Variation in nutrient limitation and seagrass nutrient content in Bahamian tidal creek ecosystems

Jacob E. Allgeier [a,*], Amy D. Rosemond [a], Craig A. Layman [b]

[a] Odum School of Ecology, University of Georgia, Athens, GA 30602, United States
[b] Marine Sciences Program, Department of Biological Sciences, Florida International University, 3000 NE 151st Street, North Miami, FL 33181, United States

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ABSTRACT

The traditional model of nutrient availability in coastal estuarine ecosystems is based on predictable inputs of nitrogen (N) and phosphorus (P) via riverine and oceanic sources, respectively. But coastlines with low nutrient input from these sources may not fit into this simple framework. Here we use observational (seagrass nutrient content) and experimental (nutrient enrichment assays) data for assessing nutrient availability and limitation for primary producers along a spatial transect extending from the mouth (nearest to the ocean) to the terminal portion (boundary with the terrestrial ecosystem) of three coastal mangrove-lined tidal creeks in The Bahamas. Compiling seagrass nutrient content from all sites showed a negative relationship between seagrass nutrient limitation (either N or P) and distance from mouth, but this pattern differed across sites with respect to which nutrient was more limiting. Our experimental results demonstrated patterns of decreased response by microalgae to dual nutrient enrichment in one site with distance from the creek mouth, and increased response to single nutrient enrichment in another, with the third showing no trend along this gradient. Our findings show that Bahamian mangrove wetlands are extremely nutrient-limited ecosystems, and that the most limiting nutrient varied among sites. In general, these ecosystems deviate from the typical paradigm of spatial nutrient limitation patterns in estuaries. We suggest that various site-specific biological and physical factors may be more important than large-scale hydrologic factors in driving trends of nutrient availability in coastal ecosystems under strong nutrient constraints, such as in The Bahamas. Our findings suggest that even minor changes in nutrient loading rates can have significant implications for primary production in subtropical oligotrophic systems.

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1. Introduction

Biogeochemical pathways that determine the availability of nutrients provide a basis for understanding key factors that constrain primary production (Elser et al., 2007; Hecky and Kilham, 1988; Tank et al., 2007). In coastal ecosystems, the traditional model of nutrient availability predicts that the primary sources of phosphorus (P) are through the delivery of nutrient rich (at a low ratio of N:P) waters from coastal upwelling (Fourqurean and Zieman, 2002; Howarth et al., 1988; Smith, 1984). Nitrogen (N) is predicted to enter the system through multiple pathways, including riverine transport of water with high N relative to P (high N:P ratio) and via N-fixing microbes that may be common in the shallow benthos (Fourqurean and Zieman, 2002; Howarth et al., 1988; Smith, 1984). Thus, in coastal systems, the predicted trend is that P limitation increases with proximity to land and N limitation increases with proximity to the open ocean. This model has been supported in many temperate and subtropical coastal ecosystems (Fourqurean and Zieman, 2002; Howarth et al., 1988; Howarth and Marino, 2006; Paerl, 2009; Smith, 1984; Smith and Atkinson, 1984).

Various characteristics of The Bahamas suggest that this model may not hold for coastal waters in the archipelago. The Bahamas are characterized by little topographic relief, a lack of riverine networks, porous karst geology, and extremely nutrient poor soils, factors that typify many non-volcanic islands (Buchan, 2000). These factors suggest that the primary source of N inputs (i.e., rivers) may be lacking. The Bahamas are also flanked by large shallow banks that may preclude oceanic upwelling (Buchan, 2000). Further, P has strong tendencies to adsorb to calcium carbonate sediments (Lapointe and Clark, 1992), which may occlude the delivery of this nutrient via tidal exchange, especially in shallow ecosystems where the sediment surface area to water volume ratio is relatively high. Alternative models may be needed to explain patterns of nutrient limitation in systems with such characteristics.

Here we use observational (seagrass nutrient content) and experimental (nutrient enrichment assays for benthic algae) data for assessing nutrient availability and limitation along a spatial gradient in shallow Bahamian coastal ecosystems. Specifically,

* Corresponding author.
E-mail address: jeallg@uga.edu (J.E. Allgeier).

seagrass nutrient content was quantified in conjunction with nutrient limitation assays of microalgae, in three tidal creek ecosystems, along a transect extending from the mouth (i.e., the confluence of the creek and nearshore ocean) to the landward margin of each creek (herein referred to as the tidal creek terminus). In general, given that P has less potential inputs to the system than N, we predicted that P limitation would dominate relative to N limitation throughout the tidal creeks, but that the degree of P limitation would increase (or that P availability would decrease) with distance from the ocean. Due to the lack of riverine input, and thus the lack of landward source of N, we predicted that the degree of N limitation would not be related to the distance from the open ocean. In this context, our research seeks understanding of nutrient limitation regimes in these exceptionally nutrient poor ecosystems (Allgeier et al., 2010) that remain less studied than temperate and subtropical systems with higher levels of nutrient input.

2. Methods

2.1. Site description

The study was conducted between March 3 and 29, 2008, in three relatively pristine mangrove-dominated wetlands on Abaco Island, Bahamas, locally known as "tidal creeks": Barracuda Creek (BC, ~4.2 km^2), Jungle Creek (JC, ~0.28 km^2) and Sucking Fish Creek (SF, ~0.7 km^2) (Fig. 1). These creek systems are typically characterized by a relatively narrow creek mouth (Fig. 1) that is the primary conduit for tidal exchange (~0.8 meter tidal amplitude, 6 hour tidal regimes). Creeks typically broaden moving landward from the mouth, grading into expanses of shallow (<0.5 m at low tide) wetlands with red
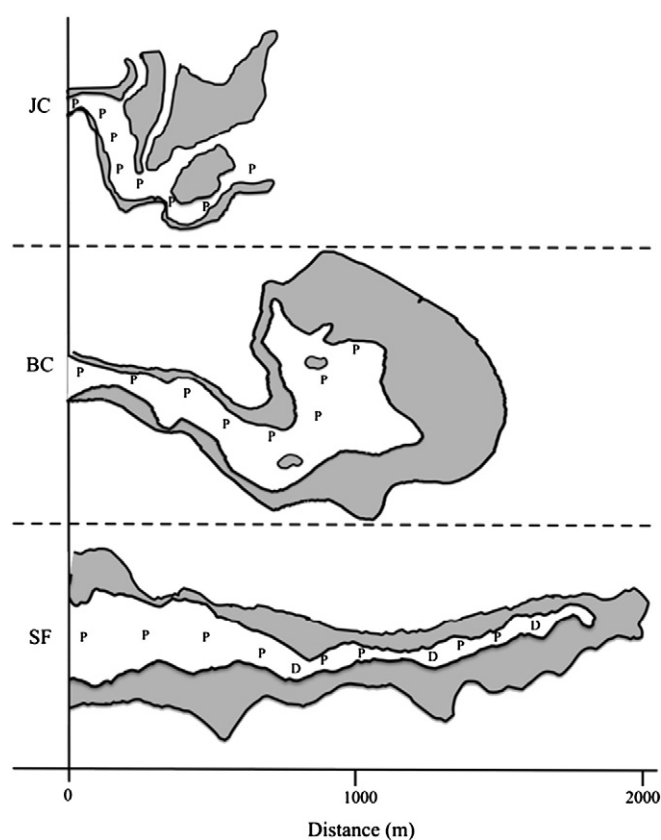

Fig. 1. Schematic of the three focal tidal creeks. The outline represents the mangrove fringe for each creek. Note that JC lacks a true terminus found in the other sites. The X-axis indicates distance from mouth. The letter 'P' indicates the location of plots within each creek. The letter "D" indicates relatively deep pools (>0.5 m at low tide – see text for more details).

Table 1
Mean values ($\pm$ SE) of ambient water column nutrients (µg/L) and salinity (parts per thousand) for the three sites. Each value represents the mean of eight samples taken at each plot. Samples were collected in March 2008. Samples below the detection limit of the machine (<0.5 µg/L) are indicated by "bd". For calculation of mean values, "bd" values were assumed to be zero.

Site	SRP	RDP	NO$_3^-$-N	NH$_4^+$-N	Salinity
BC	0.9 ± 0.9	4.3 ± 3.3	3.0 ± 1.3	4.9 ± 0.5	34 ± 0
JC	bd	2.9 ± 1.6	3.03 ± 1.4	5.2 ± 0.3	34 ± 0
SF	0.9 ± 0.5	bd	0.3 ± 0.9	8.1 ± 0.9	40 ± 0
Means	0.60 ± 0.3	2.4 ± 1.27	2.13 ± 0.91	6.06 ± 1.03	36 ± 2

mangrove (*Rhizophora mangle*) as the primary above-ground vegetation. The tidal creeks selected for this study were all characterized by the same habitat types (i.e., mangrove fringe, seagrass beds and sandy substrates), but were not completely uniform in physical characteristics (i.e., shape, size and diameter of main creek channel) (Fig. 1). The only freshwater input to these systems is via direct rainwater (as opposed to rivers). The creeks were surrounded by land that is devoid of residential, industrial or agricultural land-use, and thus were assumed to have relatively low anthropogenic nutrient inputs ("north bight" site from Stoner et al., 2011). Ambient nutrient concentrations are extremely low and show little spatial variation (e.g., change very little across the gradient from creek mouth to terminus, Table 1), presumably because of rapid uptake of nutrients from the water column by producers. More information on these tidal creeks can be found in Layman et al. (2004), Valentine-Rose et al. (2007), Allgeier et al. (2010) and Hammerschlag-Peyer et al. (2010).

2.2. Experimental Design

We investigated nutrient limitation observationally and experimentally using two important primary producer groups within tidal creek ecosystems, seagrass and benthic algae. First, nutrient content of seagrass blades has been widely used to assess nutrient availability in coastal ecosystems (Atkinson and Smith, 1983; Duarte, 1990, 1992; Fourqurean and Zieman, 2002). Seagrass nutrient content is believed to reflect ambient nutrient conditions over a relatively long time frame (i.e., months) as compared with ambient water nutrients.

While observational data is useful for assessing relative nutrient availability on this time scale, experimental enrichment of benthic algae can provide a complementary approach for understanding which nutrient(s) limit primary production. Nutrient diffusing substrates (NDS) have been widely used to determine nutrient limitation for primary production of benthic algae (Allgeier et al., 2010; Tank et al., 2007; Tank and Dodds, 2003). NDS are short-term enrichment experiments using benthic microalgae biomass accrual as the response variable. The nutrient treatment that elicits the largest algal biomass response, indicates the nutrient(s) that is most limiting to this suite of producers. Because of the relatively short colonization time of benthic algae, NDS are predicted to measure nutrient limitation for a time frame on the order of days. Benthic algae are presumed to be especially important for nutrient uptake and primary production, and to be a critical energy source to upper trophic levels in these systems, and thus are a useful proxy to measure aspects of nutrient limitation in these systems (Allgeier et al., 2010; Johnson et al., 2006; Layman, 2007). It is important to note that these two producer groups may acquire nutrients via differing means (see discussion), and thus may reflect different aspects of nutrient limitation in an ecosystem.

Our experimental design consisted of three sites, each with eight plots. At each plot, NDS experiments were conducted (Fig. 2), and samples of seagrass (where available) and water for nutrient analysis were collected. Seagrass, *Thalassia testudinum,* was collected (~20 blades) within a 10 m radius of each plot, taking care to use live leaves of similar size for consistency. Comparing nutrient content is most
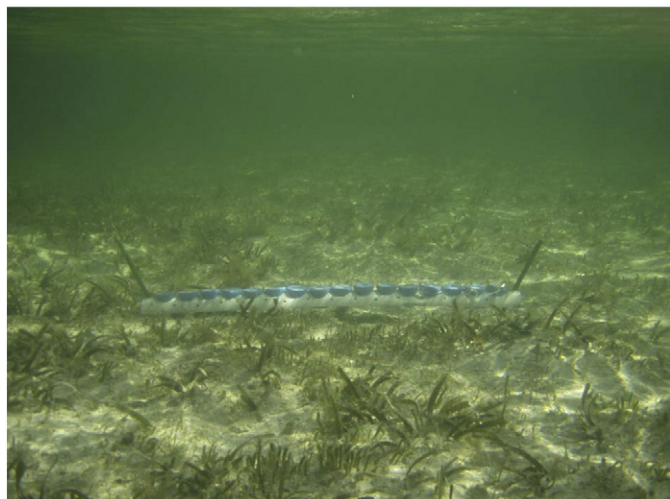
Fig. 2. Nutrient diffusing substrates during deployment.

useful with the same species of seagrass (Duarte, 1990), thus our analysis was restricted to *T. testudinum*. However, because *T. testudinum* was not present at every plot, each site was restricted to only 6 of the total 8 plots for seagrass nutrient analysis. Samples were frozen and transported to the University of Georgia for processing. All leaves of *T. testudinum* were scraped to remove epiphytes, rinsed in deionized water, dried in drying oven at 65°F for 72 h and then ground to a powder with a ball mill grinder. Ground samples were analyzed for N content with a CHN Carlo-Erba elemental analyzer (NA1500) and for P using dry oxidation-acid hydrolysis extraction followed by a colorometric analysis (Fourqurean et al., 1992). Elemental content was calculated on a dry weight basis.

NDS experiments were conducted in small cylinder canisters (~5 cm diameter × ~5 cm height) filled with agar and amended with the given treatment: N addition (0.5 M NH_4Cl), P addition (0.5 M KH_2PO_4), N + P addition (0.5 M NH_4Cl + 0.5 M KH_2PO_4), and a control (agar only) (see Allgeier et al., 2010; Tank et al., 2007 for further details). They were then capped with a porous ceramic crucible cover (Leco #528-042) for algae colonization. At each plot, 4 replicates of each of the 4 treatments (16 individual nutrient diffusing assays plot^{-1}; 384 assays total) were fixed to a long plastic bar and staked to the substrate, suspending the NDS directly above the substrate (Fig. 2). Each treatment was incubated at each plot for 24–26 days, after which little or no nutrient remains in the agar (Tank et al., 2007). In each of the three creeks, plots (n = 8 each) were evenly spaced along a transect from the mouth to the terminal end of the tidal creek (Fig. 1). The three creeks measured 600, 1300 and 1900 m, thus, there were different distances among plots. After incubation, NDS were collected and their respective crucible covers were placed in plastic bags, wrapped in foil, transported on ice and frozen for analysis. All experiments from a given site were collected on the same day. Chlorophyll *a* content ($\mu g\ cm^{-2}$ Chl *a*) of each sample was determined spectrophotometrically (Shimatzu 2100) for pheopigment corrected Chl *a* (APHA, 1995).

To determine background ambient nutrient concentrations (NH_4, NO_3, SRP and total dissolved phosphorus (TDP)) at each of the 8 plots within the 3 sites, water nutrient samples were taken at the end of the NDS incubation period. All samples were filtered through a 0.45 Whatman nylon membrane filter and all but NH_4, were frozen and transported to University of Georgia for analysis. NH_4 was analyzed within 12 h of collection fluorometrically, following Holmes et al. (1999) as modified by Taylor et al. (2007). Total dissolved phosphorus (TDP) was analyzed using the persulfate digestion method and SRP and NO_3 were determined using continuous flow colorimetry.

2.3. Data analysis

2.3.1. Seagrass nutrient content

To assess strength of nutrient limitation with respect to relative distance from the mouth of the tidal creek, irrespective of the nature of limitation (i.e., either N or P limited), we applied the Limitation Index (LI = |30–N:P|) (Campbell and Fourqurean, 2009). This index has been used to quantify the degree of divergence from the theoretically ideal 30:1, referred to as the Seagrass Redfield Ratio (Atkinson and Smith, 1983; Duarte, 1990), whereby the larger the LI value, the greater the degree of limitation by either single nutrient (Campbell and Fourqurean, 2009). By taking the absolute value, the metric allows the determination of overall limitation by highlighting the imbalance in the quantity of N relative to P, irrespective of which nutrient was relatively lower in nutrient content.

A multivariate analysis of covariance (MANCOVA) was used to assess the relative importance of site and distance (covariate) on the three response variables associated with *T. testudinum* nutrient content (%N, %P) and LI. Data were transformed to meet assumptions of normality and homoscedasticity. Because there was a significant effect of distance in the model, linear regressions were used to assess the relative importance of distance for each of these variables within each site as well as across all sites.

2.3.2. Microalgal nutrient limitation

A multi-factor analysis of covariance (ANCOVA) was used to test for effects of site, treatment (N, P, N + P) and distance from mouth as the covariate (including all interactions with treatments and distance) on Chl *a* from the NDS experiments (Tank et al., 2007). Tukey HSD post hoc tests were conducted to assess differences between treatments within and among sites. Chl *a* data were log_{10} transformed to meet assumptions of normality and homoscedasticity. Linear regressions were used to compare the relationship between the response for each NDS treatment and distance within each site and across all sites. To normalize for differences in control values among sites for the NDS treatments, we calculated the response ratio RR_x as an effect size metric, where $RR_x = ln\ (X/Cont)$ and X is the response of a given treatment (here N, P or NP) and Cont is the response of the control (Hedges et al., 1999; Tank and Dodds, 2003). RR_x values were regressed against distance (their relative location in the tidal creek) for all sites combined as well as each site separately. All analyses were performed using R software (R Development Core Team 2008).

In environments with low ambient nutrients, the primary producer response to NDS allows inference as to both nutrient

Table 2
Summary table of mean values (± 1 SE) for (1) seagrass nutrient content reported as percent of total mass (%N, %P), (2) the Limitation Index (LI), and (3) RR_x (response ratio) of a given treatment for the NDS experiments. For clarity, an RR_x of 1 indicates that the response to treatment X was 2.7 times greater than the response to the control treatment. Max Dist indicates the total length of the study tidal creek (m).

Site	%N	%P	LI	RR_N	RR_P	RP_{NP}	Max dist
BC	2.02 ± 0.04	0.06 ± 0.01	21.80 ± 7.00	0.35 ± 0.17	0.16 ± 0.12	2.61 ± 0.16	1162
JC	2.01 ± 0.08	0.11 ± 0.005	11.26 ± 1.28	0.24 ± 0.12	0.62 ± 0.13	1.98 ± 0.06	635
SF	1.65 ± 0.24	0.05 ± 0.009	8.15 ± 2.68	0.15 ± 0.08	0.32 ± 0.17	2.06 ± 0.15	1892

Table 3
Results from MANCOVA analysis used for seagrass nutrient content [%N, %P (as % total mass), and Limitation Index (LI)]. The global MANCOVA model results are first, followed by the subsequent ANCOVA model results for each independent response variable.

Global	Df	Pillai	F value	P-value
Site	2.00	1.37	5.38	<0.001
Distance	1.00	0.73	6.07	0.01
Site distance	2.00	1.14	3.33	0.01
Error	12.00			
LI	Df	Mean Sq.	F value	P-value
Site	2.00	1.21	5.05	0.03
Distance	1.00	4.05	16.90	<0.001
Site distance	2.00	0.70	2.92	0.09
Error	12.00	0.24		
%N				
Site	2.00	0.02	1.94	0.19
Distance	1.00	0.10	10.00	0.01
Site distance	2.00	0.05	4.64	0.03
Error	12.00	0.01		
%P				
Site	2.00	0.00	30.05	<0.001
Distance	1.00	0.00	16.13	<0.001
Site distance	2.00	0.00	3.64	0.06
Error	12.00	0.00		

limitation and the relative ambient availability of nutrients. The typical interpretation of nutrient limitation experiments is that a greater response to enrichment by a single nutrient indicates greater limitation of that nutrient. However, we suggest a complimentary interpretation that may hold true in ecosystems with extremely low ambient nutrient availability. Under these conditions, if the producer is provided with just one nutrient (e.g., in the N treatment), it would be expected that the response would be minimal given the lack of ambient availability of the other nutrient needed for growth (i.e., P in this example). However, if the availability of the other nutrient (in

this case P) increases, the producer response to single nutrient enrichment (N treatment) also would be expected to increase in proportion to the amount of available P. In other words, limitation of one nutrient should be positively correlated with increased availability of the other. In this case, increased RR_N could be interpreted as both increased nutrient limitation by N and/or increased availability of P. Importantly, such an interpretation is only valid in those ecosystems with extremely low ambient nutrient availability.

The term nutrient co-limitation has been subject to various interpretations and also requires specific clarification (Allgeier et al., 2011). Co-limitation implies that the producer or producer assemblage is functionally limited by both nutrients (Arrigo, 2005; Davidson and Howarth, 2007), and is demonstrated when the response to dual nutrient enrichment is greater than either single nutrient treatment (Allgeier et al., 2011). Conceptually co-limitation is a logical response given the need of both N and P for primary production. The proposed mechanism that leads to a co-limited response suggests that when producers are under conditions of dual nutrient enrichment, limitation may oscillate between the two single nutrients because the supply of one nutrient is sufficient to shift demand toward that of the other, next most limiting nutrient (Allgeier et al., 2011; Davidson and Howarth, 2007). This interplay continues until either another factor becomes limiting or a saturation state is reached (Allgeier et al., 2011; Davidson and Howarth, 2007). This interaction is often more prevalent (i.e., greater producer response to dual nutrient enrichment) under conditions of low ambient nutrient concentrations.

3. Results

3.1. Seagrass nutrient content

T. testudinum nutrient content varied within and among sites for %N and %P (Table 2). Percent N in seagrass was similar in BC and JC, but much lower in SF, whereas %P was much higher in JC than either BC or SF (Table 2). The range of values for %N were similar in comparison to the values of compiled data from the literature for percent nutrient
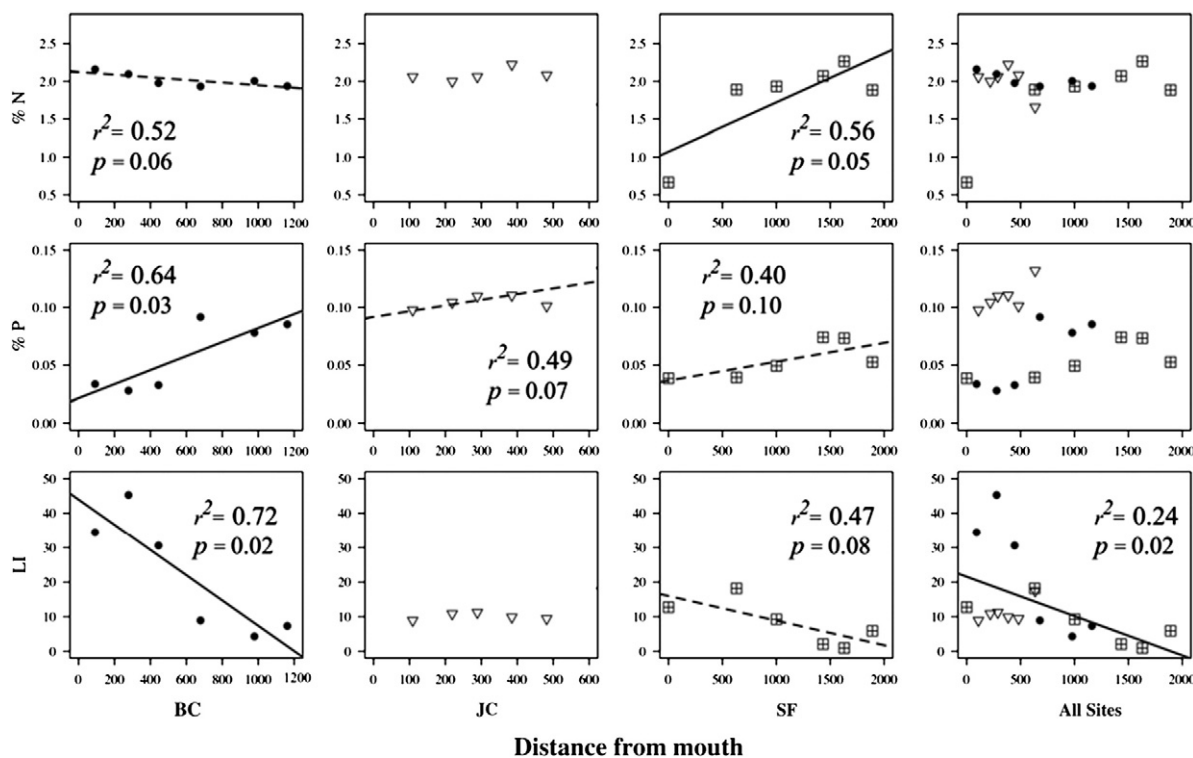

Fig. 3. Linear regressions for %N, %P, and Limitation Index (LI) and distance from mouth of tidal creek for sites (Barracuda Creek — BC, Jungle Creek — JC, and Sucking Fish — SF), as well as all sites combined (all sites). Solid lines indicate significance of $P<0.05$, and dashed lines indicate $0.05<P<0.1$.

Table 4
Results from the three-way analysis of covariance (ANCOVA) model used for NDS experiments.

	Df	Mean Sq.	F value	P-value
Site	2	2.16	15.49	<0.001
N	1	26.33	188.87	<0.001
P	1	32.85	235.64	<0.001
N P	1	15.42	110.60	<0.001
N distance	1	0.04	0.29	0.59
P distance	1	0.04	0.28	0.60
N P distance	1	0.26	1.87	0.17
Site distance	2	0.39	1.42	0.25
Distance	1	0.71	5.12	0.03
Error	84	0.14		

content of *T. testudinum* (mean ~1.9% for all studies, overall mean ~1.89 from this study), whereas %P values were substantially lower (~0.24% for all studies ~0.07 for this study) (Duarte, 1990) (Table 2).

There was a significant effect of site and distance, and their interaction on seagrass nutrient concentration in the MANCOVA model (Table 3). The effect of distance was significant for LI, %N and % P. The effect of site was significant for LI and %P, but not %N. Together, these findings suggest that nutrient availability varied both among and within sites. The interaction of site and distance was only significant for %N, suggesting that nitrogen availability differed with distance from mouth among sites (Table 3).

For the regression analyses, when combining all sites, there was a significant negative relationship between distance and LI. Within sites, the same relationship was found in BC and SF (marginally significant $p = 0.08$), but not JC. When comparing trends across all sites for %N or %P, no significant relationship was found ($p > 0.1$) (Fig. 3). Within sites, the only significant relationship between distance and %N was positive in SF (Fig. 3). A positive significant relationship between distance and %P was found at BC; in SF and JC this relationship was positive but only marginally significant (Fig. 3).

3.2. Microalgal nutrient limitation

We found strong nutrient co-limitation for microalgal production at every NDS experimental plot in our study (Table 2), with RR_{NP} ranging from 1.75 to 3.22 across creeks. With respect to the response to single nutrient assays, RR_N and RR_P were small in comparison to RR_{NP}, but varied substantially across creeks (−0.03 to 1.25 and −0.51 to 1.23, for RR_N and RR_P, respectively).

Among sites, and across the spatial gradient, there was a significant overall effect of nutrient enrichment on microalgae, but there were no significant interactions between distance and the individual nutrient treatment, nor between distance and site (Table 4). The response to NP was significantly larger than all other treatments within and among all sites (Supplementary Material 1), thus we concluded that all sites were nutrient co-limited (sensu Allgeier et al., 2011). A significant positive relationship between distance and RR_N, and distance and RR_P was found in BC (Fig. 4). A significant negative relationship was found in SF between distance and RR_{NP} (Fig. 4). No significant regressions were found with all sites combined for RR_N, RR_P or RR_{NP} versus distance (Fig. 4).

4. Discussion

We found variability in spatial trends of nutrient availability and limitation within and among three subtropical tidal creek ecosystems. While ambient nutrient concentrations in the water column provided only a snapshot view, nutrient diffusing substrates (NDS) and plant nutrient content reflect nutrient availability and limitation over longer time frames (i.e., days to weeks and weeks to months, respectively) (Allgeier et al., 2010; Duarte, 1990, 1992). On one hand, our findings consistently demonstrate strong co-limitation (demonstrated by the NDS results) in all sites, supporting the idea that these ecosystems are extremely nutrient poor across the spatial scales examined. Yet, given the oligotrophic nature of these
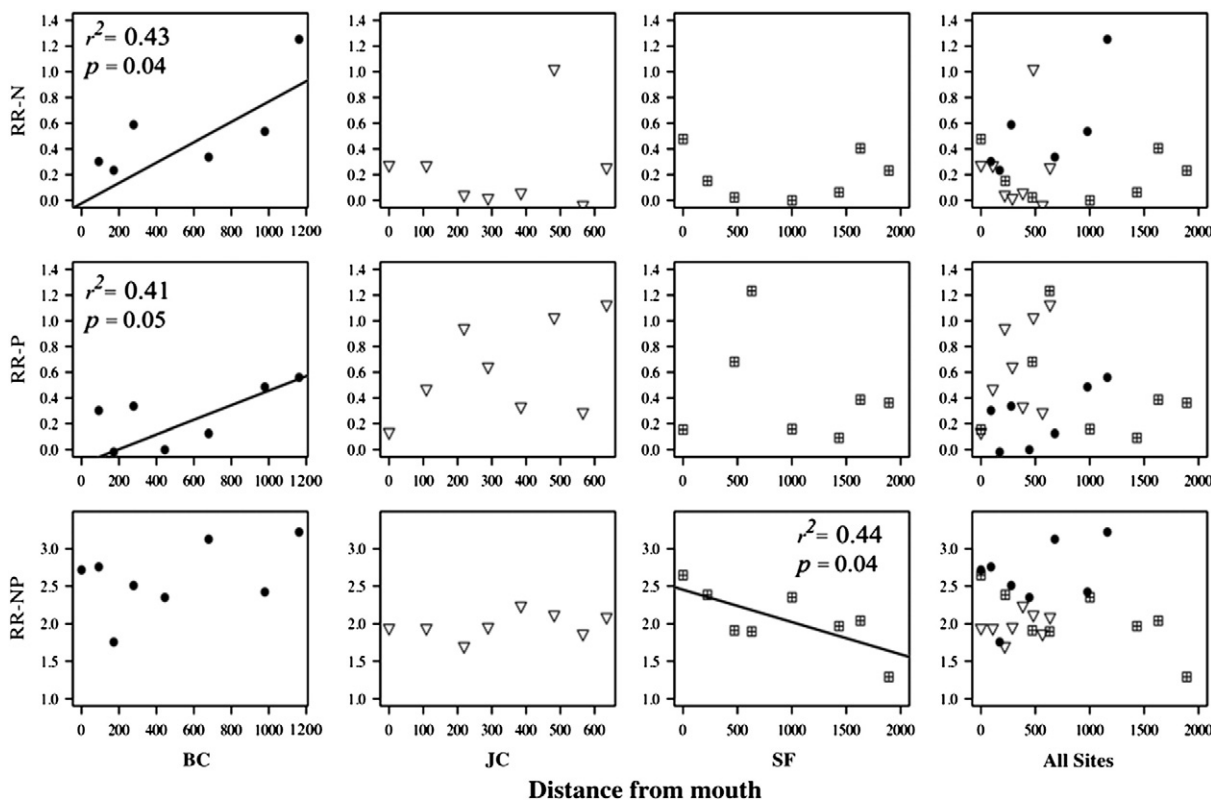

Fig. 4. Linear regressions for the response ratio for NDS treatments (RR_N, RR_P and RR_{NP}) and distance from mouth of tidal creek (Barracuda Creek — BC, Jungle Creek — JC, and Sucking Fish — SF), as well as all sites combined (All sites). Solid lines indicate significance of $p < 0.05$.

ecosystems, co-limitation would be expected. As such, interpretation of the single nutrient treatments in conjunction with the seagrass nutrient content data may be revealing as to the relatively availability of each nutrient in these ecosystems. The trends for seagrass nutrient content and single nutrient limitation (i.e., found with the single nutrient NDS treatments) observed were not uniform across sites, suggesting substantial spatial heterogeneity in nutrient availability and supply, though some support was found for increased nutrient availability with distance from the open ocean. Our findings demonstrate that these subtropical tidal creek ecosystems may deviate from traditional models of nutrient supply to coastal ecosystems (Fourqurean and Zieman, 2002; Howarth et al., 1988; Howarth and Marino, 2006; Paerl, 2009; Smith, 1984; Smith and Atkinson, 1984).

The strongest support for the pattern of increased nutrient availability with distance from the open ocean is illustrated by the *T. testudinum* nutrient content data. The Limitation Index (LI) demonstrated that nutrient limitation across all sites was negatively related with increased distance from ocean (Fig. 3), and similar negative relationships were found in two of the three sites (BC and SF, $p = 0.02$ and $p = 0.08$, respectively). Interestingly, the nutrient that seemed to increase in availability with distance differed between these two sites (%P for BC and %N for SF; SF was also marginally significant for %P), as the LI essentially provides a measure of the magnitude of limitation, but it does not distinguish which nutrient is limiting. While various mechanisms may contribute to these divergent patterns, we suggest that site-specific biological and physical characteristics were critical for regulating local nutrient dynamics, as we discuss below.

Findings from the *T. testudinum* nutrient content data were somewhat consistent with NDS experiments. For example, an increase in nutrient availability with increased distance, as suggested by the LI at both SF and BC, may be predicted to be accompanied by a decrease in RR_{NP}. Yet, this was only seen in SF. In BC, however, there was a significant positive relationship with both RR_N and RR_P, and distance. In JC no relationships were apparent. It is possible that spatial trends in percent nutrient content of seagrass and NDS are not consistent with one another because physiological differences may underlie fundamental differences in the nature of nutrient limitation between these primary producer groups. For example, in addition to nutrient uptake through leaves, seagrasses acquire a substantial amount of nutrients through their root structures via pore water and thus have access to a fundamentally different pool of nutrients than epibenthic algae (Short, 1987; Touchette and Burkholder, 2000).

Our findings from the NDS experiments across sites at first seems to be contradictory, as nutrient limitation increased with distance in BC (for both RR_N and RR_P) and decreased with distance in SF (for RR_{NP}). However, distinctions between nutrient limitation and availability may be subtle (Allgeier et al., 2011). Because the ambient availability of nutrients in these ecosystems is so inherently low, and primary producers are constrained relatively by both not just one nutrient (i.e., as suggested with the relatively high RR_{NP}), an increased response to single nutrient enrichment may indicate greater availability of the other nutrient. This mechanism has been demonstrated previously whereby increased availability of P (in this case via excretion from fishes) enhanced the response to N enrichment (Layman et al., 2011). Though this interpretation diverges from typical interpretations of such results, we suggest this possible explanation should be considered in extremely nutrient poor ecosystems such as tidal creeks in The Bahamas.

We propose two primary factors may drive differences in spatial trends of nutrient availability among sites: consumer-mediated nutrient cycling and nutrient release associated with primary production. Consumers have been shown to provide critical supplies of nutrients to ecosystems via excretion in both freshwater and marine environments (Layman et al., 2011; Meyer et al., 1983; Vanni,

2002). As such, the presence of animals may provide a consistent supply of nutrients at a local scale in the creek ecosystems. For example, many tidal creek ecosystems have deeper pools that provide refugia for fishes during low tide events, and thus fishes are found concentrated in these areas for much of the tidal cycle (Hammerschlag-Peyer and Layman, 2010; Rypel et al., 2007). This situation is likely in SF, where back reaches of the creek have multiple deep pools that host resident fishes (Allgeier, *pers obs*; Fig. 1). These findings are consistent with higher %N and %P in seagrasses at sites far from the creek mouth, which indicates that there is a greater availability of ambient nutrients for luxury uptake by these producers near fish refugia pools (Fig. 3). The overall decrease in RR_{NP} with distance in this site (Fig. 4) also supports this idea because increased availability of nutrients in the water column via fish excretion may decrease benthic algal net response to dual nutrient enrichment relative to the control (Layman et al., 2011).

Primary producers can also provide a significant source of nutrients to ecosystems (Capone et al., 1979; Valiela, 1995; Wetzel, 2001). For example, N fixation via associated epiphytic algae and microbes has been shown to provide an important source of N in shallow seagrass ecosystems (Atkinson and Smith, 1983; Capone et al., 1979; Touchette and Burkholder, 2000). Similarly, biogeochemical interactions in the rhizosphere of aquatic plants (i.e. respiration and other oxidative processes) in carbonate environments can liberate hydrogen ions, decrease pH, and release sorbed phosphate making it available for uptake (Jensen et al., 1998). Large stands of mangroves (primarily *Rhizophora mangle*) and dense seagrass beds may facilitate release of P from sediments providing an additional source of that limiting nutrient. BC in particular, had extensive mangrove wetlands that extend well beyond the terminus of the primary channel (Fig. 1). In BC the increase in availability of P with distance from ocean was pronounced as both %P (~5 fold increase from mouth to terminal end) in seagrass and the algal response to enrichment by N (potentially indicating increased availability in P, see above) increased substantially with distance from the ocean. We feel that the mechanism of biogeochemical release of P via primary producers, as opposed to consumer nutrient supply, is particularly important in BC because it lacks the low tide refugia pools for fishes (discussed above).

The apparent lack of spatial trends found at site JC is noteworthy with only one regression (%P, $p = 0.06$; Fig. 3) suggestive of a trend. We attribute this to the lack of "boundedness" (sensu Post et al., 2007) of this creek system. Though JC did have a distinct linear spatial gradient of increasing distance from the open ocean, there was no clear 'terminus'. Instead, the landward portion of the creek splits into two branches running parallel to the land margin, with each branch connecting back to other creek channels and ultimately to the open ocean (Fig. 1). Tidal flushing in this site occurs through the mouth of the creek and these secondary channels. Further, this tidal creek is connected to a larger system in which multiple blue holes, deep fissures that hydrologically connect these nearshore environments to groundwater, are present. This intrusion of groundwater may provide an important source of nutrients to the system (Lapointe et al., 2004) and given the close proximity, may influence nutrient availability in JC. We suggest that these factors enhance connectivity allowing for a more uniform or mixed distribution of nutrients throughout, potentially explaining the lack of clear spatial patterns. Further, the increased hydrologic connectivity (relative to other sites) found in JC illustrates the potentially important role of hydrology in affecting nutrient limitation regimes.

Our focus on nutrient limitation largely ignores other factors that may limit primary production, for example, light availability and grazing intensity. Given the shallow nature of each tidal creek (only one plot in JC was at a depth greater than 0.75 m and most plots were ~0.5 m) we suggest that light limitation was negligible. Likewise, the role of grazing is an important factor to consider, especially given the relative importance of benthic algae for upper trophic levels (Johnson et al., 2006; Layman, 2007; Valentine-Rose et al., 2007). However,

because all of our plots were located in similar habitat types, depth and, with the same community of mesograzers (Allgeier, *pers obs.*), we believe that while grazing certainly occurred, there is no reason to expect substantial variation in grazing intensity across study plots.

Our findings characterize a heterogeneous, yet exceptionally nutrient poor, ecosystem that diverges from the traditional model of nutrient supply to coastal ecosystems. We suggest that numerous local biological and physical factors mediate nutrient supply in these tidal creek systems, which in turn, dictate the heterogeneous and variable nature of nutrient limitation across sites. Further, despite the apparent trends associated with single nutrient limitation within sites, nutrient co-limitation nonetheless was dominant throughout, indicating that none of the pathways of nutrient supply were sufficient to fundamentally shift producers away from co-limitation to single nutrient limitation. Because these ecosystems are under such strong nutrient constraints, even minor increases in nutrient inputs could fundamentally alter primary producer community structure and associated ecosystem functions. Further research is needed in these nutrient limited ecosystems that are relatively understudied compared to coastal ecosystems characterized by more predictable nutrient inputs (i.e., continental temperate coastal ecosystems).

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