



Anthropogenic versus fish-derived nutrient effects on seagrass community structure and function

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Abstract. Humans are altering nutrient dynamics through myriad pathways globally. Concurrent with the addition of nutrients via municipal, industrial, and agricultural sources, widespread consumer exploitation is changing consumer-mediated nutrient dynamics drastically. Thus, altered nutrient dynamics can occur through changes in the supply of multiple nutrients, as well as through changes in the sources of these nutrients. Seagrass ecosystems are heavily impacted by human activities, with highly altered nutrient dynamics from multiple causes. We simulate scenarios of altered nutrient supply and ratios, nitrogen:phosphorus (N:P), from two nutrient sources in seagrass ecosystems: anthropogenic fertilizer and fish excretion. In doing so we tested expectations rooted in ecological theory that suggest the importance of resource dynamics for predicting primary producer dynamics. Ecosystem functions were strongly altered by artificial fertilizer (e.g., seagrass growth increased by as much as 140%), whereas plant/algae community structure was most affected by fish-mediated nutrients or the interaction of both treatments (e.g., evenness increased by ~140% under conditions of low fish nutrients and high anthropogenic nutrients). Interactions between the nutrient sources were found for only two of six response variables, and the ratio of nutrient supply was the best predictor for only one response. These findings show that seagrass structure and function are well predicted by supply of a single nutrient (either N or P). Importantly, no single nutrient best explained the majority of responses—measures of community structure were best explained by the primary limiting nutrient to this system (P), whereas measures of growth and density of the dominant producer in the system were best explained by N. Thus, while our findings support aspects of theoretical expectations, the complexity of producer community responses belies broad generalities, underscoring the need to manage for multiple simultaneous nutrients in these imperiled coastal ecosystems.

Key words: biodiversity; diversity–productivity relationship; ecological stoichiometry; fish nutrient supply; nitrogen; non-additive; phosphorus; ratio; Redfield; *Thalassia testudinum*.

INTRODUCTION

The relative importance of different nutrients for primary production has been a central issue in the biological sciences at least since Justus Von Liebig popularized the “Law of the Minimum” in the mid 19th century (van der Ploeg et al. 1999). The central idea was that primary production should be dependent on the most limiting nutrient, and was subsequently implemented through fertilizer application to increase agricultural production in the wake of the industrial revolution. Nearly a century later, Redfield (1958) extended this line of thinking to consider the interactive nature of multiple nutrients when he hypothesized that the ratio of nitrogen to phosphorus (N:P) found in oceanic seston was consistent with the ratio of nutrient supply from deep seawater (Redfield 1958). Since, research sub-disciplines and frameworks such as the Resource Ratio Hypothesis (Tilman 1982) and Ecological Stoichiometry (Sterner and Elser 2002) have focused on understanding the importance of multiple simultaneous nutrients for explaining ecological

processes. Yet despite these efforts, predicting ecological response to changing resource dynamics remains a critical challenge in ecology and conservation (Davidson and Howarth 2007, Conley et al. 2009).

Resource supply to producer communities is being altered via municipal, agricultural and industrial waste, and is considered to be one of the leading anthropogenic stressors to ecosystems globally, particularly in coastal systems (Smith et al. 2006). A primary concern is that enrichment of multiple nutrients, typically increasing availability of both nitrogen (N) and phosphorus (P), can have non-additive effects on ecosystems (Elser et al. 2007, Allgeier et al. 2011a, Harpole et al. 2011). Non-additive effects are difficult to predict, and thus can hinder our ability to forecast ecological responses, impeding conservation and management efforts (Darling and Cote 2008). As such, a critical pursuit in ecology and conservation is understanding how producer populations and communities respond to the independent and interactive supply of multiple nutrients (Davidson and Howarth 2007).

In addition to nutrient enrichment, changes in fish communities due to exploitation (Lotze et al. 2006, Barbier et al. 2008) have been implicated in altered ecosystem-scale nutrient dynamics in coastal ecosystems (Layman et al. 2011,

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Allgeier et al. 2016). Through the excretion of nutrients, fishes provide critical resources for marine primary producers (Allgeier et al. 2013, Burkepille et al. 2013, Layman et al. 2013) and coral (Meyer et al. 1983, Shantz et al. 2015). At the ecosystem scale, fish communities can represent one of the largest sources of nutrients to coastal ecosystems in the Caribbean (Burkepille et al. 2013, Allgeier et al. 2014). Fish communities have also been shown to provide nutrients to coral reefs at ideal ratios of N:P for coral development (Allgeier et al. 2014). Yet it remains unclear how shifts in fish-mediated nutrients, e.g., through fishing pressure, may interact with nutrient supply from anthropogenic sources to affect primary producer community structure and function.

Here we experimentally test the effects of different nutrient supply rates from different sources (fish, fertilizer) on seagrass community structure and function. We do so through a long-term (>2.5 yr), full factorial, experiment on artificial reefs. We manipulate the rate and ratio of nutrient supply through both anthropogenic nutrient enrichment and altered fish biomass. These treatment effects provide both categorical (high and low fish densities crossed with fertilizer and no fertilizer) and continuous (estimated supply rates and ratios of nutrients from these treatments) predictors of ecological function (growth rate, %N and %P tissue content, and their ratio) for the dominant producer species in the system (the seagrass, *Thalassia testudinum*), and aspects of diversity (richness and evenness) of the producer community. Specifically, we ask three primary questions:

1. How do shifts in fish communities and anthropogenic nutrient enrichment alter nutrient supply rates and ratios?
2. How do different sources of nutrient enrichment act independently and interactively to alter the function of a dominant seagrass species and/or the diversity of the entire producer community?
3. Is enrichment by nitrogen or phosphorous (or both) a more important driver of function and structure in these seagrass communities?

We predicted that anthropogenic nutrient enrichment and altered fish communities would have interactive (non-additive) effects on seagrass function and structure.

METHODS

The study was conducted in The Bight of Old Robinson, Abaco, Bahamas (N26 20.735W77 00.016), a semi-enclosed bay with a mosaic of seagrass, sand, and hard-bottom habitats (Yeager et al. 2011). Sixteen artificial reefs were constructed (December 2010) from 30 cinder blocks (~40 cm × 20 cm × 20 cm) in a pyramid shape (~100 cm × 80 cm at base, 60 cm height) on sparse seagrass habitat (~3 m deep) dominated by common turtle grass, *Thalassia testudinum*. Artificial reefs are useful for experimentation because they provide replicable units of discrete size from which the ecological responses to the local ecosystem can be measured (Hixon and Beets 1989, Carr and Hixon 1997). Ambient background nutrients in this systems are extremely low (~3.5 µg/L Soluble Reactive Phosphorus, ~3 µg/L NO₃⁻, and ~7 µg/L NH₄⁺; Allgeier et al. 2010, Stoner et al. 2011).

Nutrient supply to seagrass surrounding the reefs was altered in two primary ways: (1) nutrient enrichment via the addition of fertilizer, (2) altered fish-mediated nutrient supply via manipulation of the reef structure. These two treatments were imposed on the artificial reefs in a 2 × 2 factorial design. Nutrient enrichment was simulated using PVC diffusers filled with slow release (8 month) fertilizer (Florkan 18-6-8 NPK, type 270). Seven diffusers filled with ~500 g were suspended around each reef on glass fritted poles ~0.5 m above the substrate. Diffusers were changed every 3 months for the duration of the study. Nutrient release rates from fertilizer diffusers were estimated by determining the percent loss whereby we calculated the total loss of fertilizer on a subset of diffusers ($n = 7$) after deployment (2.7 ± 0.3 SD, 0.9 ± 0.1 SD g reef⁻¹ d⁻¹, for N and P, respectively; Appendix S1).

Fish-mediated nutrient supply was altered by reducing the physical complexity on the reefs by filling in the holes of the cinder blocks creating a smooth-sided structure. In doing so fish biomass and community composition was substantially altered (see *Results* section and Appendix S1 for detailed methods of reef manipulation, and for images of reefs). Reefs were placed >150 m apart to minimize among-reef movements of more transient fish species (Carr and Hixon 1997). Cross-reef enrichment among reefs due to fertilizer is not a concern due to the highly oligotrophic nature of the system and thus high rates of nutrient uptake by producers. This is supported by previous tests that show that fish excretion and nutrient enrichment have little to no effect on water column nutrients (Appendix S1). The experiment was a block design ($n = 4$ blocks), each consisting of the four treatment types. Reefs were affected by local fishers only through the occasional removal of Caribbean spiny lobster (*Panulirus argus*); no direct removal of fishes by humans occurred on these reefs. Note, given the difficulties of generating high levels of treatment replication within our experimental system nutrient supply rates and ratios per treatment are somewhat confounded whereby high rates of nutrient supply (associated with fertilizer treatments) also have lower ratios (Fig. 2).

Underwater visual census was used to estimate fish abundance ($n = 5$ surveys on each reef between 2011 and 2013, four of which were in 2013; 80 surveys total). All fishes were identified to the lowest possible taxonomic level, and size (total length) of each individual was estimated to the nearest cm (see Appendix S2: Table S1 for mean biomass per species within the fish community on each treatment type). Previous work on similar reefs in this system has shown that fish community assembly plateaus at ~120 d post reef construction (Yeager et al. 2011). All of our surveys were conducted after this time period. Repeatability surveys to test for variation of fish community biomass through time were conducted on an entire block (one reef of each treatment) five different times over the course of 1 yr—confirming that fish communities were relatively consistent in total N supply, P supply and biomass (i.e., in all tests there was no significant effect of the date of the survey— P -value <0.05 in ANOVA; Appendix S2: Table S2). To estimate fish nutrient supply rates and ratios for N and P, we applied models generated by Allgeier et al. (2014, 2015) to the survey datasets from this study (Appendix S3). We did not account for nutrient input from egestion from fishes, thus we are likely underestimating the net nutrients

provided by the fish community—though this is likely small relative to excreted nutrients (Schreck and Moyle 1990).

To assess the drivers by which different loading rates and ratios of nutrients affect attributes of seagrass characteristics and diversity, we measured a suite of response variables in a spatially explicit fashion along three transects radiating from each reef (June 2013). Response variables were in one of two categories: population- and community-level responses, and were taken at 7 and 6 distances, respectively: 0.5, 1, 2, 3, 4, 6, and 8 m ($n = 336$ per *T. testudinum* response with ~10,000 seagrass blades measured for growth; $n = 288$ measurements for diversity metrics—distances 0.5 and 1 were pooled) representing a total of 1,920 measurements. Previous work in this system has shown that artificial reefs create biogeochemical hotspots that extend into the surrounding seagrass bed, typically up to ~3–4 m (Layman et al. 2013). As such, taking measurements at distances exceeding the spatial extent of this biogeochemical hotspot (e.g., 8 m; Layman et al. 2013, 2016) provides a control value for response variables at ambient nutrient conditions.

Population-level responses focused on parameters associated with the dominant seagrass *T. testudinum* (growth rate mm^2/d , %N and %P in blade tissues and the density of short shoots- m^2). Relative seagrass growth rates were estimated by using the standard blade hole-punching protocol (Zieman 1974) in August 2013. Nutrient content of seagrass blades has been widely used to assess nutrient availability in coastal ecosystems (Atkinson and Smith 1983, Fourqurean and Zieman 2002) and reflects ambient nutrient conditions over a relatively long time frame (i.e., months). In the lab, *T. testudinum* short shoots and photosynthetic living tissue were rinsed with deionized water then blades were scraped to remove epiphytes. Blades were dried in at 65°C for 72 h and ground to a powder with a mortar and pestle. Ground samples were analyzed for carbon and nitrogen content with a CHN Carlo-Erba elemental analyzer (FISON NA1500) and for phosphorus using dry oxidation-acid hydrolysis extraction followed by a colorometric analysis (Fourqurean and Zieman 2002). Elemental content was calculated based on proportion of sample dry weight.

Our community-level responses consisted of two measures of diversity: richness and evenness. We surveyed all benthic producer species within the seagrass beds, including: three seagrass species, 17 macroalgal species, and a cyanobacterial mat complex. Producers were identified to the lowest taxonomic level possible (Appendix S4: Table S3, for list of organisms) always at least to genus-level, with the exception of the cyanobacterial mat complex which likely consisted of multiple species of cyanobacteria that we were unable to determine with a high degree of specificity. Responses were determined using the modified Braun–Blanquet method (Fourqurean et al. 2001). From these surveys we quantified richness, the number of unique species per survey, and the evenness, as measured by the reciprocal Simpson's D: $1 - \sum(n - N)^2$, where n is the abundance of that species per survey and N is the number of total species per survey (Simpson 1949).

Statistical analysis

Question 1: How do shifts in fish communities and anthropogenic nutrient enrichment alter nutrient supply rates and ratios?

To assess the efficacy of our treatments differences in fish abundance, biomass, and fish-mediated nutrient supply (both N and P) were tested using NMDS and compared with PERMANOVA and paired contrasts ($\alpha < 0.05$) and using two-way ANOVA and TukeyHSD post-hoc tests ($\alpha < 0.05$). Differences in enrichment, including fish-mediated nutrients plus fertilizer nutrients for N, P, and N:P, were also tested using two-way ANOVA and TukeyHSD post-hoc tests ($\alpha < 0.05$).

Question 2: How do different sources of nutrient enrichment act independently and interactively to alter the function of a dominant seagrass species and/or the diversity of the entire producer community?

Treatment-level effects of nutrient enrichment (via fertilizer input; categorical—herein +N or –N), fishing pressure (via reduction of fish biomass; categorical—herein +F or –F), and/or their interaction (all categorical variables) across reefs and for each response variable were tested using Generalized Additive Models (GAMs; Zuur et al. 2009). GAMs allow a smoothing function to be applied to the distance parameter to account for distinct non-linear patterns observed in the data. Previous work on similar reefs in the same embayment have shown distinct non-linear trends in spatial patterns associated with increasing distance from the reef (Layman et al. 2013). Thus, in addition to differences among intercepts of these factors, we incorporated additional two-way interactions with distance away from reefs for each treatment, and a three-way interaction with distance and both treatments. In this context, a significant interaction effect (anthropogenic nutrients \times fish derived nutrients) would suggest differential responses in seagrass structure or function due to these different sources. A nonsignificant interaction term would suggest that the response was not affected by the specific source of nutrients. A significant block effect was found only for the short shoot density of *T. testudinum* response, and thus this term was retained only in this model.

Question 3: Is enrichment by nitrogen or phosphorous (or both) a more important driver of function and structure in these seagrass communities?

To test if supply rates or ratios were more important for various characteristics of seagrass, we ran three sets of candidate GAM models for each response variable: the supply of N, the supply of P, and their ratio (N:P molar). Each set of models included continuous predictor variables for: total fish nutrient supply or ratio, total fertilizer supply or ratio and their interaction, and all interactions with distance (Fig. 3B). To assess if the supply rate of nutrients (either N or P) or the supply ratio (N:P) best predicted each response, the top model (for each N supply, P supply and N:P) was selected and then compared using information theory (Akaike Information Criteria) (Burnham and Anderson 2002). Once the supply rate or ratio model was selected, we then determined the weighted parameter importance for all explanatory variables that were found within the top models ($\Delta\text{AIC} < 4$). Parameter weights were calculated by summing the model weight each time a given parameter was included in one of the top models ($\Delta\text{AIC} < 4$). That is, if a parameter has a weight of 1 then it was in all top

models ($\Delta AIC < 4$), if it has a weight of 0 it was in none of the top models. Due to our experimental design the ratios of nutrient supply are somewhat confounded with nutrient supply, because higher supply rates associated with fertilization are also associated with low ratios. However, because fish communities are different across all 16 reefs creating a continuous gradient of nutrient ratios (in particular with respect to the fish only treatments: $\sim 18\text{--}34$ N:P) these values can still be applied in competing models (N or P supply, or their ratio) to test the importance of supply and ratios for given responses, though caution in their interpretation is warranted. Note that this analysis differs from the analysis for Question 2 that used categorical variables of the treatments (high vs low fish density, and nutrient enrichment vs. no enrichment; explained above), in that here we use continuous predictors of nutrient supply rates allowing a direct test of the importance of fish nutrient supply vs. fertilizer.

All analyses were conducted using R software (R Core Development Team 2012). All models met appropriate assumptions. In all cases, data were modeled at the reef level whereby transect-level data was averaged per reef, reducing sample size from $n = 336$ to $n = 112$ per *T. testudinum* response and from $n = 288$ to $n = 96$ per diversity metric.

RESULTS

Question 1

Altered structural complexity of our artificial reefs had substantial impact on fish community structure in terms of abundance and biomass, as well as fish supply of N and P (PERMANOVA, with paired contrasts P -value < 0.01 ; Fig. 1). Specifically, reduced artificial reef complexity reduced the abundance, biomass, and the amount of N or P

supply from the fish community irrespective of the presence of the nutrient enrichment treatment (PERMANOVA, with pair contrasts P -value < 0.5 ; Fig. 1). As such, altered fish community composition and nutrient enrichment created four distinct treatment levels of N and P supply and supply N:P (ANOVA with TukeyHSD post hoc tests, P -value < 0.01 between all treatments; Fig. 2). Importantly, nutrient supply was substantially greater from the fertilizer addition than fish-mediated nutrients ($\sim 345\%$ and $\sim 182\%$ greater for N and P, respectively; Fig. 2), an aspect of our design that potentially confounds our ability to test the relative importance of supply rate vs. supply ratio (Fig. 2). However, the rates of nutrient enrichment from fertilizer in our study (~ 0.45 g N \cdot m $^{-2}\cdot$ d $^{-2}$, ~ 0.15 g P \cdot m $^{-2}\cdot$ d $^{-2}$; Appendix S1) are relatively low compared to other studies in similar systems (Ferdie and Fourqurean 2004 were 1.9 and 0 times higher; and Armitage et al. 2011 was 3.5 and 1.2 times higher, for N and P, respectively) which were based off regulatory estimates of maximum wastewater and stormwater discharge in similar seagrass ecosystems. Thus, while enrichment rates and ratios maybe somewhat confounded, our design still provides useful comparison of how seagrass is affected by realistic scenarios of altered nutrient dynamics.

Four response variables for the most dominant seagrass species *T. testudinum* were measured in June 2013: growth rate (34.3–93.1 mm $^2\cdot$ d $^{-1}$), short shoot density (36.7–94.2 m $^{-2}$), and %N and %P of blade tissue (1.3–3 and 0.06–0.17, respectively). Two measures of structure and diversity of the producer community were also measured: richness (3–15 species) and evenness (Simpson's Index; 0.18–0.78), providing a perspective of how benthic producer species may have been affected. Spatial trends whereby response variables changed as a function of distance from the reefs were observed for all responses, i.e., there was a significant

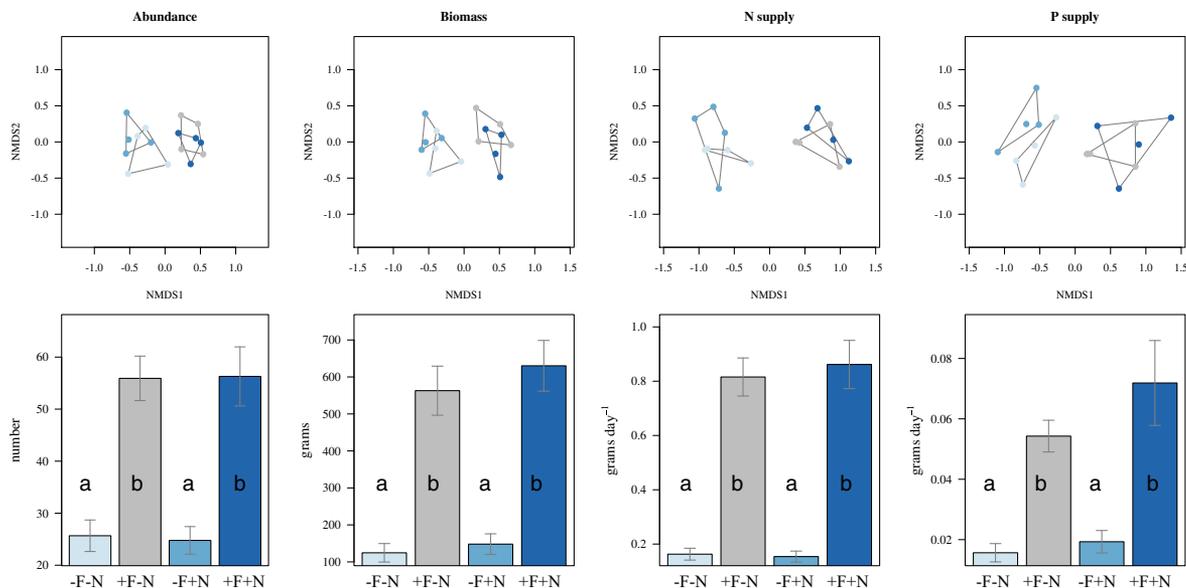


FIG. 1. (A) NMDS plots demonstrating the effect of the fish treatment (alteration of reef structure) for fish abundance, biomass (g), and N and P supply (g/d). Significant differences were found between the high and low fish community treatments. No effect was found due to the artificial nutrient treatment on fish communities. Colors representing the different treatments are same as barplot below. (B) Barplots of the same fish attributes (same columns) showing the differences in magnitudes among treatments (+F and -F indicates high and low fish density, respectively, and +N and -N indicates the presence or absence of fertilizer). Letters indicate significant differences determined by ANOVA and TukeyHSD post hoc test.

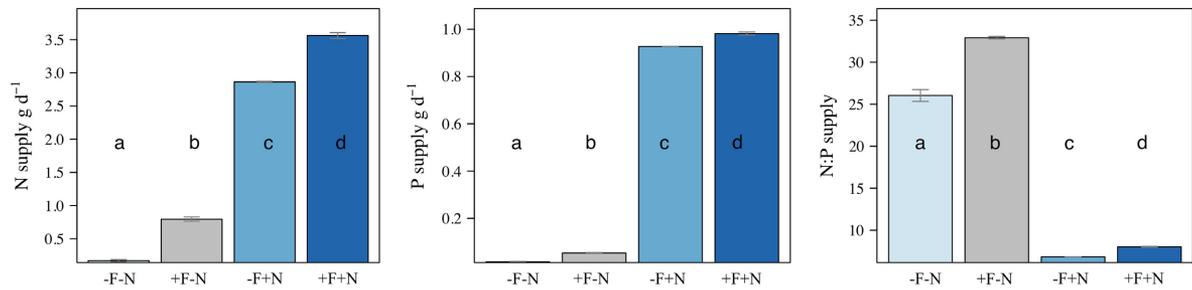


FIG. 2. Total nutrient supply rates (g/d), and ratios (molar) from the fish community at high and low density (+F, -F, respectively) and fertilizer (+N, -N), with standard deviations for all experimental treatments. Letters indicate significant differences determined by ANOVA and TukeyHSD post hoc test (Appendix S5).

effect of distance in the model. Significant spatial trends suggest that responses measured at and around the reef significantly differ from ambient or control conditions that exist 8 m away—which is beyond the influence of nutrient supply from fertilizer or fishes. Most of the spatial trends were non-linear, such that the magnitude of change is often amplified within the first 0–4 m from the reef (Fig. 3A).

Question 2

To test for the relative importance of anthropogenic nutrients vs. fish derived nutrients and their interaction we applied generalized additive models (GAMs) to the data, treating both predictors as categorical with the smoothed term being applied to the distance each measure was taken from each reef (Table 1). Reduced fish nutrient supply (FISH) had a significant positive effect on one characteristic of *T. testudinum* (short shoot density), and resulted in higher richness and evenness of the producer community (Table 1). Fertilizer addition (NUT) significantly increased four responses, growth rate, %N, %P, and evenness of the producer community (Table 1). Tissue %P showed no effect related to reduced fish nutrient supply, suggesting that even marginal amounts of fish-derived nutrients (i.e., even those supplied at low fish biomass, Fig. 1) can satiate *T. testudinum* P demand. Non-additive responses to simultaneous reduced fish nutrient supply and fertilizer enrichment, i.e., the interaction term (NUT × FISH), were only found for *T. testudinum* %N (antagonistic) and the evenness of the producer community (synergistic; Table 1). This finding indicates that, when making categorical comparisons, the relative source of the nutrients was unimportant for all other response variables, suggesting that fertilizer was functionally equivalent to fish excretion for these responses.

An effect of distance (the smoothed term in the GAMs) was found for all responses (Table 1), demonstrating that nutrient enrichment (irrespective of the source) influenced all responses relative to areas that received minimal direct nutrient input from these sources (i.e., 8 m from the reef—functionally representing a control treatment). Anthropogenic nutrient enrichment had a spatial effect with increasing distance (DIST × NUT) from reef on three characteristics of *T. testudinum* (%N, %P and growth rate), but not for either metric of community diversity. The interactions between reduced fish nutrient supply and distance from the reef (DIST × FISH) was never statistically significant (but there was a marginal effect for species richness; Table 1). No non-additive effects between the two treatments were found

across this spatial scale, i.e., there were no significant three-way interactions between the two treatments and distance from reef (DIST × NUT × FISH), suggesting that across this spatial scale nutrient enrichment effectively supplanted the loss of nutrients from reduced fish biomass (Table 1).

Question 3

To better understand the drivers by which nutrient supply rates or ratios of different sources of nutrients influence seagrass dynamics, competing models that included parameters exclusively associated with either N supply, P supply, or N:P (generated from estimating fish nutrient supply, and fertilizer enrichment on each reef; all continuous—see *Methods* section and Fig. 3) were compared for characteristics of *T. testudinum* and both community diversity metrics. Supply ratios of N:P provided the best explanatory variables for only one of the five responses (%P of *T. testudinum*, in which this model was only a slightly better candidate than the top model for N supply ($\Delta\text{AIC}_c = 2.9$) and had a lower $R^2 = 0.66$ vs. 0.68, for N:P and N, respectively; Fig. 3Biv). Instead, supply rates of N and P alone provided the best predictors for all other responses. Models that included N supply rate were chosen for growth rate and short shoot density of *T. testudinum* (Fig. 3Bii). *T. testudinum* %N and community richness was best predicted by P supply rate (Fig. 3Biii and Bv, respectively). Models that included N supply rate parameters explained variation in community evenness (Fig. 3Bvi) almost identically to models that included P supply rate parameters ($\Delta\text{AIC}_c < 2$), but P explained slightly more of the variation in the data ($R^2 = 0.66$ vs. 0.65).

Nutrient supply from fertilizer was a slightly better predictor (indicated by the height of the bars—essentially an estimate of relative parameter importance across the top models, in Fig. 3B) than fish excretion (i.e., fish nutrient supply close to the reef) for all responses except %P in seagrass tissue, which was best predicted by supply ratio. However, when considering the spatial effect of these sources, fish excretion was a better predictor—with the notable exception of richness (Fig. 3Bv). In all cases, the interaction term from the two nutrient sources was nearly or as strong of a predictor as fish excretion, suggesting that the local effect of fertilizer was to some extent always mediated by the additional input of nutrients from fish. When considering the spatial importance of these sources, strong non-additive effects (meaning the interaction term between fertilizer × fish excretion × distance was significant) were found

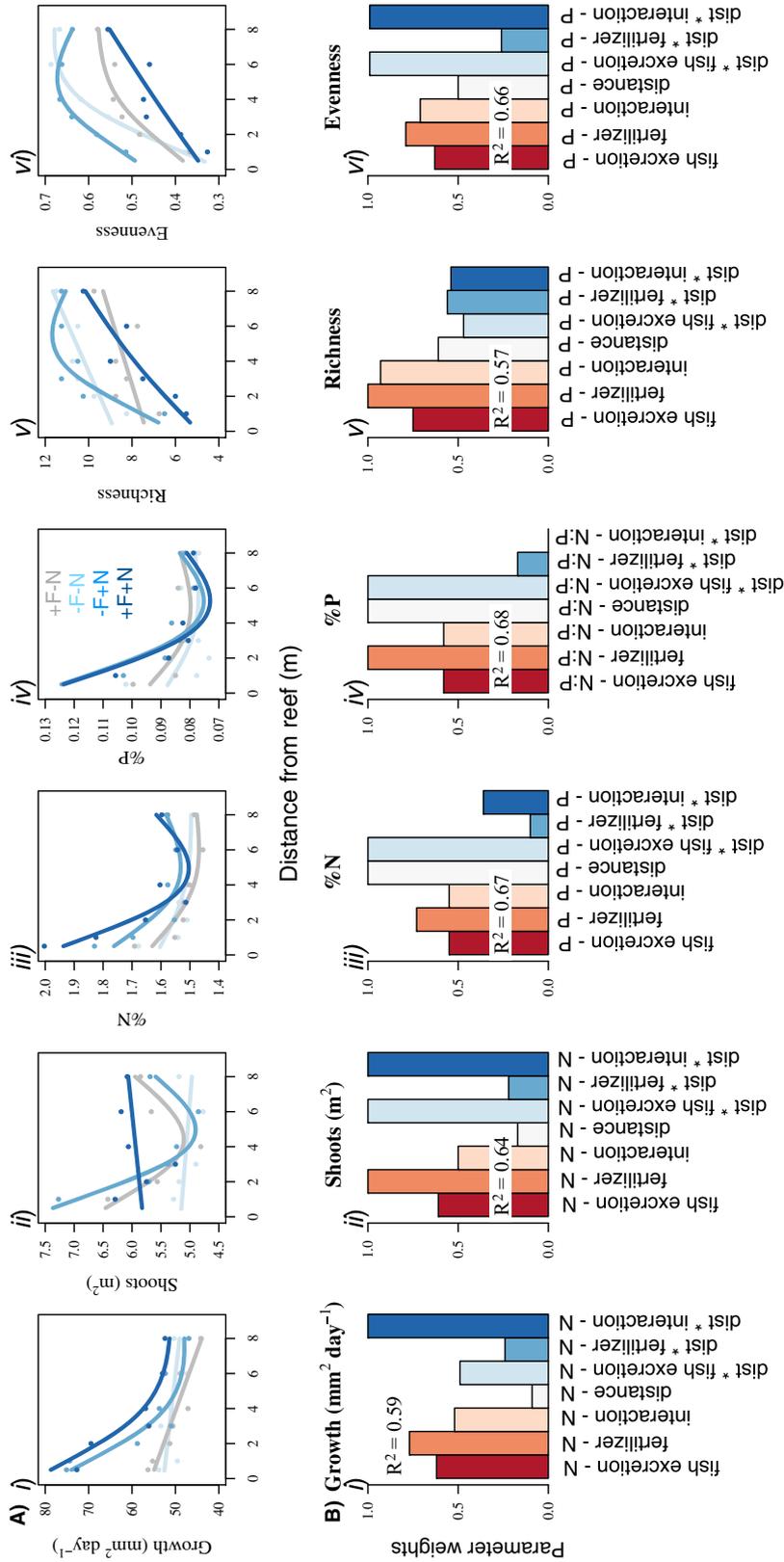


FIG. 3. (A) Generalized additive models (GAM) describing the relationships between attributes of *Thalassia testudinum* and biodiversity as a function of distance away from the reefs. (B) Parameter weights for global generalized additive models (GAM) for a continuous analysis of the same responses in panel A. Parameter weights were calculated by summing the model weight each time a given parameter was included in one of the top models ($\Delta AIC < 4$). Thus, if a parameter has a weight of 1 then it was in all top models ($\Delta AIC < 4$), if it has a weight of 0 it was in none of the top models. Predictor variables are on the x-axis and represent supply rates or ratios of N, P, or N:P from fish, fertilizer and both in conjunction. R² are the for top model for each response variable (Appendix S6).

TABLE 1. *P*-values for treatment effects for all responses including interactions with and without distance away from reef.

	Growth	%N	%P	Shoot Den.	Richness	Evenness
NUT	<0.001 (+)	<0.001 (+)	0.04 (+)	–	–	0.01 (–)
FISH	–	–	–	0.06 (+)	<0.001 (+)	0.01 (+)
NUT × FISH	–	<0.05 (–)	–	–	–	<0.01 (+)
DIST	0.02	<0.001	0.02	0.01	0.02	<0.001
DIST × NUT	0.02	<0.001	<0.001	–	–	–
DIST × FISH	–	–	–	–	0.08	–
DIST × NUT × FISH	–	–	–	–	–	–
<i>R</i> ²	0.48	0.69	0.62	0.20	0.52	0.66

Note: (–/+) indicates the direction of the response.

for growth rate, short shoot density of *T. testudinum* (both synergistic; Fig. 3Bi,ii, respectively) and the evenness of the community (antagonistic; Fig. 3Bvi).

DISCUSSION

The availability of nutrients is being altered across ecosystems globally (Smith et al. 2006). A central concern is that changes in multiple nutrient sources will interact in non-additive ways, hindering our ability to predict the consequences for the function and structure of primary producer communities (Darling and Cote 2008). Disentangling these effects requires reductive understanding of the mechanisms of these processes independently and interactively. Our 2.5 yr experimental study provides novel perspectives to address this challenge by quantifying how seagrass ecosystem function and community structure are affected by simulated changes in two prominent sources of nutrients: anthropogenic nutrient supply from fertilizer and fish-mediated nutrients. Effects on seagrass ecosystem function were varied, but dominated by independent effects from anthropogenic nutrient supply (fertilizer). In contrast, community structure was most affected by independent effects from fish-mediated nutrients, as well as synergistic interactive effects among the nutrient sources. These findings offer a step toward understanding the implications of altered resource dynamics and consequently the applicability of ecological theory for the conservation and management of these imperiled ecosystems.

One of the more interesting aspects of our study was that non-additive effects of reduced fish nutrient supply and anthropogenic nutrient enrichment, i.e., indicating different responses by the seagrass community to fish-derived and fertilizer nutrients, were found for only one characteristic of seagrass ecosystem function, the amount of N stored by the dominant seagrass species, *T. testudinum*. In this case, anthropogenic nutrient supply was related to increased N stored per unit biomass, but the size of this effect was reduced (an antagonistic effect) with reduced fish nutrient supply. The lack of non-additive responses for *T. testudinum* beyond that for %N, suggests that anthropogenic nutrient enrichment can, to a large extent, supplant the loss of nutrients from fish nutrient supply. Given the differences in relative amount of nutrients being supplied between fertilizer and fish and differences in their ratio, these findings contrast with our expectations that these differences would lead to significant effects on seagrass traits. Instead, these findings

support the idea that *T. testudinum* is a highly plastic species that can tolerate a range of nutrient conditions, a notion that is supported by the fact that, despite widespread coastal eutrophication throughout its geographic range, *T. testudinum* often still thrives in eutrophic conditions (Fourqurean and Zieman 2002).

Consistent with this finding, the ratio of nutrient supply was also a poor predictor of seagrass structure and ecosystem function. One hypothesis is that the ratio of nutrient supply by these sources are sufficiently different to promote shifts in competition and thus affect community structure. Fish communities within coastal ecosystems of the Caribbean tend to release nutrients at a relatively high N:P ratio, typically ~25 N:P molar (Allgeier et al. 2014). In contrast, certain sources of human derived nutrients, in particular sewage, typically range from ~5-15 N:P molar (Downing and McCauley 1992, Justic et al. 1995, Sterner and Elser 2002). Despite our ability to effectively simulate these nutrient ratio conditions, we found it was an important driver for only one attribute of *T. testudinum*—storage of P in tissue (even in this case the best model that included N:P explanatory variables performed only slightly better than models with N supply rate). However, this finding should be interpreted with caution because low *ratios* of nutrient supply are somewhat confounded because they are also consistent with high *rates* of nutrient supply, due to limitations associated with replicating large experimental units. Nonetheless, the supply rate of N provided the best explanatory models for the two responses of *T. testudinum* that are most indicative of rates of production: growth rate and shoot density. These findings are at first perplexing given that primary production in carbonate seagrass ecosystems is typically P limited (Short 1987), and previous research from our study system supports this claim (Allgeier et al. 2010, 2011b). A plausible mechanism would be that any level of increased P input from either nutrient source (fish or fertilizer) effectively shifted the nutrient baseline by alleviating P limitation, superficially shifting *T. testudinum* to N limitation.

The preference of *T. testudinum* for certain species of N molecules may offer a complementary mechanism. Fish release N in the form of an ammonium complex, either NH₃ or NH₄⁺, whereby the vast majority is converted to NH₄⁺ upon reaction with the aquatic environment (Anderson 2001). In contrast, more than half of the N released by fertilizer (and in particular the type used in this study) is released in the form of nitrate (NO₃[–]; Broschat and Moore 2007). The reduced nature of NH₄⁺ makes it a more labile

compound for uptake by many producers. Previous work has demonstrated substantially higher affinity for NH_4^+ over NO_3^- by *T. testudinum* and other seagrasses, with substantially greater uptake rates of NH_4^+ (Short and McRoy 1984, Terrados and Williams 1997, Cornelisen and Thomas 2004, Alexandre et al. 2014), largely due to the physiological demands associated with processing NO_3^- (Touchette and Burkholder 2000). This provides an explanation why fish nutrient supply may have additional, and often interactive effects, on production beyond that of fertilizer alone. These findings collectively support the idea that despite the much higher rate of nutrient supply by fertilizers, fish-mediated nutrients are likely a superior nutrient resource for this foundation species.

In contrast to our findings on seagrass function, we found strong effects of fish nutrient supply on producer community structure. Most notably, reduced fish nutrient supply increased the richness and the evenness of the community, whereas the interaction of the reduced fish nutrient supply and fertilizer synergistically increased species evenness (Fig. 3A; Table 1). These findings somewhat contrast expectations of biodiversity theory in that richness and evenness would have been expected to have congruent positive responses under conditions anticipated to promote highest levels of ecosystem function (high fish mediated nutrients and low anthropogenic nutrients). One possible reason for this is that altered resource supply was sufficient to shift the competitive balance between interacting species (Burkholder et al. 2007). For example, the abundance of a cyanobacterial mat complex (see *Methods* section) and two algal groups, *Acetabularia* spp. and *Dictyosphaeria* spp., increased in low fish nutrient supply and anthropogenic nutrient enrichment treatments, resulting in higher levels of richness and evenness. *Dictyosphaeria* spp. and cyanobacterial mats are known as indicator species of poor ecosystem health, in particular nutrient enrichment, in tropical seagrass beds and coral reefs (Szmant 2002, Paul et al. 2005). These findings show that although anthropogenic nutrient enrichment enhanced production of the most dominant seagrass species in these systems, its interactive effects with reduced fish nutrient supply produce less predictable outcomes for the community as a whole. The consequences of this interaction for seagrass community structure will require further study over longer durations of time to fully understand.

Our study provides findings that are relevant for conservation in various ways. First, our treatments simulated two of the greatest stressors to coastal ecosystems: fishing pressure and nutrient enrichment, thereby providing insight into how these stressors may affect aspects of community structure and ecosystem function in coastal ecosystems (Justic et al. 1995, Rabalais et al. 2002). Fish provide a critical source of nutrients to these ecosystems and fishing pressure has been shown to reduce these pathways by nearly half (Allgeier et al. 2016). However, the ubiquitous increase in nutrients from anthropogenic sources provokes the question: can anthropogenic sources supplant losses of fish-mediated nutrients? Our study shows that while the dominant seagrass species tends to respond positively in the face of reduced fish nutrients and enhanced fertilizer input, the richness and evenness of the seagrass community as a whole does not. Specifically, our study shows that the interaction of these

two stressors increases prevalence of other competing species, and thus in the long run, may ultimately alter the prevalence of *Thalassia testudinum*—the foundation species of these ecosystems. Reductions in *T. testudinum* are now common and widespread throughout its range, with substantial implications for the function of these ecosystems and the services they provide (Orth et al. 2006).

Second, there has been considerable debate regarding the importance of managing for single or multiple nutrients (typically N and P) to mitigate the effects of eutrophication. Ecological theory would generally suggest the need to mitigate both nutrients, largely due to their potential for non-additive effects. From a management perspective, this is much more challenging than focusing efforts on mitigating a single nutrient. Our study provides important insight toward this end by showing that no single nutrient best explained the majority of responses. Most notably, measures of community structure were best explained by the primary limiting nutrient to this system (P), whereas measures of growth and density of the dominant producer in the system were best explained by N. Thus, while our findings support aspects of theoretical expectations, the complexities of producer community responses belie broad generalities, underscoring the need to manage for multiple simultaneous nutrients in these imperiled coastal ecosystems.

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