



Resilience to shading influenced by differential allocation of biomass in *Thalassia testudinum*

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Abstract

Seagrasses are marine plants with fully developed leaves, roots, and rhizomes and a high degree of phenotypic plasticity. In the shallow waters along Florida's central Gulf of Mexico coast, leaf morphology of the dominant seagrass, *Thalassia testudinum*, varies along a spatial gradient in concentrations of total phosphorus (TP) in the water column. We examined ratios of aboveground to belowground biomass (AG : BG) for *T. testudinum* along this gradient to determine if they varied consistently with TP. Ratios were positively correlated with TP, indicating *T. testudinum* allocated more carbon to leaf biomass relative to belowground biomass as TP increased. To determine if this variation in AG : BG influenced resilience to shading, we carried out an 8-week, comparative shading experiment in three *T. testudinum* meadows that spanned the range of recorded ratios. The experiment showed that seagrasses employing a range of AG : BG strategies persisted for 5 weeks with ambient light reduced by ~ 93%. *Thalassia testudinum* with intermediate AG : BG exhibited the least severe impacts and strongest recovery when compared to *T. testudinum* with either high or low AG : BG. Seagrasses with high AG : BG ratios showed the most severe responses and weakest recovery. These results suggest *T. testudinum* allocates biomass such that growth and survival are maximized under the local, long-term nutrient regime, which affects the direction and magnitude of a response to a short-term reduction in light availability. In addition, we suggest that AG : BG is an important metric to monitor in *T. testudinum* meadows because of its potential to identify areas of high and low resilience.

Seagrasses are vascular plants that compete well against phytoplankton and other benthic macrophytes in oligotrophic environments because they can acquire nutrients from sedimentary sources via extensive root systems (Duarte 1995; Touchette and Burkholder 2000a). A large volume of work also suggests that belowground tissues contribute to the resilience of seagrasses because they store carbohydrates and nutrients that fuel physiological processes during periods of stress (Zieman 1975; Zieman et al. 1984; Burke et al. 1996; Lee and Dunton 1996; Touchette and Burkholder 2000a,b;

Alcoverro et al. 2001). For example, estuarine seagrasses, such as *Thalassia testudinum*, can offset periods of reduced photosynthesis due to short-term reductions in light by mobilizing sugars and starches stored in rhizomes (Lee and Dunton 1996, 1997). However, production and maintenance of large amounts of root and rhizome biomass can be metabolically expensive (Fourqurean and Zieman 1991; Hemminga 1998) and increase the risk of exposure to toxic sulfides (Pedersen et al. 2004). Large investments in belowground biomass are less necessary if *T. testudinum* leaves can obtain sufficient nutrients directly from the water column (Lee and Dunton 1999a; Touchette and Burkholder 2000a; Gras et al. 2003). Thus, variation in sediment and water column pools of nutrients could influence investment in belowground structures (Hemminga 1998; Lee and Dunton 1999b; Romero et al. 2006; Lee et al. 2007), with the ability to alter aboveground to belowground biomass ratios (AG : BG) in response to nutrient availability being an important ecophysiological adaptation. Overall, prior work presents a paradoxical role for belowground tissues that are considered both an "asset and a

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burden,” and it offers no insights into changes in resilience with variation in AG : BG (Hemminga 1998).

The seemingly paradoxical role of roots and rhizomes may arise from the fact that many shading studies do not account for morphological plasticity of seagrasses, which is extensive for *T. testudinum* (Lee and Dunton 1999b; Hackney and Durako 2004; Bricker et al. 2011; Barry et al. 2017). In addition, many studies do not place results in the context of long-term variations in water quality or the light environment experienced by seagrasses. For example, Yaakub et al. (2014) found that *Halophila ovalis* growing in turbid conditions was less resilient to additional short-term shading, whereas Maxwell et al. (2014) found the opposite result for *Zostera muelleri*, those growing in less turbid conditions were less resilient to shading. In combination, these results suggest that resilience to acute light reduction may be influenced by the history of light availability and the effect a given history creates may differ among species. Therefore, there is a need to investigate resilience to disturbances across a range of relatively stable environmental conditions and the variety of AG : BG morphologies exhibited by aquatic plants exposed to such gradients (Sculthorpe 1967; Perez et al. 1994; Barrat-Segretain 2001; Peralta et al. 2002, 2005; Puijalón et al. 2005; Maxwell et al. 2014; Thormar et al. 2016; Barry et al. 2017).

The shallow waters along the central Gulf coast of peninsular Florida are characterized by a natural south to north increase in total phosphorus (TP) concentrations in the water column, causing a related gradient in water column productivity, as measured by chlorophyll *a* (Chl *a*) concentrations (Frazer et al. 1998; Jacoby et al. 2012). *Thalassia testudinum* shoots exhibit persistent and notable morphological variation in concert with the spatial TP gradient, such that leaf area shoot⁻¹ increases by an order of magnitude and shoot height increases by a factor of ~ 5 along the gradient (Barry et al. 2017). This region of Florida represents an ideal natural laboratory for investigating differences in resilience, i.e., the ability to resist and recover from a disturbance, across different seagrass morphologies observed along a nutrient gradient. We hypothesized that the observed differences in leaf morphology along this gradient in TP (Table 1) would be accompanied by patterns in belowground biomass, to create variation in AG : BG. We further hypothesized that seagrasses with different AG : BG morphologies would exhibit different degrees of resilience to shading. We investigated these two hypotheses by collecting cores to document aboveground and belowground biomass of seagrass before and at the conclusion of a 5-week period of shading in *T. testudinum* meadows spanning the TP gradient. In addition, growth rates, number of leaves, leaf widths, leaf lengths, and leaf area shoot⁻¹ were measured weekly during the 5-week shading and 3-week recovery periods to document and compare the nature of the responses of *T. testudinum* shoots with differing allocation to aboveground and belowground biomass.

Table 1. Mean \pm SD for total water column phosphorus (TP), *T. testudinum* leaf lengths, and *T. testudinum* leaf widths for each of five estuarine systems along the TP gradient. Values for TP are the means of monthly samples collected from January 2008 through December 2013. Values for leaf morphologies are means of samples collected from the same stations as the TP data in May/June and August/September of 2010 through 2013. Estuarine systems are organized from south to north.

System	Mean TP		Mean leaf length		Mean leaf width	
	($\mu\text{g L}^{-1}$)	SD	(mm)	SD	(mm)	SD
Weeki Wachee	7.2 \pm 2.8		75.4 \pm 46.3		3.0 \pm 0.6	
Chassahowitzka	8.3 \pm 3.9		89.4 \pm 56.1		3.3 \pm 0.5	
Homosassa	10.8 \pm 6.7		152.8 \pm 102.6		4.4 \pm 1.2	
Crystal	15.6 \pm 7.2		195.6 \pm 105.5		4.9 \pm 0.9	
Waccasassa	24.2 \pm 16.6		301.6 \pm 171.8		4.8 \pm 0.8	

Modified from Barry et al. (2017).

Methods and materials

Study system

The shallow waters along the central Gulf coast of peninsular Florida provide a favorable environment for the development of seagrass meadows, and they currently support one of the largest contiguous meadows in North America (Hale et al. 2004; Mattson et al. 2007). Extensive monitoring of water quality (15–18 yr, monthly sampling) and seagrasses (4 yr, bi-annual sampling) along this coast revealed persistent spatial gradients in both the concentration of TP in surface waters and leaf morphology of *T. testudinum*, the dominant seagrass (Frazer et al. 1998; Jacoby et al. 2012; Barry et al. 2017). In addition, previous work has shown that *T. testudinum* and other seagrasses receive sufficient light to support their metabolism in all the coastal systems (Choice et al. 2014).

Sampling stations for the present work spanned the full extent of the spatial gradient in TP and leaf morphology, with stations located in the coastal waters adjacent to the Weeki Wachee, Chassahowitzka, Homosassa, Crystal, and Waccasassa rivers (Fig. 1; Table 1). Water depths in all systems were 1–2 m, and mean salinities were 24‰.

Cores

To quantify aboveground to belowground biomass ratios (AG : BG), we extracted three 10-cm diameter \times 20-cm deep cores from monospecific *T. testudinum* meadows at two stations in each of the five systems along the TP gradient ($n = 30$ cores) in August 2013 (Fig. 1). Monospecific stands of *T. testudinum* were selected to avoid potentially confounding effects of interspecific root competition (Duarte et al. 1998). Cores were placed on ice immediately after collection and stored frozen until they were processed in the laboratory.

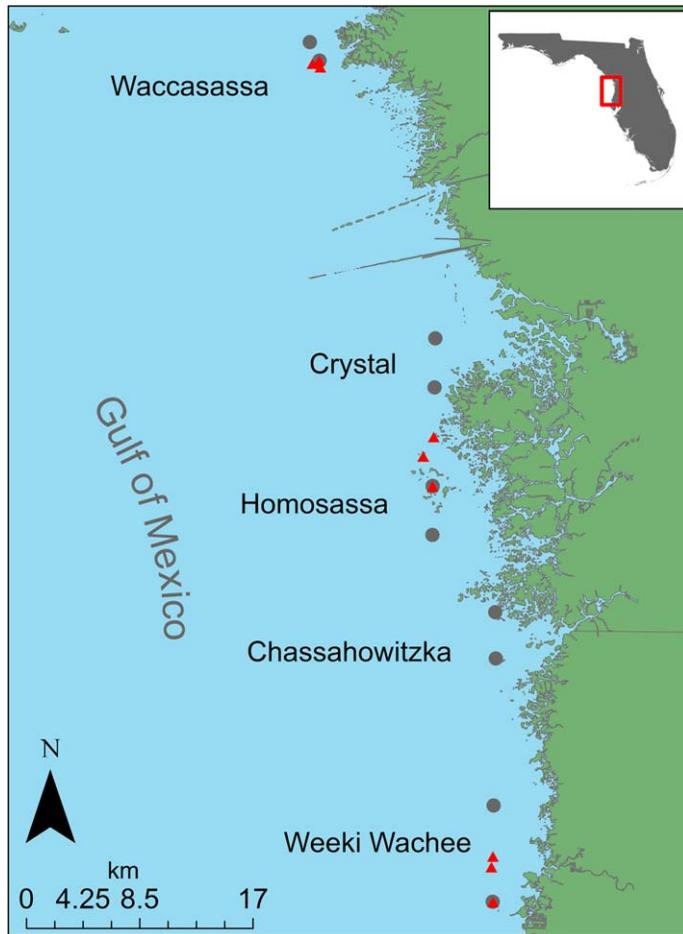


Fig. 1. Sampling stations along the central Gulf coast of peninsular Florida. Gray circles indicate seagrass biomass coring stations and red triangles represent experimental shading stations. Water column phosphorus concentration (TP) is persistently low in the south and increases toward the north of the study region.

In the laboratory, cores were placed on a 1-mm mesh screen and rinsed thoroughly with freshwater to remove sediments. Live *T. testudinum* biomass was separated into root, rhizome, and leaf fractions, which subsequently were dried for at least 72 h at 65°C. Roots and rhizomes were considered live if they were attached to living short shoots or if they were fully intact, light colored, and firm. The dry mass of leaf tissue divided by the sum of the dry mass of live root and rhizome tissues yielded AG : BG. Trends in AG : BG were identified using least-squares regression against the average TP concentration in the water column for the 24 months preceding the collection of the cores (Frazer et al. 1998; Jacoby et al. 2012). The 24-month moving average was selected because *T. testudinum* is a long-lived species that has been shown to integrate environmental conditions over such timescales (Fourqurean et al. 2005) and adjust its leaf morphology on the scale of approximately 2 yr (van Tussenbroek 1996).

Shading experiment

To explore the influence of variation in AG : BG on resilience to shading, we performed an in situ, disturbance-recovery experiment, with *T. testudinum* growth and leaf morphology monitored during 5 weeks of shading and 3 weeks of recovery from June 2014 to August 2014. The experiment was designed to compare the relative responses to shading by *T. testudinum* with differing AG : BG morphologies, with responses defined by temporal trajectories for various metrics during the periods of stress and recovery. Six 1.5-m² plots were established at nine stations (Fig. 1) along the TP gradient ($n = 54$ plots). Three stations were located in each of three systems to capture the TP gradient, i.e., low TP (Weeki Wachee [WEE]), intermediate TP (Homosassa [HOM]), and high TP (Waccasassa [WAC]). At each station, three plots were assigned haphazardly to the shading treatment and the remaining three were designated as control plots. Plastic frames covered with fabric screen (75% reduction in ambient light) were anchored above the appropriate plots, and identical frames without screen were placed at control plots. Frames with screen were open on all four sides to minimize restriction of water flow. Only shoots within the innermost 1.0 m² of the 1.5-m² area were sampled to reduce the influence of sunlight entering through the open sides of the screens. A 75% light reduction was selected because it is below the compensation irradiance for *T. testudinum* in this region (Calleja et al. 2006), and low-light events of similar magnitude have been recorded following rainfall events (Frazer et al. 2001). We aimed to create conditions under which *T. testudinum* would mobilize carbon reserves to avoid a negative carbon balance (Tomasko and Dawes 1989; Lee and Dunton 1996, 1997), but not perish due to toxic effects of sulfide accumulating in the sediment (Calleja et al. 2006). We did not sever rhizomes along the perimeter of the plots because previous work has shown severing rhizomes in *T. testudinum* has few effects on key response variables and the process can introduce undesirable artifacts (Ibarra-Obando et al. 2005). On a weekly basis, screens were scrubbed to remove fouling and afterward, we quantified photosynthetically active radiation ($\mu\text{E m}^{-2} \text{s}^{-1}$) within shade and control plots using a data logger connected to two quantum light sensors (Li-Cor Instruments, Lincoln, Nebraska) that simultaneously measured surface irradiance (above the water) and bottom irradiance in experimental plots.

As part of the experiment, we tracked changes in AG : BG and shoot densities. Cores of seagrass biomass (10-cm diameter \times 20-cm deep) were collected from the center of all plots at the conclusion of shading (week 5). Cores were processed like those collected to characterize AG : BG. Shoot density at the conclusion of the experiment was calculated by averaging shoot counts from two tosses of a 0.0625-m² quadrat in each plot.

In addition, we monitored shoot growth ($\text{mm}^2 \text{shoot}^{-1} \text{d}^{-1}$), number of leaves, leaf width (mm), leaf length (mm), and leaf area ($\text{mm}^2 \text{shoot}^{-1}$) inside experimental plots for the entire 8-week period (5 weeks shading, 3 weeks recovery). Once a week, seven shoots in each plot were marked using standard leaf marking techniques that involved punching a hole just above each shoot's basal meristem (Zieman 1974) and flagging them for later collection by gently connecting a drinking straw to the base of the shoot with a small cable tie. Shoots marked in the previous week (6–10 d of growth) were collected and frozen until processing in the laboratory. Due to inclement weather, we were not able to collect shoots from HOM (intermediate AG : BG) during week 4 of the experiment.

In the laboratory, *T. testudinum* leaves were scraped thoroughly to remove epiphytic material, and widths and numbers of leaves were recorded for each shoot. For each leaf with a hole, old growth (material above the hole) and new growth (material below the hole) were separated by cutting through the hole with a razor blade, and the lengths and widths of the resulting pieces were measured to the nearest mm. All unmarked leaves were considered new growth. Surface area of new material divided by the number of days between marking and harvesting represented growth rates for *T. testudinum* shoots ($\text{mm}^2 \text{shoot}^{-1} \text{d}^{-1}$). The width of the second youngest leaf (the younger of the two leaves adjacent to the central, youngest leaf) of each shoot was used to evaluate changes in leaf width over time. Total length (old length + new length) of all leaves on a shoot were averaged to produce estimates of mean leaf length. The surface areas of old and new leaves were summed to represent the total leaf area shoot^{-1} at each time point.

Light data collected during the first 5 weeks of the experiment (shading period) were averaged across weeks to yield estimates of the actual percentage reduction in ambient irradiance. Average percentages of ambient light were analyzed with a Kruskal-Wallis test to determine if significant differences in light reduction existed among the systems that were studied.

Estimates of AG : BG and shoot density collected in week 5 were analyzed with two-way analyses of variance (ANOVAs), wherein treatment and system were treated as fixed main effects. When ANOVAs revealed significant differences, multiple pairwise comparisons were performed using Tukey's honestly squared differences (HSD) test.

Bottom irradiance, shoot growth rate, number of leaves, leaf width, leaf length, and leaf area shoot^{-1} were initially analyzed using repeated measures multivariate analysis of variance (MANOVA) in the R package {car} (Fox and Weisberg 2011; R Core Development Team 2014), with successive weeks treated as ordered, dependent variables. When overall MANOVAs indicated significant main effects, data were further explored using hierarchical modeling (i.e., growth curve analysis, Mirman 2014) using the R package {lme4} (Bates

et al. 2014; R Core Development Team 2014). This analysis enabled us to compare the shape and magnitude of changes in the light environment and seagrass responses in shading vs. control plots over time. This combined MANOVA-growth curve analysis strategy is recommended for repeated measures data (Tabachnick and Fidell 1983; Davis 2002; Quinn and Keough 2002). We applied second-order, orthogonal, polynomial models to the data because we expected the removal of shading during week 5 would cause a single inflection point in the responses. All candidate models, therefore, contained a linear and a quadratic term for time. Fixed effects and interactions were added successively, and the effect on fit was evaluated by examining Akaike's information criterion (AIC) values and performing log-likelihood ratio tests against candidate models with fewer predictors. Random effects of sampling station on linear and quadratic terms for time were included in all models. Model fit was taken to be improved significantly when log-likelihood ratio tests returned a χ^2 value with $p < 0.05$ and AIC was minimized. The model that maximized fit and minimized complexity was chosen as the best model. Controls at HOM (intermediate AG : BG, no shading) represented the base condition, and parameters were estimated for the effects of treatment (shade or control) and system (WEE = low AG : BG, WAC = high AG : BG). Significance of individual parameters in the selected model was assessed using the normal approximation, computed with R package {lmerTest} (Kuznetsova et al. 2014; R Core Development Team 2014) and a Satterthwaite estimation of degrees of freedom. Assumptions for all analyses were checked and data transformation was applied where appropriate.

Results

AG : BG ratios

Aboveground to belowground biomass ratios (AG : BG) were significantly correlated with TP averaged over the previous 24 months ($p = 0.001$, $R^2 = 0.75$, $df = 8$). Thus, *T. testudinum* allocated more biomass to leaves than to roots and rhizomes as phosphorus became more available in the water column (Fig. 2).

Given this finding, the Weeki Wachee (WEE), Homosassa (HOM), and Waccasassa (WAC) systems were selected to represent low (0.41), intermediate (0.88), and high (1.60) AG : BG ratios, respectively. The shading experiment was conducted in these three estuarine systems.

Shading experiment

Overall mean reduction in ambient irradiance was $\sim 93\%$ during the 5-week shading period, a more intense reduction than our targeted 75%. A Kruskal-Wallis test showed that there were no significant differences in ambient light reduction among the systems ($p = 0.733$, $df = 2$), indicating seagrasses across the study region experienced similar relative reductions in available light. The overall MANOVA for

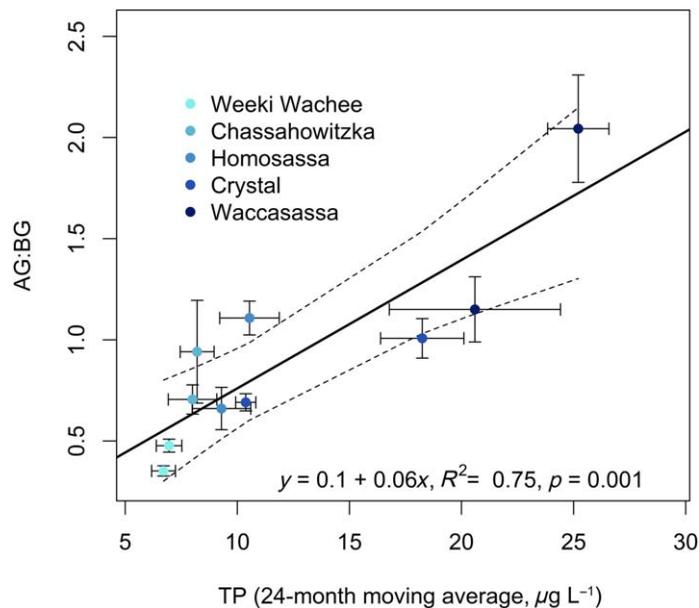


Fig. 2. Linear regression of aboveground to belowground biomass ratio (AG : BG) against total water column phosphorus concentration ($\mu\text{g L}^{-1}$ TP, 24-month moving average). Each point represents the mean value calculated from 10 stations within each system along the TP gradient. Darker blues indicate more northern latitudes and higher TP. Error bars represent standard errors.

bottom irradiance further showed that the reduction in irradiance did not differ significantly among the systems (Table 2). The effect of treatment was marginal in the overall MANOVA, likely because the last 3 weeks of irradiance data were nearly identical between shade and control plots. In addition, the irradiance MANOVA likely had lower power because fewer replicate measurements were collected than for leaf morphology parameters. Clearly, an $\sim 93\%$ reduction in ambient light was biologically meaningful and we proceeded with hierarchical modeling for irradiance despite the lack of a statistically significant treatment effect. A fully parameterized, second order, polynomial was selected as the best fitting model (Table 3). There was a significant effect of time and a significant time-by-treatment interaction (Supporting Information Table S1), both driven by the removal of the screens in week 5. Shading became more intense from weeks 1 to 5 (Fig. 3), likely due to increasing fouling of the mesh that was not removed by scrubbing. Irradiances in shaded plots converged with values for control plots upon removal of screens, and, as expected, the availability of light was effectively identical for all plots in weeks 6–8 (Fig. 3). *Thalassia testudinum* shoots persisted throughout the entire experiment in all plots, despite the initial and increasing reductions in light availability during the 5-week shading period.

Data from experimental plots in week 5 indicated that AG : BG was significantly different among systems ($p < 0.001$,

$F_{2,47} = 143.01$, Tables 4, 5). Tukey's HSD post-hoc comparisons indicated that AG : BG generally increased from south to north, as expected, but WEE and HOM control plots were not statistically different in week 5. Little evidence was found for differences in AG : BG between shade and control treatments by week 5 ($p = 0.073$, $F_{1,47} = 3.37$; Table 4). However, there was a highly significant interaction term ($p < 0.001$, $F_{2,47} = 9.40$), indicating that the response to treatment differed by system. Tukey's HSD pairwise comparisons revealed that AG : BG in shade vs. control treatments was significantly different at WAC ($p = 0.001$, AG : BG shade < AG : BG control), but not at HOM or WEE ($p = 0.684$ and $p = 0.999$, respectively). Overall mean values for AG : BG obtained in week 5 of the experiment were lower than those obtained before the experiment (Fig. 2; Table 5), likely because the collection of marked shoots throughout the experiment reduced the aboveground biomass across all plots. Despite this difference in absolute values, the general pattern of at least a doubling in AG : BG values between any estuarine system and the next system to the north was preserved in experimental plots.

Shoot density differed significantly across systems ($p < 0.001$, $F_{1,48} = 36.51$), but it did not exhibit any significant declines in response to shading within a system ($p = 0.508$, $F_{1,48} = 0.444$; Table 4). Tukey's HSD post-hoc comparisons showed that shoot density was lower overall in WAC (Table 5).

Repeated measures MANOVAs yielded significant results for both treatment and system for growth rate, number of leaves, leaf width, and leaf area shoot $^{-1}$ (Table 2). For leaf length, system was found to be a significant main effect (Table 2). Model selection procedures identified the first order model as the best fit for leaf width, leaf length, and leaf area shoot $^{-1}$ and the second order polynomial model as the best fit for growth rate and number of leaves (Table 3).

Daily growth rates declined across all plots during the 8-week study, with a significant time-by-system interaction term highlighting the fact that this effect was slightly more pronounced for WAC (Fig. 4a). Shoots at WAC and WEE had significantly higher and lower overall growth rates than HOM shoots, respectively (Fig. 4a). This result is reflected in the aboveground morphology of shoots in each system, and it is consistent with previous work that showed shoot growth rate was highly correlated with TP in this region (Barry et al. 2017; Table 1). As a result, responses to shading between systems were evaluated by comparing the relative severity of responses in shaded plots to controls within each system because initial aboveground morphologies and potential for growth differed across the region. For example, a response to shading in one system was considered more severe than responses in other systems when the change in a given metric relative to the appropriate control was more pronounced and showed less rebound during the period of recovery.

Shoots in shaded plots at both WEE and WAC showed a significant decline in growth rates compared to control plots, whereas shoots at HOM did not, resulting in the significant

Table 2. Overall repeated measures MANOVA results for irradiance and *T. testudinum* growth and morphology. Significance levels denoted by asterisks: *** = < 0.001; ** = < 0.01; * = < 0.05; . = < 0.1; blank = > 0.1; colons = interactions between factors; df = degrees of freedom; Approx. *F* = approximate *F*-ratio.

	df	Error df	Pillai's trace	Approx. <i>F</i>	<i>p</i>	
Irradiance						
Intercept	1	2	1.00	499.72	0.002	**
Treatment	1	2	0.86	12.10	0.074	.
System	1	2	0.74	5.78	0.138	
Treatment : system	1	2	0.01	0.02	0.913	
Shoot growth rate						
Intercept	1	32	1.00	10,604.35	<0.001	***
Treatment	1	32	0.57	41.75	<0.001	***
System	1	32	0.93	455.00	<0.001	***
Treatment : system	1	32	0.21	8.64	0.006	**
Time	7	26	0.91	38.13	<0.001	***
Treatment : time	7	26	0.71	7.91	<0.001	***
System : time	7	26	0.56	4.73	0.002	**
Treatment : system : time	7	26	0.46	3.13	0.016	*
Number of leaves						
Intercept	1	32	0.99	5252.99	<0.001	***
Treatment	1	32	0.59	45.32	<0.001	***
System	1	32	0.17	6.78	0.014	*
Treatment : system	1	32	0.12	4.42	0.044	*
Time	7	26	0.90	35.21	<0.001	***
Treatment : time	7	26	0.54	4.41	0.002	**
System : time	7	26	0.21	0.97	0.471	
Treatment : system : time	7	26	0.37	2.17	0.072	.
Leaf width						
Intercept	1	32	0.99	4932.69	<0.001	***
Treatment	1	32	0.24	10.26	0.003	**
System	1	32	0.79	122.32	<0.001	***
Treatment : system	1	32	0.05	1.55	0.223	
Time	7	26	0.46	3.12	0.016	*
Treatment : time	7	26	0.51	3.82	0.006	**
System : time	7	26	0.30	1.56	0.193	
Treatment : system : time	7	26	0.29	1.52	0.205	
Leaf length						
Intercept	1	32	1.00	29,135.20	<0.001	***
Treatment	1	32	0.00	0.10	0.733	
System	1	32	0.95	612.00	<0.001	***
Treatment : system	1	32	0.01	0.20	0.625	
Time	7	26	0.37	2.20	0.066	.
Treatment : time	7	26	0.20	0.90	0.515	
System : time	7	26	0.20	0.90	0.487	
Treatment : system : time	7	26	0.29	1.50	0.217	
Leaf area						
Intercept	1	32	1.00	35,149.18	<0.001	***
Treatment	1	32	0.24	9.95	0.003	**
System	1	32	0.93	439.04	<0.001	***
Treatment : system	1	32	0.07	2.27	0.142	
Time	7	26	0.66	7.23	<0.001	***

Table 2. Continued

	df	Error df	Pillai's trace	Approx. F	p	
Treatment : time	7	26	0.46	3.13	0.015	*
System : time	7	26	0.29	1.54	0.199	
Treatment : system : time	7	26	0.46	3.18	0.014	*

Table 3. Growth curve analysis model selection results for irradiance and *T. testudinum* growth and morphology. Significance levels denoted by asterisks: *** = <0.001; ** = <0.001; * = <0.01; . = <0.05; blank = >0.1; † = selected model; df = degrees of freedom; AIC = Akaike's information criterion; LogLik = negative log-likelihood; χ^2 = chi-squared statistic for negative log-likelihood ratio test.

Model	df	AIC	LogLik	χ^2	χ^2 df	p	
Irradiance							
Base	10	477.99	-229				
All fixed	13	426.22	-200.1	57.77	3	<0.001	***
All fixed with all linear time interactions	20	405.54	-182.8	34.68	7	<0.001	***
All fixed with all interactions	25	401.34	-1753	14.20	5	0.014	*,†
Shoot growth rate							
Base	10	-287.68	153.84				
All fixed	13	-378.23	202.11	96.54	3	<0.001	***
All fixed with all linear time interactions	20	-472.99	256.50	108.77	7	<0.001	***
All fixed with all interactions	25	-495.40	272.70	32.40	5	<0.001	***,†
Number of leaves							
Base	10	587.04	-283.52				
All fixed	13	474.48	-224.24	118.56	3	<0.001	***
All fixed with all linear time interactions	20	429.93	-194.96	58.55	7	<0.001	***
All fixed with all interactions	25	419.86	-184.93	20.06	5	0.001	**,†
Leaf width							
Base	10	595.99	-288.00				
All fixed	13	515.06	-244.53	86.93	3	<0.001	***
All fixed with all linear time interactions	20	490.28	-225.14	38.78	7	<0.001	***,†
All fixed with all interactions	25	496.67	-223.33	3.61	5	0.607	
Leaf length							
Base	10	-793.86	406.93				
All fixed	13	-835.77	430.88	47.90	3	<0.001	***
All fixed with all linear time interactions	20	-866.71	453.36	44.95	7	<0.001	***,†
All fixed with all interactions	25	-860.85	455.42	4.13	5	0.531	
Leaf area							
Base	10	-442.36	231.18				
All fixed	13	-482.88	254.44	46.52	3	<0.001	***
All fixed with all linear time interactions	20	-556.85	298.43	87.97	7	<0.001	***,†
All fixed with all interactions	25	-550.41	300.21	3.56	5	0.614	

treatment-by-system interaction. There was a significant interaction of treatment with the quadratic time term, indicating that shoots in shaded plots showed a significant curvilinear response not observed for shoots in control plots (Fig. 4a). This nonlinear response began when screens were removed in week 5. The most persistent response to shading was observed at WAC, with shaded shoots still growing more

slowly than controls after 3 weeks of recovery, whereas shoots in shaded plots in the other two systems were either growing as fast (WEE) or faster (HOM) than control shoots in week 8 (Fig. 4a).

Number of leaves shoot⁻¹ was initially similar in all plots, and this metric declined significantly across all systems and treatments, with the decline being more pronounced for

shaded shoots (Fig. 4b; Supporting Information Table S1). Number of leaves in shaded plots showed a significant quadratic response, whereas control plots responded in a more linear fashion (Fig. 4b). There was a significant system-by-treatment interaction term, indicating differences in the magnitude of the response of shoots across the region (Supporting Information Table S1).

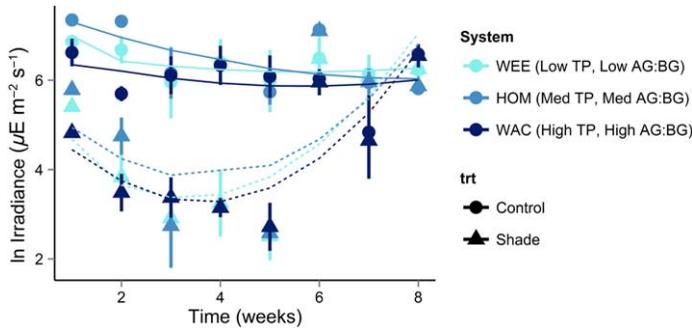


Fig. 3. Natural log of bottom irradiance over time for shade and control plots in each system. Lines represent 2nd order polynomial model predictions. Darker blues represent more northern latitudes and higher TP. Error bars represent standard errors.

Mean leaf widths were initially similar at the outset of the experiment at WAC and HOM, but lower at WEE (Fig. 4c; Supporting Information Table S1). Leaf width increased over time in HOM control plots, remained fairly constant over time in WEE and WAC control plots, and decreased over time in all shaded plots (Fig. 4c), which generated significant effects for time, time by system, and time by treatment (Supporting Information Table S1). The overall reduction in leaf width was significant for shoots at both WAC and HOM. The most severe relative reduction in leaf width was observed in WAC shaded plots (Fig. 4c). The lack of a significant quadratic response indicated that leaf width in shaded plots generally continued on a negative trajectory during the 3-week recovery period, although leaf width in HOM shaded plots increased in week 8 (Fig. 4c).

Mean leaf length was initially different across systems in all plots, with shoots at WAC and WEE having significantly longer and shorter mean leaf lengths than HOM shoots, respectively (Fig. 4d; Supporting Information Table S1). Relative to controls in the same system, mean leaf length increased primarily in HOM shaded plots, with leaf lengths remaining similar in all plots throughout the experiment at WEE and WAC as confirmed by significant treatment-by-system interactions in the growth curve analyses (Fig. 4d; Supporting Information Table S1).

Table 4. Two-way ANOVA results for aboveground to belowground biomass ratio (AG : BG) and shoot densities (shoots m⁻²) in experimental plots at the conclusion of the shading period (week 5). Significance levels denoted by asterisks: *** = < 0.001; ** = < 0.01; * = < 0.05; . = > 0.1; colons = interactions between factors; df = degrees of freedom.

	df	Sum of squares	Mean square	F value	p	
AG : BG						
System	2	8.10	4.05	143.01	<0.001	***
Treatment	1	0.10	0.10	3.37	0.073	.
System : treatment	2	0.53	0.27	9.40	<0.001	***
Residuals	47	1.33	0.03			
Shoot density						
System	2	2.20	1.10	36.51	<0.001	***
Treatment	1	0.01	0.01	0.44	0.508	
System : treatment	2	0.05	0.03	0.88	0.420	
Residuals	48	1.45	0.03			

Table 5. Mean ± SD for aboveground to belowground biomass ratio (AG : BG) and mean shoot density (shoots m⁻²) in experimental plots at the conclusion of the shading period (week 5). Systems are listed from south to north. Significant differences among systems and treatments found with Tukey’s HSD test are indicated by letter groupings.

System	Treatment	AG : BG	SD		Shoot density	SD	
Weeki Wachee	Control	0.09	0.03	ab	656	199	a
	Shade	0.09	0.02	a	796	102	a
Homosassa	Control	0.16	0.06	ab	757	282	a
	Shade	0.21	0.07	b	678	151	a
Waccasassa	Control	1.30	0.58	c	296	125	b
	Shade	0.54	0.22	d	244	518	b

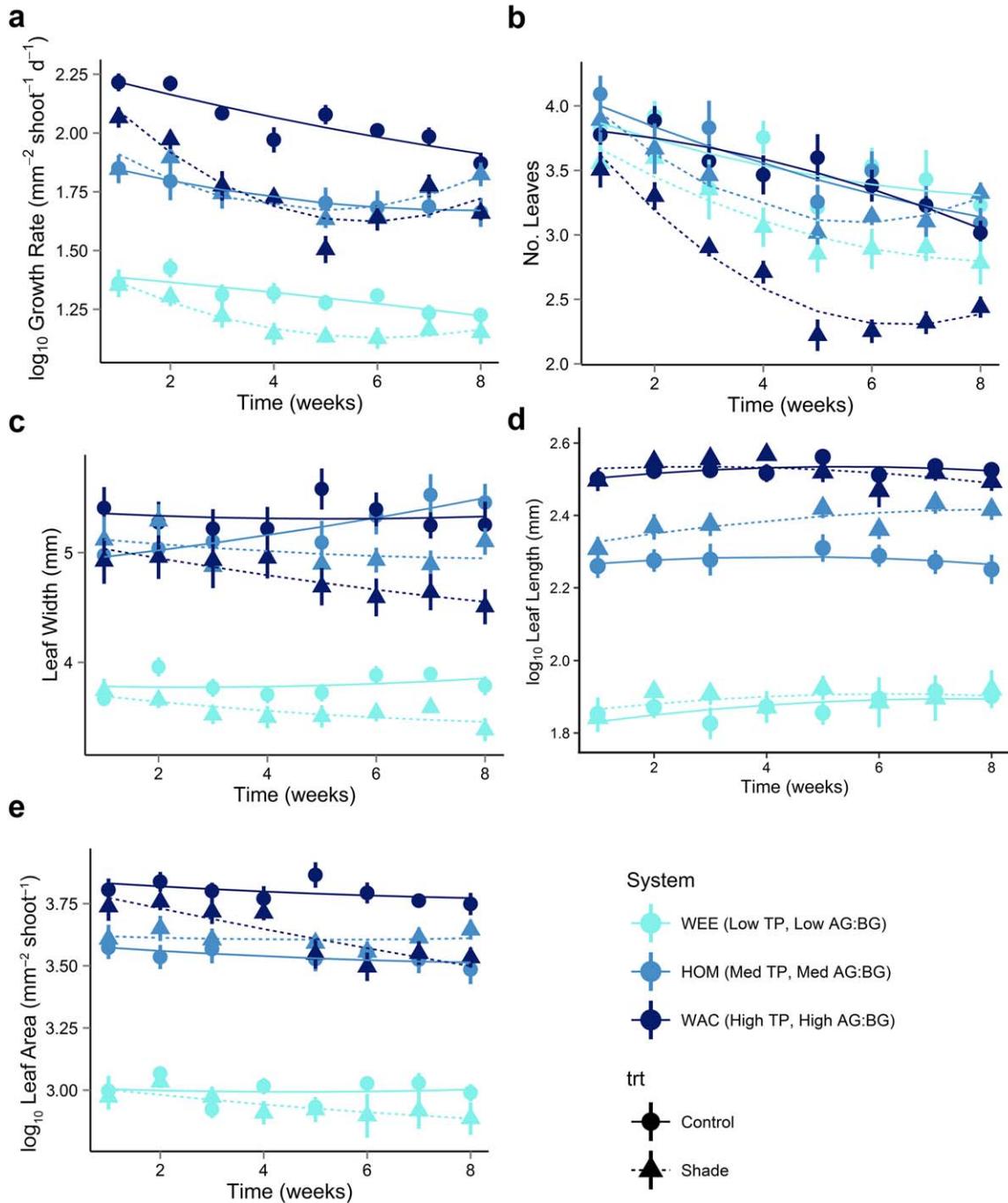


Fig. 4. Responses over time for (a) \log_{10} growth rate ($\text{mm}^2 \text{shoot}^{-1} \text{d}^{-1}$), (b) number of leaves, (c) leaf width (mm), (d) \log_{10} leaf length (mm), and (e) \log_{10} leaf area ($\text{mm}^2 \text{shoot}^{-1}$) in shade and control plots in each system. Lines represent 2nd order polynomial model fits in panels (a) and (b), and 1st order polynomial model fits in panels (c–e); darker blues represent more northern latitudes and higher TP. Error bars represent standard errors.

Finally, leaf area shoot^{-1} differed across systems at the outset of the experiment (Fig. 4e; Supporting Information Table S1), with WAC having significantly larger shoots than HOM, which, in turn, had larger shoots than WEE. Leaf area shoot^{-1} in shaded plots in all three systems diverged from

control values, which remained fairly constant for the duration of the experiment. Leaf area shoot^{-1} declined significantly in shaded plots in both WEE and WAC, with the effect being more severe in WAC plots (Fig. 4e). Conversely, leaf area shoot^{-1} increased significantly in HOM shaded

plots (Fig. 4e), resulting in a significant treatment-by-system interaction (Supporting Information Table S1). Again, the lack of a quadratic response showed that shaded WEE and WAC shoots continued to show declines in leaf area shoot⁻¹, whereas shaded HOM shoots continued to have higher leaf area than control shoots throughout the entire experiment (Fig. 4e).

Discussion

We present evidence that builds on previous work to suggest that AG : BG for *T. testudinum* varies in conjunction with concentrations of phosphorus in the water column. *Thalassia testudinum*, like many seagrass species, can acquire nutrients from sediment pore water via root tissues or from the water column via uptake through its leaves (Touchette and Burkholder 2000a; Gras et al. 2003). Nutrient ratios in *T. testudinum* leaves indicate that phosphorus limitation is present at the southern (low TP) end of the gradient and decreases in severity northward (high TP, Barry et al. 2017). Therefore, the observed alterations in AG : BG ratio likely occurred in relation to alleviation of phosphorus limitation along the gradient. As phosphorus becomes more available in the water column, the need to acquire nutrients from sediment pore waters should decline, and pressure to gather more light should increase due to increased Chl *a* concentrations that correlate with increased nutrients in the water column (Frazer et al. 2002; Hoyer et al. 2002; Jacoby et al. 2012). Our observations are consistent with the hypothesis that seagrasses alter allocation of biomass in response to environmental factors, such as light levels and nutrient availability in sedimentary and water column pools (Perez et al. 1994; Hemminga 1998; Lee and Dunton 1999a,b, 2000; Romero et al. 2006; Lee et al. 2007).

In light of the pronounced natural gradient in AG : BG in our study system, we designed an experimental manipulation to examine if seagrass meadows in different locations along the gradient would show significant variation in resilience to shading. The AG : BG gradient observed in cores taken in 2013 persisted throughout the shading experiment conducted in 2014, although the experimental manipulation likely had an effect on the absolute AG : BG values through the removal of aboveground biomass (shoots) for measurement. The overall decline in growth rate and number of leaves observed in all experimental plots likely was related to seasonal senescence because our experiment occurred in mid-summer to late-summer, when seagrass standing stock and production have been shown to decline in similar systems (Zieman 1975; Barber and Behrens 1985). Seagrasses with all three AG : BG morphologies persisted throughout the experiment, illustrating again the general ability of *T. testudinum* to survive short-term and medium-term shading disturbance (Czerny and Dunton 1995; Kraemer and Hanisak 2000; Major and Dunton 2002; Calleja et al. 2006; Lamote

and Dunton 2006). Our intent was to compare the form of the response to stress and the trajectory of recovery among differing AG : BG morphologies rather than define the length of time required for recovery; however, this body of evidence regarding resilience to shading in *T. testudinum* leads us to expect that shaded plots in all three estuarine systems would make a full recovery given sufficient time.

Importantly, seagrasses with different initial AG : BG exhibited responses that differed in direction and magnitude when confronted with relatively severe (~ 93%), short-term (5-week) reductions in ambient irradiance. In high AG : BG meadows (WAC), shading caused significant reduction in AG : BG, shoot growth rate, number of leaves, leaf width, and leaf area shoot⁻¹. Seagrasses with low AG : BG (WEE) showed fewer and less severe responses to shading, with significant reductions in shoot growth rate, number of leaves, and leaf area shoot⁻¹. Seagrasses with intermediate AG : BG (HOM) showed the fewest and least severe negative responses to shading, with significant reductions in leaf width and number of leaves, an increase in leaf length and leaf area shoot⁻¹, and no change in AG : BG. Furthermore, those seagrasses in intermediate AG : BG meadows (HOM) showed rapid recovery following removal of shading, approaching or surpassing control values by week 8 for most parameters. In low AG : BG beds (WEE), seagrasses partially recovered after screens were removed, with growth rates in shaded plots equal to controls upon the conclusion of the experiment. However, number of leaves and leaf area shoot⁻¹ had not recovered to control level by the end of the experiment in low AG : BG plots. Seagrasses in high AG : BG meadows (WAC) showed the weakest recovery, either continuing a downward trajectory or only showing partial recovery relative to controls by week 8. Shoot density did not show a significant response to shading, perhaps because the duration of shading was not long enough to elicit a detectable loss of shoots (McMahon et al. 2013). Overall, resilience to shading was lowest for seagrasses with high AG : BG, intermediate for low AG : BG, and highest for intermediate AG : BG. We present a conceptual diagram to illustrate the general differences in response across the AG : BG gradient (Fig. 5).

The physiology of seagrasses can help explain the variation in responses to shading among the three AG : BG morphologies. High AG : BG seagrasses likely were least resilient to shading because of the large amount of fast-respiring leaf tissue (Fourqurean and Zieman 1991) relative to belowground “storage” tissues. When light levels were reduced below the compensation irradiance, high AG : BG seagrasses may have exhausted carbohydrate reserves faster than low or intermediate AG : BG seagrasses because leaves have much higher respiratory demands than roots or rhizomes. The significant reduction in growth rate, number of leaves, leaf width, leaf area shoot⁻¹, and AG : BG were likely responses aimed at reducing respiratory demand by shedding leaves

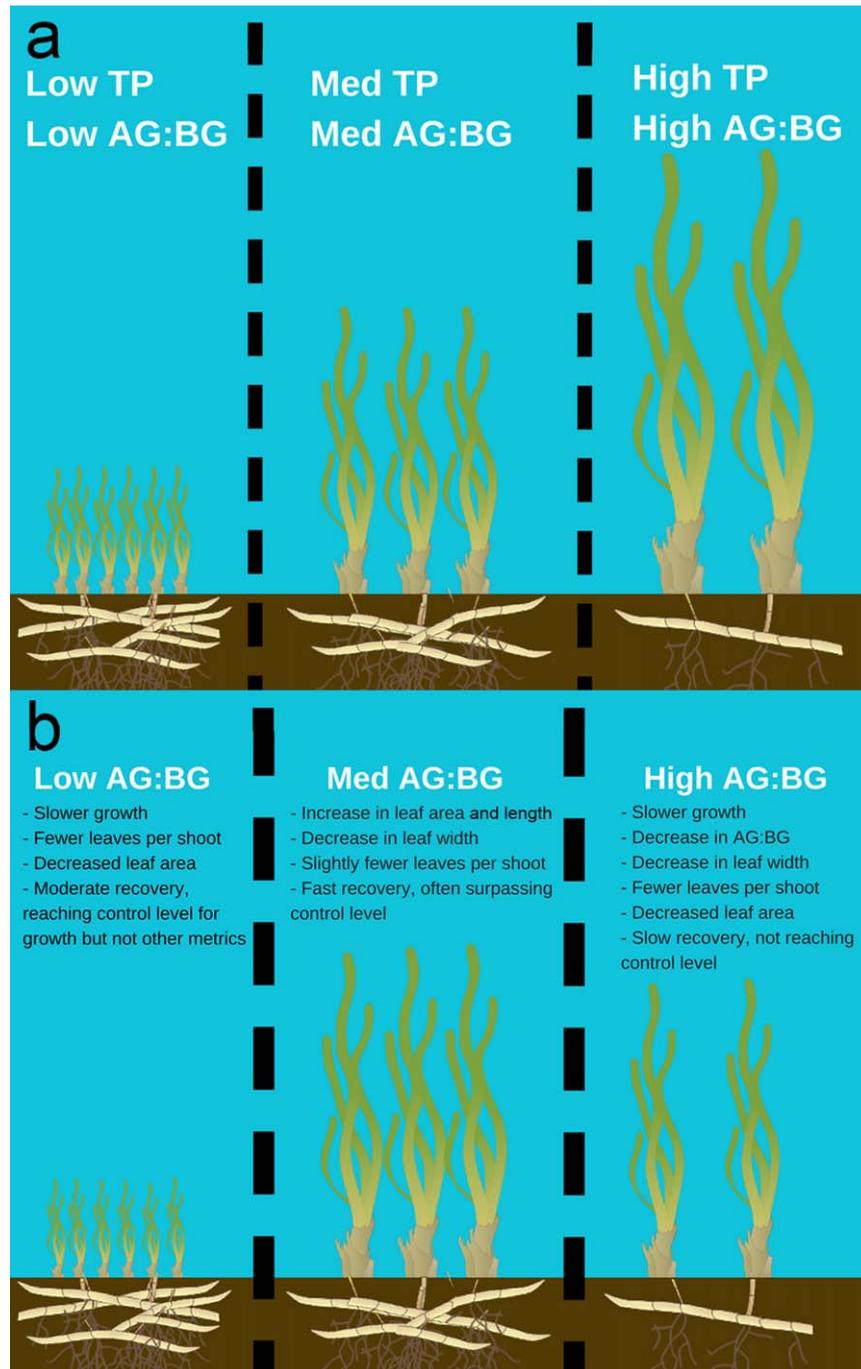


Fig. 5. Conceptual diagram illustrating (a) the general pattern in initial *T. testudinum* aboveground to belowground biomass ratio (AG : BG) along a spatial phosphorus gradient and (b) the generalized shading response of seagrasses for each AG : BG condition. Drawing not to scale. *T. testudinum* vector drawing: Tracy Saxby, IAN Image Library (<http://ian.umces.edu/imagelibrary>).

and reducing new investment in metabolically expensive leaf tissue. Similar, though less pronounced, responses in low AG : BG meadows indicated that these seagrasses also were stressed by shading, likely because they had less photosynthetic tissue to support a relatively large pool of heterotrophic belowground biomass. This result supports the

hypothesis set forth by Hemminga (1998) that seagrasses with very low AG : BG are vulnerable to entering a negative carbon balance when light is reduced. Growth rates for seagrasses with low AG : BG recovered from shading more quickly than other metrics. Previous work has shown that large belowground reserves promote fast recovery in

perturbed seagrass meadows (Zieman et al. 1984; Moran and Bjorndal 2005, 2007). In general, it appears that maintaining a positive carbon balance when light is reduced is more difficult for seagrasses with a large amount of leaf tissue (high AG : BG, WAC) than for seagrasses with a large amount of belowground tissue (low AG : BG, WEE), though seagrasses of both types seem to be more vulnerable to negative impacts than seagrasses with a more even distribution of tissue (intermediate AG : BG, HOM).

Interestingly, seagrasses with intermediate AG : BG (HOM) showed few negative responses to shading and actually increased leaf length and leaf area shoot^{-1} . Other research has shown that seagrasses sometimes increase allocation to leaf tissue in low light conditions, presumably benefiting from garnering more light through leaves that reach higher in the water column or have more photosynthetic area (Bulthuis 1983; Lee and Dunton 1997; Olesen et al. 2002; Maxwell et al. 2014). Our work suggests that this response might be advantageous only to seagrasses that have a relatively balanced AG : BG, which provides them with adequate carbohydrate reserves to support leaf metabolism and adequate leaf tissue to support belowground respiration. The increase in leaf length and leaf area in HOM in response to shading led to an increase in mean AG : BG in shaded plots, although the increase was not large enough to be statistically significant (Table 5). The increase in leaf length and leaf area is suggestive of light stress, and these changes indicate that the morphology of seagrasses in HOM shade plots was becoming more similar to the morphology observed in meadows exposed to higher TP (higher AG : BG). Thus, further shading events in these plots could elicit more severe negative responses because morphological responses to the first shading event pushed these seagrasses toward a less resilient state. Overall, the response to successive shading events by seagrasses with different initial AG : BG remains understudied.

Past shading studies have produced a relatively good understanding of the primary response of seagrasses to light reduction, but they also have resulted in several inconsistencies with respect to specific responses of different metrics of seagrass condition (McMahon et al. 2013). These inconsistencies may be due, in part, to differences in the duration and severity of experimental light reduction (McMahon et al. 2013), but our data suggest that differences in AG : BG also could produce differences in the direction and magnitude of response for a given seagrass metric within a species. For example, in our study, seagrasses with intermediate initial AG : BG responded to shading by increasing leaf area shoot^{-1} , whereas those with high initial AG : BG showed a marked decrease in leaf area shoot^{-1} . In the region we studied, AG : BG, water column phosphorus concentrations and the availability of light are linked, which in combination with the results of the shading experiment, indicates that information about background concentrations of nutrients and historical light regimes is important for predicting the

responses of seagrasses to shading. For example, seagrasses in meadows exposed to higher concentrations of nutrients may be more vulnerable to extirpation, given their allocation of biomass and the fact that competitive advantage shifts toward phytoplankton and algae that can create shade (Duarte 1995; Valiela et al. 1997). Furthermore, the influence of background environmental gradients needs to be investigated for more species of seagrasses, given that other researchers have shown that light history yielded opposite responses to further light reduction in two different species of seagrass (Maxwell et al. 2014; Yaakub et al. 2014) and the few studies that have investigated the effects of repeated shading events have yielded mixed results for a limited variety of species (Longstaff and Dennison 1999; Biber et al. 2009).

In terms of managing coastal resources, seagrasses are known to thrive in oligotrophic environments, and it is generally accepted that nutrient inputs and concomitant degradations in the light environment should be limited wherever possible. However, limited resources may preclude the equal protection of all seagrass meadows, and it is often useful or desirable to prioritize areas for restoration or protection. The present work suggests that AG : BG could be a useful metric for prioritizing management goals by helping to distinguish meadows that may be at risk of extirpation if light is reduced (high AG : BG) from those that should have higher resilience (medium AG : BG). In addition, AG : BG could be monitored in seagrass beds of interest to provide a valuable early warning of light stress if ratios begin shifting higher.

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Conflict of Interest

None declared.

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