.18*)a	4.51 (*0.25*)a	33.5 (*0.9*)a	28.0 (*5.2*)a	37.7 (*4.9*)a
5/26	426 (*22*)a	424 (*21*)a	435 (*6*)a	4.04 (*0.22*)a	4.12 (*0.10*)a	4.09 (*0.04*)a	24.3 (*7.1*)a	36.3 (*4.4*)a	36.7 (*4.1*)a
6/28	435 (*14*)a	418 (*21*)a	413 (*23*)a	4.44 (*0.15*)a	4.28 (*0.19*)a	4.22 (*0.22*)a	26.1 (*7.6*)a	31.8 (*4.3*)a	30.3 (*4.9*)a
7/26	456 (*18*)a	429 (*37*)a	431 (*30*)a	4.50 (*0.23*)a	4.37 (*0.35*)a	4.00 (*0.36*)a	35.6 (*3.4*)a	38.2 (*7.4*)a	32.6 (*5.9*)a
8/30	457 (*9*)a	444 (*25*)a	450 (*37*)a	4.41 (*0.26*)a	4.19 (*0.20*)a	4.19 (*0.32*)a	33.6 (*3.8*)a	29.0 (*1.9*)a	30.3 (*5.2*)a
10/6	498 (*24*)a	470 (*30*)a	442 (*9*)a	4.41 (*0.14*)a	4.38 (*0.11*)a	4.51 (*0.02*)a	39.5 (*2.3*)a	43.6 (*4.6*)a	40.7 (*6.4*)a
11/2	463 (*21*)a	467 (*14*)a	541 (*42*)a	4.52 (*0.14*)a	4.51 (*0.22*)a	4.61 (*0.21*)a	33.6 (*2.5*)a*	35.0 (*2.3*)a*	44.9 (*4.5*)b*
Mean	**459 (*20*)a**	**443 (*24*)a**	**454 (*28*)a**	**4.42 (*0.17*)a**	**4.31 (*0.19*)a**	**4.30 (*0.20*)a**	**32.3 (*3.9*)b***	**34.5 (*4.3*)a***	**36.2 (*5.1*)a**

1.4.3.3 POXC

Repeated measures analyses revealed that overall, POXC was similar across defoliation treatments but varied significantly across dates (Table 1.4), suggesting an important temporal effect of labile C levels in soil under Kernza.

For the period from summer harvest to the end of the season in 2015, POXC follow a pattern similar to C-min, where overall POXC values decline following the summer harvest then increase steadily through the rest of the season (Figure 1.8). As for the 2016 seasonal dynamics, POXC decreases from the beginning of the season through July then gradually increases till the end of the season (Figure 1.8). This general POXC pattern appears to inversely mirror plant growth, suggesting a loose relationship between the two whereby plant dynamics influence labile soil carbon.

For the second year of production, our average POXC values were slightly less, though for the most part comparable to those reported by Culman et al. (2013) in the second year of Kernza production. Though not statistically significant, there does appear to be a trend between defoliation treatments during the 2016 season. For a majority of dates, No Cut POXC values are greater than both defoliated treatments, with this trend consistent between July and October (Table 1.7, Figure 1.8). POXC values of the defoliated treatments also appear to closely mimic one another consistently from May through September, only to differentiate during the last two months of the season. That these trends between treatments do not appear until the second season of production, suggest that the effects of defoliation on POXC are felt in the year following the defoliations, similar to root biomass.

Permanganate oxidizable carbon reflects a more stabilized pool of labile organic matter (Culman et al., 2012) and changes in POXC have been shown to better reflect changes in carbon accumulation and stabilization in the soil (Hurisso et al., 2016). It appears from these trends and relationships between treatments that defoliation of aboveground Kernza biomass for forage harvest may potentially decrease overall seasonal POXC levels and therefore a decrease the ability of Kernza to accumulate and sequester carbon in the soil.

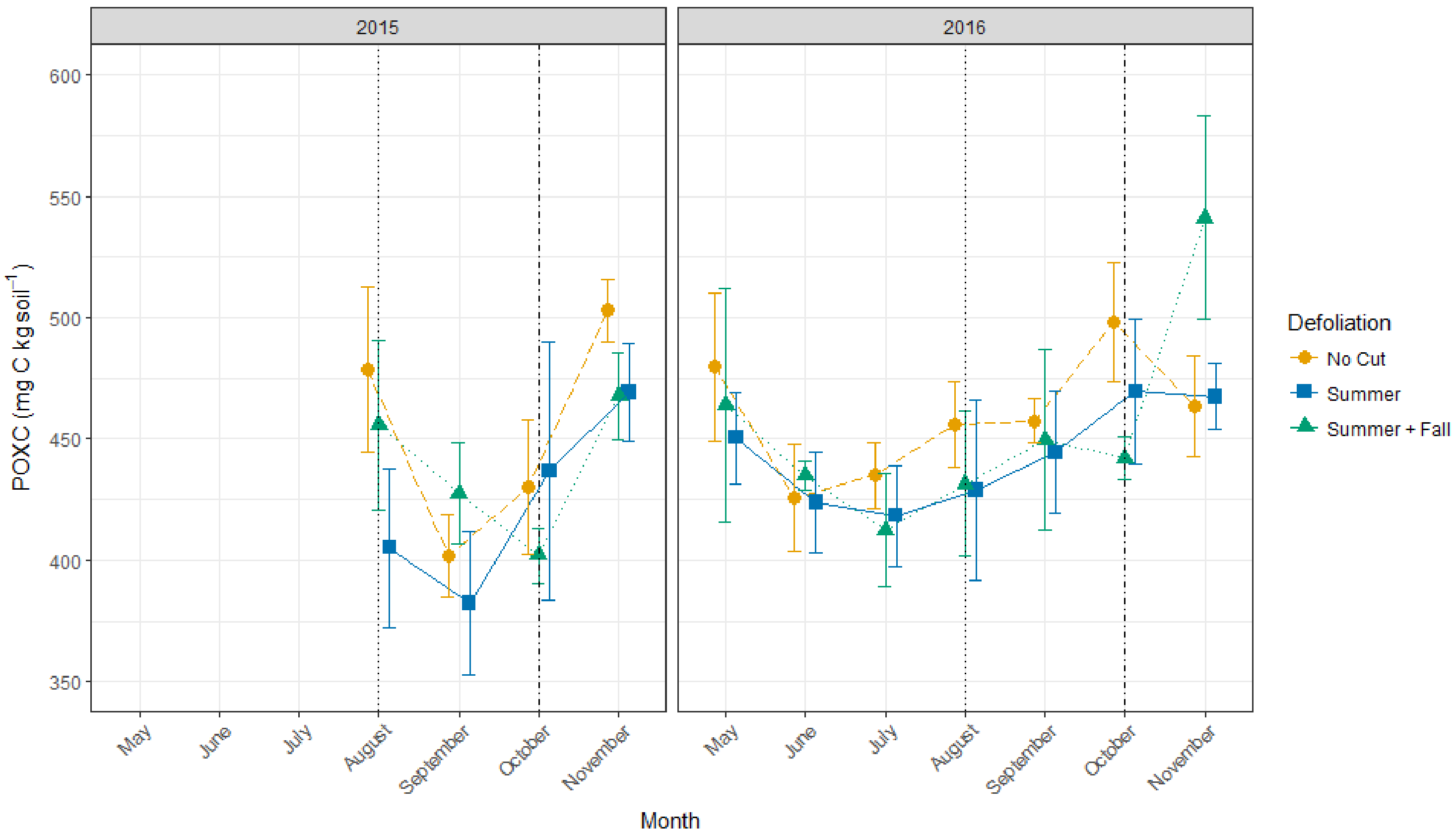

Figure 1. 8. Permanganate oxidizable carbon for No Cut (gold circle dashed line), Summer Cut (blue square solid line), and Summer and Fall cut (green triangle dotted line). Error bars represent one standard error of the mean (n=12 for each sampling date). The dotted vertical line represents the summer grain and forage harvest. The dashed vertical line represents the fall forage harvest.

1.4.3.4 Protein

Like POXC, in general, soil protein was not significantly affected by defoliation but did vary significantly by date (Table 1.4). These results, like those of POXC, suggest an important temporal dynamics of soil protein.

When years were analyzed individually, 2015 results revealed soil protein was significantly different across dates, but not defoliation treatments (Table 1.4). For the period in 2015 from August to November, soil protein values generally decline following the summer harvest, remain steady for a period, then increase slightly till the end of the season (Figure 1.9). This pattern in soil protein is similar to the patterns of C-min (Figure 1.7) and POXC (Figure 1.8) during this period.

Results of the 2016 season were different from the overall and 2015 results, in that soil protein was not significantly different between treatments or dates (Table 1.4). These statistics indicate that soil protein does not experience any seasonal dynamics, but rather remains constant and stable throughout the season. However, closer examination of 2016 trends reveal that fluctuations in soil protein do occur and patterns are generally similar across all treatments (Table 1.7, Figure 1.9). Therefore, there does appear to be some evidence for potential seasonal dynamics in soil protein.

Again, while overall differences in soil protein between treatments in 2016 were not statistically significant, there does appear to be a slight trend between them. For many of the dates in 2016, the No Cut treatment had greater levels of soil protein than the defoliated treatments (Table 1.7, Figure 1.9), similar to the 2016 treatment trend in POXC (Figure 1.8). This trend suggests that potentially, compared to not harvesting Kernza for forage, defoliation of aboveground biomass for forage may decrease soil protein levels during the following year.

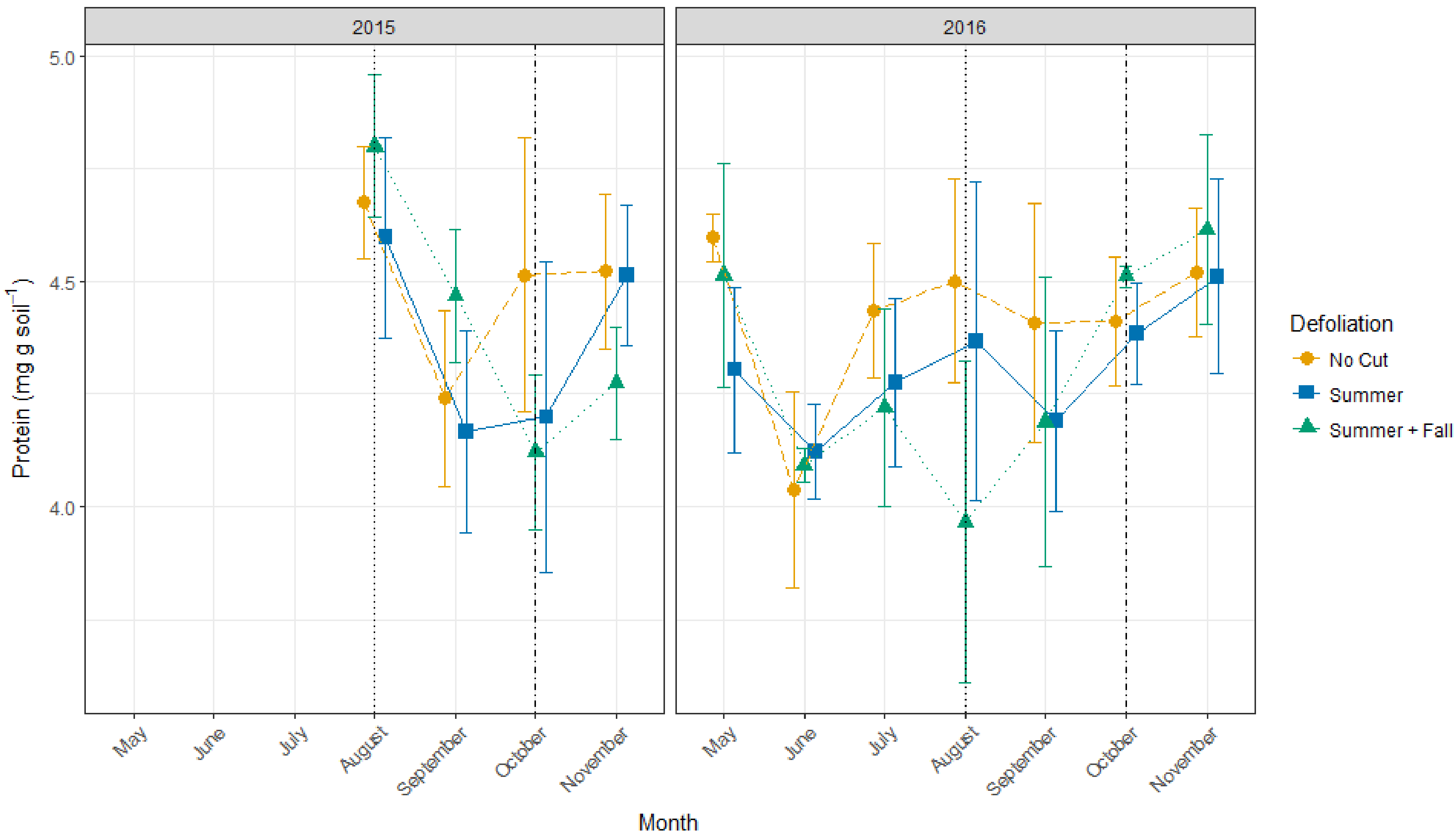

Figure 1. 9. Soil protein for No Cut (gold circle line), Summer Cut (blue square line), and Summer and Fall Cut (green triangle line). Error bars represent one standard error of the mean (n=12 for each sampling date). The dotted vertical line represents the summer grain and forage harvest. The dashed vertical line represents the fall forage harvest.

1.4.4 Relationships between Kernza and Soil Health Dynamics

Knowing the temporal dynamics of components in an agricultural system allows us to evaluate how the individual components may change throughout the growing season and therefore determine general seasonal trends for the system. An understanding of the seasonal dynamics and relationships between components will allow us to better identify the mechanisms by which defoliation impacts Kernza biomass and soil health and interpret the larger implications for the system as a whole.

The general seasonal dynamics of Kernza plant biomass and soil health variables are more appropriately determined using the 2016 data because i) the sampling period spans the full length of the growing season, ii) Kernza stands are better established and therefore more closely represent the system at maturity and iii) date was a significant source of variation for almost every variable analyzed in 2016 (Table 1.4).

The temporal dynamics of forage and root biomass provide insight into the seasonal growth patterns of Kernza. Like a true cool-season grass, Kernza experiences the most rapid and profound growth between May and June while the weather is still generally wet and temperatures are mild. Both above and belowground plant biomass experience dramatic growth during this one month period, increasing by 88 and 61%, respectively. After June, root and forage growth patterns differ (Figure 1.10). Forage does not experience much growth after June, as the plant has entered the reproductive stage and grain production takes over. Forage does undergo a small period of growth following summer grain and forage harvest, however this growth is very slight compared to the growth at the beginning of the season. Following the period of intense growth, root biomass gradually declines throughout the rest of the season. These results seemingly indicate that after June, Kernza root biomass experiences no more growth and gradually dies off till the end of the season. However, it is instead more likely that Kernza roots are continuously turning over and the rate of mortality is greater than that of growth, meaning that root growth has not ceased, it is just not enough to compensate for root die-off (Stewart and Frank, 2008). Another alternative explanation involves the sampling depth of roots. Roots were only sampled within the top 20 cm of soil and other studies have shown that perennials commonly produce large quantities of root biomass below 20 cm (Culman et al 2010, DuPont et al., 2014). Decreases in soil moisture and reductions in resource availability during the period following extreme plant growth, could likely have forced roots to grow beneath this surface layer and explore these resources at greater depths (Weaver, 1926). However, similar findings to ours were reported in studies by Gao et al. (2008) and Lopez-Marisco et al. (2015), but with measurements down to 1 meter. Therefore, even if roots did grow beneath the surface layer, it is likely they would have been subjected to the same seasonal dynamics that we observed in the top 20 cm of soil. Overall, the seasonal dynamics of Kernza forage and roots indicate that the month of May is an extremely critical time for Kernza vegetative production, and a large amount of the seasonal plant biomass is generated during this period.

The temporal dynamics of soil health characteristics allow us to better understand how they cycle within a growing season. Overall, the seasonal dynamics of POXC and soil protein are very similar and therefore, likely connected to one another (Figure 1.10), as labile soil carbon is an energy source to the microorganisms that control N cycling and availability in the soil (Drinkwater and Snapp, 2007). Mineralizable carbon fluctuates over much of the season, which appears to differentiate its seasonal dynamics from that of POXC and soil protein (Figure 1.10). However, despite its fluctuations, mineralizable carbon experiences an overall increase from July to the end of the season, making its overall seasonal pattern similar to POXC and protein. Therefore, it appears that in general, all three soil health indicators follow similar seasonal cycles whereby they decline in the first half of the season then increase throughout the second half. These results indicate that C-min, POXC, and soil protein are likely linked to one another and their overall seasonal dynamics driven by the same sources.

The seasonal dynamics of Kernza and soil health suggest that the plant and soil dynamics in this system are closely linked. These relationships are apparent at the beginning of the season during the period of rapid plant growth where plant biomass increases and soil protein and labile carbon pools decline. The increased nitrogen demand by growing plants during this period of peak growth likely caused the reduction in organically bound N (Sprent, 1987). Belay-Tedla et al. (2009) and Garcia and Rice (1994) reported similar results where organic N was lowest during the period in which plant demand was at its greatest. While POXC and protein decline, carbon mineralization remains constant between May and June, suggesting a continuously active microbial community during this period as carbon mineralization has been shown to be well correlated with microbial biomass (Franzluebbers et al., 2000; Haney et al., 2001, 2008). Therefore, the increase in plant growth, the mineralization of labile SOM by an active microbial community and the decrease in organic N levels together suggest that nutrient cycling is heavily synchronized with plant development during this time of critical growth.

As soil moisture levels begin to dramatically decrease, so does carbon mineralization, as declines in soil moisture have been reported to negatively impact carbon and nitrogen mineralization (Curtin et al., 2012). As carbon mineralization has been shown to be correlated with microbial biomass, this decline in mineralizable carbon may be indicative of declines in soil microbial community, whose turnover would add organically bound N to the soil and therefore result in the increase in soil protein we see from June to July. As roots begins to die-off after June, plant residue is added directly into the soil where they are decomposed to smaller fractions of particulate organic matter. POXC measurements, which are highly correlated to POM (Culman et al., 2012), steadily increase throughout the rest of the season indicating that dead root residue is likely being decomposed into more stable forms of soil carbon. As plant growth slows mid-season and roots continue to die-off, protein levels in the soil remain constant, reflecting a balance between plant demand for N during grain fill and the decomposition of additions of organic residue through root turnover. Overall, these findings provide strong evidence for a highly synchronized relationship between Kernza plant dynamics and nutrient cycling, and are further supported in a model of soil C and N dynamics under early to mid-succession of a perennial grain crop proposed by Crews et al. (2016), in which they maintained that the dynamics of SOM pools and soil N are intrinsically linked to plant dynamics.

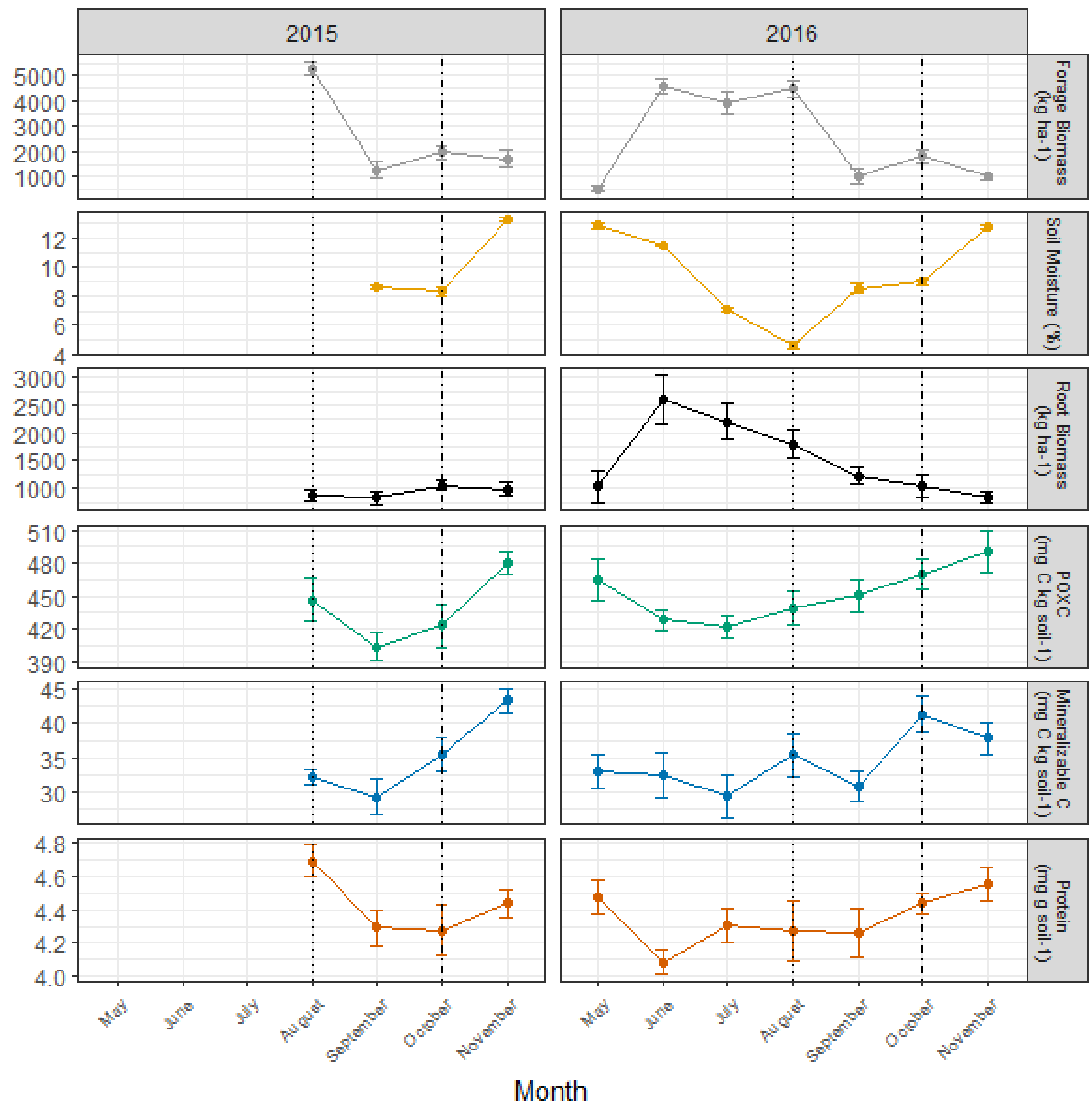

Figure 1. 10. Seasonal dynamics for 2015 and 2016 plant and soil measurements averaged across all treatments. Error bars represent one standard error of the mean. The dotted vertical line represents the summer grain and forage harvest. The dashed vertical line represents the fall forage harvest.

1.4.5 Implications of Defoliation on Soil Health

A key possible advantage of Kernza is its ability to improve soil health and it is important to determine any management or practice that abates this benefit. Managing Kernza as a dual-use crop would require the defoliation of forage biomass which has been shown to potentially effect roots. Therefore, because roots play a significant role in overall ecosystem functioning, changes in roots caused by defoliation could translate into changes in the productivity and functioning of the system and ultimately Kernza’s effect on soil health.

Overall, Kernza roots were affected by defoliation of the aboveground biomass. Our results show that defoliation did not trigger an immediate response in roots, but instead, produced an effect on overall root biomass in the following year. Lopez-Marsico et al. (2015) reported a similar response of no significant differences in the seasonal dynamics between grazing treatments, but an overall significant difference in root biomass between grazed and non-grazed stands. Therefore, our results show that Kernza root response to aboveground defoliation is not instantaneous, but instead a delayed reaction where the overall productivity of belowground biomass in the subsequent season is affected. In terms of specific effects, our findings reveal that defoliating aboveground biomass had a positive effect on the Kernza root system and this effect increased with harvesting frequency. Our study then posits that the Kernza root system is positively affected by management of Kernza as a dual-use crop.

As for the soil health indicators measured in our study, defoliation only significantly affected C-min (Table 1.4). As was the case with roots, the effects on mineralizable carbon was not an immediate result of a defoliation event, but rather a delayed response in the following season's overall levels. Therefore, defoliation's effect on soil health is heavily conferred through its effect on Kernza roots.

Although mineralizable carbon and POXC are both measures of the labile carbon pool in soil, defoliation had different effects on these two properties. Overall, defoliation had a pronounced effect on mineralizable carbon and not POXC, as differences between defoliated and non-defoliated treatments were statistically significant for mineralizable carbon but not for POXC (Table 1.4). In terms of the specific effects, mineralizable carbon was greater under defoliated stands compared to the non-defoliated stands (Figure 1.7, 1.8). The different results of defoliation effects on the two separate measures of labile carbon may be explained by a recent paper by Hurisso et al. (2016) which showed that though the measurements are related, they are differentially influenced by management practices, whereby mineralizable carbon better reflects practices which influence nutrient mineralization and POXC better reflects practices which influence carbon stabilization in the soil. Therefore, mineralizable carbon is likely more reflective of the differences in root turnover and exudation between defoliated and non-defoliated stands than is POXC. The greater rates of turnover (Lopez-Marisco et al., 2015) and therefore additions of plant residue and substrates to the soil likely increased the size of the microbial community (Stanton 1988; Wittaker, 2003) and therefore mineralization. Therefore, our findings indicate that defoliating aboveground biomass is a practice that promotes short-term nutrient availability more so than long-term carbon sequestration in soils under Kernza.

Soil protein is a measure of organic N in soils and its lower levels under defoliated stands align with our findings in C dynamics as lower levels in organic N could indicate greater N mineralization and nutrient cycling. Overall, the C and N dynamics used to evaluate soil health in this study appear to be heavily influenced by the Kernza root system and are reflective of the differences in roots under defoliated and non-defoliated managements.

Our collective findings do not indicate that overall soil health was negatively affected under management of Kernza as a dual-use crop in the first two years of production. Overall, managing Kernza as a dual-use crop stimulates greater short-term nutrient availability and cycling than managing Kernza for grain only. However, management for grain only appears to promote greater soil carbon stabilization than dual-use management. Therefore, in the long-term greater carbon may be sequestered under management of Kernza for grain only, however this system is likely still in flux and studies beyond the first two years of production are needed to fully evaluate soil health dynamics under Kernza.

1.5 Conclusions and Future Directions

This study examined the general seasonal dynamics of Kernza plant biomass and soil fractions related to soil health in order to determine interactions and relationships between system components. In general, soil dynamics appeared to be primarily driven by plant dynamics and secondarily by weather. Overall, plant and soil dynamics were closely linked to one another resulting in the synchronization of plant demand and C and N cycling. This study also evaluated the effect of dual-use management of Kernza on soil health by determining the effects of defoliation on Kernza root biomass and specific indicators of soil health. Defoliation of aboveground biomass did not produce an immediate effect on roots, rather defoliation had a delayed effected on roots that impacted overall root biomass and production in the following growing season. Harvesting forage as a part of dual-use management had a positive impact on Kernza root biomass, and this effect increased with frequency. Overall, managing Kernza as a dual-use crop did not affect the seasonal dynamics of the roots but did positively impact the overall production of roots and this effect was conferred to soil health. Overall mineralizable carbon was greater under dual-use management than grain-only management, but POXC and soil protein were not statistically different across managements. Therefore, the overall ability for Kernza to influence soil health is not negatively affected under dual-use management, however, dual-use management appears to stimulate greater short-term nutrient availability and cycling as opposed to carbon stabilization. While these results are promising, long-term research beyond the first two years of production is necessary to confirm the consistency and prevalence of trends as stands experience changes in age and weather. As our study only looked at roots and soil measures in the top 20 cm of soil, future research would benefit from exploring beneath this surface layer as Kernza roots are likely to grow deeper with time.

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Chapter 2: Evaluation of Kernza as a Dual-Use Crop Across a Variety of Regions in the United States and Canada

2.1 Introduction

2.1.1 The Necessity of Perennial Grains

Due to the extensive degradation of land and ecosystems caused by annual grain agriculture (Tilman, 1999, Foley et al., 2005; Power, 2010) recent research has focused on strategies that would increase the sustainability of these systems by balancing the tradeoffs between agricultural productivity and ecosystem functioning. Reinstituting perennial crops into cereal agriculture is a strategy that could achieve this goal (Pimental et al., 1986; Glover et al., 2010a; Crews et al., 2014). The vast range of ecosystem benefits perennials deliver compared to annuals have been well documented (Glover et al., 2010b; Asbjornsen, et al., 2013) and perennial grain breeding programs are increasing (Cox et al., 2010); both of which in combination have stimulated interest and created momentum for research in perennial grain cropping systems.

2.1.2 Kernza as a Novel Perennial Grain

One perennial species that has harnessed long-term interest in its potential for grain crop domestication is intermediate wheatgrass (IWG; *Thinopyrum intermedium*), a widely adapted, cool-season, rhizomatous grass which is most commonly grown for forage/hay across the Great Plains and Intermountain West regions (Ogle et a., 2011). Originally selected for domestication as a grain crop in the 1980's because of the crop's agronomic and nutritional properties (Wagoner, 1990; Becker et al., 1991), IWG has gone through two decades of breeding and selection initially at the Rodale Research Center (Wagoner, 1995) and more recently The Land Institute (DeHaan et al., 2013), which has trade named the IWG grain crop "Kernza." Aside from breeding, the field of Kernza research has expanded in recent years to include evaluations of ecosystems services under Kernza (Culman et al., 2013), Kernza grain and forage yield response to N rate (Jungers et al., 2017), and end-use qualities (Zhang et al., 2016). While the progress in Kernza research is promising, two major challenges continue to plague the development of Kernza as a viable perennial grain crop: i) lower seed yields relative to annual wheat and ii) declines in seed yield over time.

Though breeding efforts have succeeded in increasing the grain yield of Kernza relative to commercial forage varieties, annual Kernza grain yields remain significantly less than those of annual wheat (Culman et al., 2013; DeHaan et al., 2013; Jungers et al., 2017). In addition to lower grain yields on average, IWG experiences declines in grain yield with age (Wagoner, 1995; Weik et al., 2002; Lee et al., 2009) a problem that seems to have persisted despite the success in breeding efforts of Kernza, as a study by Jungers et al. (2017) reported declines in Kernza grain yield following the second year of production. The consequence of these problems manifests itself in the inability of Kernza to economically compete with annual cereals. Therefore, these challenges must be overcome before Kernza can be marketed as a viable alternative to annual grains.

2.1.3 A Dual-Use Solution

Utilizing Kernza as a dual-use crop for forage and grain could potentially alleviate the crop's challenges associated with grain production. Managing a perennial grain for dual-use could provide two sources of income (grain and forage), thereby relieving the economic disparity between the perennial grain and annual cereal systems (Bell et al., 2008; Reeling et al., 2012) Evaluation of other perennial grain crops under dual-use management are promising (Jaikumar et al., 2012; Larkin et al., 2014), however the potential of Kernza to perform as a dual-use crop has yet to be rigorously evaluated.

The additional defoliation of biomass for forage harvest may also help to mitigate the decline in grain production as stands mature. The disturbance caused by defoliation may help to reduce the intraspecific competition Kernza stands experience as they become sod-bound with age. Increases in plant density as stands become sod-bound have been shown in other species to reduce reproductive tillers (Casel et al., 1986) and over all grain yields (Casal, 2013; Maddonni and Otegui, 2006; Rondanini et al., 2014). Disturbance by way of cultivation (Crowle and Knowles, 1962), burning (Knowles, 1966; Canode and Law, 1978), or mechanical harvesting (Pumphrey, 1965) has been shown to help stimulate seed production of cool-season perennial forages over time (Majerus, 1988). As for specific research into the effects of cultural management on IWG declining seed yields, Canode (1965) found that any disturbance of stands by burning or mechanical removal helped to mitigate the effects of age on grain yields, and Wagoner et al. (1990) reported increased grain yields in the year following the grazing of stands post-grain harvest. However, under dual-use management Kernza forage will be harvested, and therefore defoliated, under different frequencies and timings than have been previously studied, therefore it is necessary to evaluate Kernza seed production across dual-use strategies in order to determine trends in long-term productivity.

2.1.4 Kernza Regional Assessments

Suitable conditions and locations in which to grow Kernza have yet to be determined due to a lack of evaluation across diverse regions. While bred in Kansas, Kernza field performance has only been evaluated in Michigan (Culman et al., 2013) and Minnesota (Jungers et al., 2017). Results of those studies indicated that Kernza performs well in the Upper Midwest; however, Kernza may perform differently in other regions as seed yields of IWG forage varieties have been reported to vary across regions (Wagoner, 1995) and environments (Ogle et al., 2011). IWG has traditionally been grown in the Great Plains and Intermountain West regions of the U.S. where its performed well as a forage and pasture crop, however growing Kernza for grain may require different conditions. Therefore, the approach of evaluating Kernza across multiple sites which range in climate and environment is two-fold: establishing individual site potentials for growing Kernza as well as better understanding how suited Kernza is over a climatic range. Knowing site potentials for Kernza production allows us to establish which sites and regions are suitable and optimal for growing Kernza. Knowing how Kernza performs under different conditions (i.e. drought, temperatures, soil types) allows us to better understand what and how these factors influence Kernza production and therefore adjust management practices accordingly.

2.2 Objectives and Hypotheses

The specific objectives of this study are to (i) evaluate Kernza grain and forage yields across a wide range of sites and environments (ii) assess how the defoliation of forage biomass under dual-use management affects Kernza grain and forage yields.

We hypothesize that Kernza crop yields will vary between sites with overall greater forage and grain yields at sites in cooler, northern regions. We hypothesize that the defoliation of forage biomass will not negatively impact grain yields and that defoliation will help mitigate grain yield declines commonly observed in older stands.

2.3 Materials and Methods

2.3.1 Site Descriptions

Eight sites within the United States and one site in Canada participated in this study (Table 2.1). The sites represent a wide range of environments and climate. Kernza was planted at sites during the fall of either 2014 or 2015 and depending on the year planted, sites supplied one or two years of data.

2.3.2 Experimental Design

Dual-use strategies evaluated in this study were comprised of combinations of different forage defoliation (i.e. cutting) frequencies and timings. The dual-use strategies were assessed with four forage defoliation treatments: i) Spring and Summer Cut (Sp + Su), ii) Summer Cut (Su), iii) Summer and Fall Cut (Fa + Su) and iv) No Cut, which functioned as the control in the study. All sites evaluated these four specific treatments, with the exception of Minnesota which did not incorporate a Summer Cut treatment into their research and Colorado which due to extensive lodging in the first year of production had to eliminate the No Cut treatment. The Spring Cut occurred in the spring before the plant reached first palpable node (E1 stage; Moore et al., 1991). The Summer Cut occurred in the summer after the grain harvest. The Fall Cut occurred in the fall after considerable regrowth and before the first frost. The No Cut (control) treatment was never subjected to a defoliation of forage, only the grain was harvested during the summer.

Nitrogen fertilizer was applied annually to all sites in split applications and rates ranged between 50 and 80 kg N ha^{-1}. The first application was applied in the spring, generally before spring green up. The second application was applied in the period immediately following the summer grain harvest. Sites were planted during the fall of 2014 or 2015.

2.3.3 Data Collection

Data collection methodologies varied by site, season, and year (Table 2.1). Quadrat estimates of forage and grain yields were taken when whole plot harvest measurements were not possible.

Quadrat samples were taken by randomly placing a quadrat (0.25-0.5 m^2) within each plot then following sampling protocols for the specific harvest. To estimate forage yields all forage biomass within the quadrat was cut to 10 cm above the ground and removed. Fresh weights were taken to determine moisture content then forage biomass was dried at 50-70°C for 48-72 hours. Dry weights were taken to determine final forage yields on a kg ha^{-1} basis. Seed head counts were determined by counting the number of seed heads within the quadrat. Grain yield estimates were determined by clipping the seed heads from within the quadrat and removing them. Fresh weights were taken for quadrat seed heads to determine moisture content then dried at 50-70°C for 48-72 hours. To maintain consistency among sites, seed head samples were threshed at The Land Institute. Seed heads were dried, weighed, and threshed to determine total grain weight per quadrat. After seed heads had been threshed, percent of naked seed present in the threshed sample was determined and conversely the percent of seed still in hull. Total grain values were then adjusted using the naked and in-hull seed percentages and a conversion factor of 0.7 with the following equation.

(total sample threshed grain weight x % naked seed in threshed sample) + (total sample threshed grain weight x % seed in-hull in threshed sample x 0.7)

The 0.7 conversion factor is based on the estimate that Kernza seeds in hull are typically 70% seed and 30% hull by mass (Lee DeHaan, Personal Communication). The adjusted sample grain weights were then used to calculate grain yields on a kg ha^{-1} basis.

2.3.4 Data Structuring and Terminology

In situations where sites provided two sets of data (quadrat and plot measurements) for a harvest event, only the plot level data was used for analysis. For the purposes of our research the year variable was treated according to the establishment year instead of the actual calendar year date to control for the effects of the stand age on measured plant properties. The majority of forage biomass was harvested after grain harvest, which we termed “summer forage harvest”; however, forage was also harvested in the spring and fall. Where appropriate, we added these spring and fall forage harvests to the summer forage harvest, which we termed collectively, “total annual forage harvest.”

2.3.5 Data Analysis

Data was analyzed with the PROC MIXED procedure in SAS v9.4 (SAS Institute, Cary, NC). For all analyses, harvest events were analyzed individually. Data was analyzed as repeated measures with year modeled as the repeated variable, site, year, and management as fixed effects, and block as a random effect. Data were also analyzed on an individual year basis with site and management as fixed effects and block as the random effect. Sites with two years of data were analyzed individually by site with repeated measures for only Summer Harvest data. For all analyses, significant differences were determined at $\alpha = 0.05$. Graphs were created using the ggplot2 (Wickham, 2009) package in R.

Table 2. 1. Sites, institutions and data collection methods involved in this study.

Site Name	Location	Year Planted	Factors Examined	Data Collection Method				
				Year	*Spring Forage*	*Summer Forage*	*Summer Grain*	*Fall Forage*
Alberta	Lethbridge, Alberta Ag-Canada 49° 41' N, 112° 45' W	2015	Defoliation	First	Plot	Quadrat, Plot	Quadrat, Plot	N/A
Colorado	Fort Collins, Colorado Colorado State 40° 34' N, 105° 4' W	2014	Defoliation, N Rate	First Second	Quadrat Quadrat	Quadrat, Plot Quadrat	Quadrat Quadrat	Quadrat Quadrat
Iowa	Ames, Iowa Iowa State 42° 1' N, 93° 38' W	2015	Defoliation	First	Plot	Quadrat	Quadrat, Plot	Plot
Kansas	Salina, Kansas The Land Institute 38° 46' N, 97° 33' W	2014	Defoliation, N Rate	First Second	Plot Plot	Quadrat Quadrat	Quadrat, Plot Quadrat, Plot	Plot Plot
Minnesota	St. Paul, Minnesota U of Minnesota 44° 59' N, 93° 09' W	2014	Defoliation	First Second	Quadrat Quadrat	Quadrat Quadrat	Quadrat Quadrat	Quadrat Quadrat

Table 2.1 continued on pg 78

Table 2.1 continued

New York	Ithaca, New York Cornell University 42° 27' N, 76° 28' W	2014	Defoliation, N Rate	First Second	Quadrat Quadrat	Quadrat Quadrat	Quadrat Quadrat, Plot	N/A Plot
Ohio 1	S. Charleston, Ohio Ohio State University 39° 49' N, 83° 38' W	2014	Defoliation	First Second	Plot Plot	Quadrat, Plot Quadrat, Plot	Quadrat, Plot Quadrat	Plot Plot
Ohio 2	Wooster, Ohio Ohio State University 40° 48' N, 81° 56' W	2014	Defoliation, N Rate	First Second	Plot Quadrat, Plot	Quadrat, Plot Quadrat, Plot	Quadrat, Plot Quadrat, Plot	Plot Plot
Wisconsin	Madison, Wisconsin U of Wisconsin 43° 04' N, 89° 25' W	2015	Defoliation, N Rate	First	N/A	Quadrat, Plot	Quadrat	Plot

2.4 Results and Discussion

2.4.1 Kernza Grain and Forage Yields Across Sites

Overall, Kernza grain yields varied significantly between sites and years (Table 2.2). Average grain yields in the first year of production ranged from around 500 kg ha^{-1} at the Kansas and Minnesota sites to about 1,000 kg ha^{-1} at the New York site (Table 2.3), a two-fold difference. The grouping of sites producing similar amounts of grain yield within the first year (Table 2.3) does not appear to be related to region or climate suggesting that the variation in Kernza grain yields may be influenced by something more specific to sites such as weather or management (i.e. planting dates, etc.). In the second year of production average grain yields ranged from as little as 3 kg ha^{-1} at the Colorado site to around 650 kg ha^{-1} at the Ohio 2 site. Similar to year one, second year variations between site grain yields does not appear to be related to climate or region.

The first and second year grain yield ranges reveal that the highest grain yields of the second year are only little greater than the lowest yields of the first year, suggesting an overall decrease in Kernza grain production from year one to year two. However, the presence of a highly significant site x year interaction (F-statistic = 17.27, P= <0.0001).

Table 2. 2. Plant F-statistics and significance from ANOVA of all sites with measurements for spring, summer, and fall seasons analyzed by both years and individual years.

Harvest	**Spring**		**Summer**		**Fall**
	Forage Yield	*Forage Yield*	*Grain Yield*	*SHC◊*	*Forage Yield*
			First and Second Year		
Site (S)	29.11 ***	23.66 ***	15.73 ***	406.9 ***	56.09 ***
Year (Y)	13.69 **	75.05 ***	227.27 ***	642.53 ***	15.46 ***
S x Y	11.56 ***	53.82 ***	22.85 ***	628.72 ***	35.2 ***
Defoliation (D)	-	13.05 ***	0.99	0.34	-
S x M	-	0.86	2.16 **	0.58	-
Y x M	-	0.36	1.68	0.69	-
S x Y x M	-	0.88	0.52	0.84	-
			First Year		
Site (S)	12.87 ***	38.58 ***	7.26 ***	474.01 ***	29.12 ***
Defoliation (D)	-	3.84 **	1.48	0.56	-
S x M	-	0.91	1.12	0.66	-
			Second Year		
Site (S)	44.75 ***	31.04 ***	141.11 ***	69.92 ***	210.91 ***
Defoliation (D)	-	23.26 ***	7.52 ***	7.67 ***	-
S x M	-	0.82	5.36 ***	1.2	-

* Significance level: P < 0.05

** Significance level: P < 0.01

*** Significance level: P < 0.001

◊ SHC = seed head count

Table 2. 3. Site average grain and total annual forage yields across all defoliation treatments with standard error in parentheses.

	Grain	***Forage***
	-------------- ***kg ha^{-1}*** -------------	
	First Year	
Alberta	791 (*73*)	7108 (*671*)
Colorado	724 (*68*)	13063 (*1623*)
Iowa	669 (*110*)	4137 (*359*)
Kansas	526 (*26*)	5203 (*677*)
Minnesota	535 (*52*)	12058 (*1179*)
New York	1043 (*145*)	5097 (*672*)
Ohio 1	758 (*71*)	6386 (*489*)
Ohio 2	651 (*49*)	4206 (*212*)
Wisconsin	902 (*122*)	6800 (*612*)
	Second Year	
Colorado	3 *(1.09)*	2483 (*368*)
Kansas	71 (*9*)	6585 (*524*)
Minnesota	183 (*85*)	9015 (*1236*)
New York	209 (*36*)	4806 (*705*)
Ohio 1	36 (*6*)	3285 (*268*)
Ohio 2	655 (*49*)	6411 (*400*)

warrants closer examination of trends in grain yield between years on an individual site basis. All six sites with two years of production experienced decreases in grain yield, with the exception of the Ohio 2 site which managed to sustain grain yields between years (Table 2.3). The declines in individual site average grain yields between years one and two vary in magnitude causing any trend between sites present in the first year of production to change in the second year. For example, while Colorado and Ohio 1 produced some of the greater grain yields in the first year, these sites produced the lowest and second lowest yields in the second year (Table 2.3). The only site that did not seem to change in its ranking of Kernza grain production was the New York site; in both years New York produced one of the highest grain yields out of all sites. Changes in yields between years are likely due to differences in weather (Table 2.4, 2.5), as many sites experienced lower seasonal precipitation in the second year of production compared to the first (Colorado, Kansas, New York, and Ohio 1; Table 2.4) and an overall warmer summer season in the second year compared to the first (Colorado, Minnesota, New York, Ohio 1, Ohio 2; Table 2.5).

Cumulative site grain yields for the sites that had two years of production are perhaps the best tool for assessing the overall grain production potential of a given location. Total collective grain yields were 597, 718, 727, 794, 1,252 and 1306, kg ha^{-1} for Kansas, Minnesota, Colorado, Ohio 1, New York and Ohio 2, respectively. From these estimates, it appears that overall, Northeastern regions are more productive compared to sites in the Great Plains and Upper Midwest, in terms of Kernza grain.

Of the limited published studies on intermediate wheatgrass grain yields using The Land Institute developed seed under field conditions, only sites in the Upper Midwest have been evaluated. Recent research in Minnesota has reported yields averaging 848 kg ha^{-1}

Table 2. 4. Site cumulative yearly, seasonal, and monthly precipitation

		Precipitation (mm)										
Site	**Year**	*Year Total*	*Season Total*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*
Alberta	2016	395	349.5	22.1	34.9	52.3	81.1	42.4	39.9	40.3	19.7	16.8
Colorado	2015	703.6	689.6	3.1	51.6	148.3	71.4	94	116.3	128.5	50.6	25.9
	2016	561.3	547.6	33.3	40.9	44.5	39.9	109.2	199.6	32.3	10.7	37.3
Iowa	2016	955	888.2	38.6	103.6	108.7	24.4	148.6	209	200.2	11.9	43.2
Kansas	2015	880.9	739.8	4.8	41.9	282.2	92.2	68.3	90.9	67.3	18.3	73.9
	2016	669	619.8	19.3	102.4	133.1	11.4	72.9	178.8	31.8	55.1	15
Minnesota	2015	860.8	794.5	18	52.6	125.5	84.1	157.2	70.9	97	72.9	116.3
	2016	1058.4	980.2	54.6	93	52.1	92.7	151.6	251.5	131.8	84.3	68.6
New York	2015	936.2	785.80	47.2	59.7	141.2	203.2	71.1	26.9	131.6	72.4	32.5
	2016	811.3	635.4	47.5	48.3	50.8	18.8	48.3	116.8	55.9	200.7	48.3
Ohio 1	2015	1042.8	804.1	88.2	119.5	60.6	177.1	135.2	47.6	48.2	70.2	57.5
	2016	894	702.8	89.1	67	72.9	40.6	103.2	138.4	122.9	45.5	23.2
Ohio 2	2015	851.3	668.2	63.4	71.3	110	137.9	84.7	19.9	90.9	58.5	31.6
	2016	784.8	629.9	103.7	67.9	63.9	34	73	99.8	60.6	96.5	30.5
Wisconsin	2016	987	102.7	109	37.3	87.4	104.1	164.8	138.7	156.5	85.6	41.1

Table 2. 5. Site average month, seasonal, and yearly temperature.

		Temperature (C)										
Site	**Year**	*Year Average*	*Season Average*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*
Alberta	2016	7	11.4	3.9	10.7	13.4	17.3	17.4	17.6	13.5	2.7	6.4
Colorado	2015	9.7	13.3	6.4	8.3	10.7	20.1	20.4	20.9	18.7	11.9	2.2
	2016	9.9	13.7	4.7	8.2	11.6	21.1	22.5	19.9	16.9	12.2	6.3
Iowa	2016	10.7	15.7	6.2	10.4	15.7	23.5	22.9	22.2	19.8	13.3	7.2
Kansas	2015	14.8	19	9	14.4	17.8	26.3	28.1	24.8	24.8	16.3	9.5
	2016	15	19.4	10.8	14.5	17.8	26.8	28	25.8	22.6	17.5	10.7
Minnesota	2015	7.8	13	-0.1	8.5	13.8	19.8	21.9	20.1	18.8	9.8	4.3
	2016	8.4	13.8	3.5	7.7	14.8	20.4	22.5	21.4	17.5	10.2	5.8
New York	2015	8.5	13.5	-3.6	7.2	17.2	17.9	20.4	19.8	19.2	9.8	7.2
	2016	9.5	14.4	3.7	4.8	13.6	18.6	22.1	22.8	18.2	11.5	5.9
Ohio 1	2015	11	15.3	2.6	11.2	19	21.1	22	20.7	19.8	12.5	8.4
	2016	12	16.3	8.3	10.5	15.9	22.6	23.7	23.9	20.4	14.5	7.3
Ohio 2	2015	10.4	14.6	1.5	10.6	17.7	20.1	21.4	20.8	19.1	11.6	8.2
	2016	11.7	15.4	7.4	8.7	15.1	21.2	23.3	23.6	19.1	13.3	7
Wisconsin	2016	8.8	13.8	3.6	7.1	14.3	20.3	21.8	21.4	17.7	11.3	6.3

in the first year of production using cycle 2 (TLI-C2) seed in unfertilized conditions and yields peaking at 996 kg ha^{-1} at an 80 kg N ha^{-1} fertilizer rate when evaluated across a fertilizer gradient (Jungers et al., 2017). A study in Michigan reported grain yields of cycle 1 (TLI-C1) seed ranging from 112 to 157 kg ha^{-1} in the first year, and 1390 to 1662 kg ha^{-1} in the second year of production when evaluated across a N fertilizer gradient, however the authors attributed the low first year grain yields to a very late fall planting (Culman et al., 2013). Both sites' grain yield estimates are telling of the potential for Kernza grain yields in the Upper Midwest region. First year Minnesota grain yields in our study are slightly lower than those previously reported in Minnesota by Jungers et al. (2017) and second year Minnesota grain yields in our study are dramatically lower than reported yields from both Minnesota and Michigan (Culman, et al., 2013; Jungers et al., 2017). Wisconsin first year grain yields in our study are a little more comparable to first year Kernza grain yields reported from this Upper Midwest region (Table 2.3).

In general, all sites' first year grain yields were greater than grain yield averages reported for IWG forage varieties in dryland conditions which average 280-392 kg ha^{-1}, and all sites performed as well if not better than IWG forage varieties in irrigated conditions which yield 504-616 kg ha^{-1} on average (Ogle et al., 2011). However, the majority of sites in the second year of production did not sustain this trend and yielded less grain than IWG in either dryland or irrigated conditions. When individual site grain yields were averaged across years, only New York (711 kg ha^{-1}) and Ohio 1 (653 kg ha^{-1}) produced average yields greater than those of IWG forage cultivars, the other sites yielded between

300 and 400 kg ha^{-1} on average. While decreases in second year grain yields have been reported in some instances for forage cultivars (Weik et al., 2002), this trend does not compare to those reported for Kernza grain cultivars in which grain yields generally increase between years one and two (Culman et al., 2013; Jungers et al., 2017).

Similar to grain yields, all forage yields (Spring, Summer, and Fall Harvests) in general differed significantly between sites and years (Table 2.2). During the first year of production, average total annual forage harvest yields ranged from as low as 4,000 kg ha^{-1} at the Iowa and Ohio 2 sites to as high as above 12,000 kg ha^{-1} at the Colorado and Minnesota sites, a three-fold difference (Table 2.3). The second year after planting showed significant variation in collective forage yields amongst sites (Table 2.2, 2.3). The Minnesota location again produced one of the greatest average cumulative forage yields reaching around 9,000 kg ha^{-1}, while Colorado, in direct contrast to the first year, produced the lowest cumulative forage yields around 2,500 kg ha^{-1} (Table 2.3). As with grain yields, for both years one and two the grouping of sites that produced comparable forage yields does not seem to be based on region or climate, but again the result of something more specific to individual sites.

Between year one and year two of production, the size of the range of site total annual forage harvest yields remained the same but the general values in the range decreased. While this seemingly implies an overall general decline in cumulative forage yields, closer examination of individual site averages reveals that not all sites experience a decrease in forage yields between years (Table 2.3). Site differences in the direction as well as the magnitude of change in forage yields between years results in the lack of a consistent trend in forage yield production between sites. The only trend that remained between years was that of Minnesota forage production, as it was one of the greatest in both years.

As with the grain yields, total annual forage harvest yields over both years of production can help us better assess the true forage production potentials of individual sites. Total cumulative forage yields were 9,671, 9,903, 10,617, 11,788, 15,546, and 21,073 for Ohio 1, New York, Ohio 2, Kansas, Colorado, and Minnesota, respectively. According to these estimates, it would appear that overall, sites located in the Upper Midwest and Great Plains regions are more productive than sites in the Northeastern region, in terms of forage production. These results are not that surprising as intermediate wheatgrass has traditionally been grown for commercial forage production in the Great Plains and Intermountain West regions (Ogle et al., 2011; Hendrickson et al., 2005; Karn et al., 2006) and recent Kernza forage biomass evaluations in the Upper Midwest have shown the ability of this region to produce forage. Research in this region reported yields similar to those at the Minnesota site in the first year of production (Culman et al., 2013; Jungers et al., 2017), however Minnesota second year forage yields in our study are lower than those reported for this region.

2.4.2 Kernza Grain and Forage Yields under Dual-Use Strategies

Overall, management did not influence grain yields, however a site x management interaction was significant and indicates that sites responded to management differently (Table 2.2). First year grain yields were not significantly different between managements (Table 2.2). However, the possible dual-use management effects on grain yields in the first year of production could only be assessed with the Sp + Su treatment because at the time of grain harvest only the Sp + Su management had received any forage defoliation treatments (Spring Cut). First year results indicate that in general, Sp + Su grain yields were no different from the grain yields of other managements; although, there were individual sites (Iowa, Kansas) that experienced significant declines with the Sp + Su management relative to other managements (Table 2.6). At the Iowa location, the Sp + Su yielded significantly less than the No Cut treatment, while at the Kansas site, the Sp + Su management yielded significantly less than all other managements. While these two sites do provide evidence for the possibility of a Sp + Su strategy to negatively affect grain yields, this trend is not shared by the majority of sites.

Contrary to the first-year results, individual analysis of the second-year data yielded highly significant differences in grain yield between defoliation strategies (Table 2.2). When averaged across all sites, the No Cut treatment produced the least amount of grain out of all treatments during the second year (Table 2.6). Therefore, it appears that

Table 2. 6. Average management grain yields and standard error in parentheses by site and year. Different letters in the same row denote significant differences between treatments.

	Spring and Summer Cut	***Summer Cut***	***Summer and Fall Cut***	***No Cut***
	-------------------------------- kg ha^{-1}			***--------------------------------***
		First Year		
Alberta	779 (77)a	815 (67)a	779 (76)a	-
Colorado	728 (92)a	518 (57)a	798 (79)a	853 (45)a
Iowa	430 (146)b	701 (97)ab	559 (63)ab	987 (134)a
Kansas	421 (27)b	553 (28)a	564 (29)a	567 (19)a
Minnesota	561 (52)a	-	442 (52)a	605 (NA)a
New York	934 (172)a	1102 (157)a	1092 (106)a	-
Ohio 1	757 (60)a	801 (80)a	772 (78)a	701 (66)a
Ohio 2	628 (63)a	682 (58)a	715 (47)a	578 (29)a
Wisconsin	-	960 (139)a	747 (70)a	998 (156)a
Mean	**655 (86)**	**767 (85)**	**719 (67)**	**756 (75)**
		Second Year		
Colorado	3 (0.89)a	1 (0.58)a	4 (1.45)a	4 (1.44)a
Kansas	72 (11)a	80 (6)a	62 (10)a	-
Minnesota	243 (93)a	-	243 (76)a	63 (NA)a
New York	190 (29)a	256 (45)a	152 (34)a	238 (36)a
Ohio 1	53 (3)a	41 (8)a	34 (8)a	17 (3)a
Ohio 2	642 (78)b	817 (36)a	777 (38)ab	384 (45)c
Mean	**201 (36)**	**239 (19)**	**212 (28)**	**141 (21)**

managing Kernza as a dual-use crop produces greater grain yields in the second year compared to if Kernza were just managed for grain. The poor performance of the No Cut treatment in the second year of production was likely the result of not removing the remaining biomass after the grain was harvested, as this practice has been shown to improve yields in the subsequent year in IWG (Wagoner, 1995).

Management grain yields averaged across sites decreased between years by 69, 72, 71, and 80% for the Sp + Su, Su, Fa + Su, and No Cut treatments, respectively. These results further support the idea that remaining biomass must be removed after grain is harvested in order to obtain greater grain yields in the following year.

Overall, defoliation had a highly significant effect on Summer Harvest forage yields and the lack of significant interaction between management and other main effects (site, year, and site x year) suggests that overall, management trends in forage yield remain constant even while forage yields in general may change between sites and years (Table 2.2). Average forage yields at the Summer Harvest for years one and two suggest that the Sp + Su management yields the least forage amongst defoliation treatments (Table 2.7). In both year one and two, all the individual sites with significant differences between managements maintain this trend, with the Sp + Su management yielding significantly less summer forage than other managements (Table 2.7). From these results, it appears that harvesting forage in the spring has the potential to reduce forage yields at

Table 2. 7. Individual site first and second year treatment summer forage harvest yield averages with standard error in parentheses. Different letters within the same row denote significantly different treatments at $\alpha = 0.05$.

	Spring and Summer Cut	***Summer Cut***	***Fall and Summer Cut***	***Mean***
	---------- Mg ha^{-1} ----------			
		First Year		
Alberta	6.02 (0.60)a	7.60 (0.37)a	7.12 (0.98)a	**6.91 (0.65)**
Colorado	10.01 (0.49)a	12.64 (2.40)a	12.60 (1.95)a	**11.75 (1.62)**
Iowa	0.98 (0.12)b	2.91 (0.19)a	3.39 (0.24)a	**2.43 (0.18)**
Kansas	3.00 (0.36)b	4.60 (0.66)a	4.70 (0.49)a	**4.10 (0.50)**
Minnesota	10.47 (1.22)a	-	8.92 (0.81)a	**9.70 (1.01)**
New York	3.55 (0.44)a	5.30 (0.49)a	5.32 (0.85)a	**4.72 (0.59)**
Ohio 1	5.27 (0.20)a	6.45 (0.47)a	6.48 (0.55)a	**6.07 (0.41)**
Ohio 2	3.24 (0.15)a	3.21 (0.16)a	3.29 (0.17)a	**3.24 (0.16)**
Wisconsin	-	6.33 (0.49)	6.88 (0.34)a	**6.61 (0.41)**
Mean	**5.32 (0.45)**	**6.13 (0.65)**	**6.52 (0.71)**	
		Second Year		
Colorado	1.90 (0.34)a	1.87 (0.28)a	1.65 (0.26)a	**1.81 (0.30)**
Kansas	3.68 (0.70)a	5.18 (0.32)a	5.23 (0.22)a	**4.70 (0.41)**
Minnesota	6.58 (0.64)a	-	6.62 (1.20)a	**6.60 (0.92)**
New York	2.72 (0.43)a	5.30 (0.44)a	4.16 (0.91)a	**4.06 (0.59)**
Ohio 1	2.36 (0.60)b	3.45 (0.22)a	2.40 (0.30)b	**2.74 (0.20)**
Ohio 2	5.12 (0.34)b	6.85 (0.39)a	6.15 (0.32)ab	**6.04 (0.35)**
Mean	**3.73 (0.42)**	**4.53 (0.33)**	**4.37 (0.54)**	

the summer harvest, however this does not necessarily translate into overall lesser total annual forage yields for this management.

When averaged across all sites, total annual forage yields in both years one and two reveal a general trend amongst managements, whereby the Fa + Su treatment yielded the most forage, followed by the Sp + Su treatment, and then the Su treatment which yielded the least forage annually (Table 2.8). During the first year of production, the majority of sites generally follow this trend, yielding the most collective forage under the Fa + Su management, however ranking between the Sp + Su and Su management vary by site (Table 2.8). The greater performance of the Fa + Su treatment compared to the Su treatment appears to be caused by the additional forage harvested in the Fall, as yields of these two treatments at the Summer Harvest were comparable at most sites, which is again most likely due to the fact that neither management had received any defoliation treatments at the time of the Summer Harvest (Table 2.8). In terms of the outperformance of the Sp + Su management by the Fa + Su management, it appears to be due to the lesser yields of the Sp + Su treatment at the summer forage harvest, rather than a difference in forage yields at the Spring or Fall harvests, as even sites that yielded more forage in the Spring than the Fall ended up producing the greatest annual forage yields under the Fa + Su management (Table 2.9). Examination of individual site collective forage yields in the second year of production reveal no general adherence to the observed general trend in management forage yields, in fact trends appear to be reversed with the most collective forage being produced under the Sp + Su or Su management, and the Fa + Su

Table 2. 8. Individual site first and second year treatment average total annual forage harvest yields with standard error in parentheses.

	Spring and Summer Cut	***Summer Cut***	***Fall and Summer Cut***
	---------------------------- Mg ha^{-1} ----------------------------		
		First Year	
Alberta	6.60 (0.67)	7.60 (0.37)	7.12 (0.98)
Colorado	11.30 (0.20)	12.64 (2.40)	15.25 (2.27)
Iowa	3.65 (0.44)	2.91 (0.19)	5.85 (0.45)
Kansas	5.06 (0.67)	4.60 (0.66)	5.95 (0.70)
Minnesota	12.24 (1.30)	-	11.88 (1.06)
New York	4.67 (0.68)	5.30 (0.49)	5.32 (0.85)
Ohio 1	5.92 (0.39)	6.45 (0.47)	6.79 (0.61)
Ohio 2	3.92 (0.21)	3.21 (0.16)	5.49 (0.27)
Wisconsin	-	6.33 (0.49)	7.27 (0.74)
Mean	**6.67 (0.57)**	**6.13 (0.65)**	**7.88 (0.65)**
		Second Year	
Colorado	3.85 (0.55)	1.87 (0.28)	1.73 (0.27)
Kansas	7.58 (0.88)	5.18 (0.32)	7.00 (0.38)
Minnesota	7.58 (0.88)	-	10.45 (1.59)
New York	4.34 (0.64)	5.30 (0.44)	4.78 (1.04)
Ohio 1	3.08 (0.20)	3.45 (0.22)	3.33 (0.38)
Ohio 2	5.82 (0.44)	6.85 (0.39)	6.56 (0.37)
Mean	**5.37 (0.60)**	**4.53 (0.33)**	**5.64 (0.67)**

Table 2. 9. Site average forage yields with standard error in parentheses for the Spring and Fall forage harvests in years one and two.

	Spring Harvest	**Fall Harvest**
	--------------- kg ha^{-1}----------------	
	First Year	
Alberta	588 (66)	-
Colorado	1284 (203)	2646 (313)
Iowa	2666 (326)	2462 (205)
Kansas	2062 (310)	1251 (211)
Minnesota	1770 (79)	2953 (254)
New York	1124 (246)	-
Ohio 1	645 (189)	306 (61)
Ohio 2	684 (54)	2205 (103)
Wisconsin	-	915 (92)
Mean	**1352 (184)**	**1820 (177)**
	Second Year	
Colorado	1949 (210)	75 (5)
Kansas	3900 (181)	1765 (163)
Minnesota	999 (239)	3824 (394)
New York	1619 (206)	622 (128)
Ohio 1	721 (136)	925 (80)
Ohio 2	698 (102)	415 (56)
Mean	**1648 (179)**	**1271 (138)**

management producing the second greatest annual forage in most cases (Table 2.8). This change in trend appears to be due primarily to the decrease in summer forage yields of the Fa + Su treatment relative to the Su treatment at most sites (Table 2.7) and secondarily to an increase in Spring forage yields relative to Fall forage yields in the second year of production (Table 2.9). These results suggest that a specific dual-use management's ability to produce the greatest total annual forage yield is heavily reliant on its performance at the Summer Harvest, as well as the seasonal weather.

It is clear from these results that managing Kernza for grain and forage is a better option for producers compared to only managing Kernza for grain. Not only does a dual-use strategy create an additional source of revenue with the harvesting of forage, but it also yields more grain on average. As for specific dual-use strategies, in terms of maximizing grain production, it does not appear that in general one strategy yields significantly more than another. However, harvesting forage in the spring has the potential to decrease grain yields depending on timing, maturation, and weather (Hopkins et al., 2003). Since grain yields do not vary between dual-use strategies, strategies should then be evaluated by their potential to maximize earnings through the optimization of forage production. In order to maximize total forage yields in the first year of production, harvesting forage after grain harvest and once more in the fall is recommended. Although, based on our results, utilizing this strategy in the first year of production will likely result in a decreased ability of the system to reach its optimal forage production potential. However, the alternative option of utilizing a summer forage harvest only strategy ultimately yields the same results as the summer and fall harvest option, just in different years. Therefore, in terms of quantity, either strategy will ultimately produce the same amount of forage on average, but the economic earning potential of these two strategies may differ due to differences in quality of the forage harvested in the summer and the forage harvest in the fall, however the quality of Kernza forage under different harvest times has yet to be studied.

2.5 Conclusions and Future Directions

This study evaluated Kernza grain and forage yields in the first and second year of production across multiple sites with a range of climates and environments. Overall, Kernza crop performance varied between sites, with greater Kernza grain production on average in the Northeast region and greater Kernza forage production in the Great Plains and Upper Midwest regions. This study also examined grain and forage yields of Kernza under dual-use management across three strategies relative to grain-only management. Overall, Kernza produced more grain on average under dual-use management compared to managing Kernza for grain only. In general, Kernza grain yields were not affected by defoliation frequency or timing, as grain yields were similar between all dual-use strategies. Either a summer forage harvest strategy or a summer and fall forage harvest strategy will produce maximum total Kernza forage yields. Future research needs include determination of Kernza grain and crop yields beyond the second year of production in order to evaluate general long-term trends in Kernza crop production and more specific long-term trends of Kernza under dual-use management. Research is also needed in Kernza forage quality in order to more exactly assess the economic potentials of specific dual-use strategies as well as determine the overall economic viability of Kernza as a dual-use crop.

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