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Harvest management based on leaf stage of a tetraploid vs. a diploid cultivar of annual ryegrass

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Abstract

Leaf stage-dependent defoliation is linked to the plant's physiological status and may be a more suitable criterion than time-based intervals for harvesting forage grasses, but no reports of research with annual ryegrass (*Lolium multiflorum* Lam. var. *westerwoldicum*) were found. To address this, a 2-year field study was carried out at Raymond, MS, on a Loring silt loam soil (fine-silty, mixed, thermic Typic Fragiudalfs). Forage production, morphological characteristics and nutritive value responses to defoliation based on leaf stage (2, 3 and 4 leaves per tiller) and two residual stubble heights (RSH; 5 and 10 cm) of a tetraploid ("Maximus") vs. a diploid ("Marshall") cultivar of annual ryegrass were quantified. Forage harvested, in 2011, increased linearly as leaf stage increased from 7.3 to 8.8 Mg/ha, but during 2012 was least (7.0 Mg/ha) at 3-leaf stage and similar at the other two leaf stages (7.6 Mg/ha). Tiller density was less for Maximus (1,191 tillers/m^2) than for Marshall (1,383 tillers/m^2). Leaf blade proportion decreased with increasing leaf stage and was greater by 9% for Maximus than for Marshall. Generally, forage nutritive value became less desirable with increasing leaf stage. There was a dichotomy in forage harvested and nutritive value responses, but maximum forage productivity was achieved when annual ryegrass was defoliated at the 4-leaf stage interval.

KEYWORDS
crop growth rate, diploid, forage harvested, forage morphology, leaf stage defoliation, Marshall annual ryegrass, Maximus annual ryegrass, nutritive value, residual stubble height, tetraploid, tiller density, water-soluble carbohydrate

1 | INTRODUCTION

Defoliation creates a major encumbrance on pasture plants with its impact being dependent on the timing and severity of defoliation (Fulkerson & Donaghy, 2001). Harvest management based on the physiological status of forage crops has been proposed as a more efficient tool in utilizing pastures than harvest management based on a scheduled number of days, sward surface height or herbage accumulation (Donaghy, Turner, & Adamczewski, 2008; Turner, Donaghy, Lane, & Rawnsley, 2006). Leaf stage-dependent defoliation of forage crops is linked to the plant physiological status and it is perhaps a more suitable criterion for harvesting forage crops. Forage defoliation interval based on leaf stage more readily reflects the extent of plant recovery from harvest as it relates to the replenishment of water-soluble carbohydrate (WSC) reserve (Fulkerson & Slack, 1995).

Residual stubble height, which is affiliated with the severity of defoliation and is a measure of defoliation intensity, is another major management factor that alters regrowth potential of forage crops (Lee, Donaghy, Sathish, & Roche, 2009). Because the stubble of forage grasses is a major storage site for WSC and can interact with

defoliation height to alter plant growth, then consideration must be given to the severity at which plants are defoliated (Donaghy & Fulkerson, 1997; Lee et al., 2009). In a study with tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.], there was a strong positive relationship between stubble WSC levels and regrowth (Donaghy et al., 2008). The two most important attributes of forage supply are quantity and nutritive value and each varies with frequency and intensity of defoliation (Motazedian & Sharrow, 1990; Sollenberger & Vanzant, 2011). Therefore, judicious use of pastures requires maintaining equilibrium between forage nutritive value and quantity and this requires optimization of the plant physiological status to ensure sustained production from the pasture sward for the duration of the growing season.

The combined attributes of productivity and nutritive value associated with annual ryegrass (*Lolium multiflorum* Lam. var. *westerwoldicum*) have allowed it to serve as a valued forage resource for livestock producers during the winter–spring season in the southeastern USA (Lippke, Haby, & Provin, 2006; Nelson, Phillips, & Watson, 1997). Both traditional diploid (2n = 2× = 14) and the more recently developed and widely utilized tetraploid (2n = 4× = 28) varieties of annual ryegrass are available commercially. Tetraploids are expected to have a greater ratio of cell content to cell wall (Stewart & Hayes, 2011), thus resulting in greater digestibility, crude protein (CP) and WSC concentration. There are, however, questions as to whether these perceived advantages of tetraploids over existing diploids can be exploited under varying management systems. Information to answer this question has to come from field studies of intraspecies cultivar-specific management.

There is a considerable volume of information available on the topic of leaf stage and stubble height defoliation management but primarily on perennial forage grasses, and the majority of these studies were conducted in glasshouse environment (e.g., Donaghy et al., 2008; Turner, Donaghy, Lane, & Rawnsley, 2007; Turner, Donaghy et al., 2006). This information may be limited in its application to annual forage grasses, so it will be of utmost importance to conduct studies of these plant-related indicators on annual forage grasses like annual ryegrass as a tool to improve defoliation management efficiency at the field scale. The objective of this study was to quantify forage production, morphological characteristics and nutritive value of a tetraploid vs. a diploid annual ryegrass cultivar harvested at three different leaf stages and at two stubble heights.

2 | MATERIALS AND METHODS

2.1 | Site and treatments

A field study was carried out at the E.G. (Gene) Morrison Brown Loam Branch Experiment Station at Raymond, MS, USA (32°12′N, 90°30′W), during the winter through spring seasons of 2010–2011 and 2011–2012. The predominant soil at the experimental site is a Loring silt loam (fine-silty, mixed, thermic Typic Fragiudalfs). The mean soil pH (in water) was 5.4 and Lancaster extractable P, K, Mg and Ca were 20, 63, 227 and 1258 mg/kg respectively.

Low precipitation in September and October 2010 (Figure 1) led to a delay in seeding and subsequent late start to imposing defoliation treatments in the first year of the study. Further, average air temperature from November 2010 through February 2011 was less than the 30-year average for the corresponding months, possibly causing a further restriction in forage growth during those months (Figure 1). Precipitation and temperature are major environmental factors responsible for variation in forage production and quality, and therefore, forage response differences between years in this study can be attributed to the differences in weather conditions.

Treatments were two cultivars of annual ryegrass, "Marshall," a diploid, and "Maximus," a tetraploid, three defoliation intervals based on leaf stage (2, 3 and 4 leaves per tiller based on the time of appearance of fully expanded leaves) and two residual stubble heights (RSH; 5 and 10 cm). The treatments were arranged in a 3 × 2 × 2 factorial of a randomized complete block design experiment with four replications.

The study site was previously a bahiagrass (*Paspalum notatum* Flügge) sod that was known to not have been planted to any cool-season grasses for at least 15–20 years before. A new set of plots adjacent (10 m separation) to the previous year's plots was used in the second year. Prior to seedbed preparation, glyphosate [N-(phosphonomethyl) glycine] was applied at a rate of 1.12 kg a.i./ha. Each year, the experiment was comprised of 48 plots, each 5 m long × 1.5 m wide separated by 1-m alleys between plots and 2-m alleyways between blocks. In the first year of the study, plots were seeded in late November 2010, and in the second year, early October 2011 at a seeding rate of 30 kg/ha pure live seed for both cultivars using a small-plot planter (Kincaid Equipment Manufacturing, Haven, KS, USA). In each year, the equivalent of 60 kg/ha each of N, P_2O_5 and K_2O in a blended fertilizer mixture was applied to each plot 2 weeks after seeding. In January and again in March of each year, N was applied as urea at a rate of 60 kg N/ha giving an annual total of 180 kg N/ha.

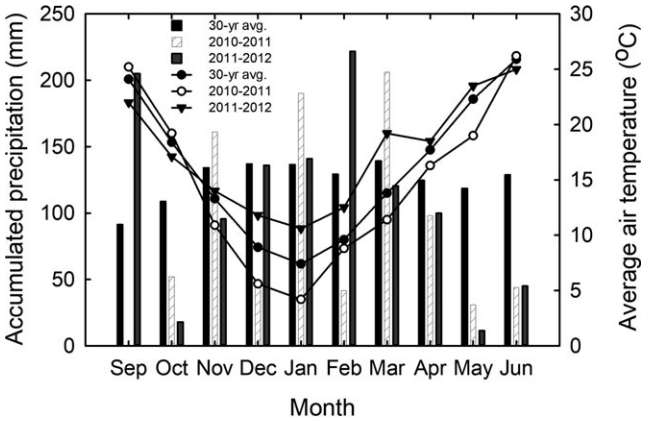

FIGURE 1 Monthly accumulated precipitation and mean air temperature at Brown Loam Experiment Station, Raymond, MS, during September to June of 2010 to 2011 and 2011 to 2012. 30-year average was calculated from 1980 to 2010 data. Vertical bars represent accumulated precipitation and lines represent average air temperatures

2.2 | Data collection

Defoliation treatments were imposed when greater than 50% of 10 randomly selected tillers attained the set number of fully expanded leaves, that is, 2, 3 and 4 leaves/tiller (Callow, Michell, Baker, Cocks, & Hough, 2005; Fulkerson & Slack, 1994; Turner, Donaghy, Lane, & Rawnsley, 2006). The expansion of each new leaf was termed "1-leaf stage" and thus the respective treatments were referred to as 2-leaf, 3-leaf and 4-leaf stages. Fulkerson and Slack (1995) suggested that the 3-leaf stage is ideal for defoliation of perennial ryegrass (*Lolium perene* L.) because optimum forage production and regrowth are attained at this stage. To test this for annual ryegrass, in addition to the 3-leaf stage, we selected 2- and 4-leaf stages to represent a gradation in the frequency of defoliation management. Further, the intensity of defoliation refers to the amount of herbage removed during the three different leaf stage harvest intervals and was represented by the two RSH (5 and 10 cm) in this study. Both frequency and intensity are known to have combined effects on forage production and this is of continued interest to forage producers as animal productivity can be altered. Forage harvested was determined by clipping a 2- × 0.6-m area in the centre of each plot at either 5 or 10 cm RSH using a hand-held battery-operated clipper. The total fresh weight of the harvested area was recorded. For dry-matter (DM) determination, a subsample of approximately 1,000 g was collected and dried in a forced-air oven at 55–60°C (usually for 72 hr) until a constant weight was achieved. Forage harvested for each treatment was reported as the total herbage accumulation during the season. In the first year of the study, there were three harvests at the 2- and 3-leaf stages and two harvests at the 4-leaf stage. In the second year, there were five, four, and three harvests at the 2-, 3- and 4-leaf stages respectively (Table 1). Crop growth rate (CGR) was calculated as the treatment herbage accumulation for each harvest period divided by the number of days for that period.

TABLE 1 Defoliation dates during the winter–spring season of 2011 and 2012

		Defoliation intervals		
		2-Leaf stage	3-Leaf stage	4-Leaf stage
Year	Harvest period	Dates of defoliation		
2011	1	23 March	29 March	05 April
	2	18 April	26 April	12 May
	3	16 May	23 May	
	Average days between harvest	28	28.5	38
2012	1	06 February	14 February	27 February
	2	14 March	20 March	30 March
	3	09 April	19 April	02 May
	4	03 May	11 May	
	5	21 May		
	Average days between harvest	27	29.7	33

Tiller density was determined from two 0.06-m^2 quadrats, initially randomly selected at sites within each plot, outside of the area identified for sampling of forage harvested and avoiding the outer rows. These sites were then marked permanently so that sampling was repeated at the same site on each occasion. Plants within the quadrats were clipped at the treatment RSH and the numbers of live tillers were counted. After sampling for forage harvested and tiller count, samples were clipped at the treatment RSH from six random sites in each plot and separated into leaf blade, pseudostem, reproductive stem and dead material. These fractions were oven-dried as described previously (55–60°C) and used to determine the relative proportion of each morphological component on a DM basis. For analysis of forage nutritive value parameters, another set of samples were collected similarly to those for plant-part separation and oven-dried (55–60°C) and then ground to pass a 1-mm stainless steel screen using a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA), and stored in airtight sterile plastic bags at room temperature until analysed. Stubble samples representing the RSH from each treatment were collected from random locations in each plot outside of the areas already sampled. Four whole plants were cut at ground level and all leaves (leaf blade) were removed from each tiller, then measured and cut to leave either 5 or 10 cm from the base, representing the treatment RSH. The tillers from each sample were counted and then dried as described above and weighed. Mean stubble weight was calculated as dry weight of each sample divided by the number of tillers in the sample. Thereafter, stubble samples were ground to pass a 1-mm stainless steel screen using a Wiley mill (Model Digital ED-5, Thomas Scientific) and stored in airtight sterile plastic bags at room temperature until analysed. Because both whole plant and stubble samples involved WSC determination, they were collected between 0800 and 0900 hr on sampling days to reduce the confounding effects of diurnal fluctuation in WSC in plant (Fulkerson & Slack, 1994) and then stored on ice in a cooler in the field. These samples were placed in the oven within 40–60 min of cutting to further inhibit respiration activity. After all sampling was completed, the remaining herbage on each harvested plot was mowed to the treatment RSH using a self-propelled mower equipped with a catch bag.

2.3 | Forage nutritive value analysis

Analysis to determine CP, NDF, ADF and WSC concentrations was carried out using near-infrared reflectance spectroscopy (NIRS) with a FOSS NIRSystems Model 6500 spectrophotometer (FOSS NIRSystems Inc. North America, Silver Spring, MD, USA) that utilized FOSS ISIScan software version 4.4 (Infrasoft International LLC, Port Matilda, PA, USA) and prediction equations developed by the NIRS Forage and Feed Testing Consortium (Hillsboro, WI, USA). The R^2 values for CP, NDF, ADF and WSC were 0.98, 0.97, 0.94 and 0.92 respectively. Stubble WSC content was derived by multiplying the WSC concentration by mean stubble weight. In vitro true digestibility (IVTD) and in vitro digestibility of the NDF fraction (NDFD) were determined using an ANKOM Daisy^{II} Incubator system (ANKOM Technology Corp, Macedon, NY, USA) using a modified version of

Tilley and Terry (1963). Forage nutritive value averaged across season was based on weighted means, that is, the concentration of each nutritive value parameter was multiplied by the forage harvested for each harvest to calculate content, and then, season total content was divided by season total forage harvested to compute the weighted concentration.

2.4 | Statistical analysis

The data were analysed by fitting mixed models using PROC GLIMMIX in SAS (SAS Institute, 2008). Responses across harvests during each growing season were treated as repeated measures in time. Leaf stage, stubble height, forage cultivar and year were fixed effects. The model used was:

$$Yijkl = \mu + Lsi + Shj + (LsSh)ij + Fck + (LsFc)ik + (ShFc)jk + (LsShFc)ijk + Yl + (LsY)il + (ShY)jl + (FcY)kl + (LsShY)ijl + (LsFcY)ikl + (ShFcY)jkl (LsShFcY)ijkl + eijkl$$

where $Yijkl$ is the dependent variable, μ is the overall mean, Lsi is the leaf stage effect, Shj is the stubble height effect, $(LsSh)ij$ is the leaf stage × stubble height interaction, Fck is the forage cultivar effect, $(LsFc)ik$ is the leaf stage × forage cultivar interaction, $(ShFc)jk$ is the stubble height × forage cultivar interaction, $(LsShFc)ijk$ is the leaf stage × stubble height × forage cultivar interaction, Yl is the year effect, $(LsY)il$ is the leaf stage × year interaction, $(ShY)jl$ is the stubble height × year interaction, $(FcY)kl$ is the forage cultivar × year interaction, $(LsShY)ijl$ is the leaf stage × stubble height × year interaction, $(LsFcY)ikl$ is the leaf stage × forage cultivar × year interaction, $(ShFcY)jkl$ is the stubble height × forage cultivar × year interaction, $(LsShFcY)ijkl$ is the leaf stage × stubble height × forage cultivar × year interaction, and $Eijkl$ is the error term. The nature of the response to leaf stage was determined using orthogonal polynomial contrasts. Correlation among stubble WSC concentration, stubble WSC content, forage harvested and CGR were performed using the PROC CORR procedure of SAS (SAS Institute, 2008). Means separation was conducted using the PDIFF option in SAS and considered different at $p \leq 0.05$ unless otherwise stated.

3 | RESULTS

3.1 | Forage harvested and crop growth rate

There was a year × leaf stage interaction effect ($p = 0.011$) on forage harvested. The interaction occurred partly because as leaf stage increased, season-long total forage harvested increased linearly 7.3–8.8 Mg/ha in 2011, but in 2012, total forage harvested was similar at the 2- and 4-leaf stages and less at the 3-leaf stage (Table 2). Also, there were trends for main effects of forage cultivar ($p = 0.100$; $S_{\bar{x}} = 0.22$) and stubble height ($p = 0.074$; $S_{\bar{x}} = 0.22$). Forage harvested tended to be greater for Maximus (7.8 Mg DM/ha) than for Marshall (7.5 Mg DM/ha) and at the 5 cm (7.9 Mg DM/ha) than at the 10 cm RSH (7.5 Mg DM/ha).

For CGR, there were main effects of cultivar ($p = 0.007$; $S_{\bar{x}} = 2.0$) and leaf stage ($p < 0.001$; $S_{\bar{x}} = 2.0$). Crop growth rate was greater for Maximus (50.0 kg DM ha^{-1} day^{-1}) than for Marshall (45.0 kg DM ha^{-1} day^{-1}) and increased from 47.0 kg DM ha^{-1} day^{-1} at the 2-leaf stage to 57.0 kg DM ha^{-1} day^{-1} at the 4-leaf stage (Table 2). Between years, CGR tended to be greater ($p = 0.073$; $S_{\bar{x}} = 3.0$) in 2011 (52.0 kg DM ha^{-1} day^{-1}) than in 2012 (43.0 kg DM ha^{-1} day^{-1}). There was no stubble height ($p = 0.286$) main effect (47.0 and 48.0 kg DM ha^{-1} day^{-1} at 5 and 10 cm, respectively) or year × cultivar × stubble height interaction ($p = 0.380$) on CGR.

TABLE 2 Forage harvested and crop growth rate (CGR) for Maximus and Marshall annual ryegrass defoliated at three different leaf stages and two residual stubble heights (5 and 10 cm) during the winter–spring season of 2011 and 2012

	Leaves/tiller stage			Contrast[†]	
	2-	3-	4-		
	Forage harvested				
Factors	Mg DM/ha			L	Q
Year 2011	7.3[a‡]	7.7[a]	8.8[a]	<0.001	0.233
Year 2012	7.7[a]	7.0[b]	7.5[a]	0.732	0.046
$S_{\bar{x}}$	0.4	0.4	0.4		
	CGR				
	kg DM ha^{-1} day^{-1}				
Leaf stage	47.0	38.0	57.0	<0.001	<0.001

[†]Orthogonal polynomial contrast, L = linear and Q = quadratic.
[‡]Within columns, means followed by same lowercase letter superscripts are not different ($p > 0.05$).

3.2 | Tiller density

There were main effects of forage cultivar ($p = 0.003$; $S_{\bar{x}} = 35.0$), stubble height ($p = 0.008$; $S_{\bar{x}} = 35.0$) and year ($p = 0.008$; $S_{\bar{x}} = 35.0$) on tiller density. Maximus had a lower tiller density (1,191 tillers/m^2) than Marshall (1,383 tillers/m^2). Harvesting at 5 cm RSH resulted in greater tiller density (1,373 tillers/m^2) than harvesting at 10 cm RSH (1,201 tillers/m^2). Tiller density was greater in 2011 (1,451 tillers/m^2) than in 2012 (1,123 tillers/m^2). There was no effect of leaf stage ($p = 0.394$; $S_{\bar{x}} = 44$) on tiller density (2-leaf stage, 1,316 tillers/m^2; 3-leaf stage, 1,238 tillers/m^2; 4-leaf stage, 1,307 tillers/m^2) or year × cultivar × stubble height interaction ($p = 0.462$).

3.3 | Stubble weight

There was a year × leaf stage × stubble height interaction effect ($p = 0.014$; $S_{\bar{x}} = 2.0$) and a cultivar × stubble height interaction effect ($p = 0.004$; $S_{\bar{x}} = 1.2$) on stubble weight (Figure 2). The three-way interaction occurred partly because in 2011, stubble weight increased with increasing leaf stage at both the 5 and 10 cm RSH (Figure 2). In 2012, however, there was no stubble weight response to leaf stage at 5 cm RSH, but at 10 cm RSH there was a linear decrease as leaf stage increased (Figure 2). Stubble weight of Maximus was greater than that of Marshall at both 5 (50.4 vs. 46.4 mg/

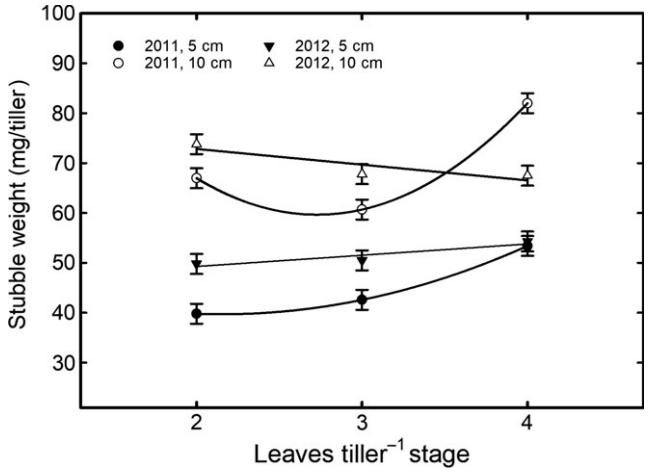

FIGURE 2 Stubble weight of tillers for Maximus and Marshall annual ryegrass defoliated at three different leaf stages and two residual stubble heights (RSH) during the winter–spring season of 2011 and 2012

tiller) and 10 cm (74.8 vs. 64.6 mg/tiller) and greater across cultivars at the 10 than at 5 cm RSH ($p < 0.001$). The interaction was due mainly to magnitude of differences, with more than a twofold difference in stubble weight between cultivars at the 10 compared to the 5 cm RSH.

3.4 | Stubble WSC concentration and content

There were main effects of cultivar ($p < 0.001$; $S_{\bar{x}} = 2.0$), leaf stage ($p < 0.001$; $S_{\bar{x}} = 3.0$) and stubble height ($p = 0.012$; $S_{\bar{x}} = 2.0$) on stubble WSC concentration (Figure 3). Stubble WSC of Maximus (125.6 g/kg DM) was greater than that of Marshall (118.8 g/kg DM). Stubble WSC concentration increased linearly ($p < 0.001$) as defoliation interval increased from 2- to 4-leaf stage and was greater at the 5 cm than at the 10 cm RSH (Figure 3). There was no effect of year ($p = 0.251$; $S_{\bar{x}} = 2.0$) on stubble WSC concentration (2011, 121.0 g/kg DM; 2012, 116.4 g/kg DM) or any interactions ($p = 0.315$).

There was a main effect of cultivar on stubble WSC content ($p < 0.001$; $S_{\bar{x}} = 0.21$) with Maximus (7.9 mg/tiller) greater than Marshall (6.2 mg/tiller) and there were no interactions involving cultivar ($p = 0.651$). Also, there was a year × leaf stage × stubble height interaction effect on stubble WSC content ($p = 0.008$). Inspection of Figure 4 shows that the interaction is primarily determined by an unusual value for the 4-leaf stage, 10 cm RSH, 2012 data point at the extreme right of the bar graph which is much lower than would be predicted from the patterns across the other bars. In addition, differences between RSH within years fluctuated with the largest margin of difference occurring at 4-leaf stage in 2011, a 4.2 mg/tiller greater WSC content at 10 cm compared to 5 cm RSH.

3.5 | Forage morphology

There was a year × cultivar × leaf stage × stubble height interaction effect on the proportion of leaf blade ($p = 0.019$). The interaction occurred partially because in 2011, there were quadratic ($p = 0.011$) responses for both cultivars at both RSH but in 2012, response of Maximus to defoliation interval at the 5 cm RSH tended to be quadratic ($p = 0.089$), and at 10 cm, it was quadratic ($p = 0.006$) (Table 3). For Marshall, however, there was a linear decrease in the proportion leaf blade as defoliation interval increased at both RSH (Table 3).

For the proportion of pseudostem responses, there was a three-way interaction effect of cultivar × leaf stage × RSH ($p = 0.021$) and two-way interaction effects of year × leaf stage ($p < 0.001$) and year × cultivar ($p = 0.005$; $S_{\bar{x}} = 0.7$). For the three-way interaction, Marshall had a quadratic response ($p = 0.033$) to leaf stage at 10 cm RSH but only tended to be quadratic ($p = 0.059$) at the 5 cm RSH (Table 3). For Maximus, however, there was no effect of leaf stage (Table 3). Generally, Marshall tended to have a greater proportion of

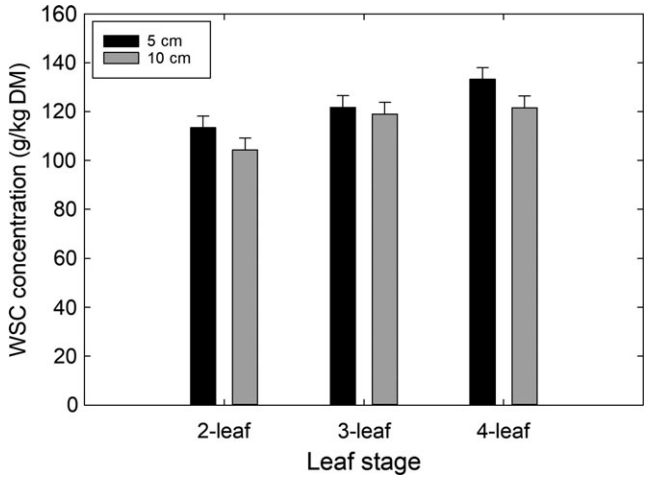

FIGURE 3 Stubble water-soluble carbohydrate (WSC) concentration of tillers for Maximus and Marshall annual ryegrass defoliated at three different leaf stages and two residual stubble heights (RSH) during the winter–spring season of 2011 and 2012

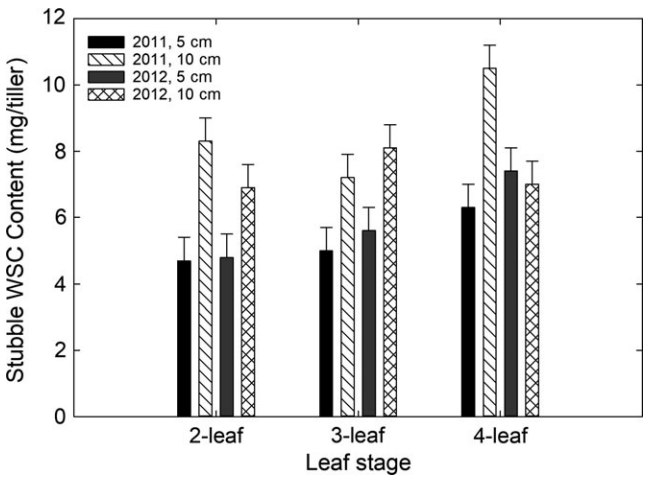

FIGURE 4 Stubble water-soluble carbohydrate (WSC) content of tillers for Maximus and Marshall annual ryegrass defoliated at three different leaf stages and two residual stubble heights (RSH) during the winter–spring season of 2011 and 2012

TABLE 3 The proportion of leaf blade, pseudostem, reproductive stem and dead material for Maximus and Marshall annual ryegrass defoliated at three different leaf stages and two residual stubble heights (RSH) during the winter–spring season of 2011 and 2012

	Leaves/tiller stage			Contrast†	
Factors	2-	3-	4-	L	Q
	% Leaf blade				
2011, Marshall 5 cm RSH	$48.4^{Aa‡}$	44.2^{Ba}	30.1^{Aa}	<0.001	0.042
2011, Marshall 10 cm RSH	47.5^{Ba}	47.3^{Aa}	28.1^{Ba}	<0.001	0.007
2011, Maximus 5 cm RSH	51.2^{Aa}	51.0^{Aa}	33.1^{Aa}	<0.001	<0.001
2011, Maximus 10 cm RSH	55.0^{Aa}	51.8^{Aa}	34.9^{Aa}	<0.001	0.009
2012, Marshall 5 cm RSH	66.6^{Aa}	62.7^{Ba}	53.2^{Bb}	<0.001	0.157
2012, Marshall 10 cm RSH	64.7^{Ba}	65.3^{Ba}	59.5^{Aa}	0.029	0.109
2012, Maximus 5 cm RSH	66.6^{Ab}	69.2^{Aa}	63.2^{Aa}	0.229	0.089
2012, Maximus 10 cm RSH	72.2^{Aa}	73.4^{Aa}	59.9^{Aa}	0.004	0.007
$S_{\bar{x}}$	1.7	1.7	1.7		
	% Pseudostem				
Marshall 5 cm RSH	$25.8^{Aa§}$	33.2^{Aa}	31.2^{Aa}	0.058	0.058
Marshall 10 cm RSH	25.4^{Aa}	31.3^{Aa}	26.7^{Ba}	0.650	0.033
Maximus 5 cm RSH	24.5^{Aa}	27.1^{Ab}	24.6^{Ab}	0.960	0.220
Maximus 10 cm RSH	20.4^{Bb}	24.3^{Bb}	23.5^{Ab}	0.220	0.281
$S_{\bar{x}}$	1.0	1.0	1.0		
	% Pseudostem				
Year 2011	$26.7^{a¶}$	35.6^{a}	25.9^{a}	0.399	<0.001
Year 2012	21.3^{b}	22.3^{b}	27.0^{b}	<0.001	0.280
$S_{\bar{x}}$	0.8	0.8	0.8		
	% Reproductive stem				
Year 2011	$14.5^{a¶}$	8.5^{a}	33.6^{a}	<0.001	<0.001
Year 2012	6.3^{b}	4.3^{b}	5.6^{b}	0.499	0.082
$S_{\bar{x}}$	1.0	1.0	1.0		
	% Dead material				
Leaf stage	6.6	6.5	8.7	0.002	0.014

†Orthogonal polynomial contrast, L = linear and Q = quadratic.

‡Within columns, means followed by same uppercase letter superscripts are not different within years and residual stubble heights (RSH) between cultivars ($p > 0.05$) and means followed by same lowercase letter superscript are not different between years within cultivars and RSH ($p > 0.05$).

§Within columns, means followed by same uppercase letter superscripts are not different within RSH between cultivars ($p > 0.05$) and means followed by same lowercase letter superscripts are not different between RSH within cultivars ($p > 0.05$).

¶Within columns, means followed by same lowercase letter superscripts are not different ($p > 0.05$).

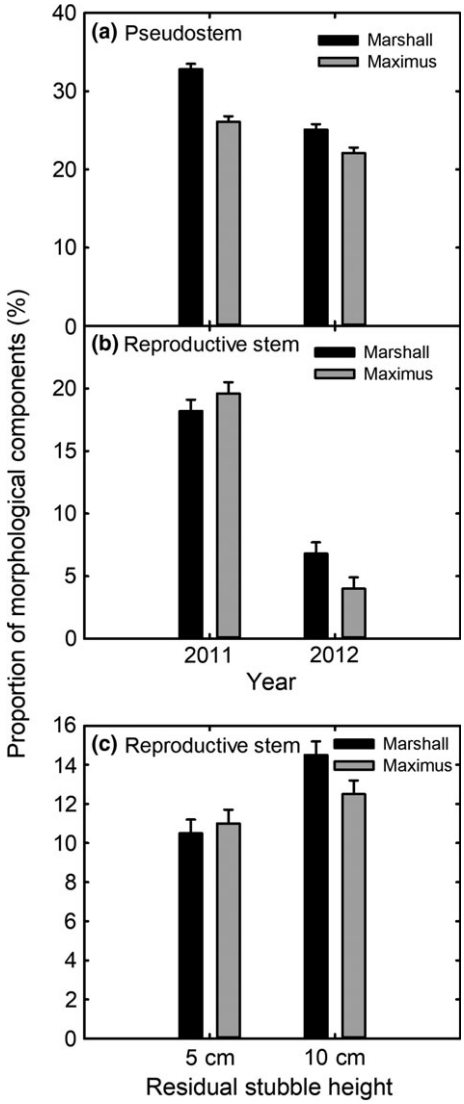

FIGURE 5 The proportion of (a) pseudostem, and (b and c) reproductive stem for Maximus and Marshall annual ryegrass defoliated at three different leaf stages (2, 3 and 4 leaves/tiller) and two residual stubble heights during the winter–spring season of 2011 and 2012

pseudostem than Maximus. The year × leaf stage interaction occurred partially because the proportion of pseudostem in 2011 was greatest at the 3-leaf stage, but in 2012 it increased linearly with increasing leaf stage (Table 3). Maximus had less proportion of pseudostem than Marshall in both years, but the magnitude of the difference was greater in the first year (Figure 5a).

There were two-way interaction effects of year × leaf stage ($p < 0.001$; $S_{\bar{x}} = 1.0$), year × cultivar ($p = 0.005$; $S_{\bar{x}} = 0.9$) and cultivar × RSH ($p = 0.016$; $S_{\bar{x}} = 0.7$) on the proportion of reproductive stem. For the year × leaf stage interaction effect, in 2011 the proportion of reproductive stem at the 4-leaf stage was more than two-fold greater than at the 2-leaf and close to fourfold greater than at the 3-leaf stage, but in 2012, the proportion of reproductive stem

was less than in 2011 and not different among leaf stages (Table 3; Figure 5b). The year × cultivar interaction occurred partly because there were cultivar differences ($p = 0.002$) in 2012, but not in 2011 (Figure 5b). The proportion of reproductive stem was similar between Maximus and Marshall (Figure 5c) at the 5 cm RSH, but at 10 cm, it was less for Maximus than for Marshall.

There were main effects of leaf stage ($p < 0.001$; $S_{\bar{x}} = 0.4$), RSH ($p < 0.001$; $S_{\bar{x}} = 0.4$) and year ($p = 0.007$; $S_{\bar{x}} = 0.4$) on the proportion of dead material, but there was no cultivar effect ($p = 0.615$) or any interactions ($p = 0.554$). The proportion of dead material was similar at the 2- and 3-leaf stages and greatest at the 4-leaf stage (Table 3), and was greater at the 5 cm (8.2%) than at the 10 cm RSH (6.3%).

3.6 | Forage nutritive value

There were main effects of cultivar ($p = 0.027$; $S_{\bar{x}} = 2.9$) and leaf stage ($p < 0.001$; $S_{\bar{x}} = 3.5$) and a year × RSH interaction effect ($p = 0.035$; $S_{\bar{x}} = 4.1$) on CP concentration. Maximus (211.6 g/kg) had greater CP concentration than Marshall (203.1 g/kg). There was a linear decrease in CP concentration as defoliation interval increased (Table 4). The year × RSH interaction effect was due partially to the magnitude of difference in CP concentration (Figure 6a).

There were three-way interaction effects of year × cultivar × leaf stage ($p = 0.007$) and year × cultivar × RSH ($p = 0.033$) on NDF concentration (Table 4; Figure 6b). Generally, NDF concentration was greatest at 4-leaf stage and the interactions were due mainly to differing patterns of response to leaf stage across years (Table 4). Overall, there was an average 11.5% reduction in NDF concentration at 2- and 3-leaf stage harvest intervals compared to 4-leaf stage (Table 4). In 2011, both Maximus and Marshall had less NDF concentration at 10 than at 5 cm RSH. In 2012, NDF concentration of Marshall was not different between RSH, but NDF concentration of Maximus was greater at 5 than at 10 cm RSH. Within cultivar and stubble height, NDF concentration was less in 2012 than in 2011 (Figure 6b).

There was a year × leaf stage interaction effect ($p = 0.044$; $S_{\bar{x}} = 15.0$) on ADF concentration (Table 4). In both years, ADF concentration was greatest at 4-leaf stage and the interaction was due mainly to differences in patterns of response (Table 4). The ADF concentration at 4-leaf stage harvest was an average 22.5% greater than at 2- and 3-leaf stage harvest intervals. There were no main effects of cultivar ($p = 0.304$; $S_{\bar{x}} = 10.0$; Maximus, 308.6 g/kg DM vs. Marshall, 299.3 g/kg), or RSH ($p = 0.408$; $S_{\bar{x}} = 10.0$; 5 cm, 307.7 g/kg vs. 10 cm, 300.2 g/kg), or any interactions ($p = 0.591$) involving these two variables on ADF concentration.

There was a year × leaf stage × RSH interaction effect ($p = 0.018$) on whole-plant WSC concentration (Table 4). There was no cultivar effect ($p = 0.222$; $S_{\bar{x}} = 3.6$; Maximus, 91.8 g/kg vs. Marshall, 95.8 kg^{-1}) or any interactions ($p = 0.765$) involving cultivar. Although there were statistical differences during 2011, there were no clear patterns of biologically distinct differences due to leaf stage. In 2012, WSC concentration at the 5 cm RSH was greatest at the 4-leaf stage, but at the 10 cm RSH it was greatest at the 3-leaf stage (Table 4).

There were two-way interaction effects of year × leaf stage ($p < 0.001$; $S_{\bar{x}} = 5.4$) and year × RSH ($p = 0.013$; $S_{\bar{x}} = 4.6$) on IVTD (Table 4; Figure 6c). There was no cultivar effect ($p = 0.173$; $S_{\bar{x}} =$

TABLE 4 Mean crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrate (WSC), in vitro true digestibility (IVTD) and neutral detergent fibre digestibility (NDFD) concentrations of Maximus and Marshall annual ryegrass defoliated at three different leaf stages and two residual stubble heights (RSH) during the winter–spring season of 2011 and 2012

	Leaves/tiller stage			Contrast†	
Factors	2-	3-	4-	L	Q
	g/kg				
	CP				
Leaf stage	229.8	212.0	180.2	<0.001	0.083
	NDF				
2011, Marshall	$483.9^{Aa‡}$	529.4^{Aa}	567.0^{Aa}	<0.001	0.454
2011, Maximus	480.9^{Aa}	518.4^{Aa}	571.5^{Aa}	<0.001	0.147
2012, Marshall	460.4^{Ab}	455.9^{Ab}	514.6^{Ab}	<0.001	<0.001
2012, Maximus	465.9^{Ab}	443.4^{Bb}	490.7^{Bb}	<0.001	<0.001
$S_{\bar{x}}$	4.0	4.0	4.0		
	ADF				
Year 2011	$289.7^{a§}$	318.6^{a}	347.6^{a}	0.004	0.994
Year 2012	262.7^{a}	260.1^{b}	345.3^{a}	<0.001	0.002
$S_{\bar{x}}$	15.0	15.0	15.0		
	WSC				
2011, 5 cm RSH	$91.7^{Aa¶}$	81.0^{Ab}	90.1^{Ab}	0.778	0.043
2011, 10 cm RSH	95.6^{Aa}	83.3^{Aa}	93.6^{Aa}	0.718	0.022
2012, 5 cm RSH	84.1^{Aa}	103.5^{Aa}	133.4^{Aa}	<0.001	0.528
2012, 10 cm RSH	78.1^{Aa}	100.8^{Aa}	90.2^{Ba}	0.215	0.054
$S_{\bar{x}}$	6.7	6.7	6.7		
	IVTD				
Year 2011	$801.1^{a§}$	778.5^{b}	729.8^{a}	<0.001	0.0371
Year 2012	766.1^{b}	818.2^{a}	743.3^{a}	0.0018	<0.001
$S_{\bar{x}}$	5.4	5.4	5.4		
	NDFD				
Year 2011	$882.6^{a§}$	865.9^{b}	814.3^{b}	<0.001	<0.001
Year 2012	858.9^{b}	905.7^{a}	831.6^{a}	<0.001	<0.001
$S_{\bar{x}}$	3.8	3.8	3.8		

†Orthogonal polynomial contrast, L = linear and Q = quadratic.

‡Within columns, means followed by the same uppercase letter superscripts are not different within years between cultivars ($p > 0.05$) and means followed by the same lowercase letter superscripts are not different between years within cultivars ($p > 0.05$).

§Within columns, means followed by same lowercase letter superscripts are not different ($p > 0.05$).

¶Within columns, means followed by same uppercase letter superscripts are not different between residual stubble heights (RSH) within years ($p > 0.05$) and means followed by same lowercase letter superscripts are not different within RSH between years ($p > 0.05$).

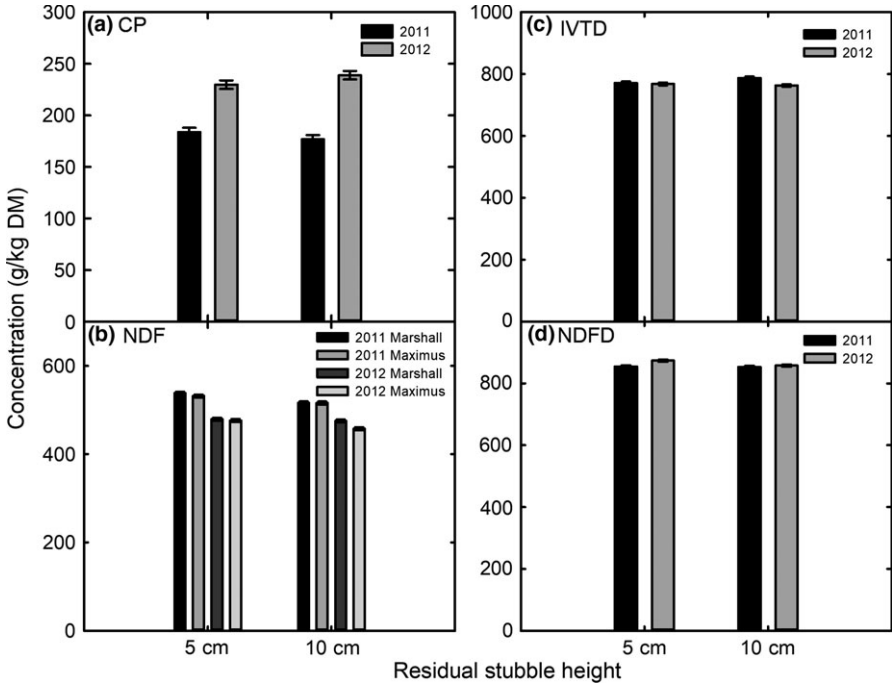

FIGURE 6 Mean (a) crude protein (CP), (b) neutral detergent fibre (NDF), (c) in vitro true digestibility (IVTD) and (d) neutral detergent fibre digestibility (NDFD) concentrations of Maximus and Marshall annual ryegrass defoliated at three different leaf stages (2, 3 and 4 leaves/tiller) and two residual stubble heights during the winter–spring season of 2011 and 2012

3.3; Maximus, 776.0 g/kg vs. Marshall, 770.0 g/kg) or any interactions ($p = 0.634$) involving cultivar. In 2011, IVTD decreased with increasing leaf stage, but in 2012, there was an increase from the 2- to the 3-leaf stage and then a decrease (Table 4). There was no effect of RSH ($p = 0.569$) on IVTD in 2011, but in 2012 IVTD was greater at 5 than at 10 cm (Figure 6c).

There were two-way interaction effects involving year × cultivar ($p = 0.008$; $S_{\bar{x}} = 3.3$), year × leaf stage ($p < 0.001$) and year × RSH ($p = 0.005$) on NDFD (Table 4; Figure 6d). In 2011, Maximus had greater NDFD (862.9 g/kg) than Marshall (845.6 g/kg), but in 2012 there was no cultivar effect ($p = 0.401$; average = 865.5 g/kg). Within cultivar, there was no year effect ($p = 0.399$) on NDFD of Maximus, but NDFD was greater in 2012 than in 2011 for Marshall. The year × leaf stage interaction occurred partly because in 2011, NDFD decreased with increasing defoliation interval, but in 2012, there was an increase from the 2- to the 3-leaf stage and then a decrease at the 4-leaf stage (Table 4). Within RSH, NDFD was greater in 2012 than in 2011 at 5 cm, but at 10 cm, there was no year effect ($p = 0.447$).

4 | DISCUSSION

This study evaluated responses of several forage parameters to defoliation frequency and intensity based on three different leaf stages and two RSH of two cultivars of annual ryegrass. Several studies have used number of leaves/tiller as a criterion when to harvest forage crops for example, Fulkerson and Slack (1995), Donaghy and Fulkerson (1997), Turner, Donaghy et al. (2006), Lee et al. (2009) using perennial ryegrass (*Lolium ryegrass* L.), Callow et al. (2005) annual ryegrass (*Lolium rigidum* Gaud.) and Italian ryegrass (*Lolium multiflorum* Lam.), Turner, Donaghy et al. (2006), Turner, Donaghy et al. (2006), Turner, Donaghy, Lane, and Rawnsley (2007) prairie grass (*Bromus willdenowii* Kunth.), Turner, Donaghy et al. (2006), Turner et al. (2007) cocksfoot (*Dactylis glomerata* L.) and Donaghy et al. (2008) tall fescue (*Festuca arundinacea* Schreb.). On the other hand, other studies have used a fixed number of days to harvest forage crops for example, Motazedian and Sharrow (1990) perennial ryegrass and subterranean clover (*Trifolium subterraneum* L.), Cuomo, Blouin, Corkern, McCoy, and Walz (1996) bahiagrass (*Paspalum notatum* Flügge), Brink, Casler, and Martin (2010) meadow fescue [*Schedonorus pratensis* (Huds.) P. Beauv.], tall fescue and orchardgrass (*Dactylis glomerata* L.), Inyang et al. (2010) Mulato II brachiariagrass (*Brachiaria* sp.), Tessema, Mihret, and Solomon (2010) using Napier grass [*Pennisetum purpureum* (L.) Schumach] and Giambalvo, Amato, and Stringi (2011) working with berseem clover (*Trifolium alexandrinum* L.). Irrespective of which defoliation criterion was used in these studies and forage species involved, plants defoliated infrequently produced greater herbage DM than those defoliated frequently. The results from these studies concurred with our study of greater herbage production for infrequent harvest (4-leaf stage) compared to frequent harvest (2- and 3-leaf stages). Further, forage production response based on defoliation using leaf-based indicator suggested a universal trend of increased herbage production (5–42% increase) for each successive increased in leaf defoliation stage (Callow et al., 2005; Donaghy & Fulkerson, 1997;

Donaghy et al., 2008; Fulkerson & Slack, 1995; Lee et al., 2009; Pembleton, Lowe, & Bahnisch, 2009; Turner, Donaghy et al., 2006; Turner, Donaghy et al., 2006; Turner et al., 2007). In this study, there was a quadratic response of crop growth rate to defoliation interval, and while the difference between 2- and 4-leaf stages was normal, the greater crop growth rate at 2- compared to 3-leaf stage was somewhat unusual. This response cannot be explained by either stubble WSC concentration or content because there was no correlation between stubble WSC concentration and crop growth rate ($r = 0.216$; $p = 0.141$) and WSC content between 2- and 3-leaf stages was not different. The results for crop growth rate in our study were similar to that reported by Turner, Donaghy et al. (2006) harvesting prairie grass and cocksfoot at the 4-leaf stage of regrowth resulted in greater crop growth rate compared with a 2- or 3-leaf stage. The overarching factors responsible for this trend are that forage plants defoliated frequently are often left with little or no leaf area compared to those defoliated infrequently and are, therefore, unable to meet the energy demands necessary for regrowth and respiration solely through photosynthesis during the immediate post-defoliation period (e.g., Lee et al., 2009). Further, there is sufficient empirical evidence that shows frequent and intense defoliation depletes the energy reserves in the tiller base (stubble) of forages, hence limiting their overall productivity (e.g., Donaghy & Fulkerson, 1997; Donaghy et al., 2008).

Generally, Maximus had greater forage harvested than Marshall and this was different from the cultivar effect on tiller density because Marshall had a greater tiller population density than Maximus. Typically, tiller weight of grasses decreased as plant density increases (e.g., Davies & Thomas, 1983; Lonsdale & Watkinson, 1982; Matthew, Hernandez-Garay, & Hodgson, 1996; Simons, Davies, & Troughton, 1972; and Smit, Tas, Taweel, & Elgersma, 2005) and tiller density and mass are responsible for forage yield (Hernández Garay, Matthew, & Hodgson, 1997; Muir, Sanderson, Ocumpaugh, Jones, & Reed, 2001). Based on observations in the field, Maximus had larger stems (tiller mass) and leaf size (parameters that were not evaluated) than Marshall and even though tiller population density was less than Marshall, the mass of Maximus tiller possibly accounts for the greater forage harvested compared to Marshall. Both stubble WSC concentration and content were greater for Maximus than for Marshall and there was a positive, but weak correlation between WSC content and crop growth rate ($r = 0.299$; $p = 0.039$) in our study, and therefore, the role of WSC on the difference in forage harvested between Maximus and Marshall is unclear. Other studies have reported a strong positive linear relationship between WSC concentration and content on the regrowth of several forage grass species (Donaghy & Fulkerson, 1998; Donaghy et al., 2008; Lee et al., 2009; Turner, Donaghy et al., 2006; Turner, Donaghy et al., 2006; Turner et al., 2007). Yet, other reports have pointed to the inconsistency in this relationship (Donaghy & Fulkerson, 1998; Richards & Caldwell, 1985; White, 1973) and that other factors such as N reserve in the stubble (Thornton & Millard, 1997; Turner et al., 2007), the contribution of stored WSC in the roots (Caldwell, Richards, Johnson, Nowak, & Dzurec, 1981) and the concurrent occurrence of photosynthesis (leaf area post-defoliation) at the canopy level (Donaghy & Fulkerson, 1997; Richards & Caldwell, 1985) are equally important in forage grass regrowth. In our study, we did not measure the N concentration of the stubble, root WSC concentration, nor leaf area index (photosynthetic activity), but observation of the morphological traits in the field, that is, tiller and leaf size (leaf area), resulting in differences in their photosynthetic capacity (Turner, Humphreys, Cairns, & Pollock, 2001) may have been the contributing factors as both tiller and leaf were of greater size for Maximus than for Marshall annual ryegrass. Further, the results reported in the literature have been mixed in relation to the ploidy level effect on forage harvested for tetraploid and diploid annual ryegrass. Among six entries of annual ryegrass evaluated during the 1997–1998 and 1998–1999 growing seasons across four locations in Louisiana, Redfearn, Venuto, Pitman, Alison, and Ward (2002) reported no differences in forage harvested, but in a 12-year (1987–1998) variety testing trial using 30 entries at five locations across Louisiana, there were differences among several entries (Redfearn, Venuto, Pitman, Blouin, & Alison, 2005). Parish (2010) in variety testing trials across four locations in Mississippi reported no difference between Maximus and Marshall. Nelson, Crowder, and Rouquette (2011) reported that across two locations in Texas, forage harvested was greater for Maximus than for Marshall at Beaumont but was similar between these cultivars at Overton. These mixed trends are suggesting to us that Genotype × Environment seems to be the greatest contributing factor for differentiation in forage harvested among ryegrass cultivars and ploidy level; hence, site-specific interpretation of productivity among cultivars may be the best approach.

Forage harvested generally was greater at 5 than at 10 cm RSH, indicating that defoliation intensity is an important consideration in harvest management. Harvesting at a greater depth in the canopy may have played a partial role in this response. Brink et al. (2010) reported that grasses cut at 5 cm RSH produced greater annual forage harvested than those cut at 10 cm RSH. Also, similar results of greater forage harvested at 5 cm than at 10 cm RSH for both perennial ryegrass and tall fescue were reported by Hamilton, Kallenbach, Bishop-Hurley, and Roberts (2013). Contrary to findings in our study, however, Volesky and Anderson (2007) studying defoliation effects on several perennial grasses (smooth bromegrass [*Bromus inermis* Leyss.], orchardgrass, creeping foxtail [*Alopecurus arundinaceus* Poir.] and meadow bromegrass [*Bromus riparius* Rhem.]) reported a 27% reduction in total forage harvested averaged across species from plant defoliated at 7 cm compared to 14 cm RSH. Further, Inyang et al. (2010) using "Mulato II" brachiariagrass (*Brachiaria* sp.) reported a quadratic decrease in herbage accumulation with increasing stubble height harvest from 2.54 to 12.7 cm RSH. In a study with bahiagrass (*Paspalum notatum* Flügge.) harvested at either 4 or 8 cm RSH, stubble height did not affect total forage harvested (Interrante et al., 2009). These varying responses indicate that in different experiments, the combination of residual stubble height and defoliation frequency may have differing responses on forage parameters as a result of the time leaf senescence occurred post-defoliation, nodal

branching (tillering) and root initiation (e.g., Fulkerson & Donaghy, 2001; Hamilton et al., 2013; Lee et al., 2009). These responses can all be altered by residual stubble height, forage species involved and environmental conditions under which forage crops are grown. Based on the studies cited above, it appears that perennial forages have reduced forage mass with more intense defoliation, probably due to reduced persistence and slower crop growth rate. Lee et al. (2009) suggested that a minimum post-defoliation stubble threshold of 6.5 mg WSC/tiller is required for normal forage growth, and if stubble WSC content falls below this threshold, herbage accumulation was negatively affected. In our study, it was only during the first year at 2-leaf stage (Table 3) that WSC content of tillers fell below this threshold.

The structure of the sward canopy (e.g., tiller density, leaves per tiller, the size of leaves and stems) and morphogenetic traits (e.g., tiller appearance, leaf appearance rate and leaf extension rate) has a direct influence on forage productivity (e.g., Hirata & Padkiding, 2004). The tiller is the growth unit of grasses and constitutes the bulk of forage yield compared to the leaf component, and it also plays a vital function in the persistence of forage grasses (e.g., Sartie, Easton, & Matthew, 2009). Ultimately, awareness of tiller dynamics helps grassland managers understand the variation in dry-matter production among forage species, persistence and forage management approaches that can be utilized to ensure sward productivity and sustainability (e.g., Interrante, Sollenberger, Blount, White-Leech, & Liu, 2010). In our study, there was greater tiller density for Marshall compared to Maximus annual ryegrass and there was a 12% greater tiller density when defoliated at 5 compared to 10 cm RSH. Further, defoliation interval (2-, 3- and 4-leaf stages) had no effect on tiller density. The results reported in the literature of variations in tiller population density among forage species and intraspecies (cultivar differences, e.g., diploid vs. tetraploid perennial ryegrass) are quite compelling (Barre et al., 2006; Cheplick, 2008; Hume, 1991; Neuteboom, Lantinga, & Wind, 1988; Sartie et al., 2009; Smit et al., 2005). On the other hand, reports on tiller density variations as a result of defoliation intensity (i.e., RSH post-defoliation) are inconsistent among forage species. Among ryegrasses, instituting a forage management approach of low post-defoliation residual stubble height resulted in a consistent greater tiller population density compared to ryegrasses harvested at higher residual stubble surface height (e.g., Grant, Barthram, Torvell, King, & Smith, 1983; Matthew, Lemaire, Sackville Hamilton, & Hernandez-Garay, 1995; Yu, Nan, & Matthew, 2008). Other studies using different forage species have reported increased tiller density at intense defoliation (lower RSH) compared to lenient defoliation (higher RSH post-defoliation) (e.g., Malinowski, Hopkins, Pinchak, Sij, & Ansley, 2003; Sbrissia et al., 2010) while others have reported contrasting results of greater tiller density at lenient compared to intense defoliation (D'Angelo, Postulka, & Ferrari, 2005; Hamilton et al., 2013; Kalmbacher, Martin, & Pitman, 1986; Volesky & Anderson, 2007). Yet, others have reported no effect of residual stubble height post-defoliation on tiller density (e.g., Hamilton et al., 2013; Lee et al., 2009; Montagner et al., 2012). Pertaining to the effect of intervals between defoliation based on leaf stage and tiller density, the results reported are mixed, for example, Turner, Donaghy et al. (2007) reported no effect of defoliation intervals on tiller density. Contrastingly, other studies have reported greater tiller density for infrequent harvest compared to frequent harvest based on leaf stage defoliation interval (e.g., Donaghy & Fulkerson, 1998; Turner, Donaghy et al., 2007). It is difficult to juxtapose the cited studies with results obtained in our study pertaining to tiller density as both the environmental and management conditions were different under which these studies were performed. Thus, several studies have highlighted some important factors altering tiller density of grasses, stressful environment (e.g., drought), leaf appearance rate (longer phyllochron), reduced bud site usage, leaf area index, limitation in N nutrition, self-shading and genotypic variability among and within forage grass species (Akmal & Janssens, 2004; Davies & Thomas, 1983; Neuteboom & Lantinga, 1989; Simon & Lemaire, 1987; Skinner & Nelson, 1992). However, it seems that the main factor responsible for the difference in tiller density between Maximus (tetraploid) and Marshall (diploid) ryegrass in our study is morphogenetic, as tetraploid ryegrass has a slower leaf appearance rate, which is driven by the level of phytochrome activity resulting in reduced tiller density compared to diploid ryegrass (Davies & Thomas, 1983; Neuteboom et al., 1988; Skinner & Nelson, 1992).

Water-soluble carbohydrates are a major contributor to the regulation of growth and development in temperate grasses, and compared to other plant parts, the stubble of these grasses is known to contain the greatest concentration of WSC (Sandrin, Domingos, & Figueiredo-Ribeiro, 2006). The magnitude of reduction in reserve levels of WSC varies with the frequency and severity to which plants are defoliated. For example, the combined effects of lower stubble height and frequent defoliations have resulted in an eightfold reduction in stubble WSC concentration and a 17-fold reduction in WSC content (Donaghy & Fulkerson, 1998). In our study, stubble WSC concentration was 5% greater for Maximus than for Marshall and stubble WSC concentration at the 2-leaf stage was 13% less than at 3- and 4-leaf stages. Donaghy and Fulkerson (1998) also reported less WSC concentration at more frequent defoliation compared to less frequent defoliation. In our study, there was a 7% greater WSC concentration at the 5 cm stubble compared to 10 cm stubble, corresponding to results reported by Turner, Donaghy et al. (2007) of a decline in stubble WSC concentration of prairie grass from 5- to 10-cm stubble segments. Donaghy and Fulkerson (1998), however, reported greater WSC concentration at a higher stubble height (5.0 cm) compared to a lower stubble height (2.0 cm). Also, contrary to our results, Sandrin et al. (2006) found no difference in the WSC concentration in the upper and lower stubble of annual ryegrass. Stubble WSC content, which is a better indicator of forage crop regrowth potential than concentration (Donaghy & Fulkerson, 1998), was greater for Maximus than for Marshall, and this can be explained by both the greater WSC concentration and stubble weight of Maximus than Marshall. Also, the greater WSC content at 4-leaf stage than at 2- and 3-leaf stages in 2011 (Figure 4) can be explained by the greater WSC concentration and stubble weight at

4-leaf stage relative to 2- and 3-leaf stages. The greater WSC content per stubble at 4-leaf stage (Figure 4) in our study has been supported by similar results reported by Turner, Donaghy et al. (2006), Turner, Donaghy et al. (2007), using several perennial grasses. Further, Donaghy et al. (2008) reported a 33 to 43% greater WSC content for stubble of tall fescue harvested at 4-leaf stage relative to 2- and 3-leaf stages. The range of difference in WSC content in our study was 21 to 35% greater at 4-leaf stage than at 2-leaf stage. Similar to results of our study, Donaghy and Fulkerson (1998) reported that WSC content of perennial ryegrass stubble was less at 2 compared to 5 cm RSH. Whole-plant WSC carbohydrate concentration were 12% greater at 4-leaf stage compared with 2- and 3-leaf stages. In addition, harvesting at 5 cm RSH resulted in 7% greater whole-plant WSC concentration than harvesting at 10 cm RSH (Table 4). The trends in this study of greater stubble and whole-plant WSC at longer intervals between defoliation (3- and 4-leaf stages) compared to shorter defoliation interval (2-leaf stage) are indicative of the adequate time plant has to recover post-defoliation, thus allowing for maximum photosynthetic activity and full replenishment of stubble reserve carbohydrates. Therefore, the accumulation of these carbohydrates is reliant on photosynthesis and sink demand (Humphreys et al., 2006) of which time is essential for maximum irradiance interception a vital factor in photosynthesis. The greater concentration of WSC closer to the stem base is a common occurrence compared to further away from the stem base where dilution effect is considerable (Lee et al., 2009; Sandrin et al., 2006), thus decreasing the whole-plant WSC when defoliated at greater canopy height. In contrast, the WSC content is generally greater because of tiller mass when forage grasses are harvested further away from the stem base compared to close to the stem base. In studies that contrast the results of our study, environmental factors (e.g., nutrient and water availability, irradiance and temperature) may have played a significant role (e.g., Humphreys et al., 2006). Variation in concentration of WSC due to genetic differences has been reported for ryegrass (Humphreys, 1989; Smith et al., 2001) and that tetraploids generally have greater WSC concentration than diploid ryegrass (e.g., Hume, Hickey, Lyons, & Baird, 2010; Smith et al., 2001) because of the larger cells and higher ratio of cell content to the cell wall of tetraploids compared to diploids (Stewart & Hayes, 2011).

Overall, Maximus had an average 9% greater proportion of leaf blade than Marshall and there was no difference in the proportion of leaf blade at 2- and 3-leaf stages, but there was an average of 13.3% decline in the proportion of leaf blade when harvested at the 4-leaf stage (Table 3). There was a 3% greater proportion of leaf blade at 10 cm relative to the 5 cm RSH. Variation in plant morphological characteristics for cultivars within the same forage species is quite common, as other researchers have reported differences in the proportion of leaf blade for several cultivars of perennial ryegrass (Gilliland, Barrett, Mann, Agnew, & Fearon, 2002; Smit et al., 2005). Cuomo et al. (1996) studying plant morphology and nutritive value of three bahiagrasses reported a frequency effect on plant morphological characteristics and that the proportion of leaf declined by 8.3% when bahiagrass was harvested at 40 days (infrequent defoliation) compared to 20 days (frequent defoliation). Hurley, O'Donovan, and Gilliland (2009) reported that both 3- and 4-leaf stages overall had a greater proportion of pseudostem than 2-leaf stage, reflective of an increase in stem elongation rate as plant maturity increased. From a cultivar perspective, the higher proportion of leaf for Maximus (tetraploid) compared to Marshall (diploid) can be accounted for by the genetic compensatory relationship between tiller size and density (Griffiths, Matthew, Lee, & Chapman, 2016). Griffiths et al. (2016) using several diploids and tetraploids perennial ryegrass reported the existence of a one-to-one relationship between tiller size and density and therefore suggested that larger tillers would be leafier which was the case for tetraploid ryegrass in our study. The decrease in the proportion of leaf at 4-leaf stage compared with 2- and 3-leaf stages is a typical response associated with infrequent harvest and it is linked with tiller mass increased and leaf senescence observed in the field. Other morphological components such as pseudostem, reproductive stem and dead material are inversely proportional to the leaf blade proportion.

In our study, Maximus had a 4% greater CP concentration than Marshall and harvesting in the upper portion of the canopy (10 cm RSH) relative to the lower depth (5 cm RSH) did not influence CP concentration. The forage cultivar difference was in contrast to the difference in CP concentration of diploid and tetraploid perennial ryegrasses reported by Balocchi and López (2009). Harvesting at 2-leaf relative to 3- and 4-leaf stages resulted in 8–22% reduction in CP concentration (Table 4). The NDF and ADF concentrations at 4-leaf stage increased by an average of 11.5–22.5% relative to 3- and 2-leaf stages. These differences may be attributed to lesser proportion of leaf and a greater proportion of senescent material at the 4-leaf stage (Table 3). In vitro true digestibility of forage harvested at 4-leaf stage declined by 7% and NDFD by 6% compared with 2- and 3-leaf stages (Table 4). Similar to the results of our study, Donaghy et al. (2008) reported a decrease in CP and digestibility and an increase in NDF and ADF when tall fescue was harvested at 4-leaf stage relative to 2- or 3-leaf stage. Lee et al. (2009) also reported a defoliation interval effect on CP, NDF, ADF and digestibility and all had a similar response to that observed in our study. Volesky and Anderson (2007) reported that both CP and digestibility were less at 7 cm relative to 14 and 21 cm RSH defoliation. In contrast to Volesky and Anderson (2007), CP concentration was not affected by stubble height in our study, but there was a difference in IVTD. The overall trends of increased in WSC, NDF and ADF and decreased in CP, IVTD and NDFD concentrations were expected as these nutritive value parameters typically decline with advanced plant age as a result of an accelerated increase in cell wall carbohydrate and lignin concentrations (Donaghy et al., 2008).

5 | SUMMARY AND MANAGEMENT IMPLICATIONS

Harvest management based on leaf stage defoliation interval had a definitive effect on responses in the first year of the study, with

greater forage harvested at the 4-leaf stage compared with 2- and 3-leaf stages. In the second year of the study, however, there was no effect of leaf stage on forage harvested. Forage harvested generally was greater at the 5 than at the 10 cm RSH. Tiller density was greater for Marshall than for Maximus and at 5 cm RSH than at 10 cm RSH, but leaf stage had no effect on tiller population density. Stubble WSC concentration and content were greater at 4-leaf stage than at 2- and 3-leaf stages. Also, WSC concentration was greater at 5 than at 10 cm RSH, but content was greater at 10 than at 5 cm RSH. Maximus had both greater stubble WSC concentration and content than Marshall. Forage morphological characteristics responded to forage cultivar, leaf stage and stubble height effects with less proportion of leaf blade, and greater proportion of pseudostem, reproductive stem and dead material at 4-leaf stage compared to 2- and 3-leaf stages. Leaf blade proportion was greater in Maximus than in Marshall and at the 10 vs. 5 cm RSH.

Harvesting annual ryegrass at the 4- vs. the 2- and 3-leaf stages results in greater forage harvested, NDF, ADF, WSC concentrations and WSC content of tiller but reduces the proportion of leaf blade and ultimately forage nutritive value by decreased CP, IVTD and NDFD. Water-soluble carbohydrates are major sources of energy for dairy cattle and energy intake is usually a limiting factor for milk production in grass-based dairy operations (Cosgrove et al., 2007). There has been a positive response of milk yield and milk protein yield of dairy cattle fed forages containing greater WSC concentration (Miller et al., 2001). Further, Oba and Allen (1999) reported that forages of greater NDFD fed to dairy cows increased dry-matter intake and milk yield. In our study, there was a dichotomy in annual ryegrass response to defoliation intervals based on leaf stage and to stubble height. Based on annual ryegrass response to defoliation frequency (leaf stage) and intensity (post-defoliation RSH) in our study, producers should harvest (silage and haylage) or employ a rotational stocking scheme that coincides with the 4-leaf stage of regrowth at 10 cm residual stubble height to maximized productivity of annual ryegrass pastures over the duration of the growing season. What is not clear from the results of this study is whether the range of differences in nutritive value (CP, NDF, ADF, IVTD and NDFD) that favours 2- and 3-leaf stage defoliation interval may be transformed into any biological impact on the performance of grazing animals. Further, because responses observed in clipping studies can vary from those in grazing systems, conducting a grazing study using a rotational stocking scheme based on these defoliation intervals and a range of grazing intensities will provide valuable insights to authenticate the most suitable defoliation interval and intensity for annual ryegrass utilization in grazing pastures. Findings from grazing studies may ultimately validate appropriate management options and mitigate injudicious utilization of annual ryegrass pastures.

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