CHESTNUT OAK (*QUERCUS PRINUS*) RESPONSE TO BROWSING BY WHITE TAILED DEER: IMPLICATIONS FOR CARBON AND NITROGEN ALLOCATION

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Overabundant white-tailed deer (*Odocoileus virginianus*) populations threaten potential forest regeneration in many parts of the northeastern U.S.A. Growth reductions in forest trees associated with high herbivory have been attributed to limitations in carbon (C), nitrogen (N), and carbohydrate storage in various plant parts. To assess the potential for regrowth of forests subjected to long-standing deer herbivory, this study evaluated the tissue chemistry, biomass accumulation, and ages of chestnut oak (*Quercus prinus*) seedlings growing in locations with different deer densities. We compared low, medium, and high herbivory intensities with the tissue chemistry of plants protected in 25-year old deer exclosure plots. Seedlings of similar height located at plots with higher herbivory were older, had greater total biomass, and had greater non-structural carbohydrates in roots compared to stems. In deer exclosure treatment plots, differences in the tissue chemistry of plants between fenced and unfenced plots were insignificant for all plant parts at all herbivory intensities. This study suggests that future forest regeneration will depend on deer management strategies including the time that regenerating plants will need to be protected in accordance with previous herbivory intensities.

INTRODUCTION

Increased herbivory by white-tailed deer (*Odocoileus virginianus*) has altered vegetation density and biodiversity in the Northeast in the past several decades (Russell et al. 2001; Rooney & Waller 2003; Goetsch et al. 2011). Alterations in plant diversity and density occur when preferred species are eaten, resulting in an increase in more resistant or lower preference species (Horsley et al. 2003; Kain et al. 2011; Slater & Anderson 2014). Constant browsing of preferred plants leads to a reduction in total understory cover (DiTommaso et al. 2014) and hinders the ability of these plants to form new growth unless they are protected from deer browse (Pendergast et al. 2016). Although previous literature indicates a negative relationship between deer browse and vegetation dynamics (Russell et al. 2001; Rooney & Waller 2003; Horsley et al. 2003; Goetsch et al. 2011; Kain et al. 2011; DiTommaso et al. 2014; Slater & Anderson 2014; Pendergast et al. 2016), conflicting studies such as Barrett & Schmitz (2013) suggest that white-tailed deer herbivory has no effect on forest regeneration. And Lucas et al. (2013) point to an increase in the growth of mature trees through increased nutrient availability with addition of fecaes contributed by high deer abundance.

Plants subjected to browsing pressure can respond in multiple ways. Decreased growth in above ground versus below ground biomass from heightened herbivory can contribute to changes in plant tissue chemistry. Several studies have attributed decreased growth in browsed plants to the removal of plant tissue C that is necessary for photosynthesis (Li et al. 2002; Wiley et al. 2013; Deslauriers et al. 2015). Some studies indicate the increase in C stress through removal of plant tissue causes a plant to favor C storage over growth (Hoogesteter & Karlsson 1992; Palacio et al. 2012; Wiley et al. 2013; Wiley et al. 2016). In addition to C stress from deer browse, browsed plants lose substantial N resources necessary for regrowth after plant part removal (Millard 1996). To replenish C and N resources, plants allocate C and N differentially between plant parts in the form of stores for continued growth (Cerasoli 2004). However, herbivory removes multiple storage components for plants, resulting in N limitation, in conjunction with

C limitation, and reduced growth, but Piper et al. (2015) point to reductions in growth from severe defoliation with no changes to N or C availability. Other studies such as Willaume & Pagès (2006) connect increased defoliation with reductions in root biomass which affects plant growth, and these reductions follow fluctuations in starch concentrations in the below ground biomass (Willaume & Pagès 2011).

The purpose of this study is to evaluate C and N in leaves, stems, and roots of chestnut oak (*Quercus prinus*) found in and around deer exclosure plots subject to three levels of herbivory: low, medium and high. Tissue chemistry examinations and age determinations from these samples will help answer the following questions: (1) how do different levels of herbivory affect C and N allocation in the leaves, stems, and roots of plants, (2) do plants use C for storage (carbohydrates) or growth (biomass accumulation) under different levels of herbivory (3) if plants are storing C when browsed, where do they store those carbohydrates (leaves, roots or stems) (4) do the ages of the trees in each herbivory intensity vary with the degree of herbivory. We hypothesize that, in general, under higher herbivory seedlings will have higher C:N ratios, lower above and below ground biomass, larger stores of starch in the below ground biomass, and reductions in growth that allow for older ages of small stature seedlings.

Improving our understanding of the interaction between deer herbivory and forest regeneration is important to identifying the time required for forest regeneration under different deer maintenance strategies. This study aims to provide a link between deer herbivory and the rate of growth associated with low to high herbivory levels to better inform land managers of the length of time needed for certain forests to regrow when deer herbivory is controlled.

METHODS AND MATERIALS

Location

The study took place in deer exclosure plots from three separate locations nearby Millbrook, NY: the Cary Institute of Ecosystem Studies (41.80N, 73.3W), the Rockefeller University Field Research Center (41.76N, 73.75W), and the Mid-County Rod and Gun Club (41.69N, 73.7W). The Cary Institute of Ecosystem Studies and the Rockefeller University Field Research Center are approximately 5 km apart and the Mid-County Rod and Gun Club is 16km from the other two locations. All forest locations were previously used as woodlots or pastures. Logging has not occurred at the locations within the past 50 years.

The locations represent varying degrees of herbivory due to specific deer maintenance strategies. Cary has had a deer management program in place since 1976 that permits doe hunting (Winchcombe 1993) resulting in low deer densities and low rates of herbivory, 70%-95% understory browse, for the location. More moderate levels of herbivory, about 95% understory browse, occur at Mid-County where buck hunting is permitted. High herbivory rates, 95% browse or above, occur at Rockefeller where hunting is prohibited resulting in a greater abundance of deer.

Deer exclosure plots were established in 1992 at four sites at each of the three locations. The exclosures were set up as a pair of fenced and unfenced 10 m x 10 m plots placed within 10 m of each other for each site. Each site was selected so that topography, light conditions, and vegetation cover were similar across all three locations. Plot data collected in 2012 was evaluated to confirm that light availability and C and N in the soils of all three locations did not vary significantly between treatments and locations.

Field and Laboratory Methods

Samples were collected from chestnut oak (*Quercus prinus*) seedlings about 50 cm in height. Seedlings of

this species were chosen due to their relative abundance at each location and their high browse preference among white-tailed deer. Leaf and stem samples were collected from three *Quercus prinus* seedlings within each fenced and unfenced plot for two sites per location (total of xx samples). Root samples were collected from 8 fully uprooted *Quercus prinus* seedlings located 3-5m outside of unfenced plots at each site (total of 24 seedlings). Due to various ongoing studies being conducted inside these permanent plots, entire plants could not be removed from fenced and unfenced plots. The uprooted plants were selected so that they accurately represent the seedlings within the permanent plots in terms of location, amount of defoliation, and size.

Samples were stored at 4°C after collection. Collected leaf and stem samples from treatment plots were weighed, dried at 60°C for three days in an oven, and ground using a ball mill (GENO-GRINDER 1700). The 24 uprooted plants collected from the locations were individually separated into leaves, stems, and roots and each plant part was weighed. Leaf and stem masses from each uprooted plant were combined to account for the above ground biomass and compared to the root mass or below ground biomass of the same plant. Uprooted plant parts were then dried at 60°C for three days and ground. Age determinations of the uprooted trees were evaluated by tree stem bisections prior to grinding. Tree rings were counted using a dissecting microscope (Leica Research 10x).

Leaf, stem, and root samples were analyzed for C, N, and carbohydrate concentrations. Total C and N was determined for all plant parts using a Carlo Erba combustion analyzer. Carbohydrate concentrations were separated into simple sugars and starch. Methods outlined in Chow and Landhäusser (2004) were used to evaluate plant parts for carbohydrate concentrations. To summarize, dried plant material was extracted in 80% ethanol boiled at 95°C and centrifuged. The supernatant was used for the simple sugar analysis using a phenol-sulfuric acid assay and wavelength absorbance to determine glucose, fructose, and sucrose concentrations. The remaining residue from the centrifuged plant extraction was used to determine starch concentrations using enzyme digestion and wavelength absorbance.

Statistical Analysis

Plant parts collected in plots were pooled to identify one average concentration of C, N, and carbohydrates per treatment per site and location. R software was used for statistical analysis to perform three-way ANOVA tests.

RESULTS

C and N Allocation in Plant Parts

Herbivory intensity did not affect C concentrations in leaves and stems of seedlings, however Mid-County supported the highest C concentrations in roots compared to the other herbivory intensities (Fig. 1). Conversely, N concentrations in leaves and roots did not vary with the degree of herbivory, but stem N concentrations were significantly lower at Mid-County than at Cary, which favored the highest N stem concentration mean at 0.781 N (Fig .1). Differences between deer exclosure plot C and N concentrations for plant parts were insignificant between all herbivory intensities and treatment types (Table 2).

C:N ratios between seedling stems were significantly higher at Mid-County than Cary, but ratios for seedling leaves and roots did not vary with herbivory intensity (Fig. 1). All herbivory levels had the lowest C:N ratios in seedling leaves and significantly higher C:N ratios in stems than leaves, but only Cary and Rockefeller supported significantly higher roots C:N ratios than leaves and stems (Fig. 1). Similar to C and N concentrations, plant part C:N ratios between deer exclosure plots indicated no differences for both treatment types in each herbivory intensity (Table 2).

Seedling Ages

Seedlings ages varied with the degree of herbivory intensity. Low herbivory conditions at Cary favored younger plants (~6 years) and high herbivory conditions favored older plants (~9 years). The moderate herbivory conditions at Mid-County warranted plants of similar age to Cary (~7 years), however only Cary and Rockefeller ages were significantly different ($P= 0.038$).

Biomass Accumulation

Biomasses for plant parts (leaves, stems, and roots) of seedlings were not affected by the degree of herbivory (Table 3), however seedling total biomass at Rockefeller was significantly higher than at Cary and Mid-County (Fig. 2). Out of the total biomass for seedlings, roots held the highest plant part biomass percentage for all herbivory intensities. Root biomass percentages ranged from 68%-72% total biomass with the lowest percentages at Rockefeller and the highest at Cary (Table 3). Furthermore, herbivory intensity did not affect above ground biomass (leaves and stems) and below ground biomass (roots) percentages (Table 3), however, below ground biomass was the highest percent of total biomass for all locations (Fig. 2).

Carbohydrate Allocation

Glucose, fructose, and galactose (GFG) concentrations in leaves, stems, and roots were unaffected by herbivory intensity (Table 1). However, within Cary, seedling leaf and stem GFG concentrations were significantly lower than concentrations in roots (Fig. 3). Only stems had lower GFG concentrations than roots at Rockefeller (Fig. 3), and no seedling plant parts had significantly different GFG concentrations within moderate herbivory conditions at Mid-County (Fig. 3). In the deer exclosure plots, comparisons between fenced and unfenced leaves and stems generated GFG concentrations at about (Table 2). Leaf and stem GFG concentration differences were insignificant between all fenced plots in the three locations and all unfenced plots in the three locations (Table 2).

Starch concentrations for seedling leaves did not vary with the degree of herbivory (Table 1). However, Mid-County seedling stem starch concentrations were significantly higher than Cary and Rockefeller concentrations with Cary having the lowest stem starch concentration at 5.275 mg/L (Fig. 3). Furthermore, seedling root starch concentrations were significantly higher at Cary compared to Mid-County, but Rockefeller root starch concentrations were not different from either locations with a mean concentration slightly lower than Cary (Fig. 3). All herbivory intensities warranted higher concentrations of starch in roots than in leaves and stems (Fig. 3). The deer exclosure plots at each herbivory level indicated no difference in leaf or stem starch concentrations between fenced and unfenced treatments (Table 2). Similar to GFG concentrations, leaf and stem starch concentrations were similar between all fenced plots in the three locations and all unfenced plots in the three locations (Table 2).

DISCUSSION

Deer management strategies did not have strong effects on C and N allocation in chestnut oaks. We expected higher herbivory intensities to induce higher C:N ratios, however, C:N ratios did not vary with herbivory intensity. Our results can be explained by differences in the sources of herbivory between this study and most previous studies. Many studies manually defoliated plants so that exact percentages of defoliation, from 50%-100% defoliation, could be surmised (Hoogesteter & Karlsson 1992; Li et al. 2002; Millet et al. 2005; Palacio et al. 2008; Wiley et al. 2013; Wiley et al. 2016). While this study accounted for the degree of herbivory a plant was exposed to based on its location, it could not account for the exact amount of defoliation a plant had received. Although all uprooted seedings at each herbivory intensity showed clear signs of browse, moderate amounts of foliage were still present at the time of uproot. Thus,

resource availability was not depleted from a 100% loss in foliage for any herbivory intensity, and foliage was not necessarily removed differentially between herbivory intensities to warrant significant differences in C:N ratios.

C and N percents of total mass per plant part were also not found to vary with herbivory intensity. In terms of total N in browsed plants, previous studies have concluded that deciduous species effectively store N in woody tissues in the winter (Millard et al. 2001), allowing for quick N remediation after later season browse and overall N to remain unaffected by plant part removal (Millard et al. 2001; Millet et al. 2005; Palacio et al. 2008). Similarly, C partitioning in previous studies involving deciduous trees was unaffected by herbivory (Palacio et al. 2008; Piper et al. 2015). The consistent C and N allocation and regrowth of chestnut oaks regardless of herbivory intensity supports Piper & Fajardo (2014) findings of a resiliency in deciduous trees to increased herbivory.

Larger stores of starch were expected to be found in the roots of seedlings in the highest herbivory intensity, however, there were no significant differences in root starch storage between locations. Although, all herbivory intensities had significantly higher starch stored in roots than the other plants parts of seedlings specific to each location. Previous studies found starch stores in roots of plants to drop directly after defoliation, indicating a shortage in carbohydrates after defoliation (Willaume & Pagès 2011). Our results could not be assessed for fluctuations in starch after browse since uprooted seedling starch concentrations could not be monitored before and after browse. So, it is unknown whether chestnut oak starch reserves fluctuate in the presence of browse as other studies suggest (Wiley et al. 2013; Wiley et al. 2016). In general, our results suggest that regardless of herbivory intensity, chestnut oaks store starch in the roots to support growth after loss of plants parts to herbivory.

The ages of the uprooted seedlings were expected to increase with increasing herbivory, and our results supported our hypothesis. We also expected that trees in higher herbivory intensities would have lower above and below ground biomass, however older seedlings at Rockefeller had significantly higher total biomass than the other two herbivory intensities. The biomass and ages of seedlings in each herbivory intensity suggests (1) the older seedlings had more time to accumulate biomass or (2) the architecture of the Rockefeller seedlings changed in response to continued herbivory. It is likely that the older plants at Rockefeller increased their biomass radially or increased the density of their biomass instead of growing vertically. The possible architectural changes in more heavily browsed seedlings would support Cooper et al. (2003) findings where shrubs subjected to increased herbivory over time increased stem and leaf area in response to increased herbivory. However, since Rockefeller seedlings did have significantly higher biomass than seedlings at other locations, our results do not support the storage over growth conclusions made in previous studies (Hoogesteter & Karlsson 1992; Palacio et al. 2012; Wiley et al. 2013; Wiley et al. 2016).

CONCLUSION

Deer management strategies do not strongly affect C and N allocation or starch storage in chestnut oak, however they do appear to affect chestnut oak age and architecture. The findings of this study suggest that previous herbivory intensities need to be considered so that forest regeneration can take place at suitable durations. In general, chestnut oaks appear to be resilient to herbivory.

ACKNOWLEDGMENTS

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APPENDIX

TABLE 1. C, N, C:N, and carbohydrate concentrations by plant part per location for uprooted seedlings. Significant values labeled with a (*) are comparisons between all locations.

Location	Plant Part	C%	N%	C:N	Sugar(mg/L)	Starch(mg/L)
Cary	leaves	46.01	2.112	21.82	80	7.5385
	stems	46.58	0.7817	60.01	73.38	5.275
	roots	43.47	0.5698	80.05	124.43	134
Mid-County	leaves	48.05	2.512	19.14	108.5	49.692
	stems	46.82	0.6054*	78.75**	66.05	33.96*
	roots	47.02*	0.5262	92.21	105.02	117.8
Rockefeller	leaves	47.53	2.504	19.07	106.69	26.54
	stems	47.95	0.6942	70.54	83.22	15.962
	roots	45.24	0.5003	93.67	101.44	129.2

$P \leq 0.05$ * ≤ 0.01 ** < 0.001 ***

TABLE 2. Fenced and unfenced plant part C, N, C:N, and carbohydrate concentration. There were no significant differences.

| Location	Plant Part	Treatment	C%	N%	C:N	Sugar(mg/L)	Starch(mg/L)
Cary	leaves	fenced	46.69	2.358	19.8	80.59	6.616
	stems		46.5	0.7556	62.05	65.47	61.692
	leaves	unfenced	46.89	2.278	20.61	88.36	9.154
	stems		46.28	0.6314	74.1	51.72	38.846
Mid-County	leaves	fenced	47.95	2.523	19.2	88.26	33.847
	stems		46.92	0.7535	64.45	64.39	104.38
	leaves	unfenced	47.7	2.447	19.54	74.51	53.77
	stems		45.87	0.7197	63.72	63.5	60.77
Rockefeller	leaves	fenced	47.18	2.502	18.87	81.35	32.54
	stems		46.43	0.727	63.98	70.34	59.46
	leaves	unfenced	47.44	2.165	21.93	98.95	45
	stems		47.1	0.7441	63.26	70.12	37.69

$P \leq 0.05$ * ≤ 0.01 ** < 0.001 ***

TABLE 3. The means of total biomass, plant part percent of total biomass, above ground biomass, and below ground biomass for uprooted seedlings per location. Above ground biomass is the sum of the leaves and stems percent biomass and below biomass is the percent of root biomass.
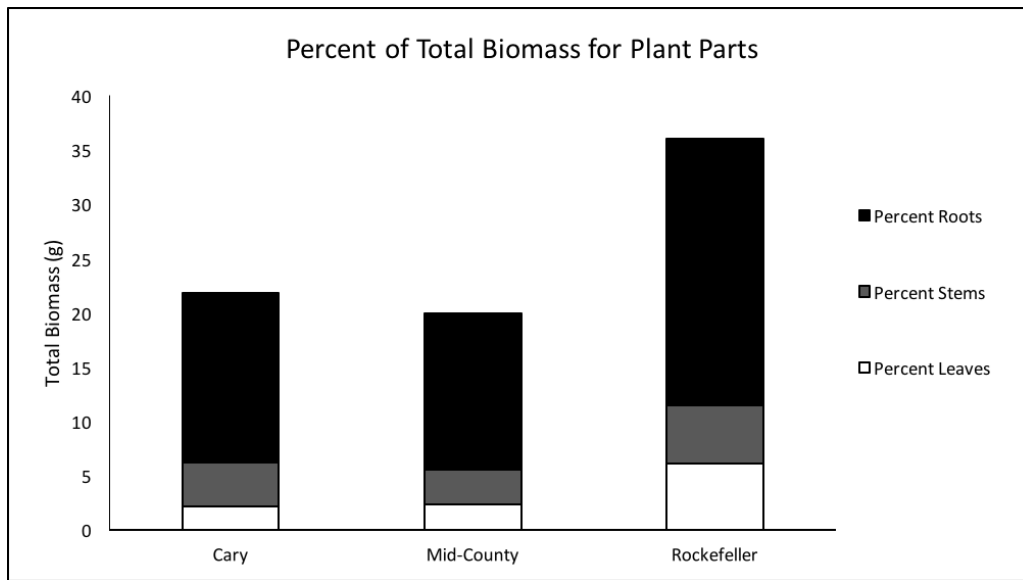
| Location	Total Biomass (g)	Plant Part	% of Total Biomass	% Above Ground Biomass	% Percent Below Ground Biomass
Cary	22	leaves	11	29	71
		stems	18		
		roots	71		
Mid-County	20	leaves	12	27	73
		stems	15		
		roots	73		
Rockefeller	36 *	leaves	17	32	68
		stems	15		
		roots	68		

$P \leq 0.05$ * ≤ 0.01 ** < 0.001 ***
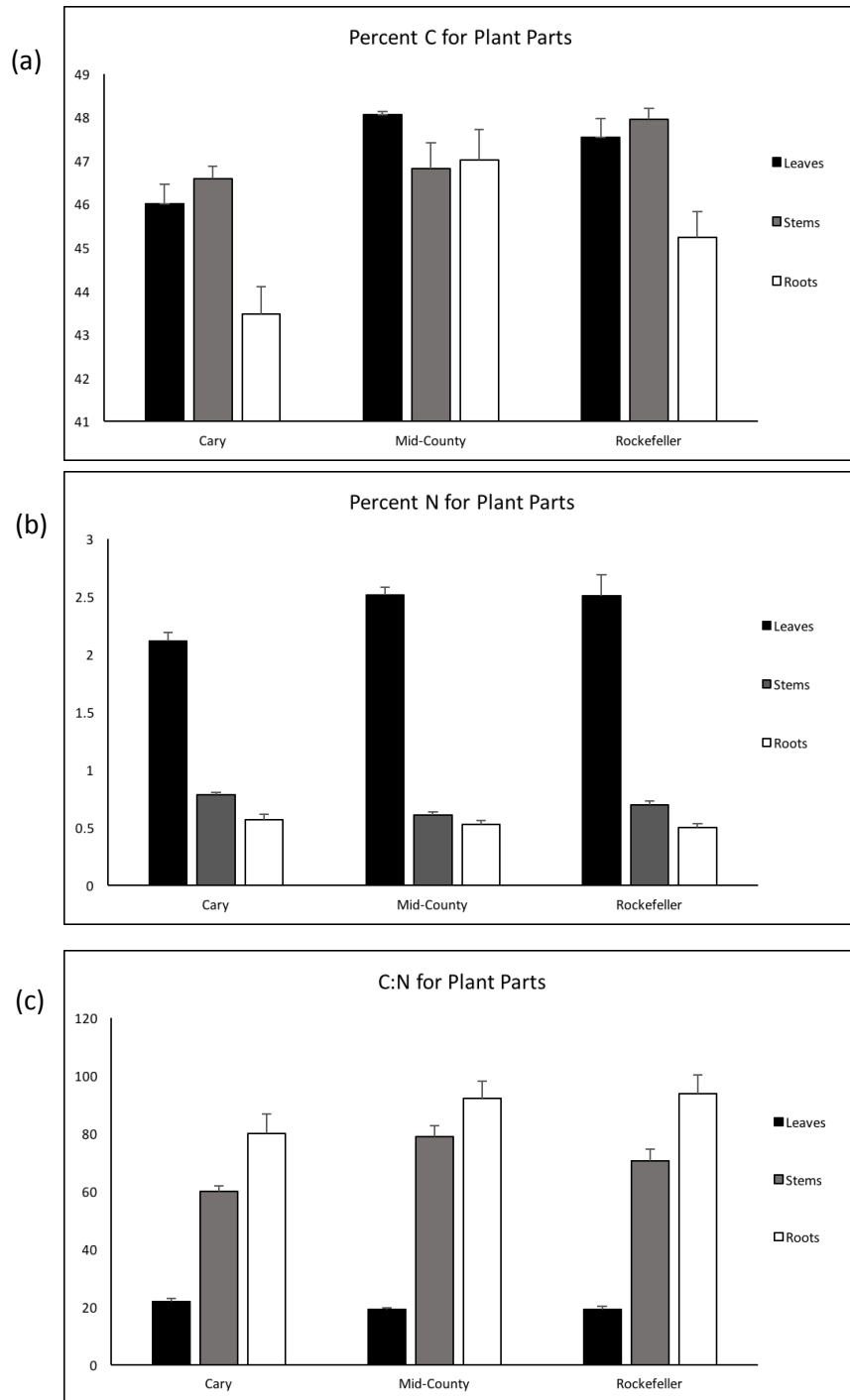
FIGURE 1. The means and standard errors for percent C (a), N (b), and C:N ratios (c) for all plant parts in all locations. Percentages were calculated from direct combustion of total mass.

FIGURE 2. The total biomass means for each location with the percent of each plant part making up the total biomass represented within each total biomass mean. Total biomass means were 22g (Cary), 20g (Mid-County), and 36g (Rockefeller).

(a)

Sugar Concentrations for Plant Parts

(b)

Starch Concentrations for Plant Parts
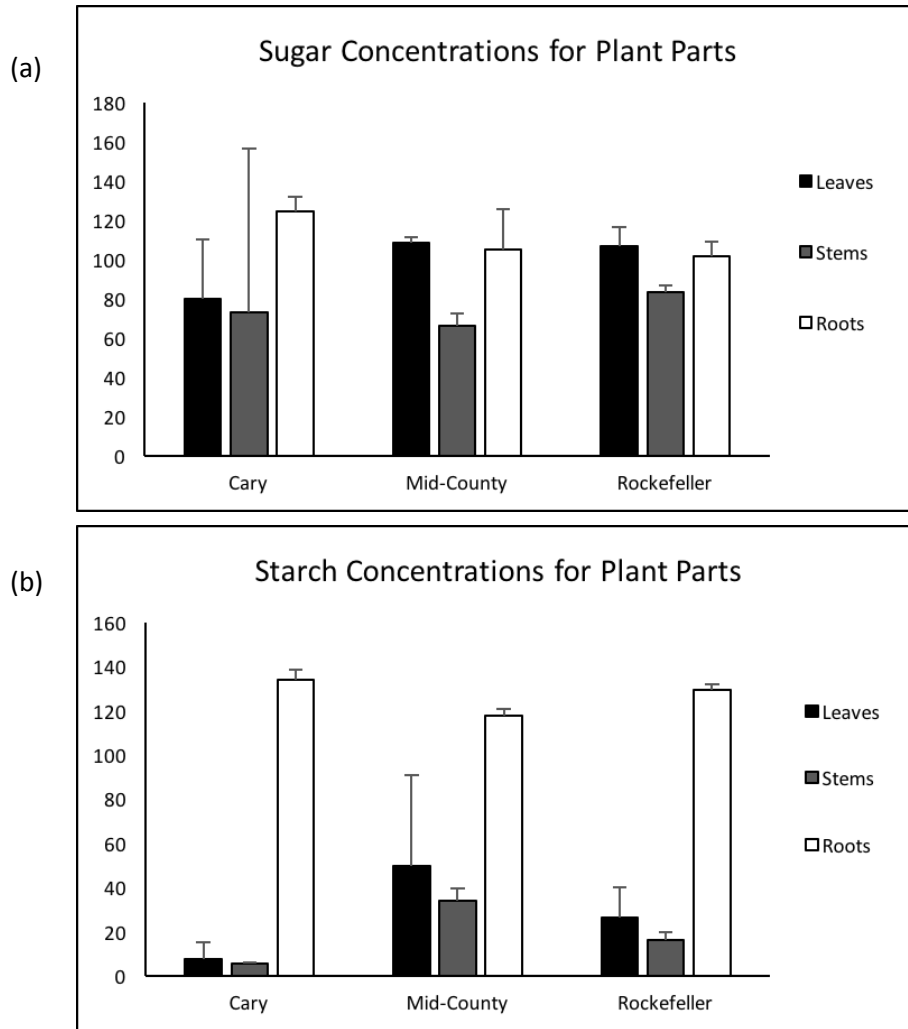
FIGURE 3. The means and standard error for sugar concentrations (a) and starch concentrations (b) for all plant arts in each location. Sugar concentrations were collected as total glucose, fructose, and galactose. Dissolved carbohydrates concentrations were in analyzed as mg/L.