

RESEARCH ARTICLE

Herbivore community determines the magnitude and mechanism of nutrient effects on subtropical and tropical seagrasses

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Abstract

1. Numerous studies have examined the combined effects of nutrients (bottom-up control) and consumers (top-down control) on ecosystem structure and functioning. While it is recognized that both can have important effects, there remains a limited understanding of how their relative importance shifts across large spatial scales where consumer functional types can vary.
2. Using seagrasses as a model ecosystem, we explore the effects of ambient variation in herbivore functional composition on the relative importance of bottom-up and top-down forcings. Distributed experiments were conducted across four Western Atlantic sites that encompassed a gradient in consumer composition. Herbivores at two subtropical sites were predominantly comprised of small crustacean invertebrates (mesograzers that consume epiphytic algae), while herbivores at two tropical sites were dominated by large macrograzers (sea urchins and herbivorous fishes that directly consume seagrass biomass).
3. To test the relative importance of bottom-up and top-down factors at each site, we manipulated nutrient supply, mesograzers abundance, and the presence of macrograzers (mainly herbivorous fishes) in a fully factorial design over a 14-week growing season. Seagrass above-ground biomass, shoot density, canopy cover, leaf productivity and epiphyte mass were measured as indicators of habitat structure and productivity.
4. Overall, nutrient addition elicited a strong response across sites, causing widespread loss of seagrass biomass and shoot density. However, the mechanisms driving these declines strongly varied as a function of resident herbivore identity. Seagrass loss at tropical sites was attributable to macrograzers, which increased their direct consumption of fertilized, nutrient-rich seagrass. Conversely, at the subtropical sites, nutrient loading caused seagrass declines associated with the proliferation of epiphytic algae, but only in locations where mesograzers abundance was low.

5. *Synthesis*. Our results confirm that nutrient enrichment generally has negative effects on seagrasses, but that the underlying mechanisms vary and may depend upon herbivore presence and functional identity. Along a subtropical to tropical gradient, the adverse effects of nutrient loading may switch from competitive algal overgrowth to a stimulation of seagrass consumption. Thus, in the tropics, top-down and bottom-up factors can act in combination, and in the same direction, to contribute to habitat loss.

KEYWORDS

bottom-up, comparative experimental approach, eutrophication, macrograzers, mesograzers, plant–herbivore interactions, sea urchins, *Thalassia testudinum*, top-down

1 | INTRODUCTION

Investigating the ecological forces underlying the functioning of plant communities is critical for understanding the health and resilience of many foundational habitats. While resource supply (bottom-up) and consumer pressure (top-down) often interact to affect ecosystem function, challenges remain in elucidating what factors govern the interplay of these processes across large spatial scales (Gruner et al., 2008). Since many foundation species span considerable geographic ranges, variation in resource limitation and the community composition of consumers can influence that interplay, restricting the generality of localized experiments and their applicability to broadly implemented management practices. Thus, there is a need to develop a holistic perspective on the functioning of plant communities, particularly at scales that encompass a suite of environmental contexts (Borer et al., 2014).

It is increasingly recognized that the separate and interactive effects of top-down and bottom-up control vary geographically, thus the forces structuring foundational habitats are likely context dependent (Burkpile & Hay, 2006; Duffy et al., 2015; Menge et al., 2002). Theory suggests that spatial gradients in biotic interactions can explain a wide range of ecological processes, yet there remains a poor understanding of this variability due to a limited number of experiments conducted across appropriate scales (Freestone, Osman, Ruiz, & Torchin, 2011; Pennings & Silliman, 2005). For instance, while some studies suggest that primary producers in warmer climates can experience higher consumer pressure (Coley & Aide, 1991; Gaines & Lubchenco, 1982; Menge & Lubchenco, 1981), other studies reveal weak evidence for latitudinal variation in consumer effects on producer biomass (Poore et al., 2012). Interactive effects, whereby top-down control is fuelled by bottom-up processes (Bertness, Crain, Holdredge, & Sala, 2008; Menge, 1992; Silliman & Zieman, 2001) have also been documented, and can further depend upon geographic location (He & Silliman, 2015). Given that the ecological forces regulating the structure of primary producers can vary geographically, large-scale standardized (i.e. distributed) experiments are emerging as a robust method for elucidating emergent mechanisms from the idiosyncrasy of isolated sites. While these methods have been employed in terrestrial grasslands (Borer et al., 2014), rocky shorelines (Menge et al., 2002) and

temperate seagrasses (Duffy et al., 2015), few studies have attempted similar approaches across subtropical or tropical marine ecosystems.

Seagrasses are foundational marine plants that provide key ecological services such as primary production, habitat provisioning and shoreline stabilization (Altieri & Van De Koppel, 2013; Orth et al., 2006). As many of these meadows are under threat (Waycott et al., 2009), the need to understand how these systems function has made seagrasses one of the leading models for examining the interplay between bottom-up and top-down processes. Seagrass vigour is generally attributed to variation in bottom-up forcing, whereby suspended sediment limits light availability, or nutrients can increase phytoplankton and/or macroalgae, also limiting light and reducing seagrass growth (Duarte, 1995; Hauxwell, Cebrian, Furlong, & Valiela, 2001). While these factors are clearly important, recent evidence suggests that in certain instances, top-down consumer effects are often of comparable significance, in part because of their potential to interact with bottom-up processes (Duffy et al., 2015; Heck, Pennock, Valentine, Coen, & Sklenar, 2000; Heck & Valentine, 2006; Hughes, Bando, Rodriguez, & Williams, 2004; Jones & Jeppesen, 2007; Östman et al., 2016). Small crustacean invertebrates (mesograzers) can benefit seagrasses by consuming epiphytic algal overgrowth, thereby buffering against nutrient effects (Heck et al., 2000; Hughes et al., 2004; Orth & Van Montfrans, 1984; Reynolds, Richardson, & Duffy, 2014; Whalen, Duffy, & Grace, 2013). In coordinated field experiments conducted across the geographic range of the temperate seagrass *Zostera marina*, nutrient fertilization had minor effects on the accumulation of epiphytic microalgae, whereas mesograzers diversity and seagrass genetic diversity instead served as the best predictors of algal abundance (Duffy et al., 2015). Likewise, seagrass-mesograzers mutualisms have also been documented by other temperate studies (Baden, Bostrom, Tobiasson, Arponen, & Moksnes, 2010; Moksnes, Gullstrom, Tryman, & Baden, 2008). Can these findings that highlight the potential for consumers to alter the magnitude and direction of nutrient effects also be generalized to tropical and subtropical regions?

Tropical waters tend to be dominated by large-bodied herbivores, all of which have fundamentally distinct interactions with seagrasses as compared to small-bodied mesograzers. Parrotfishes, sea urchins, turtles, sirenians and waterfowl all directly consume seagrass biomass,

largely altering pathways of trophic transfer within these systems (Heck & Valentine, 2006). For example, grazing by green sea turtles (*Chelonia mydas*) can shift seagrass habitat structure and species composition (Christianen et al., 2014), altering plant–herbivore interactions and broader ecosystem functioning. Furthermore, due to nutrient limitation of many herbivores (Mattson, 1980), fertilization may stimulate herbivory by macrograzers, potentially resulting in seagrass loss. Nutrient-stimulated herbivory has also been observed in other systems such as saltmarshes (He & Silliman, 2015). The contrasting functional groups of herbivores between systems raise the question whether the models of mutualistic grazing often invoked from temperate systems translate to the tropics. Do systematic differences in herbivore traits (small-bodied invertebrates vs. large-bodied vertebrates) lead to predictable differences in structuring forces and responses to nutrients? How does variation in herbivore identity modify seagrass response to nutrient enrichment? To date, no study has attempted to address these questions by concurrently manipulating top-down and bottom-up factors within tropical seagrass beds across multiple biogeographical provinces of varying consumer composition.

Here, we present the results of a coordinated experiment examining the interplay between nutrient loading, mesograzers and macrograzer activity across subtropical and tropical seagrasses within the Western Atlantic. We tested the effects of bottom-up and top-down control, and their interactions, on seagrass canopy structure, leaf productivity and epiphyte mass. As herbivore assemblages varied across subtropical-tropical boundaries, we hypothesized that trends of increasing macrograzer abundance (with decreasing latitude) would alter the direction of consumer effects towards increased consumption of seagrass biomass, particularly in conjunction with nutrient enrichment. Our findings reveal that as herbivore identity transitions from small mesograzers (at our subtropical sites) to large macrograzers

(tropical sites), the mechanisms driving nutrient effects in seagrass beds shift from algal proliferation to accelerated herbivore consumption. Hence, the specific nature of top-down and bottom-up processes within seagrasses varies spatially, and this context dependence is likely driven by changes in consumer functional composition.

2 | MATERIALS AND METHODS

2.1 | Site locations

Experiments of the same design were replicated at four sites across the subtropical-tropical waters of the Western Atlantic during the 2014 growing season (May to October; Figure 1): Indian River Lagoon (IRL), Florida, USA (27°32.24'N, 80°20.92'W); Florida Keys (FLK), Florida, USA (25°7.58'N, 80°24.29'W); Carrie Bow Cay (CBC), Belize (16°48.15'N, 88°04.95'W); and Bocas del Toro (BDT), Panama (9°21.01'N, 82°15.51'W). All sites were located in shallow water (1–3 m) and dominated by the tropical seagrass *Thalassia testudinum*, with minor abundances (<5% cover total) of other seagrasses (*Syringodium filiforme* and *Halodule wrightii*) and macroalgae (green algae). Seagrass sites were located in protected nearshore meadows, with the exception of the CBC site, which was located c. 18 km offshore, yet also within a protected meadow positioned on the leeward side of a reef. A description of physical environmental characteristics at each site is provided in the supplementary material (Table S1). Prior work has identified BDT and IRL as being relatively eutrophic (Carruthers, Barnes, Jacome, & Fourqurean, 2005; Davis, 1985), both with a history of environmental impacts resulting from human/agricultural developments and altered watersheds (Cramer, 2013; Duncan, Larson, & Schmalzer, 2004). In the current study, we used seagrass leaf tissue nitrogen (N) and phosphorus (P) concentrations as proxies

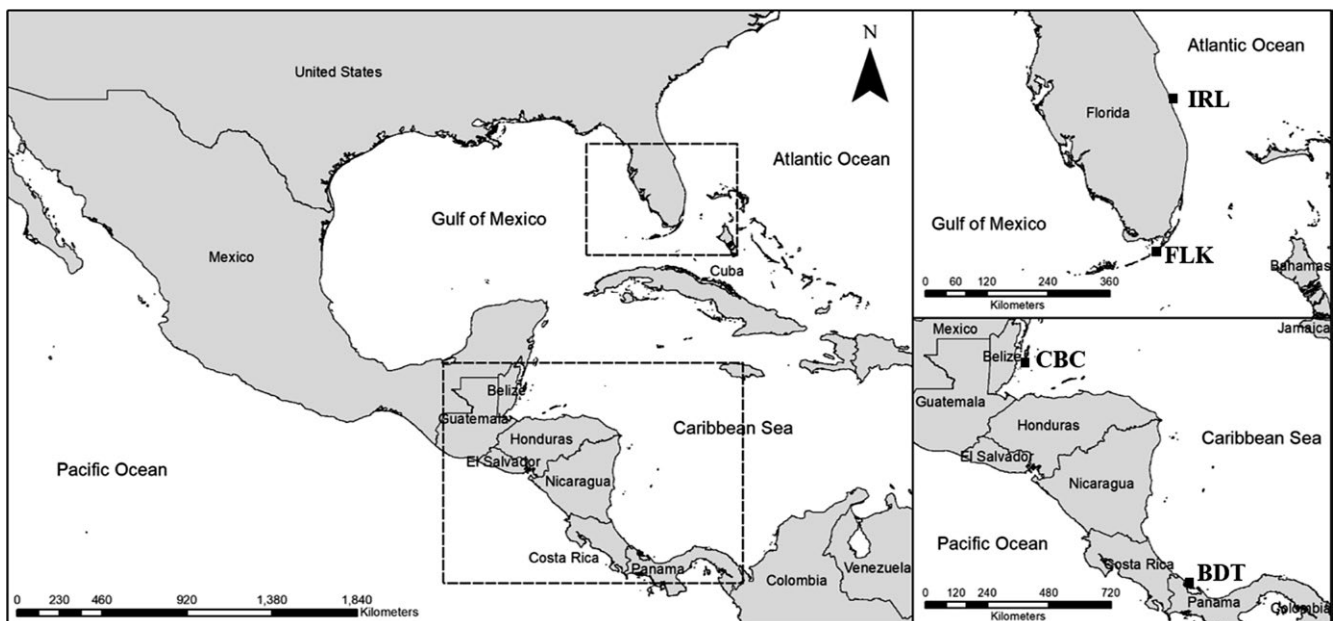


FIGURE 1 Location of the seagrass sites. Indian River Lagoon, FL (IRL), Florida Keys, FL (FLK), Carrie Bow Cay, Belize (CBC), Bocas del Toro, Panama (BDT)

for time-integrated environmental nutrient availability, and relative to the other sites, our findings confirm elevated nutrient levels in the IRL and BDT (see Section 3). The other sites (CBC and FLK) displayed lower leaf N and P concentrations, and appeared relatively pristine. Many coastal regions across the Caribbean have further been subjected to the effects of overfishing, with long-term trends towards the loss of top predators and other large-bodied fishes (Jackson, Donovan, Cramer, & Lam, 2014; Jackson et al., 2001).

2.2 | Experimental design

Individual seagrass plots (0.25 m²) were used to establish treatment combinations of three factors at each site in a fully factorial design: nutrient enrichment (added fertilizer or control), mesograzers reduction (deterrent block or control) and macrograzer exclusion (full cage, partial cage, open plot). The 12 treatments ($n = 5$ each) were randomly distributed across 60 plots at each site, for a network total of 240 experimental plots (Figure S1). All plots at each site were arranged in a uniform grid (across a 25 × 15 m area), separated by a minimum distance of 2 m, and individually trenched around all borders to limit nutrient and carbohydrate translocation. Nutrient-enriched plots received 350 g of slow-release Osmocote fertilizer (NPK 14:14:14) enclosed in fibreglass mesh bags. This fertilizer was selected because of its balanced nutrient supply, and prior effectiveness in the field (Duffy et al., 2015; Reynolds et al., 2014). Each bag was affixed to a PVC post positioned in the centre of the plot, and was replaced every 4 weeks to ensure consistent nutrient delivery. After each replacement, a random subsample of fertilizer bags ($n = 10/\text{site}$) was reweighed to quantify nutrient release (mass loss × fertilizer %N or %P/time). Mesograzers reduction was achieved using a chemical deterrent method that targets crustaceans (Whalen et al., 2013). A biodegradable pesticide (Carbaryl, 1-naphthyl-N-methylcarbamate) was incorporated (10% by weight) into a slowly dissolving plaster block that was affixed to a centre post in the mesograzers reduction plots and replaced every 2 weeks. All other plots received a control block consisting of plaster without deterrent. The 2 m distance was sufficient to limit contamination of both fertilizer and pesticide between adjacent plots. Prior to the start of the experiment, preliminary seagrass samples were collected at each site and analysed for nutrient content (see methods below). At the end of the experiment, seagrasses in the unfertilized plots displayed nutrient concentrations similar to the concentrations of these initial samples, indicating that the nutrient content of the unfertilized plots did not increase (from contamination) during the study. The only exception was in the IRL, where there was an increase in the nutrient content of the unfertilized plots as compared to the initial samples. However, this was likely attributable to seasonal increases in nutrient availability as related to regional hydrology and agricultural practices specific to this site (Li et al., 2016). For the mesograzers deterrent, prior work has demonstrated that the Carbaryl blocks used in this experiment are effective up to a distance of 60 cm, beyond which, their effectiveness is drastically reduced (Whalen et al., 2013).

Macrograzer exclusion was accomplished by enclosing designated plots in cube-shaped cages (50 cm to a side) made of plastic extruded

mesh (2.5-cm mesh size). Partial cages as a procedural control were of similar construction, but had two of the side panels removed, and open plots were marked with four corner PVC posts. Cages were scrubbed as necessary (usually biweekly) to prevent fouling. Temperature, irradiance and salinity were periodically measured (Table S1) at all sites over the course of the experiment with calibrated handheld meters or loggers (YSI Pro 20, HOBO Pendant UA-002-64; Walz diving-PAM).

Fish community structure was assessed with quantitative visual surveys at BDT, CBC and FLK sites. Belt transects (25 × 2 m, $n = 3$) were conducted within the experimental array, and a team of divers recorded the abundance and identity of all fishes along each transect. Due to turbid waters in the IRL, fish abundance was quantified using a haul seine following prior methods (Kupschus & Tremain, 2001), in which a 183 × 3 m centre-bag seine (38-mm stretch mesh) was deployed from a small skiff to sample a rectangular area (103 × 40 m) oriented parallel to the shoreline. After deployment, the net was manually pulled to shore, and all fishes in the catch were counted and identified to the species or genus level.

2.3 | Plot sampling

After 14 weeks, each plot was sub-divided into four quadrants to assess above-ground biomass, shoot density, mesograzers abundance, leaf productivity and epiphyte mass. In the first quadrant, above-ground biomass was assessed within a core (15 cm diameter) in which all vegetative biomass was collected and separated according to species. Seagrass shoots were counted, scraped free of epiphytes, pooled by species, dried to a constant weight in a 60°C oven, weighed and reported as dry mass per m². In the second quadrant, mesograzers samples were collected by enclosing a portion of the seagrass canopy (c. 15 cm diameter) within a mesh drop net (500-µm mesh size) and harvesting all above-ground biomass and associated organisms. In the laboratory, mesograzers were gently rinsed from seagrass material, stored in 70% ethanol, counted and identified. Total mesograzers abundance was standardized to the dry mass of harvested seagrass in each sample (individuals per g dry seagrass biomass). Mass-specific leaf productivity and epiphyte loading were measured across the remaining undisturbed quadrants using modified leaf punch protocols (Zieman, 1974). Approximately 10 days prior to the final sampling (which varied from September to October depending upon the start date at each site), four individual shoots were marked with a needle at the base of the leaf sheath and gently tagged with a cable tie for relocation. Marked shoots were harvested, transferred into sealed bags, gently rinsed and individually measured for relative productivity (mg g⁻¹ day⁻¹), epiphyte mass (mg/g) and shoot morphology (length and width to nearest mm). Crescent-shaped bite marks characteristic of parrotfish grazing were also counted on all harvested productivity shoots. Epiphyte mass was quantified by gently scraping all leaves with a razor blade, transferring all contents into pre-weighed 20-ml scintillation vials, and freeze-drying to achieve a dry weight. Values measured from separate shoots were averaged to obtain a plot mean. Nutrient analysis was conducted on plant material from the biomass core within each plot, as prior work has demonstrated that leaf tissue

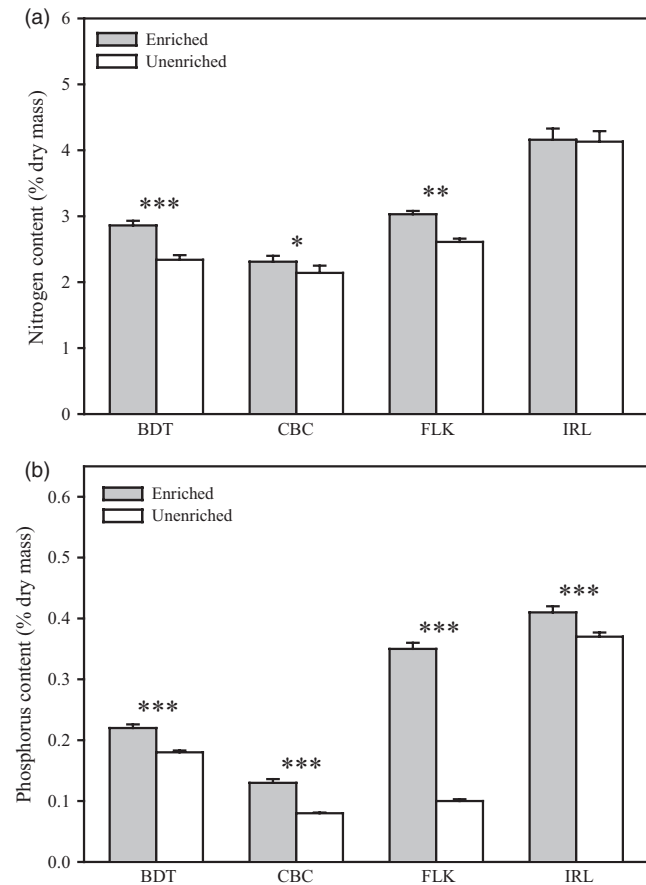


FIGURE 2 Effects of nutrient enrichment on seagrass nitrogen (a) and phosphorus (b) content ($M \pm SE$) after 14 weeks. Bars represent leaf nutrient concentrations (% dry weight) pooled across caging and deterrent treatments. Significant effects within each site are indicated with an asterisk (* $p < .05$, ** $p < .01$, *** $p < .001$)

concentrations closely reflect environmental availability (Fourqurean & Zieman, 2002; Fourqurean, Zieman, & Powell, 1992). After drying and homogenization, nitrogen content (% dry mass) was measured via CHN analysis (Fisons NA1500 elemental analyzer). Phosphorus content (% dry mass) was measured colorimetrically after a dry-oxidation, acid hydrolysis extraction (Fourqurean et al., 1992). As an additional metric for sites with sufficient water clarity (BDT and CBC), visual seagrass per cent cover was estimated from photographs taken of each plot at the end of the experiment prior to sampling. Images were analyzed with point count software (Kohler & Gill, 2006), whereby 50 points were randomly superimposed across the plot area, and the macrophyte taxa underneath each point was recorded.

2.4 | Statistical analyses

Univariate and multivariate analyses of variance (ANOVA/MANOVA) were used to examine the site-specific effects of nutrient enrichment and meso/macrograzer exclusion on seagrass response variables. Multivariate analysis of variance (MANOVA) was used to examine treatment effects on above-ground biomass and shoot density, both of which are correlated and constitute two components of seagrass

canopy structure. In this analysis, a multivariate composite index (hereafter referred to as “canopy structure”) was derived as a linear combination (weighted sums) of the two dependent variables, above-ground biomass and shoot density. Fixed factors included site (BDT, CBC, FLK, IRL), nutrients (\pm NP), deterrent (\pm Carbaryl) and caging (full, partial, open). Homogeneity of the covariance matrices was assessed with a Box’s test of equality, and omega-squared (ω^2) effect sizes were calculated to examine the proportion of the population variance attributable to experimental factors (Olejnik & Algina, 2003). Univariate ANOVAs were used to test for treatment effects on the response variables of leaf productivity, nutrient content, epiphyte mass and epifaunal abundance. Variance homogeneity was tested with a Levene’s test and omega-squared (ω^2) effect sizes were calculated. Prior to all analyses, data were tested for normality with the Shapiro–Wilks test and a visual inspection of quantile plots. Strong deviations from normality were corrected with log or square root transformations. For distributions that continued to violate test assumptions, analyses continued on transformed values with a readjusted alpha ($\alpha = 0.01$) to avoid type I error.

Some analyses were restricted to the tropical sites (BDT and CBC), which had sufficient water clarity (for photographic analysis of seagrass per cent cover) and active macrograzers (for quantifying grazing marks on seagrass blades). Seagrass per cent cover was analysed at each site with an ANOVA, and grazing marks were analysed using an ANOVA with leaf length as a covariate.

To further illustrate cross-site variation, log-transformed response-ratio effect sizes (Hedges, Gurevitch, & Curtis, 1999) were calculated for the effects of nutrients, deterrent and caging on above-ground biomass, shoot density, epiphyte mass and leaf productivity. Response ratios represent ratios of treatment over control group means, weighted by pooled standard deviations and sample sizes.

Fish community structure was compared across sites by calculating non-Euclidian metrics of dissimilarity based upon species abundances (Bray–Curtis index) and presence–absence data (Jaccard index). Fish abundance data were log transformed and standardized to a uniform benthic area prior to analysis. All analyses were conducted with *SPSS* (version 23).

3 | RESULTS

3.1 | Nutrient enrichment

Seagrass nitrogen (N) content within the unenriched plots ranged from a minimum of 2.14% in CBC to a maximum of 4.13% in the IRL (Figure 2). Phosphorus (P) content in the unenriched plots ranged from 0.10% in FLK to 0.37% in the IRL. After 14 weeks of fertilization, seagrass leaf nutrient concentrations increased, although variation among sites was evidenced by a site \times nutrient interaction for seagrass N ($p = .017$) and P ($p < .001$). N content increased with fertilization by 22%, 8% and 16% for the BDT, CBC and FLK sites, respectively, but remained unaltered in the IRL. Fertilization increased phosphorus content across all sites, with the greatest response in FLK (244% increase), followed by a 24%, 55% and 11% increase at the

BDT, CBC and IRL sites respectively. Fertilizer dissolution rates were relatively consistent across the sites (indicating similar exposure/wave energy), with mass losses ranging from 14% to 23% over the 4-week deployments. Average nutrient delivery was calculated as 0.32 g N and 0.13 g P per plot per day.

3.2 | Mesograzer abundance

Mesograzer abundance (predominantly gammarid amphipods) was low at the tropical sites, and increased with latitude by three orders of magnitude (Figure 3). Bocas del Toro and CBC displayed the lowest abundances ($M \pm SEM$) with 0.02 ± 0.02 and 0.58 ± 0.46 individuals per g dry seagrass biomass respectively. FLK and the IRL displayed higher abundances of 1.92 ± 0.44 and 24.51 ± 2.67 individuals per g dry seagrass biomass respectively. The effects of mesograzer deterrent varied across the network and were most apparent at sites with the highest mesograzer abundance (site \times deterrent: $p < .001$; Figure S2; Table S2). Significant mesograzer reductions only occurred at the CBC, FLK and IRL sites. Mesograzer abundance was likely too low at BDT to observe a significant deterrent effect (no mesograzers were found in deterrent plots). Across the network, neither nutrient enrichment nor caging had any effect on mesograzer abundance.

3.3 | Fish community structure

Quantitative surveys revealed distinct fish community structure across sites (Figure 3, Figure S3; Table S3). Tropical sites (BDT and CBC) featured high abundances of both herbivorous parrotfish (*Sparisoma radians*, *Sparisoma aurofrenatum* and *Scarus iserti*, c. 10–15 cm) and invertebrate-feeding wrasses (*Thalassoma bifasciatum* and *Halichoeres bivittatus*, c. 10–15 cm), while the FLK site was dominated by omnivorous pinfish (*Lagodon rhomboides*, c. 15 cm) which consume both invertebrate mesograzers and algal epiphytes (Heck et al., 2000; Stoner & Livingston, 1984), and carnivorous juvenile barracuda (*Sphyrna barracuda*, c. 40 cm). Fish abundance was low within the IRL, largely comprised of pinfish (c. 9 cm), grunts (*Orthopristis chrysoptera*, c. 10 cm)

and puffers (*Sphoeroides nephelus* c. 13 cm). Overall, only the tropical sites (BDT/CBC) had herbivorous fishes, while subtropical sites (FLK/IRL) were comprised of omnivorous species. Indices of dissimilarity (ranging from 0 to 1, with higher values indicating increased dissimilarity) were lowest between the two tropical sites (Bray–Curtis = 0.43), and the two subtropical sites (Bray–Curtis = 0.64). When comparing subtropical to tropical sites, pairwise dissimilarity indices increased, ranging from 0.92 to 0.99.

3.4 | Seagrass responses to nutrients and grazers

Nutrients reduced seagrass canopy structure (the multivariate composite index of above-ground biomass and shoot density) at all sites except for the IRL (site \times nutrient: $p < .002$; Figure 4, Figures S7 and S8; Tables 1, Tables S4 and S5). Structural declines in BDT and CBC were driven by reductions in above-ground biomass (due to grazing, Figure S6; Tables S6 and S7), while declines in FLK were driven by reductions in shoot density (due to algal proliferation, Figures S8 and S9; Table S8). Nutrient effects on seagrass canopy structure were undetectable in the IRL, where ambient nutrient concentrations and mesograzer abundance were highest (Table S9). Nutrient-induced grazing by herbivores was evident at both tropical sites; however, due to varying macrograzer groups (predominantly sea urchins in BDT and parrotfish in CBC), these interactions were manifested in distinct ways. In BDT, there was a significant nutrient \times cage interaction ($p = .013$) for seagrass per cent cover, as grazing reduced seagrass cover in the plots with nutrients + macrograzers (i.e. open plots and partial cages), relative to plots with nutrients + macrograzer exclusion (full cages; Figure 5, Figure S6; Table S10). In CBC, there was no significant nutrient \times cage interaction for seagrass per cent cover (Figure S4), likely due to the presence of herbivores (e.g. *S. radians*) small enough to access seagrass inside the full cages. However, there were significantly more crescent-shaped grazing marks ($p = .041$), and shorter closely cropped shoots ($p = .014$) inside the CBC plots with added fertilizer, indicative of nutrient-induced grazing by parrotfishes (Figure 6, Figure S5). Grazing marks of any kind were undetected at FLK and IRL.

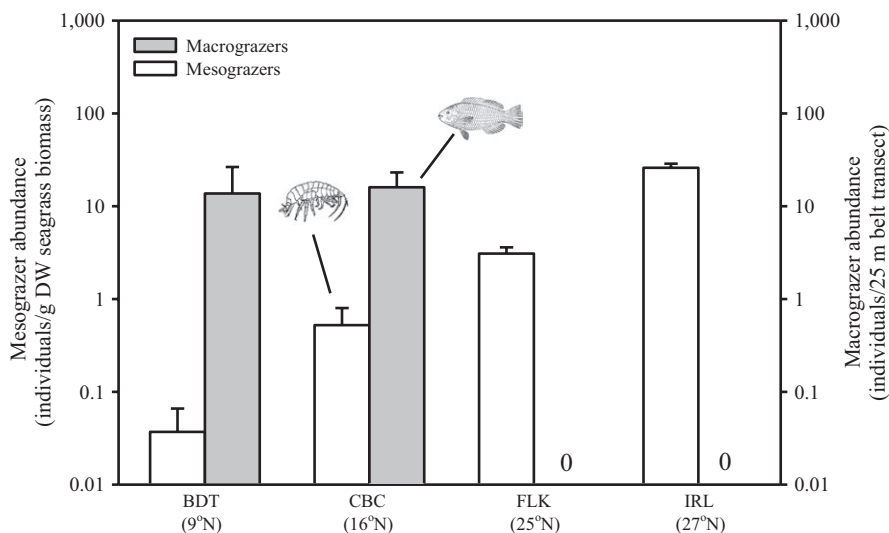


FIGURE 3 Grazer abundance across all sites ($M \pm SE$). Mesograzer data were obtained from the unmanipulated open plots at each site and macrograzer data were obtained from either visual surveys (BDT, CBC and FLK) or beach seine (IRL). Mesograzers (e.g. gammarid amphipods) are reported as individuals/seagrass biomass (g dry weight). Macrograzers (e.g. parrotfish—*Sparisoma* spp.) are reported as individuals/25-m belt transect. Values have been plotted on a log scale. Sites are indicated with their latitudinal position

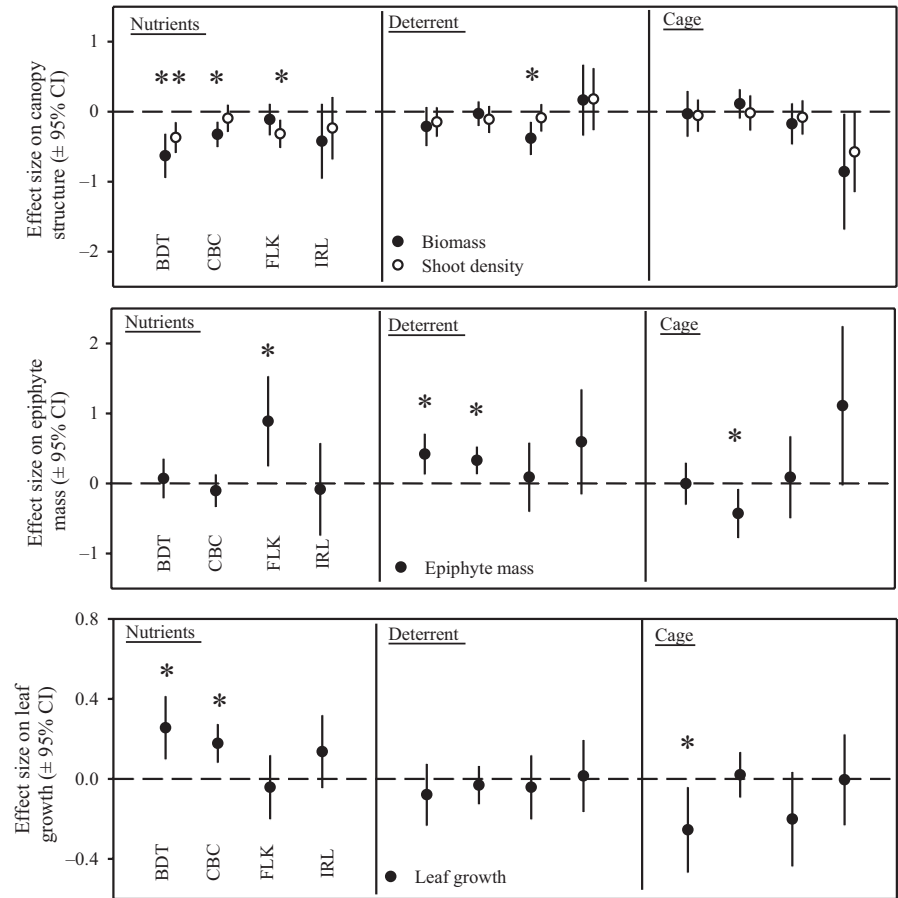


FIGURE 4 Log-transformed response ratios (\pm 95% CI) of the main effects of nutrients, deterrent and caging on seagrass canopy structure (top panel), epiphyte mass (middle panel) and leaf productivity (lower panel). Within sites, treatment interactions were uncommon. Response ratios for caging were calculated from the full and partial cage treatments. Confidence intervals that do not overlap zero indicate significant effect sizes, as indicated by asterisks ($p < .05$). BDT, Bocas del Toro; CBC, Carrie Bow Cay; FLK, Florida Keys; IRL, Indian River Lagoon

Nutrients increased leaf productivity at the tropical sites (by 19%–29%), but not at the subtropical sites (site \times nutrient: $p = .022$; Figure 4, Figure S10; Table 1). Exclusion of macrograzers also affected productivity differently among sites (site \times cage: $p = .008$), with declines in leaf growth inside the full cages at BDT. Mesograzers exclusion (deterrent) had no effect on leaf productivity at any site (Table 1).

3.5 | Epiphyte responses to nutrients and grazers

Epiphyte cover on seagrass leaves was primarily comprised of crustose coralline algae (BDT and CBC), cyanobacteria (FLK) and red algae (IRL). Nutrients increased epiphyte mass only at FLK, where both macro- and mesograzers abundance was low (site \times nutrient interaction: $p < .001$; Figure 4, Table 1). Mesograzers reduced epiphyte mass across most sites ($p < .001$), yet these effects were most evident in the IRL where mesograzers reduction increased epiphyte mass by 82% (Figure S9). Macrograzer exclusion produced variable effects on epiphyte mass (site \times cage: $p < .001$), primarily due to effects in the IRL site where epiphyte mass increased in the plots with full cages (Figure 4, Table S9).

4 | DISCUSSION

Within subtropical and tropical seagrass beds of the Western Atlantic, nutrients generally negatively impacted seagrass structure, but the

magnitude and mechanisms of nutrient effects differed and were largely influenced by site-specific herbivore identity. As macrograzer abundance increased, and mesograzers abundance decreased, the mechanisms driving nutrient effects transitioned from increased competition from epiphytic algae towards the direct effects of herbivore consumption of fertilized, nutrient-rich seagrass (BDT and CBC). These findings of nutrient-fuelled grazing are congruent with studies from other ecosystems (rocky intertidals and saltmarshes), where N enrichment can increase herbivore grazing activity (Bertness et al., 2008; Gruner et al., 2008; Silliman & Zieman, 2001). Nutrient-stimulated algal proliferation only occurred at a single subtropical site (FLK), where both mesograzers and macrograzer abundances were low. Thus, while small-bodied temperate/subtropical herbivores (i.e. amphipods) may counteract eutrophication mutualistically via indirect mechanisms of consuming algal competitors, direct seagrass consumption by large-bodied tropical herbivores (e.g. sea urchins and parrotfish) can act in the same direction as algal proliferation to reduce seagrass habitat structure. The comparative nature of our experiment presents the unique opportunity to gauge how geographic location (and local herbivore communities) influence seagrass-nutrient interactions.

At the tropical sites BDT and CBC, seagrass leaf N and P increased with fertilization, indicative of increased nutrient availability. General models suggest that such nutrient loading can promote algal overgrowth and seagrass decline (Duarte, 1995; Hauxwell et al., 2001), however, we did not find evidence of increases in epiphytic algal

TABLE 1 Treatment effects on seagrass canopy structure (MANOVA, $\alpha = 0.01$), epiphyte mass (ANOVA, $\alpha = 0.05$) and leaf productivity (ANOVA, $\alpha = 0.05$) after 14 weeks across all sites. Canopy structure represents the combined multivariate index of seagrass above-ground biomass and shoot density. Significant values are indicated in bold. Three- and four-way interactions were not significant and are excluded from the table

Canopy structure	<i>df</i>	Wilks' λ	<i>F</i>	<i>p</i>	ω^2
Site	6	0.286	54.72	<.001	0.575
Nutrients	2	0.837	18.44	<.001	0.127
Deterrent	2	0.970	2.95	.055	0.016
Cage	4	0.972	1.33	.259	0.005
Site × nutrients	6	0.899	3.45	.002	0.058
Site × deterrent	6	0.922	2.62	.017	0.039
Site × cage	12	0.929	1.18	.297	0.008
Nutrients × deterrent	2	0.521	0.52	.595	–
Nutrients × cage	4	0.324	0.32	.862	–
Deterrent × cage	4	0.972	1.33	.257	0.005
Epiphyte mass	<i>df</i>	SS	<i>F</i>	<i>p</i>	ω^2
Site	3	25.261	132.77	<.001	0.528
Nutrients	1	0.485	7.64	.006	0.008
Deterrent	1	1.37	21.6	<.001	0.028
Cage	2	0.636	5.012	.008	0.011
Site × nutrients	3	1.25	6.57	<.001	0.022
Site × deterrent	3	0.489	2.568	.056	0.006
Site × cage	6	3.567	9.373	<.001	0.067
Nutrients × deterrent	1	0.063	1.001	.318	0
Nutrients × cage	2	0.239	1.887	.154	0.002
Deterrent × cage	2	0.097	0.763	.468	–
Leaf productivity	<i>df</i>	SS	<i>F</i>	<i>p</i>	ω^2
Site	3	1.643	44.408	<.001	0.323
Nutrients	1	0.153	12.375	.001	0.028
Deterrent	1	0.009	0.708	.401	–
Cage	2	0.112	4.555	.012	0.018
Site × nutrients	3	0.121	3.269	.022	0.017
Site × deterrent	3	0.022	0.582	.627	–
Site × cage	6	0.22	2.973	.008	0.030
Nutrients × deterrent	1	0.001	0.08	.778	–
Nutrients × cage	2	0.007	0.28	.756	–
Deterrent × cage	2	0.027	1.081	.341	0.001

biomass at these sites, echoing a similar lack of epiphyte response to fertilization in eelgrass *Z. marina* across a range of sites (Duffy et al., 2015). In our study, nutrient enrichment at the tropical sites accelerated herbivory and the direct transfer of seagrass to higher trophic levels, producing conspicuous shifts in plant structure. Nutrient-induced grazing of seagrass was most clearly evident in BDT, where seagrass cover declined the most in the enriched plots with macroherbivore access (+NP partial cages and +NP open plots; Figure 5, Figure S6), suggesting that resident macrograzers (predominantly sea urchins, *Lytechinus variegatus*) responded to fertilization via increased consumption. Similar caging × nutrient interactions were not detected

in CBC because the cages were not entirely effective at excluding all grazers, particularly the locally abundant herbivorous fish, *S. radians*, juveniles of which were observed to pass through the mesh of our cages. Nevertheless, we did notice more crescent-shaped bite marks characteristic of parrotfish feeding, along with shorter closely cropped leaves on fertilized seagrasses in CBC (Figure 6), suggestive of nutrient-induced grazing (Goecker, Heck, & Valentine, 2005; Holzer, Seekell, & McGlathery, 2013). Analogous findings have been demonstrated in other systems, where fertilization can increase herbivory and resultant grazer biomass (Boyer, Fong, Armitage, & Cohen, 2004; McGlathery, 1995). In CBC and BDT, fertilization (and the associated

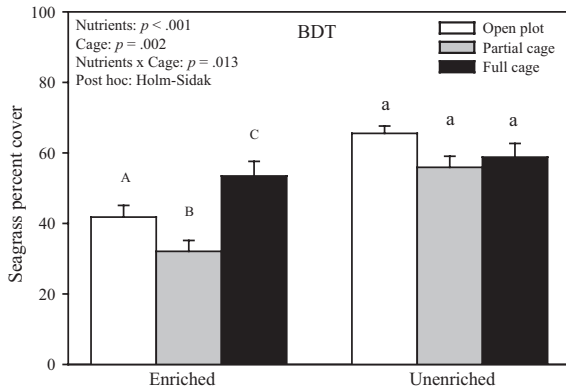


FIGURE 5 Effects of nutrient enrichment and caging on seagrass per cent cover ($M \pm SE$) at Bocas del Toro (BDT). Deterrent effects were not significant ($p > .05$). This site displayed a significant nutrient \times caging interaction. Within each level of nutrient enrichment, Holm-Sidak post hoc comparisons were conducted (distinct letters indicate significant differences between caging treatments)

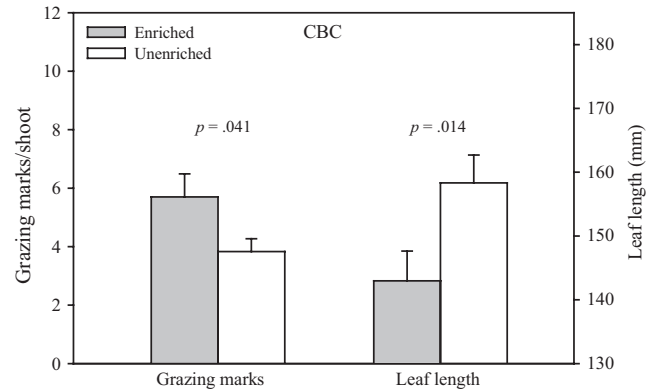


FIGURE 6 Effects of nutrient enrichment on seagrass grazing marks and leaf length ($M \pm SE$) at Carrie Bow Cay, Belize (CBC). p -values indicate the significance of the main factor of nutrient addition (ANOVA). Interactions with other treatments were not significant

increases in herbivore activity) also elevated leaf growth. Thus, while the top-down effects of direct herbivory can reduce canopy structure, leaf growth can display opposing trends and alternatively respond via increases in productivity. We note that these increases in mass-specific productivity are not exclusively driven by absolute increases in new growth, but rather by changes in relative growth (i.e. shifts in the amount of newly produced leaf material relative to older seagrass biomass; Holzer & McGlathery, 2016). Mechanisms of grazer-stimulated productivity have been previously documented in seagrasses (Kuiper-Linley, Johnson, & Lanyon, 2007; Moran & Bjorndal, 2005; Valentine, Heck, Busby, & Webb, 1997), and may result from an increased reallocation of stored carbohydrates towards leaf growth to replace lost tissue. These trends are not universal, as some studies find no evidence of stimulated productivity in cases of extended heavy grazing (Fourqurean, Manuel, Coates, Kenworthy, & Smith, 2010; Hernandez & van Tussenbroek, 2014).

Responses at the Florida Keys site followed more classical expectations of eutrophication, largely established by research with temperate seagrasses, where nutrient enrichment can increase epiphytic biomass and decrease canopy structure (Duarte, 1995; Hauxwell et al., 2001; Valiela et al., 1997). While the overall negative outcome of fertilization for seagrass canopy structure in the FLK was similar to CBC and BDT, the mechanisms behind these responses were wholly distinct. Unlike CBC and BDT, where nutrients stimulated grazer-induced declines in canopy structure, nutrient enrichment in the FLK promoted epiphytic algal proliferation, shading seagrasses and reducing shoot density. The canopy in the Keys shifted towards longer individual shoots (Figure S5) and lower areal shoot density, a response characteristic of seagrasses experiencing light limitation (Abal et al., 1994; Enriquez & Pantoja-Reyes, 2005). Herbivore abundance was low at FLK, potentially contributing to algal proliferation and supporting the idea that grazer absence and nutrient enrichment can contribute to algal proliferation (Burkepile & Hay, 2006). While pinfish

were present, and have previously been shown to consume epiphytes (Heck, Fodrie, Madsen, Baillie, & Byron, 2015; Heck et al., 2000; Stoner & Livingston, 1984), we found no evidence of pinfish grazing at this site. Algal overgrowth was primarily driven by an unidentified cyanobacterium, which can produce unpalatable secondary metabolites that inhibit grazing (Capper et al., 2016; Nagle & Paul, 1999). Mesograzer densities at FLK were also low and likely insufficient for epiphyte control, thereby suggesting the existence of a critical density below which the functional role of mesograzers may be limited (Whalen et al., 2013).

In the IRL, nutrient enrichment imposed minor effects on seagrass canopy structure and leaf productivity, likely due to (1) historically elevated nutrient concentrations within the lagoon (Davis, 1985; Heffernan & Gibson, 1983) and (2) relatively high abundances of mesograzers which consume algal epiphytes and buffer against eutrophication effects. As compared to our other sites, deterrent application was effective at decreasing amphipod abundance (Figure S2) and increasing epiphyte mass (Figure S9; Table S9), as epiphytic algae comprise 41%–75% of the diet of common amphipods within the lagoon (Zimmerman & Livingston, 1979). Similar results have been shown for other subtropical meadows, where amphipods can reduce epiphyte loading (Baggett, Heck, Frankovich, Armitage, & Fourqurean, 2010) and benefit seagrasses (Myers & Heck, 2013). In the current study, while mesograzers reduced epiphyte loading, there were no downstream benefits on seagrass canopy structure and/or leaf productivity, contrasting with prior studies with other seagrasses (Ebrahim et al., 2014; Myers & Heck, 2013; Reynolds et al., 2014). These differences might result from the substantial carbohydrate reserves of *T. testudinum*, which may continue to maintain shoot biomass and levels of productivity for extended durations, despite moderate increases in shading by epiphytes.

Our experiment represents the first to concurrently manipulate bottom-up and top-down forcings at multiple seagrass sites across lower latitudes. By working along a gradient in consumer composition (mesograzers/macrograzers), our findings illustrate the role that functional traits and herbivore identity play in influencing seagrass

responses to nutrient enrichment. In comparison to other standardized experiments (Baden et al., 2010; Duffy et al., 2015; Moksnes et al., 2008), which demonstrate the importance of temperate mesograzers, we found that the mosaic of herbivore identities across subtropical-tropical boundaries increases the diversity of seagrass responses to nutrient loading, with declines due to eutrophication being alternatively driven by either epiphytic algal proliferation or macro-grazer consumption. As suggested by prior syntheses (Duffy, Hughes, & Moksnes, 2013; Heck & Valentine, 2006, 2007), direct top-down control of seagrasses appears more important in tropical than in temperate settings, as predicted for consumer interactions in general, yet, these effects are spatially dependent and can be increasingly triggered by bottom-up factors.

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AUTHORS' CONTRIBUTIONS

J.E.C., A.H.A., V.J.P. and J.E.D. designed the experiment and contributed to drafting the manuscript; J.E.C., A.H.A., L.N.J., C.D.K., R.P. and V.J.P. collected the data; J.E.C. analysed the data.

DATA ACCESSIBILITY

Data are deposited in Figshare <https://doi.org/10.6084/m9.figshare.5346451.v1> (Campbell et al., 2017).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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