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REGULAR ARTICLE

Harvesting forage of the perennial grain crop kernza (*Thinopyrum intermedium*) increases root biomass and soil nitrogen cycling

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Abstract

Background and aims Emerging perennial grain crops yield less grain than annual crops, but the economic viability of these perennial systems could be improved if both forage and grain are harvested. However, the belowground consequences of forage removal in perennial grain systems are unknown. This study aimed to determine the effect of the additional harvest of forage biomass on overall plant biomass allocation and labile soil C and N dynamics within a perennial grain dual-use system.

Methods Plant biomass and associated soil samples of a perennial grain [Kernza (*Thinopyrum intermedium*)] were taken monthly over the first three growing seasons under three harvest regiments: No Cut (0x), Summer Cut (1x), and Summer and Fall Cut (2x).

Results The harvesting of forage biomass significantly increased both above- and belowground biomass. The once and twice forage-harvested treatments averaged 39% and 73% greater root biomass in 2016 and 39% and 49% greater root biomass in 2017 relative to the treatment not harvested for forage. Soil indicators of carbon and nitrogen storage were not affected by forage harvest but mineralizable carbon, an indicator of nutrient cycling, was greater under the forage harvested treatments.

Conclusions The harvest of forage and grain promoted nutrient availability and overall productivity (forage, root and grain biomass) relative to harvesting for grain only. Our findings suggest dual-use management of Kernza can provide a productive and profitable pathway for perennial grain adoption.

Keywords Soil health · Perennial grain · Root biomass · Dual-use · Forage harvest · Permanganate oxidizable carbon · Mineralizable carbon · Soil protein

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Introduction

Herbaceous perennial ecosystems often provide greater belowground ecosystem services such as nutrient cycling (Crews 2005), carbon (C) sequestration (Beniston et al. 2014), soil food web diversity (Culman et al. 2010; DuPont et al. 2010), and water retention and cycling (McIsaac et al. 2010) relative to annual grain systems (Glover et al. 2010; Crews et al. 2016). Perennial systems can excel at providing such services in large part because of their year-round ground cover and expansive and pervasive root systems (Kell 2011; Asbjornsen et al. 2014; DuPont et al. 2014).

Intermediate wheatgrass (*Thinopyrum intermedium*) is a cool-season, rhizome-producing, perennial grass

that produces a grain similar to wheat but significantly smaller in size (Wagoner 1995). Breeding efforts have been underway for the past 25 years to domesticate intermediate wheatgrass into a viable perennial grain crop (Wagoner 1990; DeHaan et al. 2005, 2013; Zhang et al. 2016). The new grain has been trade named 'Kernza' (DeHaan et al. 2018).

While breeding efforts have progressed, Kernza continues to yield less grain compared to annual cereals (DeHaan et al. 2013; Jungers et al. 2017), representing a substantial barrier to producer adoption. There is interest to increase the economic viability of Kernza through managing it as a dual-use crop: harvesting both forage and grain (Ryan et al. 2018). Existing studies on Kernza have focused mainly on aboveground properties such as forage yields (Wagoner 1990; Liebig et al. 2008; Wang et al. 2014; Jungers et al. 2017), grain yields (Lee et al. 2009), grain quality (Zhang et al. 2015), and forage quality (Karn et al. 2006; Jungers et al. 2017), although a few studies have reported belowground properties of Kernza (Culman et al. 2013; Sprunger et al. 2018a, b). Lack of research on the effects of aboveground management on belowground biomass is a significant knowledge gap because the production and maintenance of belowground biomass is critical to sustaining a number of important soil ecosystem processes.

Roots, specifically their production and process of decay, heavily influence ecosystem services and overall soil health. Roots have a significant impact on the chemical and biological properties of soils such as soil organic carbon (SOC) (Gill et al. 1999; Rasse et al. 2005) and microbial communities (Farrar et al. 2003; DuPont et al. 2014), and also play an important role in nutrient cycling (Ruess et al. 2003; Fornara et al. 2009). Soil organic carbon pools are regulated primarily by root residues, as residues supply significantly more C to the soil than shoot residues (Balesdent and Balabane 1996; Rasse et al. 2005). Roots of grassland 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 et al. 1987; Glover et al. 2010). A recent study by Sprunger et al. (2018a) reported that Kernza root C was 15 times greater than that of annual winter wheat in surface depths. The greater transfer of C to the soil under perennials has created significantly greater soil C pools in comparison to annual cropping systems (DuPont et al. 2014) which could have major implications for climate change mitigation (cf. 4 per 1000 Initiative).

A number of field studies have examined the effects of forage harvest and defoliation on above and belowground biomass of forage and herbaceous perennial systems and have reported variable results (Pearson 1965; Lorenz and Rogler 1967; Smoliak et al. 1972; Bartos and Sims 1974; Christiansen and Svejcar 1988). Differences in belowground response to forage harvest can be attributed to a wide range of experimental conditions in these studies such as herbivore grazing (Mapfumo et al. 2002), mechanical harvesting (Turner et al. 1993), long-standing prairie (Biondini et al. 1998), and species composition (Gao et al. 2008). A quantitative review conducted by Ferraro and Oesterheld (2002) posited that two main sources of variability in the effects of defoliation were the frequency and recovery time between defoliations and nutrient availability. The variability between production and defoliation methods, and the conflicting results that exist in the literature (Milchunas and Lauenroth 1993) necessitate an independent examination of the effects of defoliation on Kernza roots in the context of dual-use management.

Harvesting aboveground forage provides an additional revenue stream to a grower, but may negatively impact grain yields, root biomass or subsequent soil C and nitrogen (N) pools. Therefore, the objectives of the study were to i) determine the effect of forage harvest timing and frequency on Kernza plant biomass allocation and quality, and ii) determine the effect that aboveground biomass removal has on root dynamics and labile soil C and N pool dynamics important in nutrient cycling processes.

Materials and methods

Study site and experimental design

The experiment was carried out at the Ohio Agricultural Research and Development Center's Schaffter Farm 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). Prior to this study the field was in a corn (*Zea mays*), soybean (*Glycine max*), and wheat (*Triticum aestivum*) rotation. The mean annual precipitation is 883 mm and the mean annual temperature is 9.8 °C. Kernza was seeded on August 27, 2014 at a rate of 16.8 kg ha^{-1} using a no-till drill. Monoammonium phosphate (MAP, 52% P_2O_5) and muriate of potash (MOP, 60% K_2O) were broadcast

applied at 67 kg ha^{-1} each and urea (46% N) was applied to the field at 45 kg N ha^{-1} on April 24, 2015. Urea was hereafter applied annually as a split application at green up in the spring and after grain harvest (36 kg N ha^{-1} on April 24, 2015, August 19, 2015, March 30, 2016, August 15, 2016, and April 4, 2017).

A randomized complete block design with four replications was established with plots measuring 1.8 by 4.5 m. Three experimental treatments of differing forage harvest timing and frequency were assigned: i) No Cut control (0x), ii) Summer Cut (1x), iii) Summer and Fall Cut (2x). All three treatments were mechanically harvested once a year for grain using a plot combine. Since Kernza plant height can be variable, the combine head was set at a height to capture the vast majority of seed heads (approximately 50 cm). The combine cut and threshed the seed heads and deposited the chaff and stems back on the plot creating a light thatch on the top of the harvested stems. For the 0x treatment, this thatch biomass was left in place. Immediately after grain removal, forage biomass was harvested from 1x and 2x treatments, removing roughly 93% of the aboveground plant biomass. The forage was removed using a mechanical hay harvester that was adjusted to cut at 10 cm above the ground. Forage harvested from these treatments was removed from the field. At the fall harvest, forage was again mechanically removed from plots prescribed to the 2x treatment only, removing roughly 71% of aboveground biomass (Table 1).

Forage, root, and soil sampling

Above and belowground biomass and soil were sampled on a monthly basis during the growing season over a period of 3 years (Table 1). At each sampling event a single quadrat (0.25 m^2) was systematically placed in an ordered and consistent pattern in each plot to avoid legacy effects caused by previous forage harvest and core sampling. All forage biomass within the quadrat (living or dead) was cut to a height of 10 cm above the soil surface, dried at 50 °C for 72 h, and weighed. Seed head measurements were additionally collected at grain harvest each year. Seed heads within the quadrat were counted, clipped, oven dried to 0% moisture, weighed, and threshed.

Belowground biomass and soils were sampled collectively. Two 5-cm diameter soil cores were taken from areas absent of crowns and tillers within each quadrat to a depth of 20 cm. The two samples were composited and a 250 g subsample was taken and stored at 4 °C for root elutriation and analysis. Remaining samples were air-dried and ground to <2 mm for soil analyses.

Final sampling to 1 m depth

At the final sampling after summer harvest on August 16, 2017, root biomass and soil were sampled to four depths, 0–20, 20–40, 40–60, and 0–100 cm. Using an AMS 9110 Ag Probe hydraulic sampler (American Falls, ID), three 5-cm diameter cores were taken from previously undisturbed areas in each plot. The three samples from each depth were composited and mixed until homogenous. Four hundred g subsamples were taken for root elutriation and stored at 4 °C, and the remaining soil was air-dried and ground to <2 mm for soil analyses.

Root processing and quantification

Separation of roots from soil was carried out using a hydropneumatic root elutriator (Smucker et al. 1982). Subsamples (250 and 400 g for monthly sampling and final 1 m depth sampling, respectively) were run for 5 min onto a 1 mm sieve. Roots and any remaining residue were then removed from sieves manually using tweezers. Due to the difficulty in distinguishing between living and dead roots of Kernza, no attempt was made to separate accordingly. Roots were then oven-dried for

Table 1 Site management activities with corresponding dates

Activity	2015	2016	2017
Root and Soil Sampling	5/8, 3/9, 13/10, 12/11	25/4, 26/5, 28/6, 26/7, 30/8, 6/10, 12/11	4/4, 3/5, 6/6, 6/7, 7/8
Grain Harvest	12/8	2/8	9/8
Summer Forage Harvest	13/8	3/8	9/8
Fall Forage Harvest	13/10	6/10	–

Dates are formatted D/M

72 h at 40 °C and weighed. Dried forage and root biomass from summer grain harvest were ground and analyzed for C and N with a Costech ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Valencia CA). The summer harvest biomass was sampled for C and N because it was the only time that all three sample types (grain, forage, and roots) were collected and the only sample time point represented in all 3 years of the study.

Soil labile C and N pools

Permanganate-oxidizable carbon (POXC, active C; mg kg^{-1} soil) 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 BioTek Epoch spectrophotometer at 550 nm. POXC (mg kg^{-1} soil) was calculated as

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

Where 0.02 mol L^{-1} is the initial concentation 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.

Mineralizable C (24 h soil respiration after rewetting soil; mg C kg $soil^{-1}$) 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 then rewetted with deionized water to 50% water-filled pore space which was previously determined gravimetrically. The tubes were then tightly caped and kept in the dark at 25 °C for 24 h. CO_2 concentrations were determined with an LI-840A CO_2/H_20 infrared gas analyzer.

Soil protein (organically bound N; mg g $soil^{-1}$) was determined using a method from Hurisso et al. (2018). Three g of soil was measured into glass screw-top tubes with 24 ml of sodium citrate buffer (20 mM, pH 7.0). Tubes were capped and shaken at 180 rpm for 5 min. Samples were then autoclaved for 30 min at 121 °C and 15 psi. After cooling, samples were shaken for 3 min then 2 ml were transferred 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. The plate was read at 562 nm. The extractable protein content of the soil was calculated by multiplying the protein concentration of the extract by the volume of extractant used and dividing that product by the number of grams of soil used.

Soil inorganic N (sum of nitrate and ammonium; mg N kg $soil^{-1}$) was determined colorimetrically using the methods of Doane and Horwath (2003) and Sinsabaugh et al. (2000) for nitrate (NO_3^-) and ammonium (NH_4^+), respectively. Soil N was extracted with 2 mol L^{-1} KCl (40 ml per 5 g soil), shaken for 30 min then centrifuged (2000 RPM) for 3 min. Samples were read at 540 and 630 nM for nitrate and ammonium, respectively.

Data analysis

Data were tested for normality and homogeneity of variances and log or square root transformed to satisfy the assumptions of the analyses. Analysis of variance was performed on plant and soil data with the PROC MIXED procedure in SAS (version 9.4, SAS Institute, Cary, NC, USA). Management and sampling date were treated as fixed effects and block as a random effect. Significant differences were determined at $P = 0.1$, due to the high spatial variability often encountered in root measurements. Sampling date was modeled as repeated measures with compound symmetry assigned as the covariance structure. For the final sampling to 1 m, depth was modeled as repeated measures using the same criterion. Means were compared with an adjusted Tukey's pairwise comparison. Graphs were created using the ggplot2 (Wickham 2009) package in R.

Results

Weather

The precipitation in years 2016 and 2017 differed dramatically (Fig. 1) as the 2016 season experienced 164 mm less rainfall than 2017 during the period from May to August. During this same period from May to August, 2015 and 2017 received 17% and 11% greater rainfall than average, respectively, while 2016 received 40% less than average rainfall (www.oardc.ohio-state.edu/weather1). All 3 years from March 1 – November 31 were warmer than the 20-year average accumulating between 60 and 90 more growing degree days than average.The 2015 growing season had slightly below average temperatures during the summer compared to the 20-year average while the 2015 spring and fall seasons were warmer than average (data not shown). The 2016 year was warmer than average with the spring season temperatures being slightly below average and the summer and fall seasons being above average (data not shown); overall 2016 accumulated that greatest amount of GDDs out of all three study years. The 2017 year had a slightly warmer than average spring followed by average temperatures during the summer and fall (data not shown).

Forage harvest effects on plant biomass allocation

Overall, forage harvest management had a significant effect on forage biomass ($P = 0.047$, Table 2). Specifically, at the summer harvest in 2016 and 2017, when forage biomass was at its peak, the 2x cut yielded 28% and 22% greater forage, and the 1x treatment yielded 53% and 45% greater forage biomass than the 0x treatment, respectively (Fig. 2). When averaged across treatments Kernza grain yielded 642 kg ha^{-1} during the first year of production (2015), 362 kg ha^{-1} in the second year of production (2016), and 380 kg ha^{-1} in the third year of production (2017). Though overall Kernza grain yields decreased from year one to two and then remained constant from year two to three, treatments that included forage harvest components experienced significantly greater grain yields compared to the treatment where forage was not removed, and while the strength of these effects varied between years (Table 2) the trends remained the same (Fig. 2). In 2016 average grain yields of the 0x treatment were 50% and 30% less than the 1x and 2x treatments, respectively. In 2017, average grain yields of the 0x treatment were around 25% less than average grain yields of the 1x and 2x treatment.

Overall, Kernza root biomass in the surface 20 cm of soil was significantly affected by removal of aboveground biomass ($P = 0.037$, Table 2). On average, no differences were found in 2015, but root biomass in 2016 was significantly different ($P = 0.009$, Table 2). Averaged root biomass differences in 2017 were close but ultimately statistically non-significant ($P = 0.108$, Table 2). Though not every date had significant differences between treatments (Fig. 3), trends persisted across 2016 and 2017 with both the 1x and 2x treatments producing greater root biomass than the 0x treatment on each of the 12 separate sampling dates (Fig. 3, Supplementary Table 1). On average the 1x and 2x treatments had 39% and 73% greater root biomass in 2016 and 39% and 49% greater root biomass in 2017 (Supplementary Table 1).

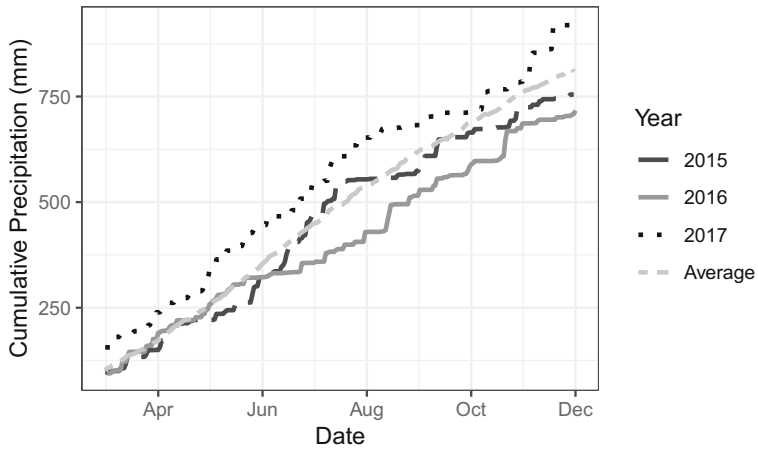

Fig. 1 Cumulative precipitation for 2015 (black dashed line), 2016 (grey solid line), and 2017 (black dotted line) growing seasons (Mar 1 – Nov 31) and the 20-yr average (light grey dashed line) at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH

Table 2 Plant and soil F-statistics and significance from repeated measures mixed-design ANOVA for all years combined and individual years

Source	Grain	Forage Biomass	Root Biomass	Soil Moisture	POXC∞	Mineralizable - C	Protein	Inorganic N◊
2015–2017								
Harvest (H)	8.63***	5.1**	5.8**	0.02	2.7	4.3*	1.8	8.7***
Date (D)	51.42***	62.3***	8.1***	432.2***	2.7***	2.3***	2.4***	25.5***
H x D	0.09	3.4***	0.9	1.1	0.9	1.1	0.5	0.7
2015								
Harvest (H)	7.7***	8.4**	1.5	1.7	0.8	5.3**	1.1	4.1*
Date (D)	–	68.1***	1.9	256.0***	2.5*	9***	3.5**	48.3***
H x D	–	3.0**	1	1.8	0.6	1	0.7	1.5
2016								
Management (M) Harvest (H)	3.7*	1.6	8.3***	0.4	1.6	4.6*	3.6*	1.3
Date (D)	–	58.4***	6.9***	299.6***	2.5**	1.5	1.5	14.4***
H x D	–	4.6***	0.4	1.2	0.7	0.7	0.4	0.4
2017								
Harvest (H)	0.76	4.8*	3.3	1.2	2.2	0.5	1.9	14.4***
Date (D)	–	77.5***	7.7***	977.1***	1.9	1.5	1	16.3***
H x D	–	2.2**	0.6	0.3	1.4	1.5	0.5	0.7

∞ *POXC* permanganate oxidizable carbon

◊Inorganic *N* nitrate + ammonium

*Significance level: $P < 0.1$

**Significance level: $P < 0.05$

***Significance level: $P < 0.01$

Root and soil variables sampled from 0 to 20 cm depth, for each variable in table $n = 4$

Seasonal dynamics of root biomass varied greatly between treatments (Fig. 3). In 2016 and 2017, forage harvested treatments had greater rates of root biomass production in the spring than the 0x treatment. In addition, root biomass declines were greatest in forage harvested treatments relative to 0x control in the summer months (June to July and July to August for 2016 and 2017, respectively). Specifically, root biomass declined by 17% in 0x, 39% in 1x and 31% in 2x between peak biomass and grain harvest in 2016. Likewise, root biomass declined by 7% in 0x, 36% in 1x and 42% in 2x from the peak biomass to grain harvest in 2017. The greater temporal variability in root biomass of forage harvested plants reflected net changes in root turnover, since no attempt was made to separate live vs. dead roots.

The proportion of whole plant biomass allocated aboveground at harvest decreased across all treatments (0x = −11%, 1x = −12%, 2x = −27%; Fig. 2) between 2015 and 2016, resulting in an increase in the proportion of whole plant biomass allocated belowground within the surface 20 cm. These trends were reversed from 2016 to 2017, with the proportion of biomass allocated aboveground increasing (0x = 7%, 1x = 7%, 2x = 15%; Fig. 2) and the proportion allocated belowground decreasing. Though the proportion of biomass allocated to grain differed between years (2015 = 9%, 2016 = 5%, 2017 = 3%; Fig. 2) it did not differ between treatments.

Forage harvest effects on plant biomass quality

The C:N ratio for forage biomass at summer harvest was significantly affected by forage harvest treatment in 2016 ($F = 19.49$, $P = 0.002$, Table 3) and 2017 ($F = 10.95$, $P = 0.004$). In 2016, C:N ratios of the 1x and 2x treatments were both significantly greater than the 0x treatment ($P = 0.008$ and 0.003, respectively); however

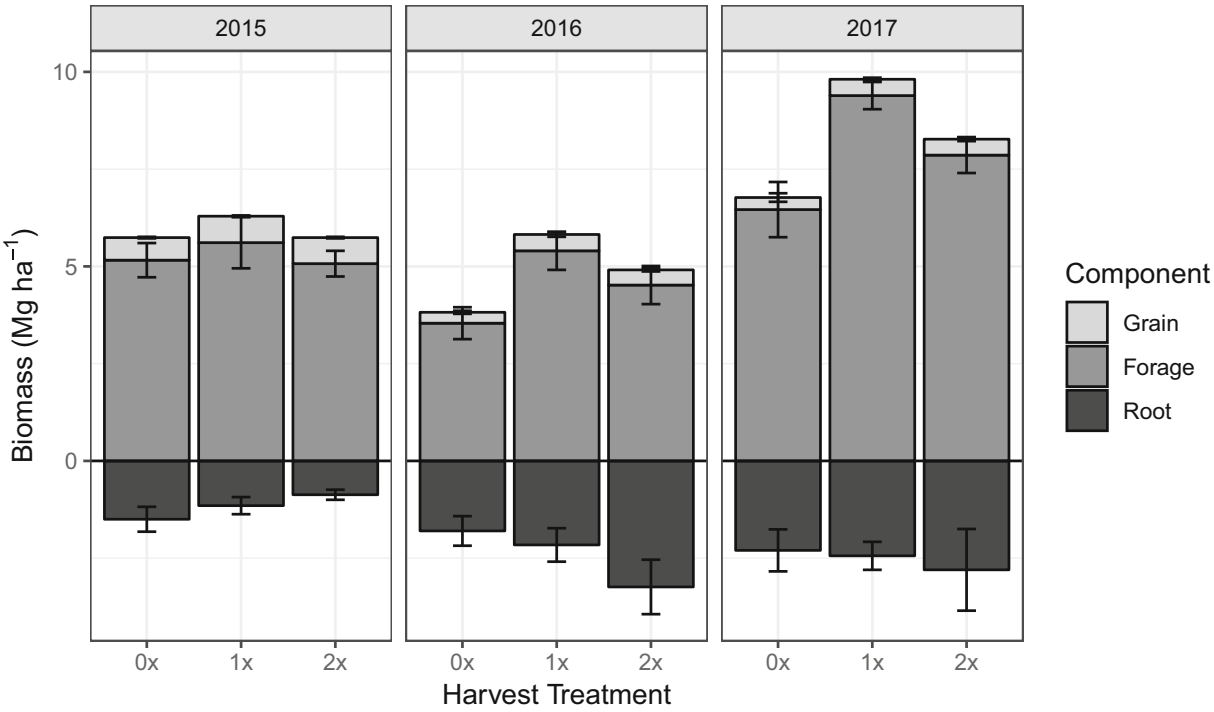

Fig. 2 Plant biomass allocation component means at summer grain harvest for three forage harvest treatments for all years. Error bars represent one standard error of the mean ($n = 4$)

in 2017 the 0x and 1x were significantly greater than the 2x treatment ($P = 0.004$ and 0.028, respectively). Forage harvest did not affect root C:N ratios at summer harvest in any year and ranged from 32.5–41.2 (Table 3).

Forage harvest effects on soil labile C and N pools

Across all years POXC was not significantly influenced by forage harvest (Tables 2, 4) and

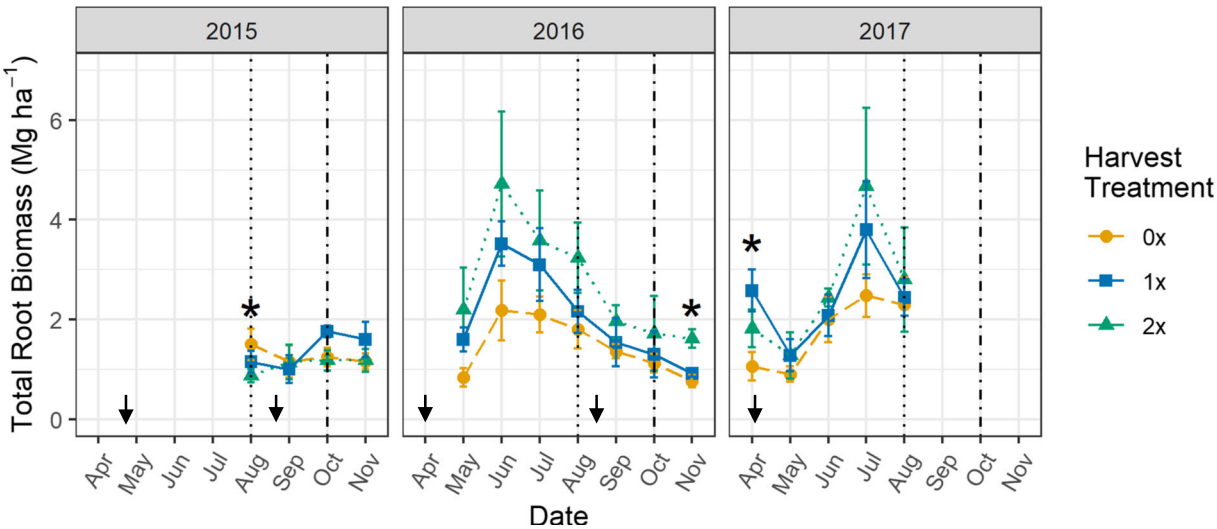

Fig. 3 Mean root biomass for 0x (No Cut, gold circle dashed line), 1x (Summer Cut, blue square solid line), and 2x (Summer and Fall Cut, green triangle dotted line) forage harvest treatments at each sampling date over 3 years at 0–20 cm. Error bars represent one standard error of the mean ($n = 4$). Vertical dotted lines represent the summer grain and forage harvest. Dashed vertical lines represents the fall forage harvest. The arrows represent nitrogen fertilization events. Asterisks indicate significant differences between treatments ($P < 0.1$)

Table 3 Mean C:N ratios (standard errors) of forage and roots at summer harvest for all years

Year	Forage			Root		
	0x	1x	2x	0x	1x	2x
	C:N					
2015	43.0 (4.4)	51.4 (2.5)	46.9 (3.2)	39.1 (3.6)	35.2 (2.2)	33.5 (2.2)
2016	55.9 (1.8)^{b}	67.4 (2.4)^{a}	70.4 (1.8)^{a}	32.5 (1.2)	34.2 (3.1)	33.3 (1.8)
2017	73.6 (1.7)^{a}	70.0 (2.2)^{a}	61.5 (1.7)^{b}	37.4 (0.4)	41.2 (1.4)	37.8 (2.0)

Different letters denote significant differences between forage harvest treatments in each year ($P < 0.1$). $n = 4$

averaged 438 mg C kg soil^{-1} with a range between 317 and 541 that resulted in no significant inter-year variability. Although not statistically different, for a majority of dates within the 2016 season, 0x POXC values trended greater than both the 1x and 2x treatments (Supplementary Table 2); this trend was not apparent in 2017.

Overall, mineralizable C was significantly affected by forage harvest and trended greater in the 1x and 2x treatments than in the 0x control treatment (Tables 2, 4). In 2015, from grain harvest to the end of the season, overall averages of the 1x treatment were significantly greater than the 0x treatment ($P = 0.051$; Table 4). In 2016, averages of the 2x treatment were significantly greater than the 0x treatment ($P = 0.053$; Table 4). Though there were significant differences between treatments within years, there was no noticeable inter-year variability in mineralizable C values. Soil protein content was significantly greater under the 0x treatment than the forage harvested treatments during the 2016 season ($P = 0.095$; Table 2) but was comparable across treatments in 2015 and 2017 (Table 2). Under all treatments soil protein annual averages decreased on average by 7% from 2015 to 2017 (Table 4).

Across all analyses inorganic N was significantly different between treatments with the exception of the 2016 season (Table 2). The 0x treatment had greater levels of inorganic N than the forage harvested treatments ($P = 0.007$) and on average was 23% and 21% greater than the 1x and 2x in 2015 and 24% and 38% greater in 2017, respectively (Table 4). Inorganic N annual averages decreased under all treatments from 2015 to 2017 (0x = −41%, 1x = −42%, 2x = −49%).

Plant and soil properties at final soil sampling to one meter

When sampled in August 2017, no significant differences in root biomass were found between treatments at any of the four depth increments to 1 m (Fig. 4). Root biomass over all 4 depths did not meet the assumption of homogeneity of variance and therefore could not be analyzed for differences, however when total summed root biomass was analyzed, no significant differences were found

Table 4 Soil property annual means (standard errors) for three forage harvest treatments at 0–20 cm depth

Year	POXC*			Mineralizable C			Protein			Inorganic N**		
	0x	1x	2x	0x	1x	2x	0x	1x	2x	0x	1x	2x
	mg C kg soil^{-1}			mg C kg soil^{-1}			g kg soil^{-1}			mg N kg soil^{-1}		
2015	453 (23)	423 (34)	438 (21)	36 (3)^{b}	39 (3)^{a}	36 (3)^{ab}	4.5 (0.2)	4.4 (0.2)	4.4 (0.2)	8.7 (1.1)^{a}	7.1 (1.0)^{ab}	7.2 (0.7)^{b}
2016	459 (20)	443 (24)	454 (28)	35 (4)^{b}	37 (4)^{ab}	38 (5)^{a}	4.4 (0.2)	4.3 (0.2)	4.3 (0.2)	6.5 (0.9)	6.7 (1.4)	5.6 (0.7)
2017	438 (34)	440 (42)	393 (36)	36 (4)	38 (7)	35 (5)	4.1 (0.2)	4.3 (0.2)	4.0 (0.3)	5.1 (0.5)^{a}	4.1 (0.6)^{b}	3.7 (0.3)^{b}

Different letters denote significant differences between forage harvest treatments in each year ($P < 0.1$). $n = 4$

*POXC permanganate oxidizable carbon

**Inorganic N nitrate + ammonium

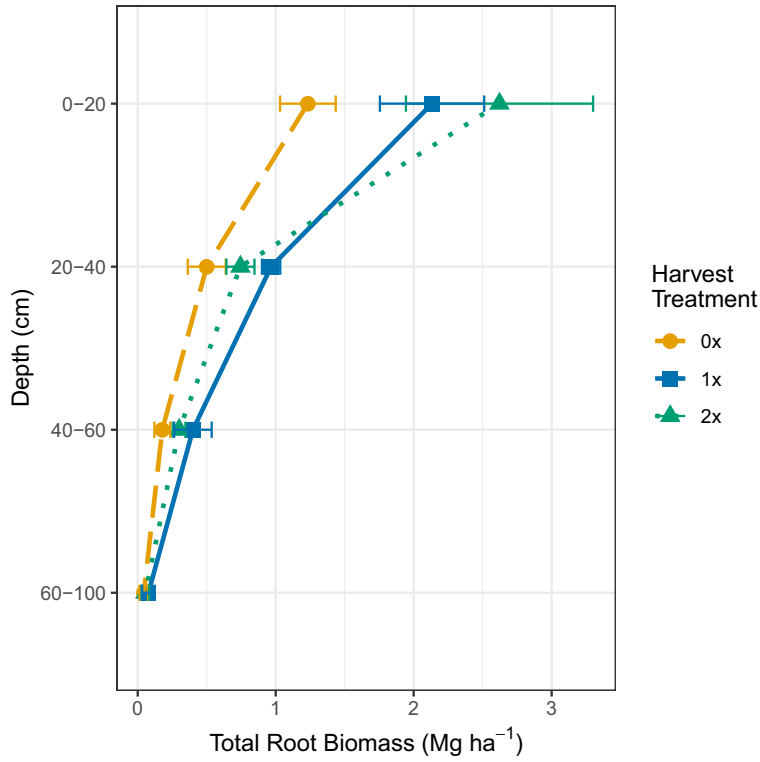

Fig. 4 Mean root biomass for 0x (No Cut, gold circle dashed line), 1x (Summer Cut, blue square solid line), and 2x (Summer and Fall Cut, green triangle dotted line) forage harvest treatments over four soil depth increments to 1 m when sampled in August 2017. Error bars represent one standard error of the mean ($n = 4$)

($F = 2.76$, $P = 0.166$). Though these findings are not statistically significant the trends between treatments are consistent with those observed across the 12 previous dates and persisted down through 60 cm depth, with the exception of the reversal between 1x and 2x treatments at 0–20 cm (Fig. 4). To one-meter depth the 0x, 1x and 2x treatments produced 2.65, 4.84, and 5.04 Mg ha^{-1} root biomass, respectively. Within the top 20 cm of soil, which contained 65% of the total root biomass, the 1x and 2x treatment produced 73% and 112% more root biomass than the 0x treatment, respectively. The 20–40, 40–60, and 60–100 cm depths each contained 24%, 9%, and 2% of the total root biomass down to 100 cm.

Permanganate-oxidizable carbon (POXC) values remained comparable across treatments down to one meter depth (data not shown), while mineralizable C was greater in both forage harvested treatments than the 0x treatment in the first 20 cm ($F = 6.36$, $P = 0.033$). Overall, soil protein was significantly greater under the 1x treatment ($F = 4.7$, $P = 0.059$) than both the 2x and 0x treatments. Inorganic N did not differ between treatments throughout the one-meter profile ($F = 0.25$, $P = 0.785$).

Discussion

Forage harvest effects on plant biomass allocation and root dynamics

A primary motivation for the development of Kernza as a perennial grain, is its ability to deliver ecosystems services within agroecosystems (Glover et al. 2010). The effectiveness of any given crop in delivering ecosystem services will be influenced by management, and therefore an assessment of forage harvest management impacts on plant biomass allocation and root dynamics is critical.

Overall, we found that harvesting Kernza forage promoted greater grain yield, seasonal forage and root biomass in years two and three of the study (Fig. 2). Kernza root response to the harvesting of forage biomass does not appear to be instantaneous; instead, the overall productivity of belowground biomass is influenced in the subsequent seasons (Fig. 3). A similar response in root biomass was observed by Lopez-Marisco et al. (2015) who reported a significant increase in root biomass of grazed stands relative to non-grazed

stands. Our findings of forage harvest effects on root biomass also align with those of previous studies which reported increased belowground biomass with removal of aboveground biomass (Milchunas and Lauenroth 1993; Pucheta et al. 2004; Lopez-Marisco et al. 2015). However, reductions in root biomass after forage removal have also been reported (Christiansen and Svejcar 1988; Biondini et al. 1998; Gao et al. 2008).

Interestingly, the increase in root biomass under forage harvested stands was not accompanied by a decrease, but rather an increase in the subsequent year's forage biomass production relative to the control (Fig. 2, Supp. Table 1). The increase in aboveground biomass production could be the result of a reduction in intraspecific competition due to the disturbance caused by defoliation or the increase in light penetration due to litter removal (Knapp and Seastedt 1986). The addition of N fertilization in our study may also have influenced biomass allocation patterns as it would have aided the plant in overcoming a resource limitation (N acquisition belowground) and shifted the allocation of resources aboveground enabling the plant to produce greater aboveground biomass (Bloom et al. 1985; Hunt and Nicholls 1986; Dietzel et al. 2015) while maintaining a greater root system.

Measuring and analyzing root biomass over the course of three growing seasons yielded insight into the effect that forage harvest management had on root production and turnover. After the crop establishment year in 2015, forage harvested treatments consistently had greater root biomass compared to the 0x control. This trend, though not statistically significant for each individual date, was especially noteworthy in 2016, where the 2x treatment had the greatest amount of root biomass at all seven samplings. Most striking was the sharp difference in root production and subsequent decline in root biomass in the forage harvested treatments relative to 0x. While no attempt was made to separate live and dead roots, the sharp decline in net root biomass can only be a function of accelerated root turnover (i.e. morality and decomposition). As with the possibility of differences between live and dead roots, changes in root morphology between years 1 and 3 are also possible; however, the decline in net root biomass from summer to autumm still represents a decrease in overall roots, regardless of size. Similar results were reported by Frank et al. (2002) in which grazing increased rates of perennial grassland root mortality and turnover, leading the authors to conclude that forage harvest was a major determinant of productivity and decomposition.

Forage harvest effects on root quality and turnover

Despite having a dramatic effect on overall root biomass quantity, harvest of aboveground forage did not affect the quality of root biomass within the first 3 years of production. Average root C:N was 36 at summer harvest (Table 3), significantly lower than the values reported by Sprunger et al. (2018a) for Kernza in the fourth year of production which ranged from about 50 to 75 for coarse roots in the surface layer. The C:N ratio is reportedly one of the primary factors in determining the rate of decomposition and turnover in roots (Silver and Miya 2001). The C:N of roots in our study falls within the intermediate range (25–75) according to classification by Heal et al. (1997) and biomass within this range can experience quick decomposition. Forage harvest influenced aboveground forage quality, however, trends among the treatments were inconsistent across the 3 years.

Differences in decomposition rates from forage harvest management may be due to an increase in root exudates as the result of plant translocation of carbohydrates from shoots to roots following aboveground biomass removal (Doll 1991; Dyer et al. 1991; Holland et al. 1996). A number of studies have reported aboveground biomass removal increasing root exudation and turnover (Tracy and Frank 1998; Paterson and Sim 1999). Hamiton and Frank (2001) provided evidence for a positive feedback mechanism between defoliation and nutrient acquisition, in which clipped plants promoted microbial activity by releasing C exudates, which resulted in greater decomposition of labile tissue and SOC. These findings were later corroborated by Hamilton et al. (2008) and Graaff et al. (2010) who reported that the quantity of exudate can be a strong mediator of the rate of decomposition.

Our results showed no evidence of differences between treatment root C:N ratios, therefore the harvesting of aboveground Kernza biomass must have an effect on another factor driving the greater rates of root-die off and decomposition amongst the harvested treatments. We reason that root exudation is the likely driver based on findings from previous literature. Therefore, harvesting the aboveground biomass of Kernza could not only be initiating a greater amount of exudation and root die-off but also greater rates of decomposition. These differences in root decomposition could have important implications for C-cycling and accumulation within the soil (Weaver et al. 1935; Gill et al. 1999) as well as overall aboveground productivity (Klumpp et al. 2009).

Forage harvest effects on soil labile C and N pools

That forage harvest management of Kernza positively influenced mineralizable C is likely a function of root quantity rather than root quality, given that forage management had no impact on root C:N ratios. Greater mineralizable C within the forage harvested treatments could be a result of increased root die-off, exudation, and decomposition posited from the steeper declines in root biomass as previously discussed. The additions of plant residue and substrates to the soil likely increased the size of the microbial community (Stanton 1988) and therefore mineralization (Franzluebbers et al. 2000; Haney et al. 2001). These dynamics between increased root mortality and mineralization under forage harvested stands provide evidence of a synchronized relationship between the perennial plant and nutrient cycling, a theory proposed by Crews et al. (2016). Therefore, we suggest that removal of aboveground biomass triggers a physiological response within Kernza that initiates root die-off and exudation to increase nutrient availability via mineralization to facilitate the reestablishment of aboveground growth. Our data support this line of reasoning, as mineralizable C was more strongly related to root biomass at the 2x treatment, than the 1x or 0x treatments (correlation coefficients: 0x = −0.19, 1x = 0.13, 2x = 0.31). Correlations were also run between mineralizable C and inorganic N (0x = −0.242, 1x = −0.135, 2x = −0.0403). Although the relationships were negative and weak, the temporal nature of these dynamics and the cumulative nature of N mineralization/immobilization dynamics likely makes these relationships difficult to detect. Similar results and processes were reported by Klumpp et al. (2009) in that grazing stimulated faster decomposition through a root mediated process of turnover and exudation, which subsequently resulted in greater aboveground productivity.

In contrast to mineralizable C, forage harvest had no influence on POXC even though both POXC and mineralizable C reflect labile C pools. Differences in forage harvest effects on these labile C pool indicators may be atrributed to the differences in the processes they reflect: mineralizable C reflects nutrient mineralization processes, while POXC often reflects more processed pools of C and is a predictor of C stabilization (Hurisso et al. 2016). Thus, despite having greater root biomass and greater C mineralization within the forage harvested treatments, increases in more processed soil C pools were not found. This lack of change in POXC between the forage harvested and non-forage harvested treatments could be a function of time, as changes in more stable C pools take several years to detect (Post and Kwon 2000). Nevertheless, increased root biomass under forage harvested stands has important implications for C sequestration within Kernza systems, since roots have a large capacity for C storage.

The steady decline of inorganic N in all three treatments overtime reflects the annual carry-over of N in belowground biomass used towards plant re-growth in perennials (Dawson et al. 2008). Thus, the larger reductions of inorganic N in the forage harvested treatments relative to 0x is likely the result of greater assimilation and remobilization of N due to greater above and belowground biomass. This is an indication that harvesting forage biomass within perennial Kernza systems could also lead to enhanced N uptake and greater N use efficiency (Pineiro et al. 2010).

Conclusions

This study evaluated the effects of forage harvest management on plant biomass allocation and labile C and N indicators related to nutrient cycling. It is the first report of forage harvest management effects on belowground processes in an emerging perennial grain crop, Kernza. Overall, our findings suggest that multiple harvests of Kernza forage stimulates forage, grain and root production. The increased biomass production was likely a result of increased nutrient cycling and availability, as forage harvest had no effect on root C:N ratios, but increased mineralizable C. This study suggests that the dual-use management of Kernza can lead to greater overall productivity and is likely a more profitable system than management of Kernza for grain only. As a summer and fall harvested system provides an additional opportunity for forage revenue (while still producing similar, if not greater, amounts of root biomass) compared to the summer-only harvested system, it may be advantageous for producers to implement a twice-harvested Kerna system, both in terms of profitability and environmental benefits.

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