

How Does Nitrogen and Perenniality Influence Belowground Biomass and Nitrogen Use Efficiency in Small Grain Cereals?

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Abbreviations: IWG, perennial intermediate wheatgrass; KBS, W.K. Kellogg Biological Station; NUE, nitrogen use efficiency.

ABSTRACT

Perennial cropping systems typically exhibit extensive root systems that contribute to important ecosystem services. However, the root systems and the distribution of roots throughout the soil profile in novel perennial grains have yet to be reported. In addition, understanding the full impact of perennial grain cropping systems on belowground processes requires knowledge of how N regimes might influence biomass partitioning and N retention. Here, we quantified root biomass distribution, crop biomass allocation, and whole-crop fertilizer N use efficiency (NUE, defined as the ratio of plant N to total N fertilizer applied) in annual winter wheat (*Triticum aestivum* L. ‘Caledonia’) and perennial intermediate wheatgrass [IWG, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] across three N treatments over a 3-yr period. Nitrogen treatments included Low N (90 kg ha⁻¹ of poultry manure), Mid N (90 kg ha⁻¹ of urea), and High N (135 kg ha⁻¹ of urea). As a perennial plant, IWG had significantly greater root biomass than annual wheat to the 40-cm depth ($p < 0.05$), but no differences were found between the crops at deeper depths. Nitrogen treatments did not affect root biomass, except for IWG in its fourth year ($p < 0.05$). Regardless of N level, IWG always had greater whole-crop NUE than annual wheat ($p < 0.05$). Results demonstrate that IWG roots represent a large pool of N that contributes to enhanced NUE and ultimately greater N retention than in annual wheat roots.

The effect of N fertilizer on aboveground biomass has received a great deal of interest in both theory and practice. Nitrogen fertilizer effects on belowground biomass and biomass allocation strategies have received far less attention. Optimal partitioning theory (Bloom et al., 1985) posits that plants will respond to nutrient-limited environments by increasing root productivity while allocating less energy to aboveground crop components. In cases where access to nutrients is adequate or excessive, optimal partitioning theory predicts a reduction in root production and an increase in aboveground (grain and shoot) production. In contrast, optimal foraging theory states that plants are expected to invest roots in highly enriched areas vs. more depauperate patches (Charnov, 1976; Loecke and Robertson, 2009; McNickle and Cahill, 2009).

There is evidence to support each of these competing theories; thus, belowground crop responses to N fertilizers in both annual and perennial crops remain poorly understood and contradictory (Heggenstaller et al., 2009; Jung and Lal, 2011; Jarchow and Liebman, 2012). In annuals, Russell et al. (2009) reported no change in corn (*Zea mays* L.) root biomass with increasing N additions, whereas Durieux et al. (1994) reported a decrease in root mass with increasing N rates at corn harvest, especially in drier growing seasons. In a perennial system, Jarchow and Liebman (2012) found that inorganic N fertilizer additions significantly reduced root biomass in a perennial C₄ grass system. At the same site, Dietzel et al. (2015) found that inorganic fertilizer significantly increased root biomass in a mixed prairie system. Contradictory

findings have also been reported with candidate perennial biofuel cropping systems, where roots responded both positively and negatively to increases in N addition, depending on crop type (Heggenstaller et al., 2009).

As perennial crops are further developed for both biofuel and grain (Glover et al., 2010; Robertson et al., 2011), assessing how N fertilization affects root biomass could have profound implications for ecosystem services that can be achieved with perennial crops. Soil C sequestration and reduced NO₃ leaching are two of the most promising ecosystem services that can be achieved within perennial grain cropping systems (Culman et al., 2013). However, these ecosystem services are primarily driven by root production. For example, perennial root systems have been shown to capture large amounts of N, which can lead to reduced NO₃ leaching (Syswerda et al., 2012; Jungers et al., 2017), and soil C accumulation is largely driven by the production and decay of fine roots (Jackson et al., 1997). Given that practitioners will need to apply N fertilizer in order for perennial grains to reach their full yield potential, it will be crucial to know how N additions influence above- and belowground biomass allocation and fertilizer N use efficiency (NUE) in perennial grain crops.

To date, it has been difficult to assess and compare NUE across different cropping systems and species with different plant-life forms, largely because belowground plant components are not quantified and are therefore excluded from NUE calculations. Nitrogen use efficiency as defined here is the ratio of N fertilizer added to a system relative to the amount of N that leaves via harvest (Robertson and Vitousek, 2009). Although there are a wide variety of methods and equations for assessing NUE in disciplines spanning from agronomy to economics (Martinez-Feria et al., 2018), rarely do they account for roots. Including roots is important because belowground plant biomass N in cereals can account for up to 60% of total plant N, whereas N rhioderivatives account for up to 71% of total assimilated plant N (Wichern et al., 2008). Given that roots represent large pools of N, neglecting to include roots within NUE equations could ultimately underestimate NUE, especially in perennial cropping systems (Dawson et al., 2008; Martinez-Feria et al., 2018).

Understanding how different rates and sources of N fertilization influence belowground productivity by depth and overall crop N content in small grain cereal crops could have important implications for agronomic productivity, whole-crop NUE, and sustainability within agroecosystems. Here, we compare crop biomass allocation, coarse and fine root vertical distributions, and NUE in annual winter wheat (*Triticum aestivum* L. 'Caledonia', referred to as "wheat" below) and perennial Kernza, a developing perennial grain crop. Kernza is the tradename of lines of intermediate wheatgrass [IWG, *Thinopyrum intermedium* (Host) Barworkth & D.R. Dewey], a cool-season grass that has been intensively bred for grain production over the last several decades (DeHaan and Ismail, 2017). The objectives of this study were (i) to quantify the amount of biomass that a novel perennial grain (IWG) allocates belowground relative to annual wheat and compare vertical root distributions between annual wheat and IWG; (ii) to determine how different sources and rates of N influence biomass partitioning and belowground dynamics; and (iii) to determine the effect of perenniality and N treatment on small-grain NUE.

MATERIALS AND METHODS

Site Description

The experiment was conducted at the W.K. Kellogg Biological Station (KBS) Long-Term Ecological Research site, located in southwestern Michigan (42°24' N, 85°24' W; 288 m asl). The mean annual precipitation and temperature at KBS are 1005 mm and 10.1°C. Soils at KBS are in the Kalamazoo (fine-loamy, mixed, active, mesic Typic Hapludalfs) and Oshtemo (Coarse-loamy, mixed, active, mesic Typic Hapludalfs) series. These soils typically have an A horizon of 30 cm, a deep Bw/Bt horizon that reaches to 80+ cm, and a BC horizon to 140 cm (Robertson and Hamilton, 2015). Prior to establishment in 2009, the field was under a corn–soybean [*Glycine max* (L.) Merr.]–wheat rotation.

Experimental Design

The study was established in 2009 as a split-plot (3.1 × 4.6 m) randomized complete block design experiment with four replicate blocks. The main factor was N treatment and the subfactor was crop type. The N treatments consisted of three different N levels that differed in source and rates of N: (i) Low N (Organic N) treatment, which received 90 kg N ha⁻¹ of poultry manure; (ii) Mid N, which received 90 kg N ha⁻¹ of urea; and (iii) High N, which received 135 kg N ha⁻¹ of urea. These treatments were chosen to represent three N management regimes that might be encountered when growing rainfed IWG in the Upper Midwest. Since N release from manure is typically slower than N release from urea and inorganic fertilizers (Rees and Castle, 2002), the N availability in this study is ordered as Low N (Organic N) < Mid N < High N.

Prior to planting, all plots were chisel plowed to 20 cm in September 2009. Every October, 2.24 Mg ha⁻¹ of pelletized poultry manure plus sawdust at 4–3–2 N–P–K (Herbruck's Poultry Ranch) was applied to the Low N (Organic N) system. This application rate delivered 90 kg N ha⁻¹. The Mid N level is the recommended rate for conventionally grown wheat in the state of Michigan, whereas the High N (135 kg N ha⁻¹) level received 50% more N than the Mid N level. Both the Mid N and High N levels received pelleted urea at three different times throughout the growing season. In the conventional systems, a starter of 33.6 kg N ha⁻¹ and 53.8 kg K ha⁻¹ as K₂O for both Mid N and High N systems were applied immediately before planting. The following spring, plots were top-dressed with urea at 28 and 50.4 kg N ha⁻¹ for Mid N and High N, respectively, typically at the beginning of April.

The crop types in this experiment were (i) annual winter wheat 'Caledonia' (soft red wheat) and (ii) Kernza (IWG), which was developed through bulk breeding and mass selection at the Land Institute in Salina, KS (DeHaan et al., 2005 Cox et al., 2010). Planting for both crops occurred on 8 Nov. 2009 at a seeding rate of 310 seeds m⁻² (1.25 million seeds acre⁻¹) for IWG and 432 seeds m⁻² (1.75 million seeds acre⁻¹) for winter wheat. Both crops were planted at a 15-cm row spacing. Subsequent plantings of winter wheat occurred on 8 Oct. 2010, 22 Sept. 2011, and 2 Oct. 2012 at the same seeding rate and row spacing. More details regarding planting and chemical application can be found in Culman et al. (2013).

Aboveground Biomass Sampling

Aboveground biomass was measured at grain maturity for both crops. In general, wheat was harvested in early to mid-July, and IWG was harvested at the end of July or early August.

Aboveground biomass and yields were determined by randomly placing two 0.25-m² quadrats in every plot and clipping the crop biomass to 10 cm above the soil. Samples were then threshed to separate grain from straw and dried at 60°C for 48 h before weighing.

Belowground Biomass and Soil Sampling

Belowground biomass and soil samples were collected near peak biomass and anthesis every June from 2011 to 2013. In 2011, belowground biomass was measured only in the Low N and High N levels. A hydraulic direct-push soil sampler (Geoprobe) was used to take three 1-m cores per plot. All cores were taken in between plant rows. The cores were 6 cm in diameter and were subsequently divided into five depths (0–10, 10–20, 20–40, 40–70, and 70–100 cm). The three cores were composited by depth interval, and a subsample of 400 mL from each depth was taken for root analyses, with the remainder used for soil chemical and physical analyses. Gravimetric soil moisture was determined by calculating the difference between the fresh weight and dry weight of a 40-g soil sample (Jarrell et al., 1999) and expressed on a dry weight basis: % soil moisture (dry weight) = 100 [(fresh weight – dry weight)/dry weight].

Roots were separated into two size classes: coarse (>6 mm) and fine (1–6 mm). Coarse roots were separated from soil by dry sieving through 6-mm sieves. Fine roots were obtained from soil sieved through 1-mm sieves by wet sieving. No attempt was made to determine live vs. dead roots. To clean roots prior to weighing and drying, roots were soaked in deionized water and hand washed. Both coarse and fine roots were dried at 60°C for 48 h prior to weighing. To report root biomass on a kilogram per hectare basis, we converted root samples from a mass per volume basis to a mass per area basis by multiplying root mass per volume by length of the soil depth interval and areal conversion factors.

Crop Carbon and Nitrogen Analysis

Dried coarse and fine roots were ground to a fine powder using a mortar and pestle. Dried grain and stem crop components were ground separately to 1 mm with a Wiley mill. Both above- and belowground crop parts were analyzed for C and N in a CHNS analyzer (Costech Analyzer ECS 4010, Costech Analytical Technologies). In 2011, biomass was not analyzed for C and N.

Fertilizer Nitrogen Use Efficiency

We used the mass balance approach to calculate fertilizer NUE. Total plant NUE [(straw kg N ha⁻¹ + grain kg N ha⁻¹ + coarse and fine root kg N ha⁻¹)/fertilizer N applied (kg N ha⁻¹)], aboveground NUE [(straw kg N ha⁻¹ + grain kg N ha⁻¹)/fertilizer N applied (kg N ha⁻¹)], and root NUE [(coarse and fine root kg N ha⁻¹)/fertilizer N applied (kg N ha⁻¹)] were determined. Ratios >1.0 are an indication that the crop took up more N than was applied in a given growing season.

Statistics

Prior to analysis, data were tested for normality and homogeneity of variance. Once assumptions were met, all crop and soil responses were analyzed using Proc Mixed of SAS 9.3 (SAS Institute, 2011). Crop, N level, depth, and year were treated as fixed effects and block as a random effect. Significant differences were determined at $\alpha = 0.05$. Double repeated measures were used to account for both depth and year in the model. Means were compared with an adjusted Tukey's pairwise means comparison.

RESULTS

Weather and Soil Moisture

Cumulative precipitation and growing degree days between the months of March and October in 2011 and 2013 varied substantially from those in 2012 (data not shown). The site at KBS received above-average precipitation during the 2011 and 2013 growing seasons, receiving 858 and 752 mm, respectively. Growing degree days were similar in 2011 and 2013 (2497 and 2435°C d, respectively), and were comparable with the 30-yr average (2431°C d). In 2012, southwest Michigan experienced drought conditions from June to August, with 557 mm of cumulative precipitation between March and October, 77% of the 30 yr average (721 mm).

Gravimetric soil moisture varied between crop types, but not across the N gradient (Table 1). Gravimetric soil moisture was heavily influenced by crop, year, and depth ($p < 0.05$). Although wheat consistently had greater gravimetric soil moisture than IWG, pairwise comparisons reveal that significant differences were mainly found at 40 to 70 and 70 to 100 cm (Table 1).

Aboveground Biomass

Aboveground biomass greatly differed between the two crops (Table 2, $p < 0.05$) when analyzed over all years. In 2011 and 2013, IWG aboveground biomass ranged from 10.6 to 19.9 Mg ha⁻¹ and was consistently greater than wheat aboveground biomass, which ranged from 8.4 to 14.7 Mg ha⁻¹ (Table 2). In the 2012 drought year, both crops had lower productivity; averaged across N levels, IWG biomass decreased by 69% and wheat decreased by 53% between 2011 and 2012. In 2013, IWG aboveground biomass increased with greater levels of applied N; however, the N level effect was not significant ($p > 0.05$).

Belowground Biomass

Intermediate wheatgrass consistently had greater coarse and fine root biomass than wheat across all years (Table 2, $p < 0.05$). Depending on the N level and year, IWG had between 3 and 12 times greater coarse and fine root biomass than wheat. There was no overall N level effect on coarse root biomass ($p > 0.05$). However, in 2013, IWG coarse root biomass under High N was significantly greater than Mid N and Low N coarse root biomass (Table 2, $p < 0.05$), reflecting a significant crop \times N level \times year interaction ($p < 0.05$).

There was a significant overall N level effect on fine root biomass ($p < 0.05$). For example, in 2013, fine root biomass of IWG under Mid and High N was significantly greater than in the Low N system ($p < 0.05$). The crop \times N level effect was significant ($p < 0.05$) because increasing levels of N influenced only IWG.

Coarse and Fine Root Biomass by Depth

Coarse root biomass of IWG was between 3.4 and 8 times greater than wheat coarse root biomass at surface depth intervals of 0 to 10 and 10 to 20 cm (Fig. 1, $p < 0.05$). There were a few marginally significant differences at mid- (20–40 cm) and subsurface (40–70 and 70–100 cm) depth intervals ($p < 0.05$), but in general, there were few differences between wheat and IWG coarse root biomass at lower depths (Fig. 1). Wheat vertical coarse root distribution was fairly consistent across the 3 yr, with 93% of the roots found in the top 40 cm. Intermediate wheatgrass

had similar vertical coarse root distributions, with 94% of roots typically found in the top 40 cm; however, IWG root biomass increased significantly in the surface depths over time (Fig. 1).

Although there were no overall N level effects on coarse root biomass, there was a four-way interaction between year, crop, N level, and depth ($p < 0.05$). Notable differences in IWG coarse root biomass across the different N levels at the surface depths likely caused this significant interaction. For example, pairwise comparisons revealed that in 2011 and 2013, High N IWG coarse root biomass at 0 to 10 cm was significantly greater than Low N coarse root biomass ($p < 0.05$). In 2012, Low N IWG had greater coarse root biomass than Mid N IWG ($p < 0.05$) but was not significantly different from High N IWG. There were no differences between N levels at subsurface depths. Greater amounts of variability occurred at surface depths than at subsurface depths, especially in 2012.

Fine root biomass distributions were very similar to coarse root biomass distributions, with the majority of roots concentrated in the top 20 cm. However, IWG allocated a greater amount of biomass to fine roots than to coarse roots below 40 cm (Fig. 1 and 2). There were large differences in fine root biomass between crops ($p < 0.05$), across N levels ($p < 0.05$), over time ($p < 0.05$), and by depth ($p < 0.05$). There was also a significant three-way interaction among depth, year, and crop ($p < 0.05$), which reflected the range of fine root production over time for IWG at surface depths compared with greater stability in wheat. Furthermore, significant differences in fine root production between IWG and wheat were typically only in the top 0 to 40 cm. At the surface, IWG fine root biomass was typically between 1.5 and 4 times greater than wheat fine root biomass. The IWG fine root biomass tended to increase with increasing levels of fertilizer, especially at 0 to 10 cm, whereas wheat fine root biomass did not. The IWG fine root biomass increased over time, with the greatest values occurring in 2013 under Mid N and High N systems.

Crop Allocation and Root/Shoot Ratios

Differences in crop biomass allocation were evident for wheat and IWG (Fig. 3). In nondrought years, IWG allocated between 23 and 50% of its total biomass to roots as compared with wheat, which allocated ~10% to roots. In 2011 and 2013, IWG root/shoot ratios were two times greater than wheat root/shoot ratios (Fig. 3, $p < 0.05$). The significantly higher root/shoot ratios evident in 2012 in comparison with 2011 and 2013 were caused by large reductions in aboveground biomass, rather than gains in belowground biomass. In 2012, IWG root biomass was equal to that in 2011 and slightly lower than that in 2013 (Table 2). There were no significant N level effects on root/shoot ratios (Fig. 3, $p > 0.05$).

Crop Nitrogen and Fertilizer Nitrogen Use Efficiency

Aboveground biomass N contents were statistically similar between the two crops. Aboveground N contents significantly differed across the N gradient, with greater N content typically found in the High N system (Table 3, $p < 0.05$) for both crops. There were significant pairwise comparisons across N levels for aboveground N in 2013, but not in 2012.

The total N contained in IWG coarse and fine roots was consistently greater than that in wheat (Table 3, $p < 0.05$). The total N contained in coarse roots generally increased with increasing N fertilizer additions (Table 3). The significant crop \times N level interaction ($p < 0.05$) along with pairwise comparisons indicate that N levels had a much stronger influence on IWG

than on wheat, especially in 2013. There was an overall N level effect on fine root N content ($p < 0.05$). For IWG in 2012 and 2013, Mid N and High N had significantly greater fine root N than the Low N system (Table 3). Although there was an overall crop by N level interaction ($p < 0.05$), wheat fine root N did not appear to be as strongly influenced by increasing N levels compared with IWG. Total N strongly differed by crop ($p < 0.05$) and N level ($p < 0.05$) and was substantially greater in IWG and always larger in the High N level.

There was a significant year effect for all crop parts, with N contents typically greater in 2013. Coarse and fine root N contents were also examined by depth (data not shown) and exhibited very similar trends to coarse and fine root biomass by depth (Fig. 1 and 2).

The fertilizer NUE for above- and belowground biomass components were also calculated separately (Table 4). In terms of total crop NUE, IWG was more efficient at using fertilizer N than wheat ($p < 0.05$) during both years. Across both year and N level, IWG NUE ratios ranged from 0.8 to 1.5 and wheat NUE ranged from 0.56 to 0.86 (Table 4).

The IWG within the Mid N system exhibited greater NUE than that in the Low N and High N systems ($p < 0.05$). The significant interaction between crop and N level ($p < 0.05$) was an indicator that N level had little effect on wheat NUE. Significant gains in NUE from 2012 to 2013 were visible in all three N levels for wheat and were most noticeable in the Mid N system for IWG. Nitrogen use efficiency increased by up to 53% in wheat from 2012 to 2013 and by up to 43% in IWG (Table 4). There was no crop effect on aboveground NUE, as wheat and IWG were statistically similar to one another ($p > 0.05$). However, there was an overall N level effect, where wheat aboveground NUE was greater in Low N systems and IWG aboveground NUE was greater under Mid N. Root NUE was substantially greater in IWG than in wheat ($p < 0.05$).

DISCUSSION

Annual versus Perennial Biomass Allocation

Annual and perennial species have different development and growth strategies (Fitter et al., 1988). Such differences are reflected in their rooting patterns, which has implications for nutrient resource availability and ecosystem function. In general, annual plants tend to have shallow root systems that are more advantageous in high nutrient conditions compared with perennials, which typically display a deeper and more dense (often herringbone) architecture (Fitter et al., 1988). Although annual crops are fast growing and have traits that allow for rapid acquisition of resources (Fitter et al., 1991), perennials prioritize allocating biomass belowground, which allows them to conserve resources for long-term survivorship (Roumet et al., 2006). Thus, our findings that IWG allocated 23 to 50% of biomass to roots, whereas annuals only allocated 10% of biomass belowground, is not surprising and aligns with a larger body of literature. Furthermore, our findings concerning above- and belowground biomass in situ over a 3-yr period provide a valuable baseline to breeders working to enhance IWG via direct domestication.

Root and Biomass Allocation Responses to Added Nitrogen

Here, we compared different rates and sources of N fertilizer that practitioners apply to small cereal grains in the Midwest to enhance our understanding of biomass allocation patterns in annuals and perennials. In particular, we wanted to determine if greater applications of N fertilizer increase or decrease root biomass, given the important role that roots have in delivering ecosystem services.

We found that the different rates and sources of N had no influence on root/shoot ratios for either annual wheat or perennial IWG over the course of 3 yr. Additionally, we found that wheat root biomass remained stable across all N fertilizer levels in all 3 yr. There was also no IWG root response to N in the first 2 yr; however, root biomass of established IWG increased with greater N additions in 2013. The lack of a root response to increased N additions in Years 1 and 2 for IWG could be due to environmental and developmental factors. In 2011, IWG stands were 2 yr old and still establishing, which could prevent observed responses to increased N fertilizer (Jung and Lal, 2011). Although we might expect to see a root response to N level during the third year, 2012 was a drought year, which may have negated a response to added N. A root response to N was observed in 2013, when IWG was mature and growing conditions were favorable. In fact, in 2013, IWG root biomass significantly increased with higher levels of N, whereas root/shoot ratios remained stable. Root biomass also remained stable in wheat across N levels. This could suggest that perhaps water was a greater limiting factor than N (Meinke et al., 1997).

Our findings are at odds with optimal partitioning theory, which posits that where access to nutrients is adequate or excessive, reductions in root production and increases in aboveground (grain and shoot) production will occur. Our findings also contrast with at least one in situ study; Jarchow and Liebman (2012) reported greater root biomass of C₄ grasses in unfertilized systems compared with their fertilized system. However, interpretation is clouded in their study by extreme nutrient limitations in the unfertilized system, which contrasts with this study, where each treatment received at least some N fertilizer. In contrast, Heggenstaller et al. (2009) reported that switchgrass (*Panicum virgatum* L.) and big bluestem (*Andropogon gerardii* Vitman) belowground biomass increased with greater N fertilizer additions, which corroborates our IWG findings in Year 3. Increases in root diameter due to greater nutrient uptake in nutrient rich environments could explain greater root biomass in systems receiving greater N additions (Ryser and Lambers, 1995).

It is often predicted that when nutrients or water are limiting, crop allocation should shift to the production of fine roots that can capture resources available at greater depths (Bloom et al., 1985). We found no evidence for enhanced fine root production in either wheat or IWG within the Low N level at any depth to 1 m. Likewise, Jarchow and Liebman (2012), who found greater root biomass in an unfertilized C₄ grass system at the surface, also found no evidence of increased root production at depth, although they did not distinguish between coarse and fine root biomass.

Thus, our findings seem to corroborate the optimal foraging theory, where plants are expected to invest roots in highly enriched areas vs. more depauperate patches (Charnov, 1976; Loecke and Robertson, 2009; McNickle and Cahill, 2009). In this study, roots increased under High N and were mainly concentrated in the top 0 to 10 cm, rather than foraging deeper in the soil profile to obtain other available nutrients. Others have suggested that biomass partitioning is a function of ontogenetic drift, wherein biomass allocation is determined by growth and development rather than shifts in reallocation due to limiting resources (Coleman et al., 1994; Reich, 2002). The growth patterns of IWG in this study are consistent with this theory, as root biomass increased over time, especially in the High N system. Perhaps in this system, the increased root biomass within the established IWG under High N is simply due to changes in development, allowing IWG to gain access to greater nutrient capture. Monitoring belowground N responses over a 3-yr period demonstrates that roots of IWG increase rather than decrease with greater N fertilizer additions. This has important implications for how perennial grains can be

managed for optimal yields while still exhibiting an extensive root system that can contribute to essential ecosystem services.

Fertilizer Nitrogen Use Efficiency in Annual versus Perennial Systems

Above- and belowground biomass responses to N additions can have profound impacts on internal and external crop N cycling. For this reason, we were also interested in determining whole-crop fertilizer NUE. As noted earlier, we consider whole-crop fertilizer NUE to be total crop N (above + belowground biomass N) per fertilizer N added (Ladha et al., 2005; Robertson and Vitousek, 2009). This mass-balance approach allows us to determine the efficiency with which wheat and IWG assimilate added N.

Since aboveground NUE was not significantly different between wheat and IWG, it is likely that the extensive roots of IWG and their large capacity for N storage are the main drivers for their high NUE values, which gives them an efficiency advantage over wheat. For example, in 2013, IWG root NUE increased by 40% in the Low N and Mid N treatments and by 87% in the High N treatments. In three cases, the IWG whole-plant NUE was >1, indicating that the crop took up more N than was applied, which demonstrates its ability to assimilate large amounts of N (Dawson et al., 2008). Traditionally, root N content has not been included in NUE calculations (Weih et al., 2011). However, excluding belowground components from NUE calculations overlooks the important pool of N that roots represent and ultimately underestimates deep-rooted crops and their ability to retain N (Dawson et al., 2008; Martinez-Feria et al., 2018).

Nitrogen use efficiency significantly differed across N level in IWG, but not in wheat. The IWG NUE was greatest at the Mid N level compared with the Low N and High N levels. One explanation for greater NUE at the Mid N level could be that biomass production and N uptake kept up with N supply, unlike in the Low N system, which always had lower above- and belowground biomass. This study highlights that regardless of which rate and source of N is applied, perennial IWG has the ability to retain large amounts of N compared with wheat, which has important implications for N retention within this newly developed crop.

Implications for Enhanced Ecosystem Services by Perennial Grain Crops

These findings demonstrate that perennial grain cropping systems can significantly enhance ecosystem services in agriculture by increasing root biomass. Under a range of N additions, IWG produced up to eight times more total root biomass than wheat in the top 40 cm of soil. No differences were found between the two crops deeper in the profile, refuting the hypothesis that perennial grain crops are likely to have greater root biomass at depth than wheat (Cox et al., 2006; Glover et al., 2010; Kell, 2011).

Increased root biomass has been shown to enhance N cycling and accumulation (Fornara and Tilman, 2008). For example, increased root biomass in perennial systems can lead to N immobilization, and the quick release of fine root N during turnover can lead to N retention and accrual (Fornara et al., 2009). These results demonstrate that increased root biomass enabled IWG to take up large amounts of N and contributed to overall greater NUE. As a result, minimal N losses likely occur in these systems; for example, relative to wheat, IWG at this site reduced NO₃ leaching by 86 to 99% in 2011 (Culman et al., 2013).

Greater root biomass could also lead to increases in soil organic matter over time (Robertson et al., 2000; West and Post, 2002). As perennial crops age, a greater standing stock of

belowground biomass is established (Craine et al., 2003). This allows C to accumulate in root biomass and soil due to root turnover, which provides between 30 and 80% of organic C inputs to soil (Kalyn and Van Rees, 2006). In this study, IWG root biomass increased by 51% from 2011 to 2013. However, despite such increases in root biomass and root C accumulation, Sprunger et al. (2018) found no evidence for early soil carbon gain under IWG at this site. This is consistent with the requirement that IWG may need to be grown for at least 4 yr to obtain observable C sequestration benefits relative to an annual cereal crop.

CONCLUSIONS

This study examined the effect that different sources and rates of N had on annual and perennial root biomass, biomass partitioning, and fertilizer use efficiency. Perennial IWG had greater root biomass than wheat in the top 20 cm, regardless of fertilizer rate or source. Overall, N availability had no influence on biomass partitioning for either crop. However, established IWG stands increased root biomass with increasing N inputs, whereas wheat root biomass remained stable despite varying levels of N. Roots of IWG enhanced N retention and NUE and appeared to have contributed to the reduction of NO₃ leaching, as reported elsewhere. Given their C and N accrual and the retention of N by their extensive root systems, perennial grain crops could contribute significantly to the environmental sustainability of cereal crops.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

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REFERENCES

- Bloom, A.J., F.S. Chapin, and A.M. Harold. 1985. Resource limitation in plants-an economic analogy. *Annu. Rev. Ecol. Syst.* 16:363–392. doi:10.1146/annurev.es.16.110185.002051
- Charnov, E.L. 1976. Optimal foraging, the marginal value theorem. *Theor. Popul. Biol.* 9:129–136. doi:10.1016/0040-5809(76)90040-X
- Coleman, J., K.D.M. McConnaughay, and D.D. Ackerly. 1994. Interpreting phenotypic variation in plants. *Trends Ecol. Evol.* 9:187–191. doi:10.1016/0169-5347(94)90087-6
- Cox, T.S., J.D. Glover, D.L. Van Tassel, C.M. Cox, and L.R. DeHaan. 2006. Prospects for developing perennial grain crops. *BioScience* 56:649–659. doi:10.1641/0006-3568(2006)56[649:PFDPGC]2.0.CO;2
- Cox, T.S., D.L. Van Tassel, C.M. Cox, and L.R. Dehaan. 2010. Progress in breeding perennial grains. *Crop Pasture Sci.* 61:513–521. doi:10.1071/CP09201
- Craine, J.M., D.A. Wedin, F.S. Chapin, and P.B. Reich. 2003. The dependence of root system properties on root system biomass of 10 North American grassland species. *Plant Soil* 250:39–47. doi:10.1023/A:1022817813024

- Culman, S.W., S.S. Snapp, M. Ollenburger, B. Basso, and L.R. DeHaan. 2013. Soil and water quality rapidly responds to the perennial grain Kernza wheatgrass. *Agron. J.* 105:735–744. doi:10.2134/agronj2012.0273
- Dawson, J.C., D.R. Huggins, and S.S. Jones. 2008. Characterizing nutrient use efficiency in natural and agricultural ecosystems to improve the performance of cereal crops in low-input and organic agricultural systems. *Field Crops Res.* 107:89–101. doi:10.1016/j.fcr.2008.01.001
- DeHaan, L.R., Van Tassel, D.L., and Cox, T. S. 2005. Perennial grain crops: A synthesis of ecology and plant breeding. *Renew. Agric. Food Syst.* 20:5–14.
- DeHaan, L.R., and B.P. Ismail. 2017. Perennial cereals provide ecosystem benefits. *Cereal Foods World* 62:278–281. doi:10.1094/CFW-62-6-0278
- Dietzel, R., M.E. Jarchow, and M. Liebman. 2015. Above- and belowground growth, biomass, and nitrogen use in maize and reconstructed prairie cropping systems. *Crop Sci.* 55:910–923. doi:10.2135/cropsci2014.08.0572
- Durieux, R.P., E.J. Kamprath, W.A. Jackson, and R.H. Moll. 1994. Root distribution of corn: The effect of nitrogen fertilization. *Agron. J.* 86:958–962. doi:10.2134/agronj1994.00021962008600060006x
- Fitter, A.H., J.D. Graves, G.K. Self, T.K. Brown, D.S. Bogie, and K. Taylor. 1998. Root production, turnover and respiration under two grassland types on an altitudinal gradient: Influence of temperature and solar radiation. *Oecologia* 114:20–30. doi:10.1007/s004420050415
- Fitter, A.H., T.R. Strickland, M.L. Harvey, and G.W. Wilson. 1991. Architectural analysis of plant root exploitation efficiency. *New Phytol.* 118:375–382. doi:10.1111/j.1469-8137.1991.tb00018.x
- Fornara, D.A., and D. Tilman. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *Ecology* 96:314–322. doi:10.1111/j.1365-2745.2007.01345.x
- Fornara, D.A., D. Tilman, and S.E. Hobbie. 2009. Linkages between crop functional composition, fine root processes and potential soil N mineralization rates. *Ecology* 97:48–56. doi:10.1111/j.1365-2745.2008.01453.x
- Glover, J.D., J.P. Reganold, L.W. Bell, J. Borevitz, E.C. Brummer, E.S. Buckler, et al. 2010. Increased food and ecosystem security via perennial grains. *Science* 328:1638–1639. doi:10.1126/science.1188761
- Heggenstaller, A.H., K.J. Moore, M. Liebman, and R.P. Anex. 2009. Nitrogen influences biomass and nutrient partitioning by perennial, warm-season grasses. *Agron. J.* 101:1363–1371. doi:10.2134/agronj2008.0225x
- Jackson, R. B., H.A. Mooney, and E.D. Schulze. 1997. A global budget for fine root biomass, surface area, and nutrient contents. *Proc. Natl. Acad. Sci. of the* 94:7362–7366.
- Jarchow, M.E., and M. Liebman. 2012. Tradeoffs in biomass and nutrient allocation in prairies and corn managed for bioenergy production. *Crop Sci.* 52:1330–1342. doi:10.2135/cropsci2011.09.0481
- Jarrell, W.M., D.E. Armstrong, D.F. Grigal, E.F. Kelly, H.C. Monger, and D.A. Wedin. 1999. Soil water and temperature status. In: G.P. Robertson, et al., editors, *Standard soil methods for long-term ecological research*. Oxford Univ. Press, New York. p. 55–76.
- Jung, J.Y., and R. Lal. 2011. Impacts of nitrogen fertilization on biomass production of switchgrass (*Panicum virgatum* L.) and changes in soil organic carbon in Ohio. *Geoderma* 166:145–152. doi:10.1016/j.geoderma.2011.07.023
- Jungers, J.M., L.R. DeHaan, K.J. Betts, C.C. Sheaffer, and D.L. Wyse. 2017. Intermediate wheatgrass grain and forage yield responses to nitrogen fertilization. *Agron. J.* 109:462–472. doi:10.2134/agronj2016.07.0438.
- Kalyn, A.L., and K.C.J. Van Rees. 2006. Contribution of fine roots to ecosystem biomass and net primary production in black spruce, aspen, and jack pine forests in Saskatchewan. *Agric. For. Meteorol.* 140:236–243. doi:10.1016/j.agrformet.2005.08.019
- Kell, D.B. 2011. Breeding crop plants with deep roots: Their role in sustainable carbon, nutrient and water sequestration. *Ann. Bot. (Lond.)* 108:407–418. doi:10.1093/aob/mcr175

- Ladha, J.K., H. Pathak, T.J. Krupnik, J. Six, and C. van Kessel. 2005. Efficiency of fertilizer nitrogen in cereal production: Retrospects and prospects. *Adv. Agron.* 87:85–156. doi:10.1016/S0065-2113(05)87003-8
- Loecke, T.D., and G.P. Robertson. 2009. Soil resource heterogeneity in the form of aggregated litter alters maize productivity. *Plant Soil* 325:231–241. doi:10.1007/s11104-009-9973-5
- Martinez-Feria, R.A., M.J. Castellano, R.N. Dietzel, M.J. Helmers, M. Liebman, I. Huber, and S.V. Archontoulis. 2018. Linking crop- and soil-based approaches to evaluate system nitrogen-use efficiency and tradeoffs. *Agric. Ecosyst. Environ.* 256:131–143. doi:10.1016/j.agee.2018.01.002
- McNickle, G.G., and J.F. Cahill. 2009. Plant root growth and the marginal value theorem. *Proc. Natl. Acad. Sci. USA* 106:4747–4751. doi:10.1073/pnas.0807971106
- Meinke, H., G.L. Hammer, H. Van Keulen, R. Rabbinge and B.A. Keating. 1997. Improving wheat simulation capabilities in Australia from a cropping systems perspective : Water and nitrogen effects on spring wheat in a semi-arid environment. *Eur. J. Agron.* 7:75–88. doi:10.1016/S1161-0301(97)00032-4
- Rees, R and K. Castle. 2002. Nitrogen recovery in soils amended with organic manures combined with inorganic fertilisers. *Agronomie.* 22:739-746. doi: 10.1051/agro:2002061
- Reich, P. 2002. Root-shoot relations: Optimality in acclimation and adaptation or the “emperor’s new clothes”? In: Y. Waisel, et al., editors, *Plant roots: The hidden half*. 3rd ed. Marcel Dekker, New York. p. 205–220. doi:10.1201/9780203909423.ch12
- Robertson, G.P., and S.K. Hamilton. 2015. Long-term ecological research in agricultural landscapes at the Kellogg Biological Station LTER site: Conceptual and experimental framework. In: S.K. Hamilton, et al., editors, *The ecology of agricultural landscapes: Long-term research on the path to sustainability*. Oxford Univ. Press, New York. p. 1–32.
- Robertson, G.P., S.K. Hamilton, S.J. Del Grosso, and W.J. Parton. 2011. The biogeochemistry of bioenergy landscapes : Carbon, nitrogen, and water considerations. *Ecol. Appl.* 21:1055–1067. doi:10.1890/09-0456.1
- Robertson, G.P., E.A. Paul, and R.R. Harwood. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gasses to the radiative forcing of the atmosphere. *Science* 289:1922–1924. doi:10.1126/science.289.5486.1922
- Robertson, G.P., and P.M. Vitousek. 2009. Nitrogen in agriculture: Balancing the cost of an essential resource. *Annu. Rev. Environ. Resour.* 34:97–125. doi:10.1146/annurev.envIRON.032108.105046
- Roumet, C., C. Urcelay, and S. Díaz. 2006. Suites of root traits differ between annual and perennial species growing in the field. *New phytol.* 170:357–68.
- Russell, A.E., C. Cambardella, D.A. Laird, D.B. Jaynes, and D.W. Meek. 2009. Nitrogen fertilizer effects on soil carbon balances in Midwestern U.S. agricultural systems. *Ecol. Appl.* 19:1102–1113. doi:10.1890/07-1919.1
- Ryser, P., and H. Lambers. 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. *Plant Soil* 170:251–265. doi:10.1007/BF00010478
- SAS Institute. 2011. *The SAS system for Windows*. Release 9.3. SAS Inst., Cary, NC.
- Sprunger, C.D., S.W. Culman, G.P. Robertson, and S.S. Snapp. 2018. Perennial grain on a Midwest Alfisol shows no sign of early soil carbon gain. *Renew. Agric. Food Syst.* 33:360–372. doi:10.1017/S1742170517000138
- Syswerda, S.P., B. Basso, S.K. Hamilton, J.B. Tausig, and G.P. Robertson. 2012. Long-term nitrate loss along an agricultural intensity gradient in the Upper Midwest USA. *Agric. Ecosyst. Environ.* 149:10–19. doi:10.1016/j.agee.2011.12.007 [erratum: 149:65].
- Weih, M., L. Asplund, and G. Bergkvist. 2011. Assessment of nutrient use in annual and perennial crops: A functional concept for analyzing nutrient use efficiency. *Plant Soil* 339:513–520. doi:10.1007/s11104-010-0599-4

West, T.O., and W.M. Post. 2002. Soil organic carbon sequestration rates by tillage and crop rotation. *Soil Sci. Soc. Am. J.* 66:1930–1946. doi:10.2136/sssaj2002.1930

Wichern, F., E. Eberhardt, J. Mayer, R.G. Joergensen, and T. Muller. 2008. Nitrogen rhizodeposition in agricultural crops: Methods, estimates and future prospects. *Soil Biol. Biochem.* 40:30–48. doi:10.1016/j.soilbio.2007.08.010

Fig. 1. Coarse root biomass values in annual winter wheat (triangles) and perennial intermediate wheatgrass (IWG, circles) for three N levels (Low N [Organic N], Mid N, and High N) over 3 yr (2011, 2012, and 2013) at five different soil depths. Error bars represent the SEM, asterisks denote significance at $p < 0.05$, and t denotes significance at $p < 0.1$.

Fig. 2. Fine root biomass values in annual winter wheat (triangles) and perennial intermediate wheatgrass (IWG, circles) for three N levels (Low N [Organic N], Mid N, and High N) over 3 yr (2011, 2012, and 2013) at five different soil depths. Error bars represent the SEM, asterisks denote significance at $p < 0.05$, t denotes significance at $p < 0.1$.

Fig. 3. Root/shoot ratios of wheat and perennial intermediate wheatgrass (IWG) in 2011, 2012, and 2013 over three N levels (Low N [Organic N], Mid N, and High N). The sum of total coarse and total fine roots was used to calculate total root biomass. Total straw and grain were summed to determine total shoot biomass. Error bars represent the SEM, and different letters denote significance at $p < 0.05$.

Figure 1

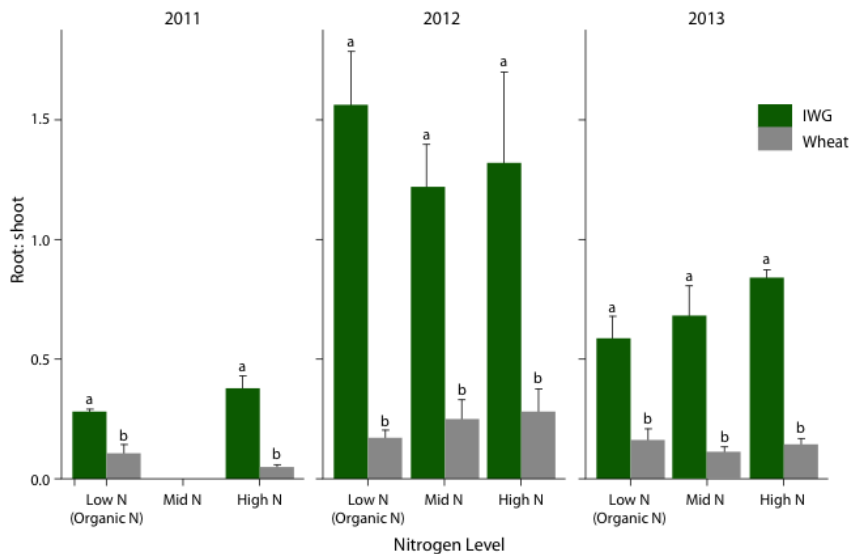


Figure 2

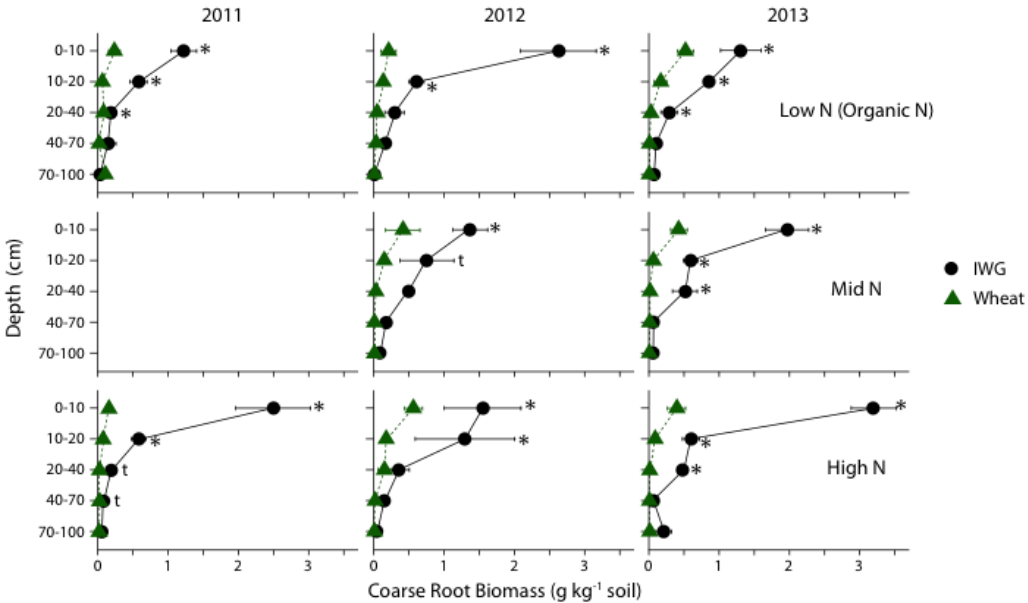


Figure 3

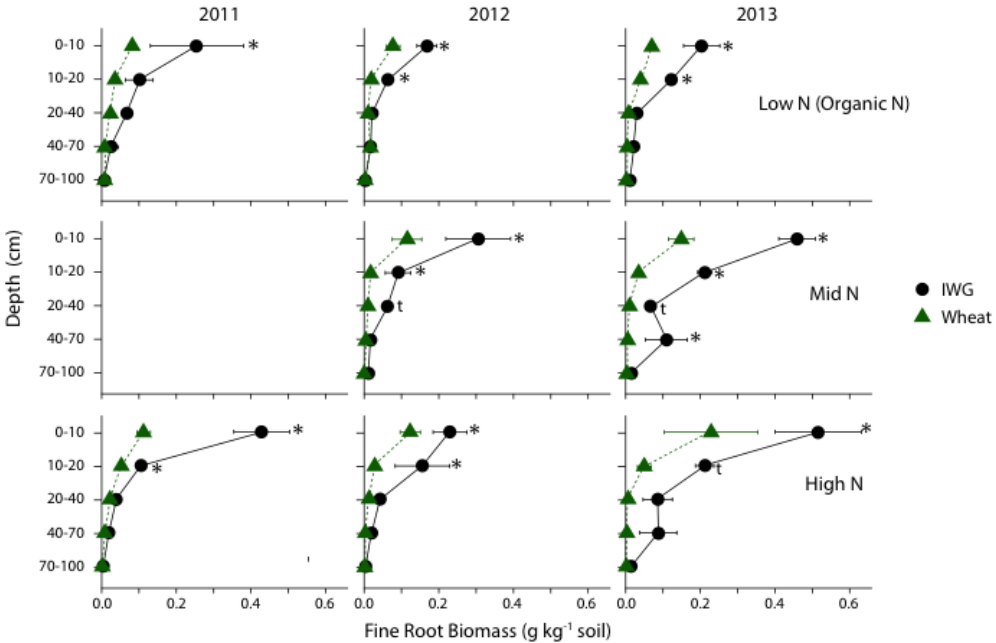


Table 1. Gravimetric soil moisture at five depths throughout the soil profile in wheat and perennial intermediate wheatgrass (IWG) in 2011, 2012, and 2013, averaged across N levels (means ± SE).

Depth cm	Soil moisture					
	2011		2012		2013	
	Wheat	IWG	Wheat	IWG	Wheat	IWG
	% —————					
0–10	9.1 (0.6)a†	8.0 (0.5)a	9.4 (0.6)a	8.4 (0.5)a	11.4 (0.5)a	10.8 (1.2)a
10–20	8.0 (0.5)a	7.3 (0.4)a	9.6 (0.5)a	8.0 (0.4)b	11.3 (0.5)a	10.2 (0.4)a
20–40	8.8 (0.6)a	8.5 (0.7)a	8.0 (0.4)a	7.3 (0.6)a	11.8 (0.6)a	10.6 (0.6)a
40–70	10.4 (0.5)a	8.5 (0.4)b	8.9 (0.5)a	7.7 (0.5)b	11.7 (0.6)a	9.5 (0.6)b
70–100	8.4 (0.6)a	7.6 (0.5)a	7.9 (0.6) a	5.6 (0.5)b	9.9 (0.9)a	7.8 (0.5)b

† Different letters within years denote significant differences between crops for each depth combination at $p < 0.05$.

Table 2. Above- and belowground biomass in annual wheat and perennial intermediate wheatgrass (IWG) across three N levels in 2011, 2012, and 2013 (means ± SE). Roots were sampled to 1 m. Root size classifications were coarse (>6 mm) and fine (1–6 mm).

N rate	Aboveground		Coarse roots		Fine roots		Total crop biomass	
	Wheat	IWG	Wheat	IWG	Wheat	IWG	Wheat	IWG
	Mg ha ⁻¹ —————							
2011								
Low N	12.63 (1.8)c†	14.9(1.1)b	1.1 (0.6)b	3.4 (0.5)a	0.31(0.02)b	0.99 (0.1)a	14.0 (2.2)b	19.29 (2.5)a
Mid N	12.53 (1.2)c	19.9 (1.2)a	NA‡	NA	NA	NA	NA	NA
High N	14.73 (0.7)b	15.9 (0.8)b	0.4 (0.1)b	5.0 (0.7)a	0.34 (0.05)b	0.99 (0.3)a	15.4 (0.8)b	21.89 (1.1)a
2012								
Low N	5.99 (0.58)a	4.3 (0.6)b	0.7 (0.2)b	5.93 (0.5)a	0.26 (0.06)b	0.43 (0.07)a	6.95 (0.7)b	10.65 (0.8)a
Mid N	5.16 (0.9)a	5.78 (0.9)a	0.9 (0.3)b	5.75 (0.5)a	0.24 (0.03)b	0.82 (0.2)a	6.25(0.8)b	12.36 (1.3)a
High N	7.4 (0.8)a	5.33 (0.5)a	1.6 (0.4)b	5.8 (1.4)a	0.27 (0.03)b	0.78 (0.2)a	9.3 (0.3)b	11.9 (1.2)a
2013								
Low N	8.4 (0.81)b	10.6 (0.7)a	1.1 (0.3)c	5.3 (0.7)b	0.21 (0.02)c	0.76(0.07)b	9.77 (0.9)c	16.6 (0.6)b
Mid N	9.68 (0.2)b	11.9 (0.8)a	0.8 (0.2)c	6.1 (1.0)b	0.33 (0.06)c	1.8 (0.33)a	10.8 (0.2)c	19.86 (1.5)a
High N	8.99 (0.6)b	12.2 (0.5)a	0.8 (0.2)c	8.45 (0.6)a	0.43 (0.2)c	1.9 (0.4)a	10.2 (0.5)c	22.54 (1.4)a

† Different letters denote statistical differences across crop and N level means within a given year for each response variable.

‡ NA, .

Table 3. Total N content in annual wheat and perennial intermediate wheatgrass (IWG) across three N levels in 2012 and 2013 (means \pm SE). Roots were sampled to 1 m. Root size classifications were coarse (>6 mm) and fine (1–6 mm).

N rate	Aboveground		Coarse roots		Fine roots		Total crop biomass	
	Wheat	IWG	Wheat	IWG	Wheat	IWG	Wheat	IWG
kg N ha ⁻¹								
2012								
Low N	53.4 (7.1)a [†]	35.9 (3.5)a	5.84 (1.6)b	41.4 (6.1)a	1.5 (0.3)c	3.2 (0.3)b	60.9 (8.1)c	80.5 (9.4)b
Mid N	53.2 (14.2)a	49.2 (6.2)a	6.86 (2.1)b	40.7(2.7)a	2.2 (0.3)c	5.24 (0.7)a	62.3 (10.1)c	95.1 (8.3)b
High N	67.6 (10.3)a	49.8 (3.0)a	13.2 (4.1)b	55.3(8.5)a	2.5(0.2)c	5.5 (1.6)a	83.3 (4.8)b	110.6 (5.0)a
2013								
Low N	58.2 (5.0)a	63.8 (5.4)ac	6.9 (1.9)c	20.0 (2.9)b	2.0 (0.2)c	5.0 (0.5)b	67 (5.8)d	88.8 (1.9)c
Mid N	68.1 (6.1)c	88.5 (10.7)b	6.1(1.6)c	34 (6.0)b	3.3 (0.5)c	12.0 (2.4)a	77.5 (5.5)cd	134.5 (8.7)a
High N	102.2 (15.8)a	76.2 (4.3)bc	6.3 (1.6)c	52.1 (3.5)a	3.9 (1.6)c	13.5 (3.7)a	112 (15.2)b	141.8 (9.9)a

[†] Different letters denote statistical differences across crop and N level means within a given year for each response variable.

Table 4. Fertilizer use efficiency in harvested N, root N, and total plant N. Nitrogen use efficiency (NUE) ratios >1.0 indicate that the plant took up more N than what was applied during the growing season.

N rate	Aboveground NUE		Root NUE		Whole-plant NUE	
	Wheat	IWG	Wheat	IWG	Wheat	IWG
2012						
Low N	0.52 (0.05)a [†]	0.4 (0.04)a	0.07 (0.01)b	0.5 (0.07)a	0.59 (0.04)c	0.89 (0.1)b
Mid N	0.46 (0.1)a	0.55 (0.07)a	0.1 (0.02)b	0.51 (0.02)a	0.56 (0.12)c	1.05 (0.09)a
High N	0.45 (0.07)a	0.37 (0.03)a	0.12 (0.04)b	0.45 (0.06)a	0.57 (0.03)c	0.83 (0.1) b
2013						
Low N	0.65 (0.06)a	0.71 (0.06)a	0.09 (0.02)c	0.27 (0.05)b	0.75 (0.06)c	0.98 (0.02)b
Mid N	0.76 (0.07)a	0.75 (0.06)a	0.1 (0.02)c	0.51 (0.09)a	0.86 (0.06)c	1.5 (0.09)a
High N	0.75 (0.1)a	0.56 (0.03)a	0.08 (0.01)c	0.49 (0.04)a	0.83 (0.1)c	1.05 (0.07)b

[†] Different letters denote statistical differences across crop and N level means within a given year for each response variable.