

Article

Root Characteristics of Perennial Warm-Season Grasslands Managed for Grazing and Biomass Production

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Abstract: Minirhizotrons were used to study root growth characteristics in recently established fields dominated by perennial C4-grasses that were managed either for cattle grazing or biomass production for bioenergy in Virginia, USA. Measurements over a 13-month period showed that grazing resulted in smaller total root volumes and root diameters. Under biomass management, root volume was 40% higher (49 vs. 35 mm³) and diameters were 20% larger (0.29 vs. 0.24 mm) compared to grazing. While total root length did not differ between grazed and biomass treatments, root distribution was shallower under grazed areas, with 50% of total root length in the top 7 cm of soil, compared to 41% in ungrazed exclosures. These changes (*i.e.*, longer roots and greater root volume in the top 10 cm of soil under grazing but the reverse at 17–28 cm soil depths) were likely caused by a shift in plant species composition as grazing reduced C4 grass biomass and allowed invasion of annual unsown species. The data suggest that management of perennial C4 grasslands for either grazing or biomass production can affect root growth in different ways and this, in turn, may have implications for the subsequent carbon sequestration potential of these grasslands.

Keywords: prairie plants; minirhizotron; roots; grazing; biofuels

1. Introduction

Forage and grazinglands in much of the humid eastern United States are dominated by cool-season (C3-photosynthesis) species. Perennial warm-season (C4-photosynthesis) grasses are rarely used intentionally for agricultural purposes in this region, but this may change in coming years. In particular, perennial warm-season grasses (e.g., switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman), etc.) that once dominated the native tall grass prairie in the central US may be especially well suited to provide biomass for biofuel or forage for grazing to complement cool-season species in eastern forage systems. Perennial C4 grasses can be highly productive while sequestering carbon (C), and these species are under increasing scrutiny for their potential to produce renewable bioenergy as well reducing greenhouse gas (GHG) emissions to mitigate global warming. Perennial C4 grasses have deep and extensive root systems with an estimated root biomass of 14 Mg ha⁻¹ [1], which contributes to the generation of a soil C pool as large as 191 Mg C ha⁻¹ in the top 3-m of grassland soils [2]. Life cycle assessments, which measure the energy and GHG balances of bioenergy production, suggest that perennial species (*i.e.*, cellulosic biomass) have a higher net energy ratio and greater reductions in GHG emissions than corn grain used for bioethanol [3]. A large part of the reductions in GHGs comes from greater soil organic carbon (SOC) storage under perennial grasses such as switchgrass than under annual cropland: Switchgrass may store more than 15 Mg C ha⁻¹ more than annual cropland, nearly a 9% increase in SOC [4].

In temperate systems, C4 grasses also have great potential to supply ample quantity and adequate quality herbage during the summer, especially during years of drought [5,6]. These grasses are well adapted for growth during the hottest part of the year and are tolerant of drought and low nutrient conditions, and can complement cool-season pastures during the summer. Although C3 grasses are generally of higher nutritive value than warm-season grasses, cattle performance may be adequate on C4 pastures, and warm-season pastures can produce over 60% more plant biomass in July and August [5]. Given the multiple use potential of C4 grasslands, it is important to understand how managing for grazing or biomass for bioenergy affects warm-season species root characteristics and their associated ecosystem services such as C storage and soil quality.

Compared with aboveground plant variables, relatively few studies have examined the belowground impacts of grazing. Following grazing or clipping, the majority of the carbon (C) is allocated to new leaf production. For example, defoliated blue grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths) may have 53% of new growth in leaves and only 18% in roots, compared to 33% and 29%, respectively, in control plants [7]. While it is traditionally hypothesized that grazing reduces root growth [8,9], other studies suggest that root growth may remain the same or even increase [10–12], sometimes after a several-week lag period [13]. Grazing can also affect C, N, and P pools and nutrient cycling by causing changes in grassland composition and animal activities that can increase the breakdown and incorporation of plant litter into soil [14,15].

Rooting distributions may shift under grazing, with root penetration depth and lateral spread decreasing in grazed areas [9]. In native tall grass prairies, mowing can increase root biomass in the

top 10 cm of soil by 36%, although there may be no significant differences in total root biomass due to the mowing treatment [16]. Grazing can increase the production of roots <0.5 mm in diameter by nearly 75%, compared to ungrazed pastures [17]. Likewise, root dynamics may be altered under grazing, with root mortality increasing by as much as 22% in soils under grazing [12]. This increase in root mortality may be partially explained by reductions in root diameter in soils under grazing as roots with larger diameters have increased longevity [18]. It may also be due to changes in species composition: Root longevity of warm-season grasses is nearly five times greater than the longevity of forbs and legume roots, while longevity of cool-season grasses is also less than that of warm-season species [19]. The objective of this study was to examine how seasonal root morphology and rooting distributions were affected in grasslands managed for either grazing or biomass production. Using minirhizotrons, root images were collected for thirteen months under grazed areas and within grazing exclosures managed for biomass production for bioenergy. We tested the hypothesis that grazing would stimulate root growth and shift rooting distributions to shallower depths.

2. Materials and Methods

The experiment was conducted at the Virginia Tech College Farm near Blacksburg in Montgomery County, VA (37°12'0"N latitude and 80°34'40"W longitude, elevation of ~540 m). Soils were moderately- to well-drained Unison and Braddock cobbly soils (fine, mixed, semiactive, mesic Typic Hapludults and fine, mixed, semiactive, mesic Typic Hapludults), Unison and Braddock soils, and Guernsey silt loam (fine, mixed, superactive, mesic Aquic Hapludalfs), with slopes ranging from relatively flat to moderately steep, 2% to 25% slopes [20]. Soil properties (particle size analysis, pH, soil bulk density) are displayed in Table 1. Soil texture analysis using the hydrometer method describes soils as loams until 30 cm soil depth, where clay loams are present. Soil pH gradually rises from 6.1 to 6.5 and soil bulk density increases from 1.0 g·cm⁻³ to 1.5 g·cm⁻³ as soil depth increases to 40 cm.

Table 1. Soil pH, particle size analysis, soil texture, and bulk density (SBD) in the established warm-season native grasslands from 0–40 cm soil depth. The SBD is recorded for both grazed areas and exclosures.

Depth (cm)	pH	Sand	Silt	Clay	Texture	SBD, Grazed	SBD, Exclosure
0–5	6.08	51.2	31.1	17.8	Loam	1.00	1.01
5–10	6.10	46.7	34.9	18.5	Loam	1.38	1.48
10–20	6.31	47.2	32.9	20.0	Loam	1.34	1.37
20–30	6.50	41.2	35.6	23.3	Loam	1.48	1.51
30–40	6.52	38.7	34.4	27.0	Clay Loam	1.51	1.42

2.1. Vegetation

Minirhizotrons were placed within four 0.3 ha fields that were sown with a mixture of native prairie grasses in June 2008. Four native grass species were sown: Switchgrass (*Panicum virgatum* L.), indiagrass (*Sorghastrum nutans* (L.) Nash), big bluestem (*Andropogon gerardii* Vitman) and Virginia wildrye (*Elymus virginicus* L.). All fields were originally in endophyte-free tall fescue (*Festuca arundinacea* Schreb.), each between 1 and 1.1 ha. Seeds were planted into killed sod using a

Haybuster[®] notill seed drill (Jamestown, ND, USA) at a seeding density of 12.4 pure live seed kg ha⁻¹; by weight, 20% was switchgrass, 40% was big bluestem, 30% was indiangrass, and 10% was Virginia wildrye. By 2011, Virginia wildrye was rare within plots (found in only 3 plots during the August 2011 sampling), so plots were largely sown with warm-season species. Weeds and tall fescue were initially controlled by a glyphosate application at 2.3 L ha⁻¹ one month prior to seeding, followed by an herbicide application in early June 2008 using Journey[®] (BASF, Research Triangle Park, NC, USA), a mixture of imazapic (8.13%) and glyphosate (21.94%), applied at 0.88 L ha⁻¹ as a pre-emergent treatment to control weeds. In 2009, Journey[®] was also used for spot-spraying to control emerging patches of thistles. No fertilizers were applied during the entire experiment.

In each pasture, a 50 m² grazing enclosure was fenced off in May 2009 and managed mimic grassland used for biomass for bioenergy. These biomass for bioenergy enclosures will be referred to as “enclosures” in this paper. Cows grazed the pastures in 2009 in early June for a length of two weeks and then again at the start of August for one month. In 2010, cows grazed the plots starting at the end of May for 16 days and the beginning of August for three weeks. In 2011 and 2012, cows began grazing in early June, with the second graze periods beginning in late August. Pastures were stocked at three cows ha⁻¹ with a stocking density of about 1630 kg live weight ha⁻¹. Grazing began when forage height reached a minimum of 60 cm and continued until canopy height was reduced to 16 cm. Warm-season pastures were mowed between graze periods to a maximum of 30 cm canopy height. Mowing was performed to even out the heights of grazed swards and remove any inflorescences that may have begun to emerge. The enclosures were allowed to grow all season and then harvested in late October.

Herbage mass was measured within grazed areas and enclosures just prior to each grazing period in early June 2011 and 2012. Species relative cover was analyzed by visually estimating the ground cover of each species within a 0.5 m² quadrat. Six or three randomly placed 0.5 m² quadrats in each grazing area and enclosure, respectively, were used to estimate relative cover. After visual estimation, standing biomass was clipped from one-half of each quadrat (0.25 m² sample area). The cut biomass was hand-sorted to sown species or unsown “weeds”, then dried and weighed. It should be noted that some “weeds” may actually be species with significant forage value such as Kentucky bluegrass (*Poa pratensis* L.) or clovers (*Trifolium* spp.), but as these were not originally sown, they are in this case termed “weeds”.

2.2. Minirhizotron Installation and Image Capture

While many root studies involve plants in pots or destructive field measurements, minirhizotrons provide an *in situ*, nondestructive, and continuous method to analyze fine root responses. Although minirhizotrons pose several advantages over other techniques such as soil coring and in-growth cores, difficulties with tube installation and time-consuming image analysis are two drawbacks for this technique [21]. Directly studying roots can allow calculations of root production and root turnover of individual roots across seasons or years.

The minirhizotrons were installed in early November 2010 to a 50 cm depth. Three minirhizotron tubes (inner and outer diameters of 5.0 cm and 5.6 cm, respectively) were installed at a 45° angle inside and outside each of the four enclosures (Figure 1). Root images were captured by a BTC I-CAP

Image Capture System (Bartz Technology Corporation, Carpinteria, CA, USA) (Figure 2). Images were collected every 30 days from May 2011 to May 2012. No data were collected in August 2011. Soil-root images (2.5 cm^2 in size) were taken at 5 cm intervals along the minirhizotron tube (3.5 cm intervals of vertical soil depth) beginning at ~ 1 cm soil depth, and extending to 45 cm in the tube, or 32 cm vertical soil depth. The images were analyzed using the software WinRHIZO Tron (Regent Instruments Inc., Quebec City, Quebec, Canada). Roots were manually traced to estimate individual root length and diameter for determination of root length, surface area, volume, and average root diameter in each image. Results were reported based on measurements within each 2.5 cm^2 image and at the 5 cm minirhizotron tube length markers (*i.e.*, reports at 5 and 10 cm tube depths are 3.5 and 7 cm vertical soil depths, respectively). No attempts were made to separate live roots from dead roots, and as such, root turnover could not be estimated.

Figure 1. Set-up of minirhizotrons in the field. A hole was dug in the soil and three minirhizotron tubes were inserted at 45° angles into the ground. The hole was then capped to prevent light and moisture from entering. To collect photographs, an imaging system was slid into each hole, collecting pictures at marked locations every 5 cm along the tube.

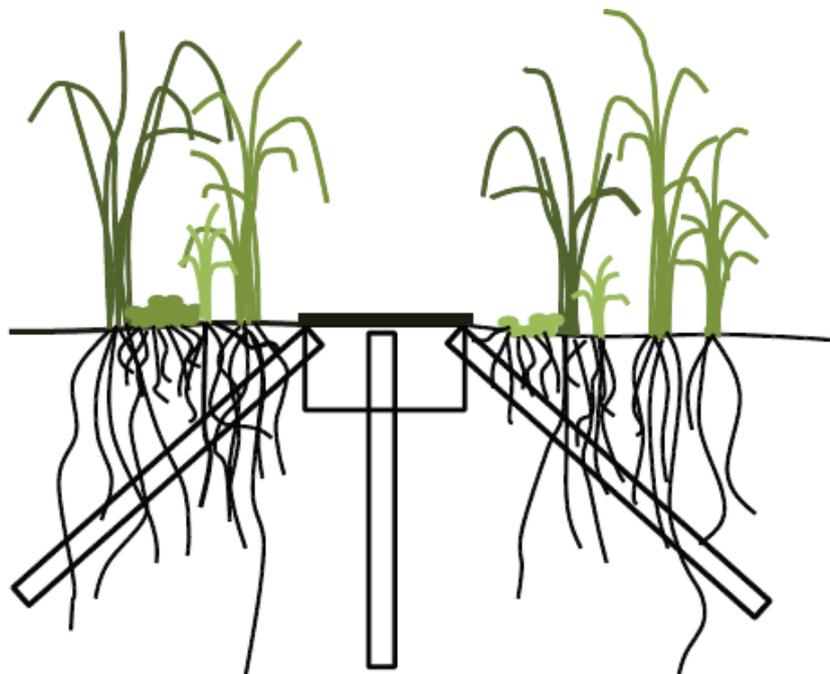
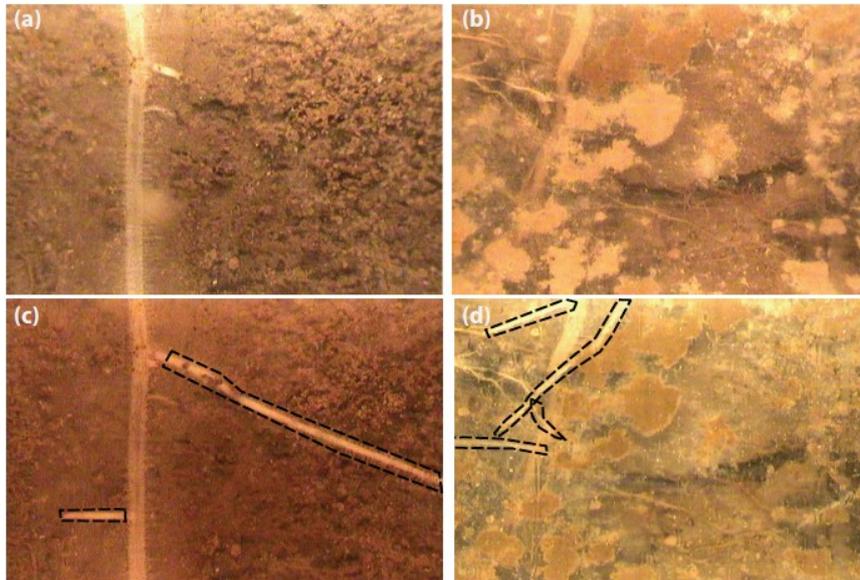


Figure 2. Images collected from the minirhizotron. One set was collected at the 25 cm minirhizotron tube depth (17 cm vertical soil depth) in (a) May 2011 and (c) June 2011. The second set was collected from the 5 cm minirhizotron tube depth (3.5 cm vertical soil depth) in (b) December 2011 and (d) January 2011. Roots boxed by dashed lines indicate newly appearing roots based on the previous month's image.



2.3. Data Analysis

Data were analyzed with SAS software 9.3 (SAS Institute Inc., Cary, NC, USA) using repeated measures ANOVA, with treatment (grazed or managed for biomass) and date (twelve months of images) as variables. The three tubes within each pasture and treatment were treated as subsamples, and the results from each set of three tubes were averaged. When all ten depths were tested, each depth was analyzed separately. The Fisher's Least Significant Difference (LSD) test was used to separate means where either treatment or date was statistically significant at $p < 0.05$. Since no interaction terms (treatment \times date) were significant, only the main effects of date and treatment are discussed in the Results section.

3. Results

3.1. Weather

Historic records from a nearby weather station show that the temperature from April through September (the primary period of growth for C4 species) averages 17.9 °C in this area, while total precipitation during this time averages 52.1 cm (Table 2). The years 2010 through 2012 were warmer than average by 1.2 to 1.7 °C. The 2010 growing season was particularly dry, with only about 70% of the historic average precipitation. In 2010, the June through August temperatures averaged 23 °C, compared to the historic average of 20.9 °C, and early season (April–June) precipitation was very low, at only 48% of historic averages. In 2011 and 2012, precipitation was closer to seasonal averages.

Table 2. Historic and monthly average temperatures and total precipitation for March through September 2008 through 2012 in the Kentland Farm region.

Month	Historic Averages		2008		2009		2010		2011		2012	
	Temp † (°C)	Precip (cm)	Temp (°C)	Precip (cm)								
March	5.7	8.5	6.7	5.1	6.2	8.6	6.5	6.5	6.3	11.7	10.7	7.2
April	10.6	7.8	11.6	9.7	11.5	6.8	12.8	3.4	13.1	12.8	11.9	7.8
May	15.6	10.2	15.0	5.3	16.4	18.9	17.6	6.3	16.5	16.1	18.1	8.7
June	19.7	10.0	20.9	5.6	20.9	9.2	22.6	3.7	21.5	2.7	20.1	5.4
July	21.9	9.4	21.2	14.1	20.2	9.4	23.6	6.1	23.5	11.2	23.5	9.9
August	21.2	7.2	19.7	7.1	21.4	7.4	22.9	8.8	22.0	4.4	21.3	7.2
September	17.7	7.9	18.0	3.4	17.4	6.9	18.5	8.3	17.9	10.3	17.3	7.3
Aver Temp	16.1	--	16.2	--	16.3	--	17.8	--	17.3	--	17.6	--
Total Precip	--	61.0	--	50.3	--	67.2	--	43.1	--	69.2	--	53.5

† Abbreviations are as follows: Temp: temperature; Precip: precipitation; Avg Temp: the average temperature from March through September; Total Precip: the total precipitation from March through September.

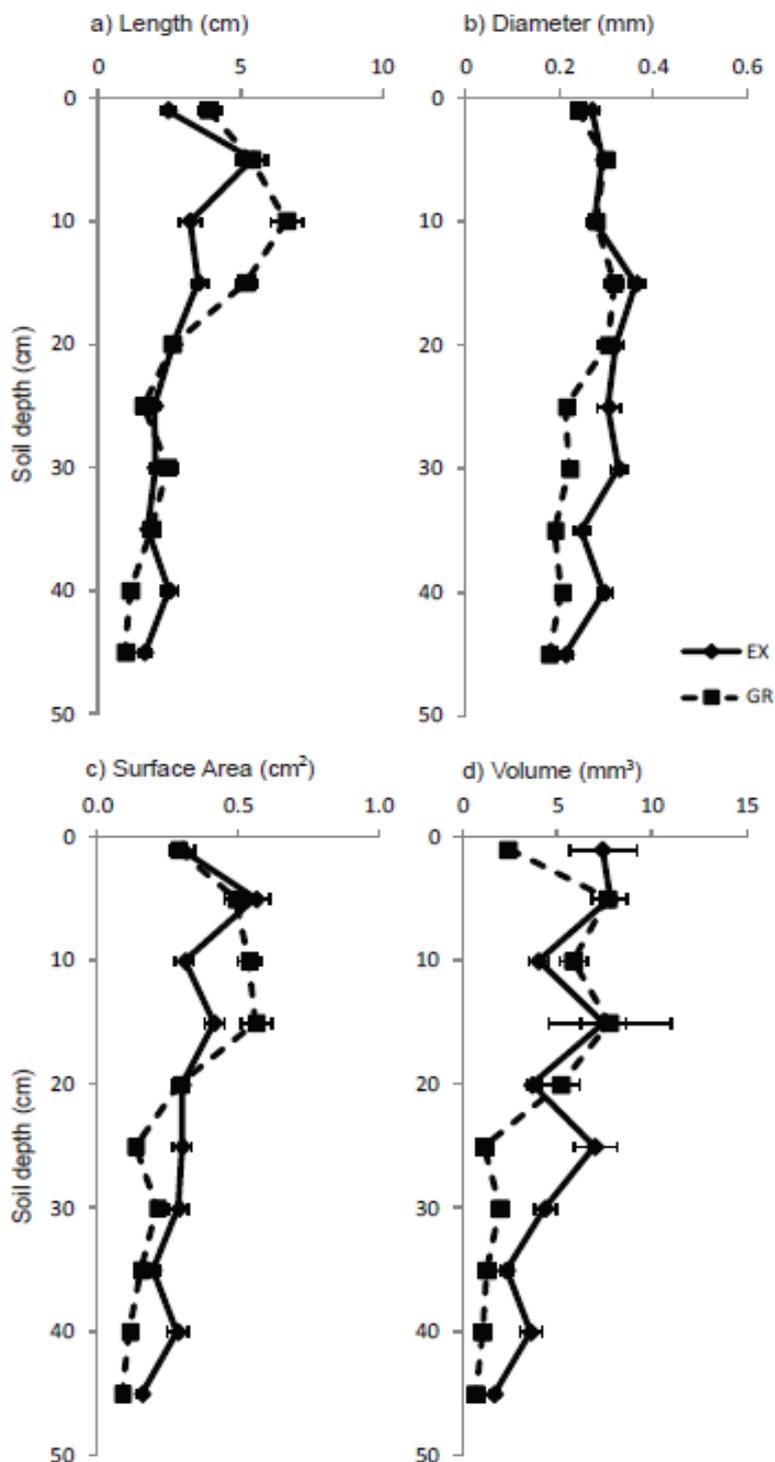
3.2. Vegetation

Although total biomass did not differ between grazed and enclosure treatments in June 2011, the biomass plots tended to contain more sown grass biomass by dry weight (2025 kg ha⁻¹ vs. 1520 kg ha⁻¹) and less weedy biomass (850 kg ha⁻¹ vs. 1140 kg ha⁻¹). Results were similar for June 2012, although sown herbage mass increased to 2745 kg ha⁻¹ for grazed areas and 4435 kg ha⁻¹ for biomass plots. In June 2012, weed biomass was nearly 3.5 times greater in grazed areas than biomass plots. From 2011 to 2012, sown grass species cover in enclosures increased from 50% to 76%, while in grazed areas sown species cover remained at 47%, with unsown species comprising the remaining cover.

3.3. Roots

The effect of month was significant for total root length, surface area, and volume within minirhizotron images when summed across all ten depths ($p < 0.0001$ for length and surface area, and $p = 0.005$ for volume). Most root growth occurred during the spring and fall periods sampled during this experiment. With the exception of root diameter, root variables also tended to decrease with increasing soil depth (Figure 3). When variables were analyzed by soil depth, sampling date was more often significant at minirhizotron tube depths ≥ 30 cm (21 cm vertical soil depth) than those depths nearer the surface. Few differences in root characteristics were noted immediately following the June and August grazing periods.

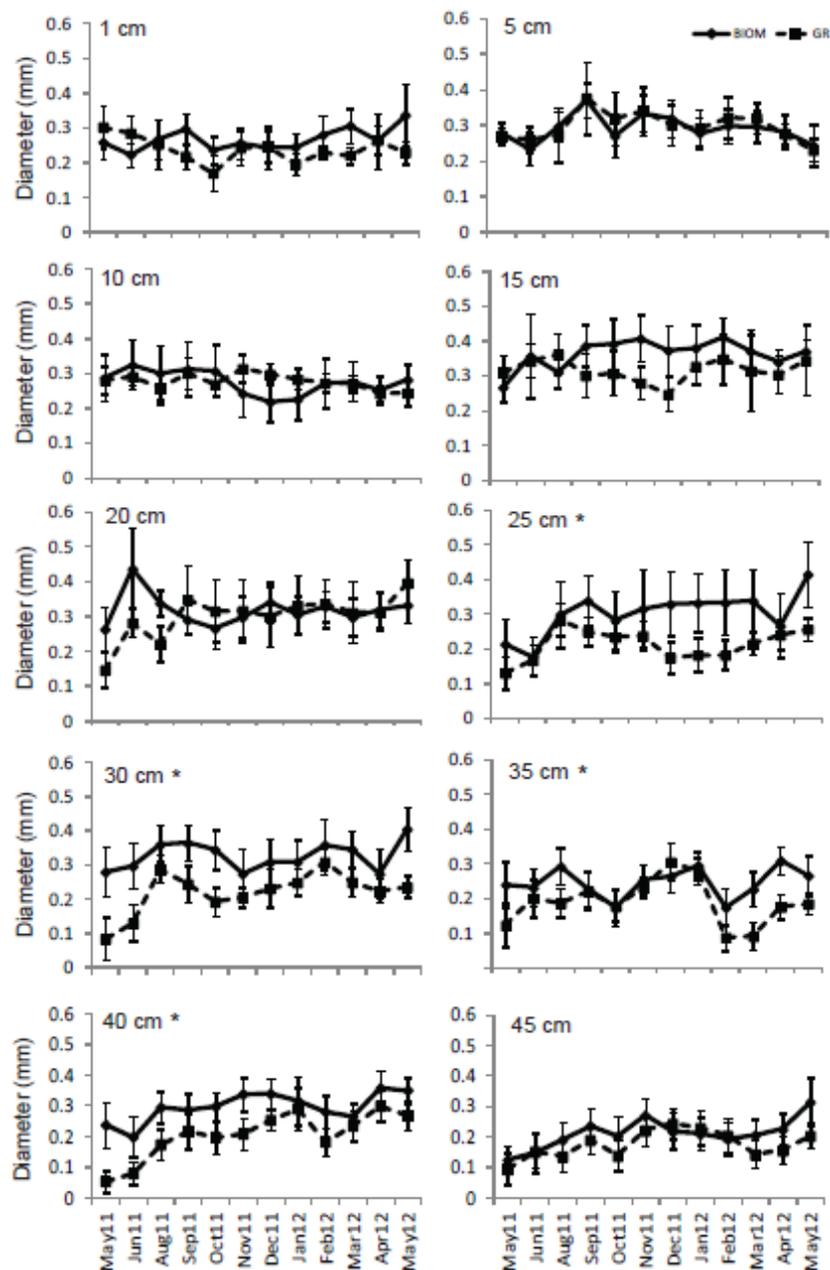
Figure 3. Thirteen-month averages by minirhizotron tube length (5 cm tube length = 3.5 cm vertical soil depth) for (a) total root length; (b) average root diameter; (c) total surface area; and (d) total root volume in soils under established native pastures managed for grazing (GR) and biomass crop exclosures (BIOM).



Total root length was the longest in the top 15 cm (*i.e.*, 10.6 cm vertical soil depth) and was the smallest at the deepest depth (Figure 3a). In exclosures, the largest root lengths occurred at the 5 cm depth. The largest root length under grazed grasses was at 10 cm. Grazing increased root length in the top 15 cm, and was 55% larger in the top 1 cm ($p = 0.035$) and 110% larger in the 10 cm depth

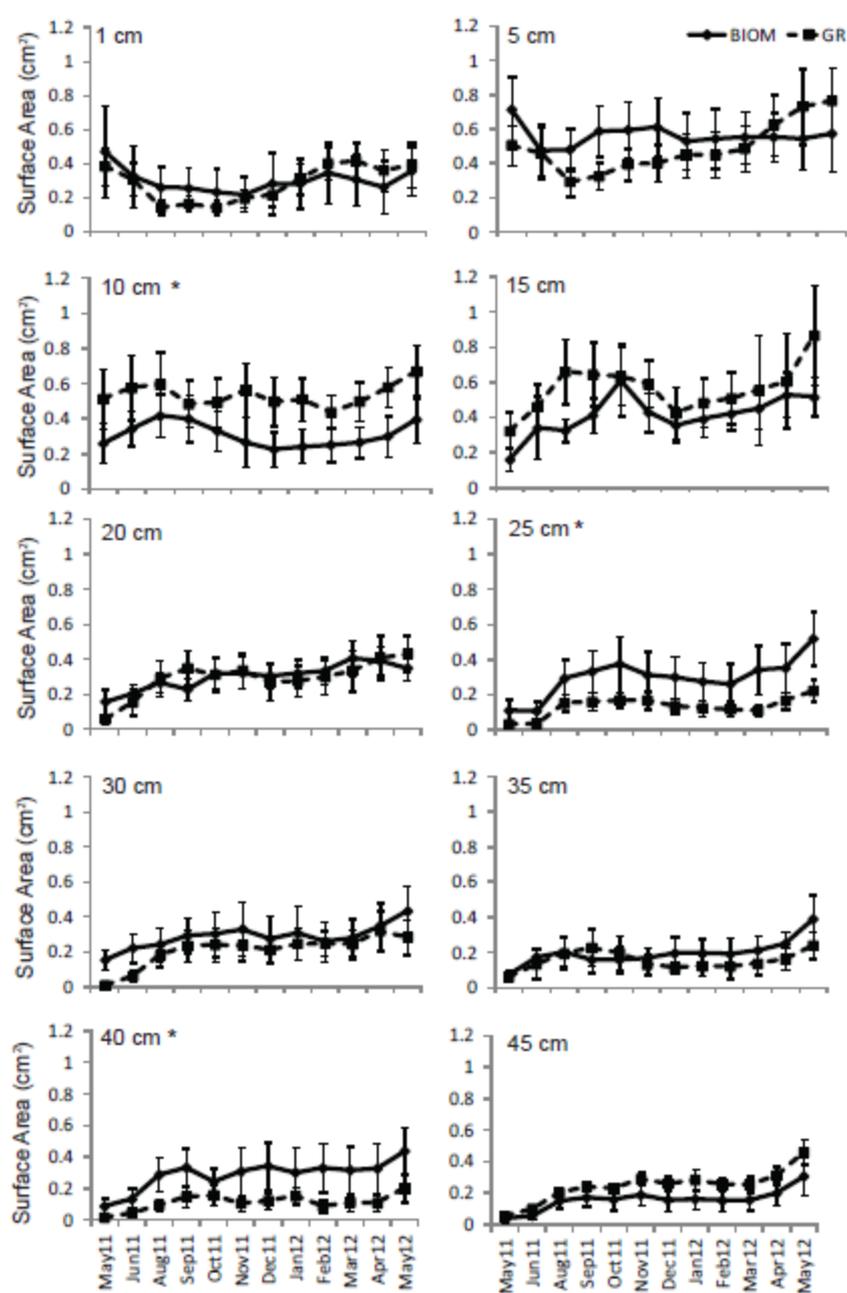
Most roots identified in this experiment were <2 mm in diameter, defined as fine roots [22]. The average root diameter was similar in soils under management for grazing or biomass in the top 20 cm of minirhizotron depths, peaking at about 0.32 mm and 0.36 mm in diameter at the 15 cm depth for biomass and grazing areas, respectively (Figure 3c). The five largest root diameter averages all occurred under exclosures, with a largest average root diameter for an individual soil-root image of 1.5 mm. Between the 25–40 cm depths (17–28 cm vertical soil depths), the root diameter under exclosures was larger ($p < 0.035$ for all four depths) than those under grazed areas (Figure 5).

Figure 5. Average root diameter by month and by minirhizotron tube length (5 cm tube length = 3.5 cm vertical soil depth) in soils under established native pastures managed for grazing (GR) and biomass crop exclosures (BIOM). Asterisks after the minirhizotron length represent depths where root diameter is different in soils under grazing and biomass production ($p < 0.05$).



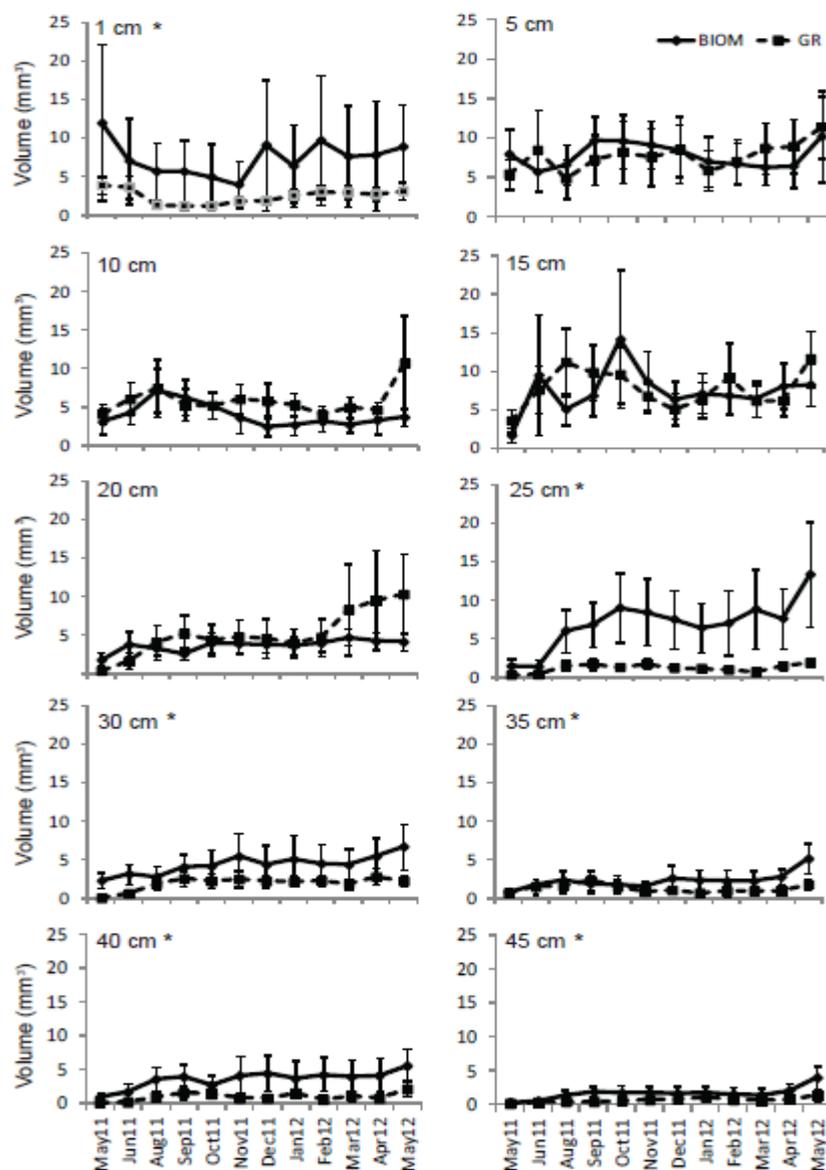
Total root surface area was highest under grazing near the soil surface, particularly at minirhizotron depths of 5–15 cm, while surface areas in exclosures only reached similar levels at the 5 cm depth (Figure 3b). Below the 15 cm depth, root surface area under grazing declined by 25% compared with peak values at the 15 cm depth. As a result, root surface area under biomass management tended to be larger than those under grazing at minirhizotron depths ≥ 25 cm (Figure 6). Summed across all ten soil depths, root surface area did not differ under grazing or exclosures ($p = 0.094$).

Figure 6. Average total root surface area by month and by minirhizotron tube length (5 cm tube length = 3.5 cm vertical soil depth) in soils under established native pastures managed for grazing (GR) and biomass crop exclosures (BIOM). Asterisks after the minirhizotron length represent depths where the root surface area is different in soils under grazing and biomass production ($p < 0.05$).



The root volume peaked at two soil depths under grazed areas and exclosures: The 5 and 15 cm depths (Figure 3d). Excluding grazing significantly increased root volume at 6 depths: The 1 cm depth ($p = 0.035$), and the minirhizotron depths of 25–45 cm ($p \leq 0.03$ for all five depths, Figure 7). The average total root volume summed from all ten depths under exclosures was nearly 40% larger than those under grazing (49 vs. 35 mm³, respectively). Over 67% of the total root volume under grazed areas occurred in the top 15 cm minirhizotron tube depths (10.6 cm vertical soil depth), compared to only 54% in exclosures. Likewise, almost 11% of total root volume under exclosures occurred in the 40–45 cm minirhizotron depths (28–32 cm vertical soil depths), compared to only 5% in soils under grazed areas.

Figure 7. Average total root volume by month and by minirhizotron tube length (5 cm tube length = 3.5 cm vertical soil depth) in soils under established native pastures managed for grazing (GR) and biomass crop exclosures (BIOM). Asterisks after the minirhizotron length represent depths where volume is different in soils under grazing and biomass production ($p < 0.05$).



In summary, averaged across all depths and dates, root length was larger under grazed areas than under exclosures ($1.27 \text{ cm}\cdot\text{cm}^{-2}$ photo image area vs. $1.09 \text{ cm}\cdot\text{cm}^{-2}$ for grazed and ungrazed roots, respectively, $p = 0.0016$). Even though root length was greater under grazed areas, exclosures had a larger average root diameter (0.29 mm vs. 0.24 mm , $p < 0.0001$), and a greater root volume (49 mm^3 vs. 35 mm^3 , $p < 0.0001$) compared to those under grazed areas. Surface area was the only variable that did not differ between the two management practices (0.3 cm^2 per image, $p > 0.10$).

4. Discussion

Grazing is often associated with species composition and cause a shift from deep-rooted, spreading root systems to shallower and less branching systems with smaller root diameters [9,23]. The data obtained in this study on root measurements are in agreement with these results of more shallowly distributed root systems with narrower root diameters.

The shift to a shallower rooting system with less total volume may be due to several changes caused by grazing, such as increased allocation of resources to shoots and changes in species composition. The plots were grazed twice each growing season and this defoliation may have shifted biomass production from root to shoot biomass and reduced root relative growth rates, resulting in a lower seasonal root biomass [24]. In the current experiment, the proportion of sown prairie grass biomass differed in grazed plots and exclosures. In June 2011, prior to beginning a third year of grazing, grasses accounted for 70% of the herbage mass in exclosures, compared with 57% in grazed areas. The difference widened in June 2012, where almost 90% of the herbage mass in exclosures was contributed by sown grasses, compared to almost 60% in grazed areas. The unsown species commonly present included clovers (red, white, hop; *Trifolium* spp.), Kentucky bluegrass (*Poa pratensis* L.), sweet vernalgrass (*Anthoxanthum odoratum* L.), and other annual and short-lived perennial weedy species. The decrease in sown native grasses in grazed areas suggests that grazing may reduce the competitiveness of native grasses, allowing cool-season species and annuals to become more abundant.

The shifts in species composition from native prairie species to non-native species due to our grazing management may have corresponding changes to rooting distributions. Nearly 70% of red and white clover biomass may occur in the top 20 cm of soil [25], and as much as 92% of Kentucky bluegrass may be in the top 33 cm [26]. In contrast, almost half of switchgrass root biomass may be present in the top 30 cm of soil [27]. Big bluestem, a relatively shallow rooting prairie grass species, may have 18% of its root mass in the 18 to 56 cm soil depth [26]. A similar change in root depth is evident in this study as non-native, shallow rooting species increased in abundance in grazed areas. It is also possible that the increased abundance of native perennial grasses in the exclosures increased competition for resources and resulted in deeper root growth. As the pastures were not irrigated or fertilized, it is possible that the plants experienced resource limitation. Skinner and Comas [28] found that in response to drought and N stress, grasses allocated more energy to deep roots while legumes and forbs did not. The data suggest that grasses are better able to tap deeper soil resources during periods of stress.

Total root volume summed across the entire 45 cm minirhizotron tube length (32 cm vertical depth) was greater in exclosures and resulted from larger root diameters rather than any difference in total root length. Other studies also demonstrate that grazing management may cause changes in root

volume by impacting root diameter [29]. Root diameter may affect a species' growth and resource acquisition by affecting root area available to take up resources. While thinner roots are more able to uptake water and nutrients, thicker roots can withstand less favorable soil conditions, penetrate more compacted soils, and have increased longevity [30]. Root diameter is generally affected by species type, with taller species having larger root diameters than shorter species [31]. In the current study, the replacement of tall native grass species by shorter cool-season species and invasive weeds in grazed areas may help explain the observed smaller root diameters measured in these plots.

Furthermore, larger amounts of root length and volume in grazing exclosures were found in deeper soils compared with grazed plots, suggesting that ungrazed grasses may be able to tap water from deep in the soil profile to better withstand moderate drought. The shift in rooting depth distribution may also impact C sequestration, as deep roots can increase C storage at deeper depths [4]. The overall larger root volume within biomass plots suggests that C4 grasslands managed for bioenergy production could have the potential to store more C than if they are managed for grazing.

5. Conclusions

Research on the effects that grazing or biomass for bioenergy production may have on belowground properties is needed to aid in making future recommendations for grassland management, since studies on root properties and dynamics in grasslands are relatively few when compared to aboveground measurements. Even though studies suggest that grazing may stimulate the growth of aboveground tissue and infrequently roots [10,32], in the current study grazing generally reduced root volume and root diameter. Although grazing increased root length near the soil surface, total root length and surface area through 45 cm minirhizotron tube length (32 cm vertical soil depth) were not significantly different under grazed areas and exclosures. In contrast, summed root volume was larger in soils in ungrazed plots managed for biomass. These changes in root characteristics and rooting distribution under grazed and biomass plots were likely caused by changes in plant species composition and reduced energy allocation to root biomass. These observed differences in root characteristics may have implications with respect to nutrient cycling and C sequestration, and more research is needed to understand better how management may impact belowground processes in these ecosystems.

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Conflict of Interest

The authors declare no conflict of interest.

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