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How does nitrogen and forage harvest affect belowground biomass and nonstructural carbohydrates in dual-use Kernza intermediate wheatgrass?

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Abstract

Intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] is a cool-season perennial forage grass, whose grain is commercialized in the United States as "Kernza." Its extensive root system may help in reducing soil erosion, water pollution, and C emissions. Nitrogen fertilization and forage harvest intensity may affect the belowground biomass and nonstructural carbohydrate (NSC) concentrations, which may affect growth in subsequent years. We compared N doses and forage harvest treatments in a replicated complete block experiment in three environments (location-years: Arlington, WI, 2016 and 2017; and St. Paul, MN, 2016). Seeds were sown in the fall, Kernza grain was harvested in the following summer, and rhizomes and roots were sampled in fall to a depth of 0.1 m over 2 yr. The water-soluble carbohydrates (WSC) accounted for 97–99% of NSC, across environments. The WSC concentration was higher in rhizomes than in roots in both years, but WSC mass was higher in roots than rhizomes due to greater root biomass. Nitrogen generally did not change NSC concentrations across years, but reduced WSC in rhizome the second year in WI. Forage harvest did not affect NSC concentrations across locations and years. Belowground biomass to 1-m depth in the fall of the second year in Wisconsin averaged 478.3 g m^{-2} regardless of treatment. Summer forage yield in the following year was positively associated with root biomass in the fall. These results suggest that harvesting forage in a Kernza dual-use system is not detrimental to intermediate wheatgrass above- and belowground productivity.

1 | INTRODUCTION

Perennial crops can improve agricultural sustainability compared with annuals due to their extensive root systems and continuous soil cover, which reduce soil erosion,

Abbreviations: NSC, nonstructural carbohydrate(s); WSC, water soluble carbohydrate(s)

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nutrient runoff, and fix C while simultaneously increasing farmer income by decreasing annual inputs and costs (Culman, Snapp, Ollenburger, Basso, & DeHaan, 2013; Jungers, DeHaan, Betts, Sheaffer, & Wyse, 2017). Intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey], is a widely adapted, high-yielding, high-quality cool-season perennial grain and forage dual-use grass (DeHaan, Wang, Larson, Cattani, & Zhang, 2013), whose grain is commercialized in the United States as "Kernza" with an interest from farmers for its dual use (Lanker, Bell, & Picasso, 2019). Despite the interest, lower grain yield of Kernza compared with the annual cereals limits widespread adoption (DeHaan et al., 2013). One way to overcome such an important economic limitation is maximization of the economic value via increasing forage production and grain yield simultaneously (Pugliese, Culman, & Sprunger, 2019). Improving early spring regrowth and regrowth after each harvest of Kernza is a relevant goal to extend its grazing or forage utilization period. Agronomic management of intermediate wheatgrass for forage has been investigated recently (Karn, Berdahl, & Frank, 2006; Lee, Donaghy, Sathish, & Roche, 2009; Liebig, Hendrickson, Berdahl, & Karn, 2008; Ogle, John, Tober, & Jensen, 2011). Furthermore, agronomic management practices of intermediate wheatgrass for optimizing both forage and grain yield in dual-use systems is an increasing area of research (Favre, Munoz Castiblanco, Combs, Wattiaux, & Picasso, 2019; Hunter, Sheaffer, Culman, & Jungers, 2020; Hunter, Sheaffer, Culman, Lazarus, & Jungers, 2020; Pugliese et al., 2019; Zimbric, Stoltenberg, & Picasso, 2020). Understanding the mechanisms underlying biomass production, carbohydrate storage and utilization, and allocation to vegetative regrowth and reproduction over time is needed to develop management recommendations for dual-use Kernza systems. In particular, it is critical to understand the role of belowground organs such as rhizomes and roots which play central roles in carbohydrate storage and regrowth and therefore impact the provision of ecosystem services like soil erosion protection and agronomic production of forage and grain year after year.

Rhizomes are horizontal underground plant stems capable of producing the shoot and root systems of a new plant (Jernstedt & Bouton, 1985; Li & Beuselinck, 1996). Rhizomes and roots are used to store carbohydrates and proteins, which can be translocated and used to form new shoots during regrowth after dormancy. Nonstructural carbohydrates (NSC) stored in rhizome and roots are important for cold tolerance, winter survival, and regeneration and spring regrowth in perennial forage grasses (Bai, Xun, Li, Zhang, & Li, 2010; Carvalho, Asega, & Figueiredo-Ribeiro, 2007; Harradine, 1980; Washburn et al., 2013; White, 1973). Two types of NSC have been identified as storage carbohydrates in grasses: starch (polymer of glucose, water insoluble) is the primary storage carbohydrate in warm-season grasses, whereas water-soluble carbohydrates (WSC) are the primary storage carbohydrate in cool-season grasses (Chatterton, Harrison, Bennett, & Asay, 1989; Pollock & Cairns, 1991; Smith, 1972). Intermediate wheatgrass WSC include fructans, fructose, glucose, and sucrose (Ogden & Loomis, 1972; Zhao, Mackown, Starks, & Kindiger, 2008).

Core Ideas

- Water-soluble carbohydrates were 97 to 99 % of non-structural carbohydrates in Kernza.
- Both rhizomes and coarse roots play an important role in carbohydrate storage.
- Forage harvest and N did not affect NSC concentrations.
- Harvesting forage in a Kernza dual-use system does not reduce belowground biomass.

The effects of forage harvest regimes and N fertilizer application on the belowground biomass and concentration and mass of NSC in various plant parts were investigated as such a role had direct implication in regrowth in other species. The results revealed that intensive defoliation or grazing reduced the root biomass particularly under dry conditions (Pearson, 1965; Smoliak, Dormaar, & Johnson, 1972) and use of N fertilizers increased the root biomass (Lorenz & Rogler, 1967). On the other hand, results suggesting no effect of defoliation on root biomass (Bartos & Sims, 1974) or contrasting differences among species were also reported (Caldwell, Richards, Johnson, Nowak, & Dzurec, 1981). When the effect of mass of NSC reserves in rhizomes were investigated in big bluestem (*Antropogon gerardi* Vitman) and indiangrass [*Sorghastrum nutans* (L.) Nash], it was found that the reserves were depleted during the regrowth in spring and restored from late summer to winter (McKendrick, Owensby, & Hyde, 1975). A negative correlation between total root biomass and NSC was reported in Caucasian bluestem [*Bothriochloa caucasica* (Trin.) C.E. Hubbard] under grazing (Christiansen & Svejcar, 1988). Harvest frequency and availability of N interact to impact forage plant growth (Ferraro & Oesterheld, 2002). Hence, analyses of the effects of forage harvest regimes and N fertilizer level on the NSC in rhizomes and roots of Kernza intermediate wheatgrass are relevant to optimize agronomic management of this new crop in dual-use systems.

Previous research on intermediate wheatgrass showed that N fertilization did not affect root biomass until the fourth production year (Sprunger, Culman, Robertson, & Snapp, 2018). In a study of forage harvest management

effects on root biomass of Kernza, multiple forage harvests (summer and fall) stimulated forage, grain, and root production, implying that the dual-use management of Kernza can lead to greater overall productivity (Pugliese et al., 2019). However, these studies did not measure NSC reserves that may affect crop regrowth ability and can be drastically altered due to the plant development stage, temperature, water stress, and N fertilization change (Moraes, Chatterton, Harrison, Filgueiras, & Figueiredo-Ribeiro, 2013; Pollock & Jones, 1979; Rosa et al., 2009). Therefore, the objective of this research was to determine the effect of forage harvest and N fertilization on NSC and biomass in rhizome and root in Kernza intermediate wheatgrass harvested for grain and forage.

2 | MATERIALS AND METHODS

2.1 | Plant materials and experimental design

Kernza intermediate wheatgrass seeds from the fourth cycle of selection for increased grain yield by the Land Institute (Kansas, USA) were used for this study. The field experiment was established at two locations with similar soil types (Mollisols and Udolls) representative of the upper midwestern United States: the University of Wisconsin–Madison Arlington Agricultural Research Farm (Plano silt loam [fine-silty, mixed, superactive, mesic Typic Argiudolls]), near Arlington, WI (43°18′ N, 89°20′ W) and the Agronomy and Plant Genetics Department Research Farm (Waukegan silt loam [fine-silty over sandy, mixed, superactive, mesic Typic Hapludolls]), St. Paul, MN (44°59′ N, 93°09′ W). In Wisconsin, plots were 1.8 by 6.1 m separated by 1-m alleys arranged in a randomized complete blocks design with three replications. Planting was on 15 Sept. 2015 at a density of 18 kg pure live seed ha^{-1} at 0.012-m depth and 0.19-m row spacing. The treatment design was a factorial arrangement of two factors: forage harvest schedule and N fertilization. Forage harvest schedule had two levels: control (only grain harvest in summer, no forage harvest) vs. twice a year in summer (after grain harvest, 3 Aug. 2016 and 23 Aug. 2017) and fall (7 Oct. 2016 and 25 Oct. 2017) forage harvests. Nitrogen fertilization had two levels: 90 vs. 134 kg ha^{-1} split applied, one half at spring green-up (6 May 2016 and 26 Apr. 2017) and the second half in late summer (18 Aug. 2016 and 25 Aug. 2017). Soil sampling to 15-cm depth and analysis were conducted in the spring of 2016 following the methods described by Peters and Laboski (2013). Soil analyses results for the site were pH = 7.3, organic matter = 3.0%, P = 36 mg kg^{-1}, K = 109 mg kg^{-1}, Ca = 1824 mg kg^{-1}, Mg = 538 mg kg^{-1}, cation exchange capacity = 15 cmol kg^{-1}, B = 0.9 mg kg^{-1}, and bulk density = 1.08 g cm^{-3}. In Minnesota, plots were 3 by 4.5 m, separated by 1.5-m alleys and arranged in a randomized complete block design with four replications. Planting was on 5 Sept. 2014 at a density of 12 kg pure live seed ha^{-1} at 0.013-m depth and 0.3-m row spacing. Treatments consisted of the two forage harvest schedules: control vs. twice a year in summer (5 Aug. 2015 and 3 Aug. 2016) and fall (20 Oct. 2015 and 5 Oct. 2016). Nitrogen fertilization was not manipulated as a treatment in Minnesota plots, and it was 56 kg N ha^{-1} applied as urea in April in all plots. In March of each year, Dual Magnum (active ingredient: *S*-metolachlor, 83.7%, 913 g L^{-1}) preemergence herbicide was applied at 1.2 L ha^{-1}. Belowground samples were taken during 2016 and 2017 in Wisconsin, which were the first and second production year of Kernza, whereas in Minnesota they were taken only in 2016, which represents the second production year of Kernza (the Minnesota experiment was planted 1 yr earlier than the Wisconsin experiment). This study therefore comprised three environments, defined as combinations of location-years: WI-2016 (first production year of Kernza), WI-2017 (second production year), and MN-2016 (second production year). In season precipitation (accumulated from March to November each year) was 980 mm for MN-2016, 924 mm for WI-2016, and 834 mm for WI-2017. Average season temperatures were 13.8 °C for N-2016 and WI-2016, and 13.2 °C for WI-2017. Detailed weather information for each environment is presented in Supplemental Table S1.

In each year following the belowground biomass sampling, spring forage aboveground biomass samples were taken with 0.5-m × 0.5-m quadrats from each plot with a uniform height of ∼5 cm, dried at 60 °C for ∼5 d, and weighed. Spring forage sampling dates were 18 Apr. 2017 and 10 May 2018 in Wisconsin, and 5 May 2017 in Minnesota. Each summer at grain harvest (dates listed above), aboveground Kernza biomass was sampled with one 0.5-m × 0.5-m quadrat per plot. Kernza spikes were separated from the remaining biomass, dried, and then threshed to obtain grain yield. The remaining biomass was also dried and weighed and reported as summer forage biomass.

2.2 | Belowground biomass sampling

To determine the suitable depth for rhizome and root sampling in October 2016 (first production year of Kernza) in Wisconsin, we excavated two 0.16-m^2 quadrats by 0.3-m depth and partitioned all Kernza belowground biomass in 0.1-m-depth increments. Aboveground biomass was 304 g m^{-2}, and total belowground biomass (0–0.3 m) was 186 g m^{-2}, of which 74% was roots and 26% was rhizomes. The top 0.1-m soil layer contained 95% of rhizome biomass and 79% of root biomass, the 0.1- to 0.2-m layer

FIGURE 1 Picture of roots and rhizomes sampled

contained 4% of rhizome and 14% of root biomass, and the 0.2- to 0.3-m layer contained 0% of rhizome and 7% of root biomass. Therefore, we decided to confine rhizome and root biomass sampling to the top 0.1 m of soil, as this accounted for most of the biomass with the minimum sampling effort.

Belowground biomass sampling occurred on 17 Nov. 2016 and 8 Nov. 2017 in Wisconsin (first and second production years), and on 28 Oct. 2016 in Minnesota (second production year only). The grass was cut to a stubble height of 0.05 m. A flat shovel with a 0.1-m marker was then pushed into the soil directly to get a soil volume 0.4 × 0.4 × 0.1 m (depth) for each sample. Subsequently, all the rhizomes and roots for each sample were collected and put in a 0.3-m × 0.5-m nylon mesh bag. The samples in the mesh bag were washed using a Rootwasher hydropneumatic elutriation system (Gillisons's Variety Fabrication) using a 2-mm sieve, retaining coarse roots (Figure 1). When the samples were clean, roots and rhizomes were separated and oven dried (60 °C) until constant weight (~5 d) was achieved. For Minnesota samples, only rhizomes were measured, dried, and processed. The dried specimens were weighed and ground to a 1-mm particle size using first a coarse grinder and then a UDY cyclone mill (Udy Corporation) for later NSC analyses.

In order to better estimate the belowground biomass in depth, on 16 Oct. 2017, we sampled each plot in Wisconsin to a depth of 1 m using a tractor-mounted Giddings hydraulic soil probe. One 72-mm-diam. core was taken in the middle row between two Kernza plants in the center of the plot. Each core was partitioned into subsections in the field (0–0.10, 0.10–0.25, 0.25–0.50, and 0.50–1.0 m) and immediately placed in plastic bags in a cooler with dry ice before being transported to the laboratory freezer until analysis. Prior to root washing (with the Rootwasher described above), samples were removed from the freezer and allowed to thaw for 24 h. Washed samples were dried and weighed using methods described above.

2.3 | Water-soluble carbohydrate determination

Nonstructural carbohydrates are composed of WSC (mainly glucose, fructose, sucrose, and other oligosaccharides) and starch. We used the ground samples from rhizome and root of each plot to analyze WSC according to the method of Hall (2014), and starch according to the method of Hall (2015).

The starch analysis included the steps that follow. We placed 0.10 g of sample, 0.09 g starch and 0.09 g glucose as controls, and a tube with no substrate as a reagent blank into individual screw cap tubes. A total of 30 ml of 0.1 M acetate buffer (pH 5.0) was added to each tube. Then, to one replicate tube for each sample, 0.1 ml heat-stable, alpha-amylase (thermostable amylase HTL, ~8,300 bacterial amylase units; BioCat) was added. To the other replicate, no enzymes were added, and these were used to measure free glucose. The tubes were incubated 1 h at 100 °C and vortexed three times at 10, 30, and 50 min. The samples were then cooled on bench (~0.5 h to ~50 °C or less). After cooling, 200 units of amyloglucosidase solution (E-AMGDF, Megazyme International) was added to the tubes with amylase, and tubes were capped, vortexed, and incubated again in a 50 °C water bath for 2 h and vortexed once at 1 h. After removal from the water bath, 20 ml of distilled water was added to each tube. Then, for each tube, 2 ml of solution was transferred to microfuge tubes and centrifuged at 1,000*g* for 10 min. The supernatant was diluted as needed to fall in standard curve for glucose analysis. Samples, glucose standard solutions, and reagent blanks were pipetted (0.1 ml) in duplicate into culture tubes, and 3.0 ml of glucose oxidase-peroxidase reagent was added to each tube. Samples were incubated at 50 °C for 20 min. The samples were then read within 30 min after the incubation on spectrophotometer at 505 nm.

For WSC analysis, 0.10-g samples were placed into 50-ml conical tubes. Distilled water (35 ml) was added to each tube, sealed tightly, vortexed, and incubated at 40 °C for 1 h in a shaking incubator. Extracts were then transferred to 1.5- to 2-ml microfuge tubes and centrifuged at 12,000*g* for 10 min at ambient temperature. Dilutions were performed with distilled water if needed. Solutions were subsequently assayed with phenol-sulfuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). About 0.5 ml of each sample and sucrose standards were pipetted into triplicate tubes (0, 33, 66, and 100 μg sucrose ml^{-1}). An amount of 0.5 ml of 5% phenol was added to each tube with repeat pipette and vortexed. A total of 2.5 ml of concentrate H_2SO_4 was then added to each tube using a bottle dispenser and vortexed immediately. All tubes were vortexed a second time and capped with glass marbles. The samples were incubated in a 30 °C water bath for 20 min in the dark.

TABLE 1 Means and errors (SE) for total organ biomass (dry matter), water-soluble carbohydrates (WSC) and starch concentration (dry matter basis) and mass in Kernza intermediate wheatgrass rhizomes and roots in the first 0.1 m of soil depth under different N fertilization treatments over two production years (stand ages) in Arlington, WI, and St. Paul, MN

Source	Location	Age	Nitrogen	WSC conc.	WSC mass	Starch conc.	Starch mass	Biomass
		yr	kg ha^{-1}	g kg^{-1}	g m^{-2}	g kg^{-1}	g m^{-2}	
Rhizome	Arlington, WI	1	90	162.5 ± 14.8a	2.0 ± 1.5b	1.7 ± 0.3b	0.0 ± 0.0b	12.9 ± 5.7b
			134	177.5 ± 14.8a	3.4 ± 1.5b	2.0 ± 0.3b	0.0 ± 0.0b	19.4 ± 5.7b
		2	90	173.0 ± 14.8a	5.6 ± 1.5a	6.2 ± 0.3a	0.2 ± 0.0a	28.0 ± 5.7a
			134	130.2 ± 14.8b	2.2 ± 1.5b	6.6 ± 0.3a	0.1 ± 0.0a	16.1 ± 5.7b
	St. Paul, MN	2	56	135.3 ± 7.9b	3.9 ± 0.9b	1.5 ± 0.3b	0.3 ± 0.0a	29.1 ± 3.8a
Roots	Arlington, WI	1	90	127.3 ± 5.0a	9.8 ± 1.0ab	3.2 ± 0.5	0.3 ± 0.1b	79.3 ± 8.6b
			134	105.3 ± 5.0b	7.5 ± 1.0b	1.8 ± 0.5	0.2 ± 0.1b	79.3 ± 8.6b
		2	90	96.0 ± 5.0b	9.0 ± 1.0ab	3.0 ± 0.5	0.3 ± 0.1ab	93.2 ± 8.6ab
			134	98.7 ± 5.0b	11.8 ± 1.0a	3.2 ± 0.5	0.4 ± 0.1a	118.1 ± 8.6a

Note. Means not sharing any letter for each variable are significantly different at $\alpha = .05$.

After incubation, the samples were removed, vortexed, and cooled for 30 min. Finally, the samples were read on spectrophotometer at 490 nm using acid-resistant cuvettes.

The NSC concentrations were reported as grams per kilogram (dry matter basis) and mass (total mass of carbohydrates in grams per square meter), and dry biomass of roots and rhizomes was reported in grams per square meter.

2.4 | Statistical analyses

Rhizome and roots variables were analyzed independently. An ANOVA was conducted using a mixed model for each rhizome variable (biomass, WSC, and starch concentration and mass) with the following fixed effects: environment (combination of location and year: WI-2016, WI-2017, MN-2016), forage harvest effect (control or two harvests), N fertilizer effect, all the two- and three-way interactions, and blocks (nested within location) as a random effect. For the root variables (measured only in the Wisconsin location), we used a similar mixed model where environment was year, or stand age (first or second production year). For the belowground biomass analyses to 1-m depth in Wisconsin in the second year, we used a mixed model with blocks as a random effect, and the fixed effects of forage harvest, N, and soil depth (0–0.10, 0.10–0.25, 0.25–0.50, and 0.50–1.0 m), and all the two and three way interactions between forage harvest, N, and depth. Depth was considered repeated measurements using plots as subjects. When interactions were detected, we estimated and compared least squared means using the slice option in the Mixed procedure of SAS software (SAS Institute, 2013). Simple linear regressions were estimated for means of aboveground biomass and grain yield of the following year against the mean concentrations and mass of biomass and NSC of belowground organs, within each location, using the Reg procedure of SAS software (SAS Institute, 2013). Statistical significance was assessed at the 5% probability level unless otherwise indicated.

3 | RESULTS

3.1 | Nonstructural carbohydrate concentration and mass

Rhizomes accounted for 17% of belowground biomass and did not differ across years or N levels in Wisconsin (Table 1). Rhizome biomass in Minnesota in the second year was similar to that in Wisconsin (Table 1). Roots biomass in the first 0.10 m of soil accounted for 83% of the belowground biomass on average and increased in the second year for the high N level to 88% in Wisconsin (Table 1).

The WSC concentration ranged from 96.0 to 127.3 g kg^{-1} in roots and from 130.2 to 177.5 g kg^{-1} in rhizomes (Table 1). The WSC mass accounted for 97–99% of the NSC mass in both organs, years, and locations (Figure 2). The WSC concentration in rhizomes was 47 and 57% higher than that in roots in first and second year, respectively, in Wisconsin (Table 1). However, given the greater biomass of roots, 76–73% of total belowground WSC mass was contributed by roots (in the first 0.1 m of soil) each year, respectively (Figure 2). The starch concentration ranged from 1.3 to 6.7 g kg^{-1}. The starch mass accounted for only 1–3% of the NSC mass across organs, years, and locations (Figure 2).

No differences were observed due to N fertilization in most variables in Wisconsin to a depth of 0.10 m. However, starch concentration in roots in the first year was reduced 43%, and WSC concentrations and mass in rhizomes were reduced 25 and 60%, respectively, in the second year in the

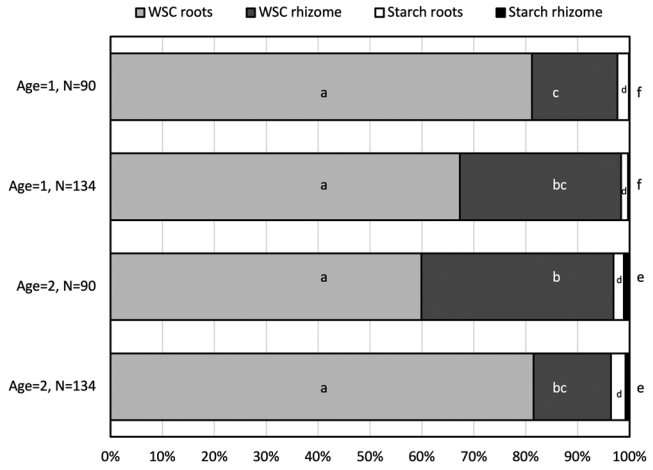

FIGURE 2 Mass of water-soluble carbohydrates (WSC) and starch for roots and rhizomes expressed as percent of total belowground nonstructural carbohydrate (NSC) mass for Kernza intermediate wheatgrass in the first 0.1 m of soil depth under different N fertilization treatments (90 and 134 kg ha^{-1}) over two production years (stand ages) in Arlington, WI. Means not sharing any letter for each variable are significantly different at $\alpha = .05$

high N treatment. Root biomass was 27% higher in the high N level in the second year (Table 1).

The forage harvest treatment did not affect root or rhizome biomass or NSC across years or locations (Table 2).

An interaction among environment (location-year) and N was detected for WSC and biomass (Table 2), so means were sliced by environment and N.

3.2 | Belowground biomass

Belowground biomass (rhizome and roots) up to 0.10 m of depth did not differ with N or forage harvest treatments, and it increased with stand age ($P < .01$) when sampled by the shovel method. By the fall of the first production year belowground biomass in the top 0.1 m of soil was 98.0 ± 7.0 g m^{-2}, and by the fall of second production year it reached 126.6 ± 7.0 g m^{-2} in Wisconsin (Table 1).

When using the soil probe sampling method in the second year in Wisconsin, a triple interaction N by forage harvest by soil depth was detected for belowground biomass up to 1-m depth ($P < .01$); therefore, this variable was further analyzed by forage harvest treatment and soil depth. In the first 0.1 m of depth, a N × forage harvest interaction was detected ($P = .04$), where higher N dose increased the belowground biomass in the summer + fall forage harvest treatment but reduced the biomass in the control treatment (Figure 3). No N or forage harvest effects were detected at other depth ranges. Belowground biomass in the first 0.1 m of depth with the soil probe method was, on average, 328.7 ± 54.4 g m^{-2}. Accumulated belowground

TABLE 2 *P* values for the main effects and interactions for water-soluble carbohydrate (WSC) concentration, WSC mass, starch concentration, starch mass, and biomass for roots and rhizomes in Kernza intermediate wheatgrass in the first 0.1 m of soil depth for three environments (E; Wisconsin 2016, Wisconsin 2017, and Minnesota 2016), two forage harvest managements (FH), and two N fertilizer doses (N)

	Rhizome					Roots				
Effect	**WSC conc.**	**WSC mass**	**Starch conc.**	**Starch mass**	**Biomass**	**WSC conc.**	**WSC mass**	**Starch conc.**	**Starch mass**	**Biomass**
E	.09	.23	**<.01**	**.01**	.21	**<.01**	.11	.31	**.03**	**.01**
FH	.46	.17	.91	.48	.18	**.02**	.22	.66	.68	.50
N	.20	.30	.37	.67	.56	**.02**	.83	.31	.89	.18
E × FH	.09	.09	.37	.97	.22	**.02**	.60	.66	.89	.19
E × N	**.01**	**.01**	.86	.41	**.05**	**<.01**	**.03**	.19	.09	.18
FH × N	.09	.24	.86	.97	.53	**.03**	.59	.88	.68	.96
E × FH × N	.25	.85	.59	.73	.35	**.02**	.93	.66	.89	.47

Note. Bolding indicates *P* values <.05.

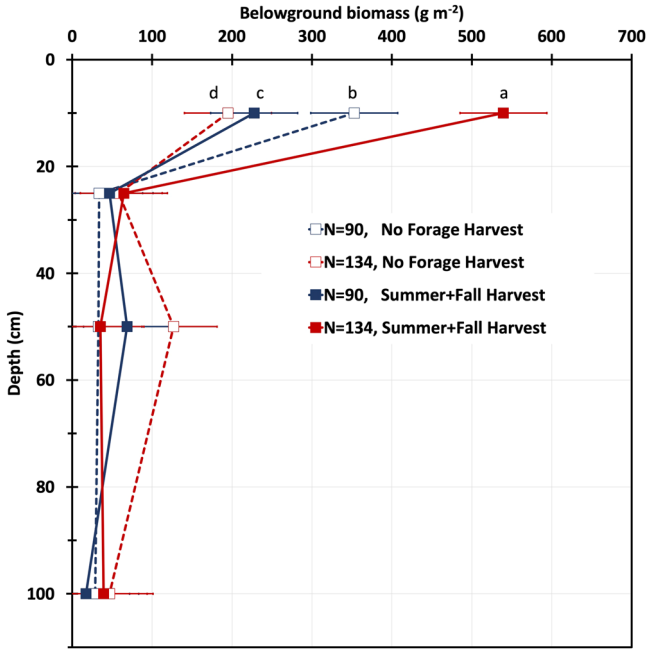

FIGURE 3 Belowground biomass in Kernza intermediate wheatgrass over four soil depth increments to 1 m (0–0.10, 0.10–0.25, 0.25–50, and 0.50–1 m) sampled in the fall of the second production year (October 2017) under two different forage harvest regimes (no forage harvest vs. harvest in summer and fall) and two N fertilizer applications (90 and 134 kg ha^{-1}) in Arlington, WI. Standard errors bars are shown. Significant differences between treatments were found only in the first 0.10 m (means not sharing any letter are significantly different at $\alpha = .05$)

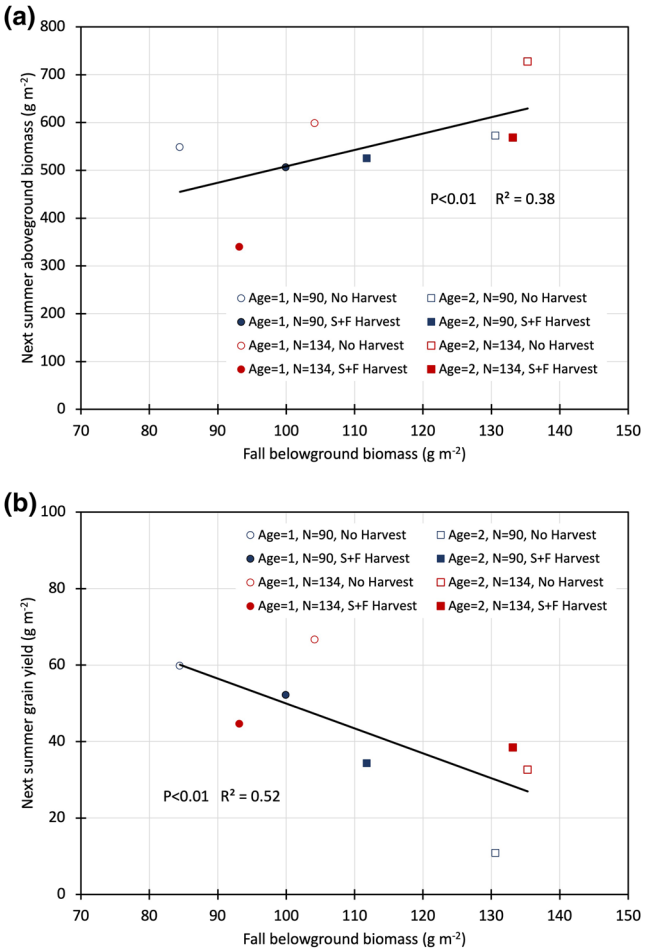

FIGURE 4 Kernza intermediate wheatgrass (a) summer aboveground biomass in the following year (g m^{-2}) and (b) summer grain yield (g m^{-2}) vs fall belowground (root and rhizome) biomass (g m^{-2}) in the first 0.1 m of soil depth under two different forage harvest regimes (no forage harvest vs summer and fall [S+F] harvest), two N treatments (90 and 134 kg ha^{-1}), and 2 yr (first and second production year) in Arlington, WI. Each data point is the mean of three replications, and it was identified with age of the stand at the belowground sampling time, N fertilization, and forage harvest treatment. Simple linear regression P values and R^2 are shown ($N = 8$)

biomass up to 1-m depth in the second year averaged 478.3 ± 117.6 g m^{-2} and did not change in response to harvest or N fertilizer treatments (Figure 3). Of the belowground biomass, 69% was in the first 0.10 m, 11% was from 0.10 to 0.25 m, 14% was from 0.25 to 0.50 m, and 7% was from 0.50 to 1.0 m.

3.3 | Association with following year forage and grain yield

Spring forage yield in the following year did not differ across treatments or years in Wisconsin, averaging 114.3 ± 12.1 g m^{-2} in Wisconsin for the 2 yr. In Minnesota, spring forage yield was 124.3 ± 21.2 g m^{-2} for the control and 262.2 ± 21.2 g m^{-2} for the two forage harvests treatment ($P = .02$). Spring forage yield in the second year in Wisconsin was positively associated with rhizome starch concentration in the first year ($R^2 = .36$), but not with any other variable in the first or second year in any location. Summer aboveground biomass in Wisconsin was 512.9 ± 30.0 g m^{-2} in the second year (2017) and 585.0 ± 30.0 g m^{-2} in the third year (2018, $P < .01$), and it did not differ with N or forage harvest treatments. In Minnesota, summer aboveground biomass in the third year (2017) averaged 1,059.6 ± 95.1 g m^{-2}, not different between forage harvest treatments. Summer aboveground biomass was positively associated with previous year fall belowground biomass ($R^2 = .38$, $N = 8$, Figure 4a), fall root biomass ($R^2 = .40$, $N = 8$), WSC mass in roots ($R^2 = .48$, $N = 8$), and NSC mass in roots ($R^2 = .42$, $N = 8$) across the 2 yr in Wisconsin.

Grain yield in Wisconsin was 63.2 ± 6.5 g m^{-2} and 46.3 ± 5.9 g m^{-2} for the control and two harvest treatment, respectively, in the second year (2017) and 21.7 ± 5.8 g m^{-2} and 37.0 ± 5.3 g m^{-2} for the control and two harvest treatment, respectively, in the third year (2018, $P < .01$ for the interaction). In Minnesota, grain yield was 66.4 ± 8.1 g m^{-2}

in the third year (2017) control treatment, and 37.6 ± 8.1 g m^{-2} in the two harvests treatment ($P = .02$). Grain yield was negatively associated with previous fall belowground biomass ($R^2 = .52$, $N = 8$, Figure 4b) and belowground NSC mass ($R^2 = .34$, $N = 8$) across 2 yr in Wisconsin.

4 | DISCUSSION

4.1 | Nonstructural carbohydrate concentration and mass

Nonstructural carbohydrates are the major subclass of the organic reserves mobilized during regrowth (Brown & Blaser, 1965; Kigel, 1980; Volenec, 1986; White, 1973). Previous studies have shown that fructans and other WSC (glucose, fructose, sucrose, and other oligosaccharides) are the main carbohydrate reserve for most temperate forage grasses (Meier & Reid, 1982; Pollock & Jones, 1979; Sullivan & Sprague, 1943; Waite & Boyd, 1953), whereas sucrose or starch are the main reserve for the tropical grasses (White, 1973). Our results show that WSC were the most abundant storage carbohydrates in both rhizomes and in roots of intermediate wheatgrass. The starch concentration was very low in comparison with the WSC concentration in both organs, as expected. This was consistent with previous research showing that 70–85% of NSC in intermediate wheatgrass vegetative structures was WSC (Zhao et al., 2008).

Biomass of roots was greater than rhizomes in both years and therefore accumulated greater mass of WSC. Nevertheless, the concentration of WSC in rhizomes was much higher than that of roots. This suggests that both rhizomes and roots play important roles in the storage of WSC and therefore in the survival and regrowth of the perennial grasses (Janeček & Klimešová, 2014). Root biomass increased significantly in the second year, and WSC mass was increased in parallel. However, the rhizome biomass or WSC mass did not change significantly over the first 2 yr. It should be noted that in our design, stand age is confounded with year (climatic conditions) because we did not have two stand ages in the same year in each location; therefore, further studies are needed to disentangle the effect of stand age and climatic year in carbohydrates storage in perennial grasses.

4.2 | Nitrogen and forage harvest effect

Nitrogen application did not have a consistent effect across years. In the first year, the higher N application did not affect WSC mass or belowground biomass, but in the second year, N reduced WSC mass and biomass in rhizomes but not roots when sampled for first 10 cm with the shovel method. Furthermore, when sampled with the probe, N did not affect total belowground biomass to 1-m depth, but in the first 10 cm increased biomass only in the two forage harvest treatments. One possible explanation of this is that belowground biomass is highly variable and hard to estimate precisely. An additional source of uncontrolled variability that may have interfered was the presence of weeds. Although weed biomass was very low in this experiment (Zimbric et al., 2020) weed root biomass would be concentrated in the first 10 cm of soil depth and could have increased sampling variability. Previous studies have also found contrasting results. Pelletier et al. (2009) found that N applications from 30 to 110 kg ha^{-1} did not affect NSC concentration in Timothy (*Phleum pratense* L.) in Canada; Klimes and Klimesova (2002) also found that N fertilization (88 kg ha^{-1}) did not affect carbohydrate reserves in three perennial grass species in grasslands in Europe (*Bromus, Molinia*, and *Calamagrostis*) compared with the unfertilized control. In contrast, Sugawara (1983) found that high application rates of N (240 kg ha^{-1}) decreased carbohydrate reserves in orchardgrass (*Dactylis glomerata* L.), and Pan, Bai, Han, and Zhang (2004) found that NSC concentrations in the rhizomes of the perennial cool-season grass Chinese wild rye [*Leymus chinensis* (Trin.) Tzvelev] increased with N application rates to 175 kg ha^{-1}, but these rhizome concentrations were reduced at application rates of 280 kg ha^{-2}. Disentangling the interactions between N, NSC, and plant growth is not simple. It is usually assumed that stored NSC fluctuates depending on the balance between supply via photosynthesis (source) and demand for growth and respiration (sink). However, NSC dynamics respond to additional roles of NSC (e.g., metabolic, osmotic) critical for plant survival, which require maintaining relatively high concentrations of WSC (Martínez-Vilalta et al., 2016).

Forage harvest treatments did not affect NSC reserves in rhizomes or roots. This suggests that the intensity of defoliation in the Kernza dual-use system (only two forage harvests, one in summer and one in fall) was not severe enough to affect NSC. Previous studies have shown that frequent defoliation of perennial ryegrass (*Lolium perenne* L.) in spring reduces its survival over summer in a subtropical environment associated with reduced WSC (Fulkerson, 1994; Fulkerson & Bryant, 1993). Frequent defoliation has been shown to lower WSC reserves in ryegrass (Davies, 1965; Fulkerson & Slack, 1995) and other grass species (Bartholomew, de, & Booysen, 1969; Bommer, 1966). However, Benot et al. (2019) found that grazing intensity did not affect the NSC concentrations in five grass species in at the end of the grazing season (fall), which is consistent with our findings. Therefore, harvesting Kernza

intermediate wheatgrass as a dual-use crop for grain and forage did not negatively affect NSC reserves.

4.3 | Belowground biomass

Although different methods for belowground biomass estimation differ in their results (Milchunas, 2009), we found values of root biomass in our study that were consistent with previous studies on intermediate wheatgrass (Pugliese et al., 2019; Sprunger et al., 2018). In the Ohio study, root biomass in the first 0.2 m of depth, sampled in November, ranged between 116 and 118 g m^{-2} in the first year, and between 76 and 162 g m^{-2} in the second year, for the control and the two forage harvests, respectively (Pugliese et al., 2019, Supplemental Table S1). These values are within the range of what we observed for sampling in the first 0.1 m of depth. Furthermore, in their sampling to 1-m depth, root biomass ranged from 265 to 504 g m^{-2}, for the control and two forage harvests, respectively (Pugliese et al., 2019), which is also within the range of what we observed. In the Michigan study, root biomass to 1-m depth in the second year ranged between 439 and 599 g m^{-2} for low (90 kg ha^{-1}) and high (135 kg ha^{-1}) N treatments, respectively (Sprunger et al., 2018). In their study, root biomass reached 1,035 g m^{-2} in the fourth year of intermediate wheatgrass growth in the high N treatment. This study provided additional evidence to support that Kernza intermediate wheatgrass produces a dense, deep root system that can deliver multiple ecosystem services like soil erosion control and C sequestration (Culman et al., 2013)

4.4 | Association with following year's forage and grain yield

There was not a consistent effect across years or locations of NSC concentration or mass on the following year's spring regrowth, forage biomass, or grain yield. However, the positive association between first-year rhizome starch concentration and second-year spring regrowth in Wisconsin is consistent with previous studies on other species (Fulkerson & Slack, 1995; Garnier, 1992). It may be hypothesized that in the first year, rhizomes play a relevant role in storing reserves for spring regrowth, but as the stand gets older, and coarse roots accumulate more biomass and NSC, rhizomes contribute less NSC, and the association between rhizome NSC and spring regrowth weakens. Summer biomass in the following year was associated with previous fall belowground (roots and rhizomes) biomass (Figure 4a), and WSC and NSC in roots. The overall greater root biomass is often linked with biomass shoot growth and performance (Sainju, Allen, Lenssen, & Ghimire, 2017). This further supports the hypothesis that coarse roots play a relevant role in the following year's growth and yield. The role of fine (<2-mm size) roots was not evaluated in this study and should be further investigated. The fact that forage harvests in summer and fall did not affect NSC dynamics in Kernza suggests that the dual-use management (for grain and forage) will not negatively affect regrowth in the next spring or persistence over the long term. However, our conclusions are limited to the first 2 yr of growth of a perennial grass, and it would be relevant to assess these relationships with data from older Kernza stands.

The negative association found between grain yield in the following year and belowground biomass and NSC in the previous fall suggests that the accumulation of reserves and carbohydrate metabolism during the fall, when new tillers are developing, could be limiting the reproductive potential in the following year. Alternatively, this association may be simply caused by the fact that as stands age, more resources are allocated towards vegetative than reproductive structures. Furthers studies are needed to explore these mechanisms and potential tradeoffs in perennial grain systems. This is extremely relevant for Kernza, given the fact that grain yield has been reported to decline as the stand ages (Pugliese et al., 2019; Jungers et al., 2017; Zimbric et al., 2020).

5 | CONCLUSIONS

Our results show that WSC are the most abundant storage carbohydrates in intermediate wheatgrass Kernza. Both rhizomes and coarse roots play an important role in the storage of WSC and therefore in the regrowth of this perennial grass. The forage harvest regimens and N application rates evaluated did not consistently affect NSC, WSC, and belowground biomass of Kernza in the first 2 yr of growth. Therefore, harvesting forage in a dual-use system is not detrimental to Kernza intermediate wheatgrass productivity above- or belowground.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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