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## DISTINCT PHYSIOLOGICAL RESPONSES UNDERLIE DEFOLIATION TOLERANCE IN AFRICAN LAWN AND BUNCH GRASSES

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*Premise of research.* African grass communities are dominated by two distinct functional types: tall, caespitose bunch grasses and short, spreading lawn grasses. Functional type coexistence has been explained by differences in defoliation tolerance, because lawn grasses occur in intensively grazed areas while bunch grasses are less associated with heavy grazing. If different responses to tissue loss explain their distribution, expectations are that biomass production and leaf-level physiology will be negatively impacted in bunch relative to lawn grasses.

*Methodology.* We tested the influence of defoliation on three lawn and three bunch grasses from Tanzania and South Africa by quantifying growth and measuring physiological response of these grasses to simulated herbivory in a glasshouse experiment. Specifically, we measured photosynthesis, transpiration, stomatal conductance, leaf dry matter content (LDMC), specific leaf area (SLA), leaf nitrogen, and leaf pigment concentrations in leaves of bunch and lawn grasses that were clipped or unclipped.

*Pivotal results.* In contrast to our expectations, clipped lawn and bunch grasses did not differ in photosynthesis, leaf nitrogen, or biomass production, and both lawn and bunch grasses upregulated photosynthesis in response to clipping. However, defoliated bunch grasses had higher rates of stomatal conductance and transpiration compared with defoliated lawn grasses. Also, leaf carotenoid concentrations increased in response to clipping for both functional types but much more in bunch than in lawn grasses. An analysis of leaf-level physiological relationships with structural equation modeling showed that lawn and bunch grasses exert control over carbon gain in different ways. In bunch grasses, net carbon gain was associated with leaf-level structural properties (LDMC and SLA) that varied in response to defoliation, while in lawn grasses, increased carbon gain was the result of increased leaf [N] subsequent to defoliation.

*Conclusions.* The varied responses of lawn and bunch grasses to defoliation appear to arise from their different investments in defense and carbon assimilation subsequent to defoliation. Bunch grasses invest relatively more in carotenoid production, likely as a mechanism to enhance regrowth and protect costly leaves from photodamage. Moreover, bunch grasses maintain efficient carbon assimilation by structural adjustments in leaves (decreasing LDMC subsequent to defoliation), while lawn grasses maintain efficient water use by increasing leaf [N] subsequent to defoliation. Thus, we conclude that a key difference between lawn and bunch grasses is not defoliation tolerance per se but physiological adaptations that constrain them to environments with different moisture availability subsequent to defoliation.

*Keywords:* assimilation rate, carotenoids, grazing, leaf nitrogen, leaf tissue density, photosynthesis, SLA, structural equation model.

### Introduction

Understanding the response of organisms to disturbance and stress underlies basic theories of adaptation (Stanton et al. 2000), species coexistence (Shea et al. 2004), and maintenance of species diversity in ecological communities (Tilman 1994). Among plants, grasses are a model system to study plant adaptations to disturbance because they are among the most widely distributed of all the plant families (Cheplick 1998),

having successfully colonized almost every biome on earth. Consequently, grasses have evolved a wide variety of traits that mitigate many of the major ecological and environmental challenges facing land plants, for example, water and nutrient limitation, hypersaline soils, extreme temperatures, and disturbance. A key innovation in the grass lineage has been the evolution of the C<sub>4</sub> photosynthetic pathway, which has facilitated their expansion into open, often stressful habitats (Sage 2004; Osborne and Freckleton 2009; Edwards and Smith 2010; Edwards et al. 2010).

While much attention has been paid to the functional adaptation of the C<sub>4</sub> photosynthetic pathway in grasses (Taylor

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et al. 2010), less attention has been paid to the evolution of other innovations that give rise to functional variation in grasses. For example, African grasses are grouped into broad categories on the basis of their morphology and reproduction: tall, caespitose bunch grasses, which largely establish from seed, and prostrate lawn grasses, which spread largely by stolons (McNaughton 1984, 1985; Archibald et al. 2005; Cromsigt and Olff 2008; Stock et al. 2010). It is often assumed that these functional types are an evolutionary consequence of variation in tolerance to defoliation by large ungulate herbivores, with lawn grasses better able to tolerate herbivory than bunch grasses (McNaughton 1984; Hartvigsen and McNaughton 1995; Benot et al. 2008; Cromsigt and Olff 2008; Stock et al. 2010). Indeed, there has been some evidence that tall and short grasses respond differently to defoliation in terms of carbon assimilation, water use, and biomass compensation (Coughenour 1985; van Staalduinen and Anten 2005; Anderson et al. 2006). Current theory suggests that spatial and temporal heterogeneity in herbivory encourages coexistence between these functional groups at both small and large scales (Coughenour et al. 1985; Augustine and McNaughton 1998; Adler et al. 2001; Cromsigt and Olff 2008). Differences in stature imply that a trade-off between the light competitive ability of the tall grasses and defoliation tolerance (or avoidance) of the short grasses promotes coexistence of both groups (Coughenour et al. 1985; Augustine and McNaughton 1998; Huisman and Olff 1998; Cullen et al. 2006). Implicit in the theory is the assumption that physiological processes in bunch grasses are negatively impacted when defoliated and that they require time to recover normal physiological functioning. For example, one expectation is that carbon assimilation ( $A_n$ ) and the ability to respond to abruptly changing light levels declines after defoliation in bunch grasses—but not in lawn grasses—because of reduced resource allocation to photosynthesis (rubisco/chlorophyll) and photoprotective pigments (carotenoids). Some tall grasses experience decreased photosynthetic rates subsequent to defoliation, but typically such observations are associated with changes in resource availability (Anderson et al. 2006) or severe defoliation (Hodgkinson et al. 1989). On the other hand, compensation—or, in some cases, stimulation—of photosynthesis subsequent to defoliation in short-stature grasses has been observed to occur via several mechanisms, including stomatal regulation (Wallace et al. 1984) and increased enzyme activity (Anderson et al. 2006).

An alternative hypothesis is that plants in herbivore-dominated ecosystems share a more general tolerance of herbivory (Tiffin 2000; Fornoni 2011); this is consistent with studies that have shown that tall grasses are able to compensate for defoliation in terms of photosynthesis or biomass production (Wallace et al. 1984; Coughenour et al. 1985). Under this hypothesis, lawn and bunch grasses evolved different strategies for compensating for tissue loss according to the specific environments in which they typically experience defoliation. For lawn grasses, these tend to be seasonally dry, high-light habitats, while for bunch grasses, these tend to be more mesic habitats in which competition for light is intense. The differences between these alternative hypotheses outlined above have consequences for understanding plant evolutionary adaptation to stress in general and grass evolution more specifically.

The objective of our study was to explore the physiological effects of defoliation on lawn and bunch grasses from African grazing ecosystems, those in which ungulate herbivores are abundant members of the faunal communities. Our a priori hypothesis was that tall grasses would experience a physiological decline in carbon assimilation and other leaf-level physiological processes, such as water use efficiency (WUE) and investment in chlorophyll pigments *a* and *b*, whereas lawn grasses would be relatively resilient. Because changes in pigment concentrations may be associated with an adaptive response to stress in grasses (e.g., protection against photodamage), we also assayed carotenoid concentration in functional types after defoliation. Our final goal was to compare leaf-level relationships among leaf tissue density, specific leaf area (SLA), [N], and carbon assimilation in lawn and bunch grasses and ask whether these functional types responded differently to defoliation. We achieved this goal by constructing separate models for lawn and bunch grasses, based on existing physiological models that mechanistically decompose carbon gain (Evans and Poorter 2001; Shipley et al. 2005), and evaluating them with observed data.

## Methods

The effects of repeated defoliation on leaf-level physiological responses of lawn and bunch grasses were measured in a 73-d laboratory study lasting from March 1 to May 12, 2009. Five species were selected for this study: three lawn and two bunch grasses from Serengeti National Park (lat. 2°19'58"N, long. 34°34'00"E; Tanzania) and Hluhluwe-iMfolozi Park (lat. 28°02'24"N, long. 32°03'36"E; KwaZulu-Natal Province, South Africa). Four species, all with the  $C_4$  photosynthetic pathway, were collected from each study site; three were common to both sites, and the remaining two were found at only one of the sites. The species from Serengeti National Park were *Digitaria macroblephara* (lawn), *Panicum coloratum* (lawn), *Panicum maximum* (bunch), and *Sporobolus pyramidalis* (bunch). The species from Hluhluwe-iMfolozi Park were *Digitaria longiflora* (lawn), *P. coloratum* (lawn), *P. maximum* (bunch), and *S. pyramidalis* (bunch). Species were selected on the basis of their importance in the ecosystems and their known ecological roles as either lawn or bunch grasses (McNaughton 1983; Anderson et al. 2007; Cromsigt and Olff 2008).

In January 2009, multiple clones of each species were collected from five replicate sites within each ecosystem, chosen along gradients of rainfall and grazing intensity. Grasses were transported to a glasshouse at the University of Groningen and grown for an acclimation period of ~1 mo. Subsequently, two clones from each species, site, and ecosystem were selected for inclusion in the study, reduced to ~5 tillers plant<sup>-1</sup>, clipped to 10 cm (roots and shoots), and planted in a 5 : 4 : 1 volumetric ratio of potting soil, sand, and clay (Anderson et al. 2006) in polyvinyl chloride pots (10 cm in diameter and 30 cm tall). One clone from each combination of species, site, and ecosystem was randomly assigned to either a clipped or an unclipped treatment. The experimental design followed a split-plot design, with functional type (lawn and bunch) × ecosystem (Serengeti National Park and Hluhluwe-iMfolozi Park) × treatment (clipped and unclipped) combinations with five replicates

(sites). All grasses were supplied with 50 mL of deionized Hoagland's solution (Anderson et al. 2006) once a week. Pots were maintained at constant soil moisture by weighing pots frequently (every 2–3 d) and adding water as needed to achieve a predetermined target weight equivalent to 42% of water holding capacity. Plants in the simulated grazing treatment were clipped to a height of 5 cm three times during the experiment: on days 20, 33, and 46. All plant material removed during simulated grazing was dried at 65°C for 48 h and weighed for inclusion with final harvest biomass. Final plant biomass, leaf tissue density, and nitrogen were measured on each of the five replicates per treatment (except in the rare case of mortality), whereas leaf-level physiological measurements were collected on three of the five plants selected at random.

#### *Plant Biomass, Leaf Structural Properties, and Leaf Nitrogen*

**Plant biomass.** Before planting, the wet weight of each ramet was established before planting as a way to ensure that no size biases existed among treatments or functional types before the start of the experiment. Our measurements confirmed that there was no biomass difference between functional groups ( $F = 0.81$ ,  $P = 0.42$ ), treatments ( $F = 0.13$ ,  $P = 0.72$ ), or functional groups by treatments ( $F = 0.002$ ,  $P = 0.97$ ) at the time of planting. On the final day of the experiment, all plants were harvested, roots washed of soil, and dried at 65°C for 48 h. Final biomass was calculated as the summed weight of all dried plant components; in the case of the clipping treatment, this included the biomass removed during the three simulated grazing events.

**Leaf dry matter content (LDMC) and SLA.** On the day of final harvest, LDMC, also called leaf tissue density, was measured as the percentage of dry leaf weight to fully hydrated wet leaf weight. SLA ( $\text{m}^2 \text{kg}^{-1}$ ) was measured by estimating the leaf area of three to five fully hydrated leaves from digital pictures with the software SigmaScan (Systat Software, San Jose, CA) and dividing by the leaf dry weight (Anderson et al. 2011).

**Leaf nitrogen.** Leaf nitrogen (%N) was estimated on harvested biomass at the termination of the experiment using a Bruker near-infrared spectrophotometer (NIR; Bruker, Ettlingen). Samples were oven dried at 70°C for 48 h and ground to a fine power on a Foss Cyclotec grinder (Foss, Hillerød) before analysis. The NIR spectra obtained from our samples were used to estimate their %N values from a multivariate calibration of African grass samples measured on the NIR and a CHNS EA1110 elemental analyzer (Carlo-Erba Instruments, Milan) to obtain the true %N values. Validation tests between the N concentrations obtained from the elemental analyzer and those predicted from the NIR using the cross-validation method showed the NIR method to be highly accurate ( $R^2 = 0.99$ ,  $n = 203$ ).

#### *Leaf-Level Physiological Measurements*

**Gas exchange.** Leaf-level rates of gas exchange per unit area—including net carbon assimilation ( $A_n$ ;  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $G_s$ ;  $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ), and tran-

spiration ( $E$ ;  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ )—were measured with the TPS-2 Portable Photosynthesis System with a  $25 \times 18\text{-mm}$  leaf cuvette fixed with a variable PLC4 LED light unit (PLC6; PP Systems, Hitchin). Leaf width in the cuvette was measured for each sample to the nearest 0.1 mm to correct for gas exchange per unit leaf area. All measurements were made in a climate-controlled chamber at a constant temperature (25°–27°C) and background light levels. Gas exchange measurements were made on the two youngest fully expanded leaves on three individuals per grass species. Measurements were made at three different light levels in the cuvette (320, 420, 990  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and in darkness to determine plant respiration rates. Multiple measures of gas exchange were taken for each leaf blade to ensure that steady-state responses were achieved. Gas exchange parameters were calculated automatically using the software provided with the photosynthesis meter. Following Shipley et al. (2005), net carbon assimilation was also expressed on a mass basis ( $A_m$ ;  $\text{mg C g}^{-1} \text{d}^{-1}$ ). WUE, defined as the ratio  $A_m : E$ , was estimated for different species to compare carbon assimilation per unit of water loss.

#### *Leaf Pigment Concentrations*

Tissue samples were extracted with a 0.4-mm<sup>2</sup>-diameter punch from the same place on the leaf where gas exchange and stomata density were measured. Leaf disks were placed in test tubes with liquid nitrogen long enough for it to evaporate, after which they were crushed with a glass rod. Three milliliters of 80% acetone was added per leaf disk, sealed, and stored for 12 h in the dark at 4°C. Subsequently, samples were centrifuged for 10 min, and 1 mL of supernatant was pipetted into a  $1 \times 1\text{-cm}$  glass cuvette. The solution was analyzed using a spectrophotometer (Shimadzu UV-1250, Tokyo) at absorbances of 663.2, 646.8, and 470 nm. Chlorophyll *a*, chlorophyll *b*, and carotenoid pigment concentrations ( $\mu\text{g mL}^{-1}$ ) were calculated using standard equations (Lichtenthaler 1987). The ratio of chlorophyll *a* to chlorophyll *b* was calculated to provide an index of N partitioning between energy transfer via photosystem II (chlorophyll *a*) and energy capture via light-harvesting protein complexes (chlorophyll *b*). The theory of optimal N allocation predicts increased chlorophyll *a* : chlorophyll *b* ratios with increasing light for a given level of N availability (Hikosaka and Terashima 1995; Kitajima and Hogan 2003).

#### *Statistical Procedures*

To determine the effects of defoliation on the final biomass and leaf-level physiological measures of lawn and bunch grass functional types, we used a linear mixed-effects model with defoliation (clipped or unclipped), grass functional type (bunch or lawn), and defoliation  $\times$  functional type interactions as fixed effects and species and sites as separate random effect. Thus, species collected within different sites were considered as replicates. Analyses were conducted in R (ver. 2.10.1) using the lme procedure (package nlme; Pinheiro et al. 2011) and a crossed random effects model on a grouped data object, as described by Pinheiro and Bates (2000, pp. 163–166). Data were evaluated for assumptions of normality and homosce-

**Table 1**  
**Linear Mixed-Effects Model Results for Various Leaf-Level Physiological Measures of African Lawn and Bunch Grasses (Functional Type) That Were Either Clipped or Unclipped (Treatment)**

Physiological parameters	df	Functional type		Treatment		Functional type × treatment	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Plant biomass and leaf properties:							
Total final biomass	1, 72	4.67	<u>.0340</u>	119.56	<u>&lt;.0001</u>	13.46	<u>.0005</u>
Leaf dry matter content	1, 72	12.59	<u>.0007</u>	16.54	<u>.0001</u>	1.61	<u>.2083</u>
Specific leaf area	1, 72	2.24	.1388	1.51	.2224	.68	.4125
Leaf nitrogen (%N)	1, 42	3.36	.0735	66.25	<u>&lt;.0001</u>	7.87	<u>.0075</u>
Leaf pigment concentrations:							
Chlorophyll <i>a</i>	1, 40	.01	.9338	1.77	.1905	.01	.9670
Chlorophyll <i>b</i>	1, 40	.11	.7464	22.00	<u>&lt;.0001</u>	2.86	<u>.0986</u>
Carotenoids	1, 33	2.56	.1192	47.42	<u>&lt;.0001</u>	10.72	<u>.0025</u>
Gas exchange:							
Net photosynthesis:							
<i>A<sub>o</sub></i>	1, 42	1.74	.1944	1.94	.1711	.58	.4517
<i>A<sub>m</sub></i>	1, 42	1.11	.2976	5.38	<u>.0253</u>	.276	.3898
Transpiration ( <i>E</i> )	1, 42	6.64	<u>.0136</u>	6.46	<u>.0148</u>	5.25	<u>.0271</u>
Stomatal conductance ( <i>G<sub>s</sub></i> )	1, 42	2.20	<u>.1457</u>	3.90	.0548	4.25	<u>.0456</u>
Water use efficiency ( <i>A<sub>m</sub> : E</i> )	1, 42	.17	.6810	.62	.4344	.76	.3894

Note. Underlined *P* values are those considered significant at  $\alpha = 0.05$ .

dasticity using Shapiro-Wilk's test and residual plots before analysis. Separate models were evaluated for each of our response variables, which included final biomass, LDMC, SLA, %N, *A<sub>o</sub>*, *A<sub>m</sub>*, *G<sub>s</sub>*, *E*, WUE, and concentrations of photosynthetic pigments (carotenoids and chlorophylls *a* and *b*). Statistical significance of main and interaction effects from the linear mixed-effects model were evaluated with a Wald test for each term in the model; subsequent contrasts between individual treatments were conducted using the R package contrast (Kuhn 2011), with differences considered significant at  $\alpha = 0.05$ .

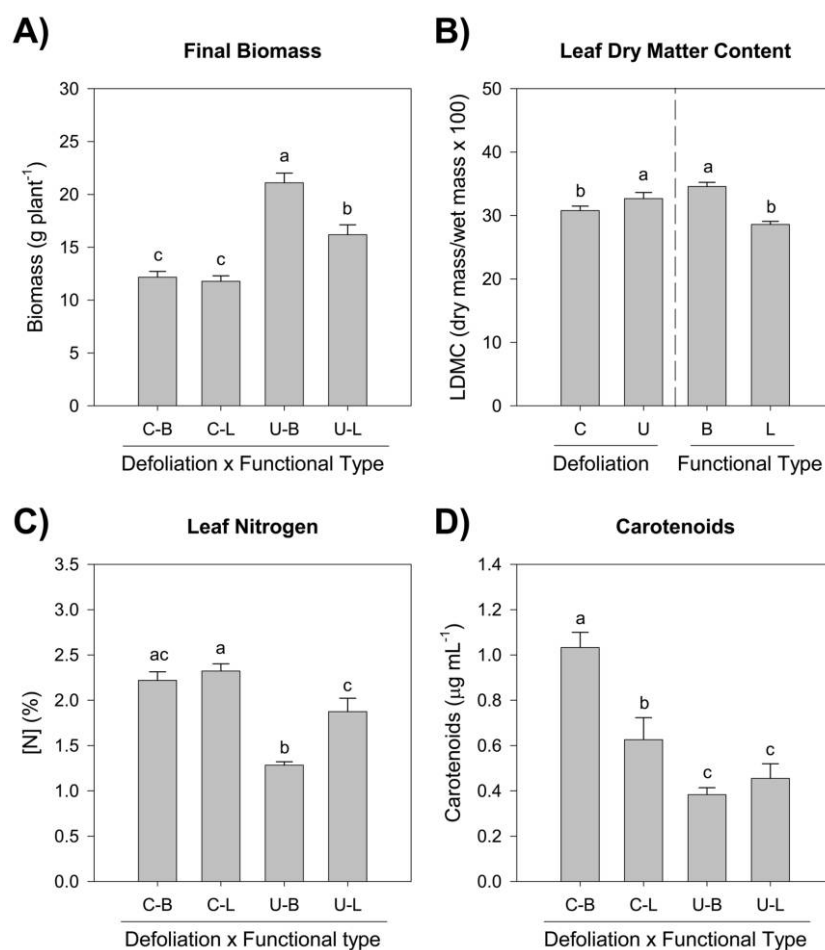
To compare leaf-level physiological relationships between lawn and bunch grasses, we used structural equation (SE) modeling (Grace 2006). Our first step was to create a conceptual model representing theoretical relationships among LDMC (mg dry mass g<sup>-1</sup> fresh mass), SLA (m<sup>2</sup> kg<sup>-1</sup>), leaf N (mmol N g<sup>-1</sup>), and net carbon assimilation (*A<sub>m</sub>*; mg C g<sup>-1</sup> d<sup>-1</sup>), as specified by Shipley et al. (2005), which then guided the analysis of the observed data using the SE model. There were two notable changes between our model and that of Shipley et al. (2005). First, we had no measure of leaf thickness. However, leaf thickness in the Shipley et al. (2005) model is a direct predictor of SLA and leaf N but only an indirect predictor of *A<sub>m</sub>*; therefore, omitting leaf thickness reduces variance explanation of upstream predictors but not for carbon assimilation itself. Second, because we were specifically interested in functional type responses to defoliation, we included it as a predictor in the model. Data from all plants (i.e., both clipped and unclipped) were included, but analyses were conducted separately for bunch and lawn grasses. As with Shipley et al. (2005), *A<sub>m</sub>* was ln transformed before analysis. Evaluation of the conceptual model with SE modeling was based on maximum likelihood procedures and was conducted in AMOS (ver. 19.0; Arbuckle 2007). We implemented a model-pruning strategy, in which nonsignificant paths ( $\alpha > 0.10$ ) were trimmed

until only significant paths remained; this  $\alpha$  value was selected because of the reduced sample size associated with analyzing lawn and bunch grasses in separate models. Model fit was assessed using the  $\chi^2$  statistic and its associated *P* value. A model was deemed adequate when the observed and expected covariances were not significantly different from one another on the basis of the critical *P* value of  $\alpha > 0.10$ . Note that values of  $P \gg 0.10$  associated with the  $\chi^2$  statistic suggest that the observed covariance structure of the data was not statistically different from the covariance structure expected from the model. Standardized path coefficients are presented because we are interested in the relative effects of different predictors.

## Results

### Final Plant Biomass

There was a statistically significant interaction between the defoliation treatment and grass functional type on final biomass (table 1; fig. 1A). This result arose because unclipped bunch grasses had a larger final biomass than unclipped lawn grasses (contrast  $\pm$  SE = 5.44  $\pm$  1.58 g,  $t_{69} = 3.45$ ,  $P = 0.0009$ ), which in turn had a larger final biomass than either of the clipped treatments (contrast  $\pm$  SE = 3.91  $\pm$  1.0 g,  $t_{69} = 3.9$ ,  $P = 0.0002$ ). Contrary to our expectations, the final biomass of clipped bunch grasses was not different from clipped lawn grasses (contrast  $\pm$  SE = 0.95  $\pm$  1.57 g,  $t_{69} = 0.61$ ,  $P = 0.54$ ). The indistinguishable final biomass of clipped lawn and bunch grasses (fig. 1A) is an important prelude to the remainder of the results because it demonstrates that defoliation did not have a more detrimental effect on the bunch grasses in terms of absolute biomass accumulation, as might be expected.



**Fig. 1** Response of final plant biomass (A), leaf dry matter content (LDMC; B), leaf nitrogen ([N]; C), and carotenoid concentration (D) to interactions between functional type bunch (B) or lawn (L) and defoliation clipped (C) or unclipped (U). Only significant main or interaction effects are shown. Bars show means + SE of treatment combinations. Bars sharing a letter are not significantly different from Wald tests performed with the package contrast in R (see “Methods”).

#### LDMC and SLA

The leaves of bunch grasses had statistically higher LDMC than did the leaves of lawn grasses (contrast  $\pm$  SE =  $6.34\% \pm 1.78\%$ ,  $t_{69} = 3.56$ ,  $P = 0.0007$ ), and clipping decreased LDMC compared with unclipped leaves (contrast  $\pm$  SE =  $-2.03\% \pm 0.50\%$ ,  $t_{69} = 4.03$ ,  $P = 0.0001$ ); these results were consistent across functional types and treatments (table 1; fig. 1B). SLA did not differ between functional types or clipping treatments, nor was their interaction effect significant (table 1).

#### Leaf Nitrogen

There was a significant functional type by clipping interaction for leaf N (table 1; fig. 1C). Leaf N of clipped lawn and clipped bunch grasses did not differ from one another (contrast  $\pm$  SE =  $0.15\% \pm 0.21\%$ ,  $t_{40} = 0.70$ ,  $P = 0.49$ ), whereas unclipped lawn and bunch grasses were significantly different from one another (contrast  $\pm$  SE =  $0.64\% \pm$

$0.22\%$ ,  $t_{40} = 2.93$ ,  $P = 0.006$ ), with unclipped bunch grasses having lower leaf N than unclipped lawn grasses.

#### Pigment Concentrations

The concentration of chlorophyll *a* did not differ across functional type or clipping treatments (table 1). In contrast, clipping reduced leaf chlorophyll *b* compared with unclipped plants (contrast  $\pm$  SE =  $1.11 \pm 0.24$ ,  $t_{37} = 4.69$ ,  $P < 0.0001$ ), and this decrease was similar for both lawn and bunch grasses, as demonstrated by the lack of a significant interaction term (table 1). Because chlorophyll *a* did not vary across treatments, the chlorophyll *a* to chlorophyll *b* ratio followed exactly the pattern of the chlorophyll *b* results (data not shown). In the case of leaf carotenoid concentration, there was a significant functional type  $\times$  clipping interaction (table 1; fig. 1D). Clipping effects were especially pronounced within the bunch grasses, which had higher carotenoid concentrations than clipped lawn grasses (contrast  $\pm$  SE =  $0.33 \pm 0.11$ ,  $t_{30} =$

3.17,  $P = 0.0035$ ) and unclipped bunch grasses (contrast  $\pm$  SE =  $0.64 \pm 0.09$ ,  $t_{30} = 7.15$ ,  $P < 0.0001$ ). Clipped lawn grasses were also higher than grasses in either of the unclipped treatments (fig. 1D), while unclipped lawn and bunch grasses had indistinguishable carotenoid concentrations (contrast  $\pm$  SE =  $0.07 \pm 0.10$ ,  $t_{30} = 0.70$ ,  $P = 0.49$ ).

#### Gas Exchange and WUE

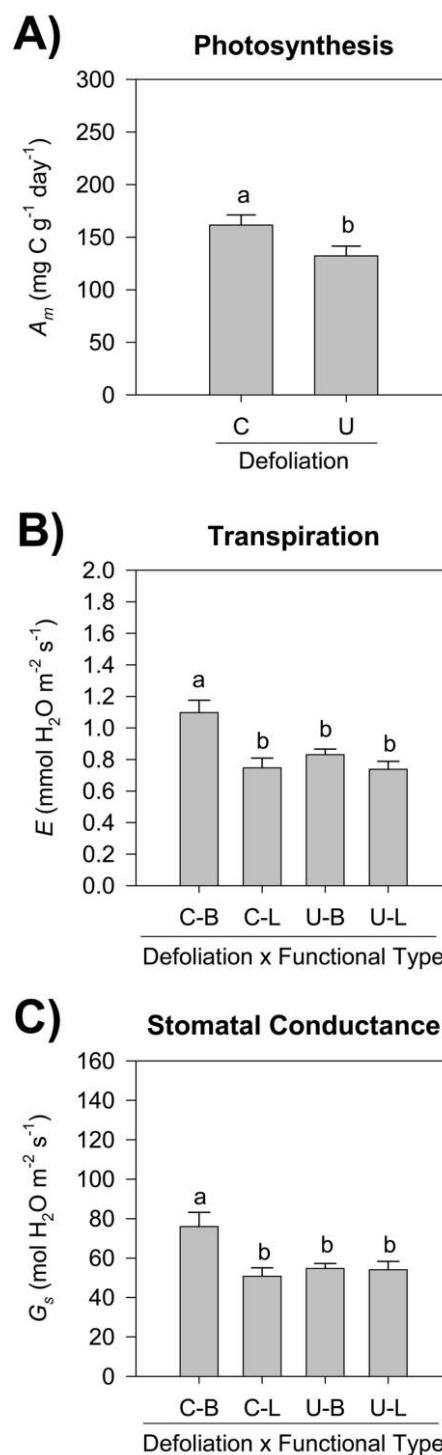
For all species,  $A_o$  was highest at PAR =  $990 \mu\text{mol m}^{-2} \text{s}^{-1}$  (results not shown); therefore, all measurements reported are at this light level. No differences were detected in carbon assimilation per unit area ( $A_o$ ) for any treatment (table 1), but carbon assimilation per unit mass ( $A_m$ ) was higher in clipped compared with unclipped grasses (table 1; fig. 2A; contrast  $\pm$  SE =  $28.19 \pm 12.11 \text{ mg C g}^{-1} \text{ d}^{-1}$ ,  $t_{40} = 2.33$ ,  $P = 0.025$ ). Bunch grasses increased  $E$  and  $G_s$  in response to clipping, whereas lawn grasses maintained stable  $E$  and  $G_s$  despite defoliation (table 1; fig. 2B, 2C). WUE did not vary consistently among any of the treatments (table 1), suggesting that increases in transpiration in clipped bunch grasses (fig. 2B) were offset by proportional increases in  $A_m$  (fig. 1A).

#### SE Model Results

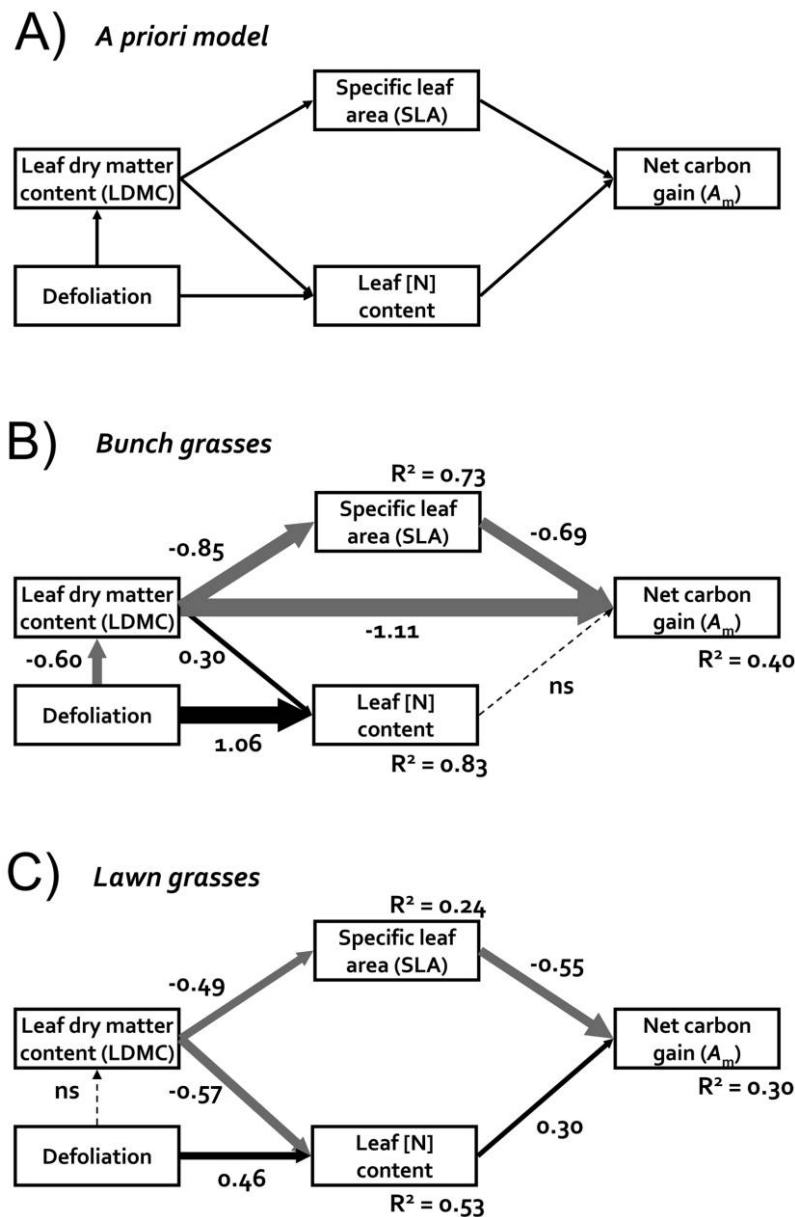
The final accepted SE models showed a good fit between the a priori model (fig. 3A) and the observed data for both bunch ( $\chi^2 = 4.53$ ,  $\text{df} = 4$ ,  $P = 0.34$ ) and lawn ( $\chi^2 = 6.24$ ,  $\text{df} = 5$ ,  $P = 0.28$ ) grasses. The final model for bunch grasses (fig. 3B) accounted for 40% of the variation in  $A_m$ ; the final model for lawn grasses accounted for 30% of the variation in  $A_m$  (fig. 3C). The variance explained in SLA was greater in bunch compared with lawn grasses (0.73 vs. 0.24), as was also the case for leaf [N] (0.83 vs. 0.53). For both functional types, the LDMC  $\rightarrow$  SLA path was negative as was the SLA  $\rightarrow$   $A_m$  path, which led to a positive indirect relationship between LDMC and  $A_m$ . However, variation between the final models suggests that different underlying physiological relationships govern carbon gain in lawn and bunch grasses. First, defoliation had stronger positive effects on leaf [N] in bunch (1.06) compared with lawn (0.46) grasses and a negative effect on LDMC in bunch grasses ( $-0.60$ ) but no effect in lawn grasses (fig. 3C). In addition, after controlling for defoliation effects, the standardized path coefficient between LDMC and leaf [N] had opposite signs for the two grass functional types: positive (0.30) for bunch grasses and negative ( $-0.57$ ) for lawn grasses. Moreover, the model for bunch grasses included a direct negative LDMC  $\rightarrow$   $A_m$  path ( $-1.11$ ), while no such effect existed for lawn grasses. Finally, leaf [N] was positively related to  $A_m$  in lawn grasses (0.30), while this path was not significant in bunch grasses.

#### Discussion

We expected that defoliation would reduce physiological functioning, resulting in reduced growth and biomass accumulation in bunch grasses compared with lawn grasses. These expectations arose because the literature suggests that lawn grasses are relatively insensitive to defoliation (McNaughton and Chapin 1985) and that the leaves of bunch grasses incur a higher relative cost (Belsky 1986). Our measurements con-



**Fig. 2** Response of net carbon gain ( $A_m$ ; A), leaf transpiration ( $E$ ; B), and leaf stomatal conductance ( $G_s$ ; C) to interactions between functional type bunch (B) or lawn (L) and defoliation clipped (C) or unclipped (U). Only significant main or interaction effects are shown. Bars show means + SE of treatment combinations. Bars sharing a letter are not significantly different from Wald tests performed with the package contrast in R (see "Methods").



**Fig. 3** A, A priori conceptual model modified from Shipley et al. (2005), which guided the structural equation (SE) modeling analysis of the data (for details, see “Methods”). Arrows represent hypothetical causal effects of one variable on another. B, Final SE model results for bunch grasses, showing positive (black) and negative (gray) direct relationships as arrows. The numbers associated with the arrows are standardized linear path coefficients; for statistically significant effects, the width of each arrow is proportional to the effect size, while nonsignificant effects are shown as dashed lines. As with Shipley et al. (2005), carbon assimilation was  $\ln$  transformed before analysis. The proportion of variance explained is shown by the  $R^2$  value associated with each response variable. C, Final SE model results for lawn grasses. Numbers, symbols, and transformations are as in B.

firmed that bunch grasses have higher LDMC compared with lawn grasses, a trait indicative of greater leaf carbon investment and leaf longevity (Garnier et al. 2001). However, in contrast to our predictions, both lawn and bunch grasses up-regulated  $A_m$  in response to clipping, and they had indistinguishable biomass and leaf [N] by the end of the experiment when clipped. Consequently, even though the ratios of clipped to unclipped biomass differed between functional types (fig.

1A), the similar response of bunch and lawn grasses in the clipped treatment was unexpected.

Our results also revealed important variation in the mechanistic processes underlying carbon and nitrogen assimilation. For example, when clipped, bunch grasses increased  $E$  and  $G_s$ , while no such increase was seen in lawn grasses (fig. 2), suggesting that bunch grasses increase transpiration as a mechanism to increase carbon assimilation, while lawn grasses favor



a strategy of water conservation subsequent to defoliation. While both functional types increased leaf [N] subsequent to defoliation, the SE models suggest that lawn grasses do so as a mechanism to increase carbon assimilation. In contrast, the SE model for bunch grasses suggest that increased leaf [N] was associated with defoliation and LDMC, but the main mechanism of influencing carbon assimilation was structural, that is, via SLA and LDMC.

Both groups increased carotenoid concentration in response to clipping, which is consistent with earlier studies of variations in pigment concentration in response to defoliation and high irradiance (Murchie and Horton 1997; Rosevear et al. 2001; Eyles et al. 2009). However, bunch grasses invested more heavily in carotenoid production after defoliation than did lawn grasses, suggesting that they rely heavily on the protection of the photosynthetic reaction center from photo-oxidative degradation (especially by ultraviolet) after defoliation (Lichtenthaler 1987; Lambers et al. 2008). Carotenoids are also known to act as photoreceptive and light-harvesting pigments, so it is conceivable that the increased concentration of carotenoids also supports a rapid regrowth in environments characterized by light competition (Havaux et al. 1998; Ritz et al. 2000).

In addition, SE model results suggest that the leaf-level physiological relationships underlying carbon gain in these two functional types differ from previously published relationships. For example, the negative  $SLA \rightarrow A_m$  paths imply unique physiological adaptations in lawn and bunch grasses that deviate from expected relationships. In their analysis of 154 leaf-level measurements from a wide variety of plants species, Shipley et al. (2005) report a strong positive relationship between SLA and  $A_m$ . Moreover, the negative path between  $LDMC \rightarrow [N]$  reported by Shipley et al. (2005) is consistent with our model for lawn grasses but not for bunch grasses. Finally, the lack of a positive  $[N] \rightarrow A_m$  path for bunch grasses is unexpected, but all our attempts to keep this path in the model resulted in an extremely poor fit. Species often show variation in the photosynthesis-nitrogen relationship because the optimal relationship depends on other leaf properties, such as leaf life span and cell wall thickness, and varies according to evolutionary history (Hikosaka 2004). The SE model suggests that after controlling for the effects of SLA on  $A_m$ , increased leaf [N] in bunch grasses is allocated in ways that do not translate into higher carbon gain. We can only speculate, but one possibility is that allocation of N in bunch grasses is structural and dedicated to improving properties such as leaf longevity and defense against herbivores (Ryser and Urbas 2000; Endara and Coley 2011). An important difference between our study and that of Shipley et al. (2005) is that they considered a broad range of plant taxa while we focused on *Poaceae*. Some of the relationships identified here may depend on taxonomic scale and may still be consistent when placed in the broader context of physiological variation across plant families.

The results of the SE models imply that defoliation initiates a cascade of physiological effects that ultimately influence carbon gain in different ways for lawn and bunch grasses. For example, changes in LDMC in bunch grasses are associated with offsetting direct and indirect (mediated by SLA) on  $A_m$ . However, it should be noted that the different slopes in the SE model largely reflect differences among species rather than phe-

notypic variation expressed within functional types. For example, in lawn grasses, SLA increases in this order: *Panicum coloratum*, *Digitaria macroblephara*, and *Digitaria longiflora*;  $A_m$  declines in the opposite order, with little overlap among species. In addition, many of these relationships seem to follow closely the heights of the species, which has also been proposed as a key life-history axis that separates seed plants (Westoby 1998). The analysis of additional species using this framework will shed considerable light on what environmental histories cause these trait relationships to arise: whether they are species specific or whether they also represent phenotypic plasticity within functional types.

Defoliation frequency (time in between events) and severity (proportion of tissue removed per event) can have important influences on individual plant species and coexistence. While we implemented a moderate defoliation regime in terms of severity and frequency (Wallace et al. 1984; Coughenour et al. 1985; Green and Detling 2000; Leriche 2003), it is possible that extremely frequent or intense defoliation may qualitatively change the responses of lawn and bunch grasses to disturbance. Rare, extreme events can have important effects on community assembly (Augustine and McNaughton 1998; Archibald 2008; Ogutu et al. 2008), and future studies should consider whether outcomes are qualitatively different under chronic defoliation, as is locally possible in Serengeti. For example, extreme defoliation would favor prostrate growing lawn grasses, which physically protect a portion of their leaf biomass within their densely matted crown (McNaughton 1984; Coughenour 1985).

A final noteworthy distinction separating lawn and bunch grass is the habitat in which they experience defoliation. Lawn grasses occur in drier, more seasonal environments in which drought stress is common, such as arid or high-salinity environments, and where they are defoliated, often repeatedly, by grazers (Anderson et al. 2006; Croomsigt and Olff 2008). In contrast, taller-stature bunch grasses are found in mesic habitats with less seasonality and where they experience strong light competition (McNaughton 1985; Briske and Derner 1998). Moreover, the productive areas dominated by tall grasses have high fire frequency, and fire may be the most common source of seasonal tissue loss (Archibald 2008; Anderson et al. 2011). This is consistent with the concept that Serengeti tall grasses, such as *Themeda triandra*, are water spenders (sensu Williams et al. 1998), and they must invest heavily in regrowth after defoliation because they will soon face fierce light limitation from competitors.

Taken together, our results suggest that lawn and bunch grasses differ not in their tolerance of defoliation per se but instead in their physiological adjustments after herbivory. This hypothesis removes the emphasis from defoliation tolerance and places it on the physiological trade-offs that generate differences in plant water use, nitrogen allocation, and photo-protection following herbivory. This modified hypothesis is consistent with the preferred habitats of bunch and lawn grasses and the environmental conditions they are likely to experience subsequent to defoliation. This study suggests that tissue-removing disturbances, such as consumption by herbivores or fire, in conjunction with environmental gradients of moisture promote coexistence of grass functional groups in

grazing ecosystems and are key drivers of grass community assemblage across grass-dominated African ecosystems.

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